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AIM AND SCOPE

Crossian Resonance, the multi-disciplinary peer-reviewed and refereed national journal launched in June 2010, strives to reach research scholars from all directions and various cross-sections of society providing a platform to resonate scientific findings. It aims to foster the spirit and aspirations of the academics and to promote a research culture among the erudite. The sustainability and success of the research journey is a step ahead in the 50th milestone of our institution playing a strategic role and nurturing the fruitful service of quality and need-based education.

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Literature and Ideology: A Marxian Study of Devi's *Outcast : Four Stories*

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ABSTRACT

Marxist literary criticism maintains that a writer's social class, and its prevailing 'ideology' have a major bearing on what is written by a member of that class. Over the years, literature and ideology, both individually and in tandem, have evolved as epistemic-discursive categories. Mahasweta Devi spearheaded the movement against the industrial policy of the earlier communist party of India (Marxist) government of West Bengal. Specifically, she stridently criticized the confiscation of large tracts of fertile agricultural lands from the farmers by the government and ceding the land to the industrial houses at throwaway prices. This paper is an attempt to showcase how 'ideology' undergoes an unprecedented expansion in the writings of Devi with reference to her female characters in her short story collection Outcast: Four Stories

Keywords: ideology, literary aesthetics, colonization, patriarchy, oppression, exploitation

Marxist literary criticism maintains that a writer's social class, and its prevailing 'ideology' have a major bearing on what is written by a member of that class. So instead of seeing authors as primarily autonomous 'inspired' individuals whose 'genius' and creative imagination enable them to bring forth original and timeless works of art, the Marxist sees them as constantly formed by their social contexts in ways which they themselves would usually not admit. Over the years, literature and ideology, both individually and in tandem, have evolved as epistemic-discursive categories.

Mahasweta Devi, a Padmashree award winner and social activist is a renowned Bengali writer who portrays the life of the tribal people and more specifically the marginalized characters especially women on social margins of class and caste. She has been studying and writing incessantly about the life and struggles faced by the tribal communities in the states like Bihar, West Bengal, Madhya Pradesh and Chattisgarh. She has wholly involved herself to work for the relief of the tribal people from these struggles. She often narrates the brutal oppression faced by the tribal people at the hands of the powerful upper caste persons comprising of landlords, money lenders and government officials.

Devi spearheaded the movement against the industrial policy of the earlier communist party of India (Marxist) government of West Bengal. Specifically, she stridently criticized the confiscation of large tracts of fertile agricultural lands from the farmers by the government and ceding the land to the industrial houses at throwaway prices. "A responsible writer,

standing at a turning point in history, has to take a stand in defense of the exploited. Otherwise history will never forgive him" (Beniwal and Vandana 84).

The endings of Devi's stories reflect another aspect of her literary aesthetics. When read on a macro level, her stories can be seen as different takes on the composite reality. Showcasing the different permutations and combinations of the same narrative trajectories, her stories seem to form a creative-critical continuum along which the different tales merge into each other, reinforcing and sedimenting the sub-strata of her literary vision. This pattern of parallels and repetition, instead of being indicative of a narrow range of concerns, signifies the expansive fluidity of Devi's vision.

A deeper probe into the supposed commonality of her stories reveals a definite progression in the evolution of Devi's activist world-view and her solutions. The stories that initially indicated a simple, linear narrative which terminated in closed endings, evolve into ambivalent and ambiguous open-ended constructions in the more mature phase of her career. This progression reflects the phases in the development of India as a nation from a homogenous, monolithic, unilinear concept to a cacophonous, diffuse entity, incorporating tensions and collisions within its ambit. The successive progression of her characters through her narratives not only closely parallels the dynamics of India as a nation in transition, but also reflects an increasingly mature response of the subaltern towards this problem of the nation.

Devi's *Outcast* showcases four stories with women protagonists, three of whom are of adivasi descent. The stories show Devi's continuing preoccupation with the plight of the tribal people.

Rape occurs as a recurring trope that underwrites the fate of the protagonists and their disenfranchisement in post-Independence India in the first three stories. Dhouli is Dusad's daughter, and a young widow. Misrilal, a Brahmin's son, falls in love with her. Dhouli knows the fate of the women of her community who fall prey to the lust of the Brahmin family and repulses him. But Misrilal convinces her of his love and promises to marry her, saying that "the government law too sanctions our marriage" (Dhouli 12). Being so young Dhouli cannot resist him and she gets pregnant. But when Misrilal's family comes to know about it, they send Misrilal away and subject Dhouli and her mother to abject humiliation. The fate of such a woman in her community depends on the attitude of the Brahmin family. If the Brahmin family takes pity on the victim and hands out doles, she is left in peace. Otherwise she is made an outcast and is compelled to become a whore. Dhouli holds out as long as it is

possible for her, but sheer hunger drives her to become a common prostitute. This invites the wrath of the Brahmin family on Dhouli and she is driven out of the village because she has brought dishonour to them, by allowing "the door through which the lion entered" to be "visited by rats and swine!" (Dhouli 30).

Shanichari is an oraon girl from Rata whose story is told against the background of the Adijati Raksha Movement. The tribal people are displaced from their area and are compelled to migrate to Kolkata to work in the brick kilns in inhuman conditions. Shanichari resists going to Kolkata but during the movement when the BMP, CRP and BSP unleashed a reign of terror, they subject her to gang rape along with the other women and leave them in the jungle without food or clothing: "Without clothes, the girls are forced to hide in the forest...'we'll get you new clothes and take you to Kolkata to work in the brick kilns. you'll work hard, eat well, make money. Come, come!'" (Shanichari 46-47). Eventually Shanichari is compelled to seek work in the brick kiln where she is raped daily by Rahmat, the brick kiln owner. His friends, Cronies, the local goons, and even the police get their fair share of the female flesh, and of course, the cuts. The brick kiln is insulated from the world outside and there is no way escape. However, the brick kiln shuts off and Shanichari returns to the village with Rahmat's child in her womb, and is made an outcast.

The "Fairy Tale" is unbearably real. It presents another face of exploitation of the tribals. Josmina is a HO tribal. Though poor she lived a contented life in Rajabasha with her husband, Sarjom Purti. But the village money lender, Nandlal Shahu who is part of an elaborate racket for supplying cheap coolie labour to Punjab, lures them with the dream of higher wages. He sells them off to Niranjan Singh. They have to work for eighteen hours a day. Niranjan rapes Josmina daily. Somehow, Josmina and Sarjom Purti are able to escape and take refuge with Karnal Singh. But the same story of rape and exploitation is repeated. When they finally escape and come back to Rajabasha,Josmina has a child in her womb. Fearing the inevitable ostracization she jumps into Koyena and drowns herself.

The situations depicted by Devi in the above stories are unbearably grim and are a devastating indictment of the social system that allows such injustice to perpetuate. It seems as though rape has become an inevitable fact of daily life for the tribal women who venture out of their natural habitat as they have to for their livelihood. One must remember that in the tribal society, rape is extremely rare and women enjoy a status of honour. What makes these incidents more culpable is the fact that the whole administrative machinery is complicit with it.

Devi's oeuvre subverts the grand narratives of colonization, patriarchy, caste system and class division. She depicts the fate of women who are twice colonized and victimized in the Indian social system but resists the term feminism as she proficiently displays the exploitation of the lower class and tribal men also, as her emphasis is on class and not on gender. Her work does not appear to be written by a middle class woman who empathizes with the tribals and the adivasis and the lower classes, but, as one who has herself suffered the oppression and exploitation by the elite. She questions and re interprets the great Indian epics and myth that are used to exploit and oppress the lower strata of the society. A fearless crusader for social justice, Devi's characters rebel against the parameters of acceptability set down by the society. Told lucidly, her stories chill the bone with their stark simplicity and brutal honesty. As a journalist, she steadfastly criticizes the failure of both the state and the central government policies through the daily newspapers. Her quarterly *Bortika* raises a voice against the violence, oppression, and exploitation of the lower classes and the adivasis. Along with educating the masses, Devi's message of revolution and change, has affected the policies of the government of India but the state government has not acknowledged her fiery spirit. In short, she has used her pen as a sword to eradicate the evils of socio - economic customs and traditions. One may need several volumes to analyze and interpret the works of Devi.

Works Cited

Beniwal, Anup, and Vandana. "Writing the Subaltern at the Interface of Fiction and Ideology: An Engagement with the Works of Mahasweta Devi." *Quest*. July – Dec. 2006: 84-92. Print.

Devi, Mahasweta. "Dhouli" *Outcast: Four Stories*. Trans. Sarmistha Dutta Gupta. Calcutta: Seagull Books, 2002. Print.

Devi, Mahasweta. "Shanichari" *Outcast: Four Stories*. Trans. Sarmistha Dutta Gupta. Calcutta: Seagull Books, 2002. Print.

History in Her Story: A Study on Chimamanda Nagozi Adichie's *Half of a Yellow Sun*

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ABSTRACT

The present paper focuses on Chimanda Ngozi Adichie's merging of history in her fiction Half of a Yellow Sun. In the *recent years most of the Nigerian writers show a resurgence of interest in* bringing in *history in their narrative and this* results in *a reconstruction of the past from a present perspective. In this light, Adichie has placed her text in the context of history to give a frame work of the life* of *her people. The story is a combination of politics and personal relationships, charged by the brutal conflicts that occur around the main characters. Adichie's tactful portrayal of the characters and events reflect her meaningful blending of history* with *fiction. Through her narrative she creates a synergy between the past and the present. This becomes imperative because it is only through this synergy that she can reconnect to the past and recreate the future.*

Key Words: New Historicism, fiction, Nigerian civil war

History in Her Story: A Study on Chimamanda Ngozi Adichie's *Half of a Yellow Sun*

New Historicism is a fascinating new critical practice which shows a resurgence of interest in history. It is the reconstruction of the past from a present perspective. As Barry describes it in his book *Beginning Theory*, new historicism might be defined as "a method based on the parallel reading of the literary and non-literary texts. . . .instead of a literary 'foreground' and a historical 'background' it envisages and practices a mode of study in which literary and non-literary texts are given equal weight and constantly inform or interrogate each other" (116). New historicism therefore gives a greater understanding of the situation in which the text was written, instead of being considered of equal importance on its own. This paper looks at the intertextuality in Adichie's fiction using the theoretical lens of new historicism.

The approach of New Historicism argues that a work of literature does not exist devoid of its conditions or circumstances. In this light, literary works are as much a product of the author's mindset as well as the conditions that surround it. The historical and the social context of the author are almost as important as anything else in assessing the construction of a literary work. In this light, New Historicism is contingent, stressing its role in the development of literature.

From the Anglo-Saxon period to till today, literary texts have always chronicled the history of the people. History is a sort of memorial to the achievements and endeavours of the

people or the community either collectivity or as an individual. The scope of history is as wide as it is complex. In literature the concept of history emerged many years ago. Historical incidents like war, have been the main focus of the earlier writers. In the works like Homer's *Iliad* and the *Odyssey*, Virgil's *The Aeneid*, Aeschylus's *Agamemnon*, Shakespeare's war tragedies like *Macbeth, Julius Ceasar, Othello* and *King Lear*, readers find the concept of war. In the most recent years readers find the merging of history into the literary text of African literature. Many writers in Africa are re-writing the history of colonization in their works. Chinua Achebe and Ngugi Wa Thiango are the African writers who reconstructed the colonial history in their writings *Thing*s *Fall Apart* and *Weep not Children* respectively. The Nigerian novelists establish a synergy between the past and the present. This becomes imperative because it is only through this synergy that they can reconnect the past and recreate the future. Most of the Nigerian novelists attempt to reawaken history though their works. The Nigerian Civil War has been featured in the contemporary Nigerian novels. The Nigerian novels in particular can be regarded as historical novels because most of the Nigerian novels are based on their own history.

Chimamanda Nagozi Adichie's *Half of a Yellow Sun* is one of the latest contributions to the Nigerian Civil War and the destructive effects of the war on the environment. It is an incomparable voice in African literature. In this novel Adichie recreates Nigeria's most recent history.

Adichie trespasses the boundary of the historical recount of events by interweaving human aspects, turning on multiple microphones for each of these voices to be heard. The theme of war opens up into the bigger theme of humanity where the characters are struggling with issues of love, class, race, profession and family, among others. *Half* of *a Yellow Sun* is an example of one of the many forms where fiction can coexist with history. One could argue that it is a literary approach to Hayden White's concept of history as a narrative. *Half of a Yellow Sun*, unrestrained by the margins of truth and untruth which the historians are bound to, produces a sincere version of the Nigerian Civil War which is not just fascinating to read but an expression of knowledge about the human kind. This novel is an expression of polyphony on the Nigerian Civil War. Adichie goes beyond the historical research and travels deep into the memory of Nigeria, going into the roots of the conflict, into the injustice, the violence and the pain of war; the irrelevance of humanity amidst these conditions.

Half of a Yellow Sun, Adichie's second novel, was published in 2006. It is set in Nigeria, and deals with two periods, the early 60s and the late 60s, which are of pivotal importance in the postcolonial history of Nigeria. In the late 60s, the country was involved in

a bloody and violent conflict, the Nigeria-Biafra War, which lasted from 1967 to 1970. Adichie shifts between these two time periods in the novel. In the parts on the early 60s, the events leading up to violent conflict are sketched, and the main characters are introduced. Adichie's tactful portrayal of the characters and the events reflect her meaningful blending of history in her fiction. Since the novel deals with the real historical events, it is useful to investigate the way in which the author chooses to portray them.

The main characters are on the Biafran side of the conflict. The story is told through three different points of view: Ugwu, a young boy who represents the people of the villages; Olanna the daughter of well to do city dwellers; and Richard, a white ex-patriot originally from England. The story is a combination of politics and personal relationships, charged by the brutal conflicts that occur around the main characters. The story begins in a peaceful and idealistic setting and moves towards the consequences of the civil war. Olanna has fallen in love with a radical Nigerian professor who rallies his colleagues, friends, and students around the idea that the Southern portion of Nigeria needs to declare its independence. A new country has formed and it is called Biafra. Many of the characters are inspired and excited about this concept. But well into the novel, the realities of war become a major factor. The story traces the various crises in the life of the major characters that synchronize with the major events and movements in the history of Nigeria.

The narrative jumps a few years ahead, when the Nigerian government is overthrown. The Northern Hausa blame the Igbo for the coup. There is then another coup, and this time many Igbo soldiers are killed. Olanna now has a child whom she calls 'Baby', and she takes her to Kano to visit her relatives. The violence against the Igbo becomes a pogrom, and Olanna's relatives are brutally murdered. She escapes on a train to Nsukka and sees a woman carrying her daughter's severed head in a basket. Meanwhile Richard watches Igbo civilians being murdered at the airport. Colonel Ojukwu, the Igbo leader, announces that Southeast Nigeria will secede and become the Republic of Biafra. Followed by Ojukwu the students asked Odenigbo to address the gathering "Odenigbo climbed up to the podium waving his Biafran flag: swaths of red, black, and green and, at the center, a luminous half of a yellow sun. 'Biafra is born! We will lead Black Africa! We will live in security! Nobody will ever again attack us! Never again!"(163).

Due to war the ethnic differences are signaled. In Lagos, Olanna and Arize have encountered an incident, which inculcates a fear within them. They have seen a crowd slapping a man for being an Igbo. The crowd also has enquired Olanna and Arize about their

identity. In order to escape from the crowd they conceal their identity and start speaking Yoruba language fluently though they are Igbos.

In chapter twelve, Adichie clearly presents how the Muslims have created trouble to Igbo. When Richard returns to Port Harcourt from Kano he meets a customs officer named Nnaemeka, an Igbo. When they both are having a conversation, a group of three men come running holding long rifles and search for Igbo people. They find Nnaemeka and compel him to say Allahu Akbar, but he refuses to do so. So the soldiers cut open the chest of Nnaemeka. This gives the reader an idea about how the Igbo suffers in a race oriented society. To live as an Igbo is not an easy thing in this society of ethnic difference. They struggle in the hands of the English, the Hausa, and the Yoruba people. They are muted. They cannot raise their voice against this difference. If they do so, the result is 'death'. When the history of Nigeria, is traced this ethnic difference is found to arise only because of the divide and rule policy of the British. This is narrated in the novel through the character Richard. Though he is an Englishman he has love for the Igbo world and its arts. He writes about the power thirst of the British colonials through his book "The Basket of Hands". He writes,

> The notion of the recent killings being the product of "age-old" hatred is therefore misleading. The tribes of the North and the South have long had contact, at least as far back as the ninth century, as some of the magnificent beads discovered at the historic Igbo-Ukwu site attest. No doubt these groups also fought wars and slave-raided each other, but they did not massacre in this manner. If this is hatred, then it is very young. It has been caused, simply, by the informal divide-and-rule policies of the British colonial exercise. (167)

The title of the novel signifies the image of the Biafran flag, which is composed of half of a yellow sun over stripes of red, black and green. In the novel Olanna teaches her students about the flag – the red symbolizes the blood of the Igbo slain in the 1966 pogrom, the black is to mourn their deaths, the green is for Biafra's future prosperity, and the yellow sun is for the country's glorious future. Adichie often points out the yellow sun on the uniforms of the Biafran soldiers, and sometimes contrasts this image of hope with scenes of violence or tragedy. The flag ultimately comes to represent the optimism of the Biafrans when they first secede from Nigeria, and then symbolizes the horrors of starvation and war that came to crush that hope.

Literary reflections on the Biafra war have a long and distinguished history from the most famous poet to have died in the war Christopher Okigbo, to Chinua Achebe, Cyprian Ekwensi and Flora Nwapa. Born in 1977, Adichie is part of a new generation writer revisiting

the history of the past in the spectacles of the present. She brings to it a lucid intelligence and compassion, and a heartfelt plea for memory. Thus, through her descriptive style she has concentrated on bringing out history in her story.

Works Cited

Adichie, Chimamanda Ngozi. *Half of a Yellow Sun*. London: Fourth Estate, 2006.Print.

Barry, Peter. *Beginning Theory: An Introduction to Literary and Cultural Theory*. 3rd ed. New Delhi:Viva, 2010. Print.

An Overview of Parsi Rituals as portrayed in *Family Matters* by Rohinton Mistry

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ABSTRACT

Parsis in India, even though a micro community, possess a distinct identity and culture. The present day Parsis descend from a group of Zoroastrians from Greater Iran who immigrated to the western borders of South Asia (Gujarat and Sindh). They immigrated during the eighth or tenth century to avoid persecution by the Muslim invaders who were in the process of conquering Iran. At the time of the Muslim conquest of Persia, the dominant religion of the region was Zoroastrianism. Iranians rebelled against the Arab invaders for almost 200 years. In Iran this period is now known as "Two Centuries of Silence". During this time, many Iranians who are now called Parsis, chose to take refuge by fleeing from Iran to India.

Being a Parsi, Rohinton Mistry clearly portrays the Parsi religion and rituals in his novels. He elaborates on the various shades of the world of the Parsi community and their problematic position within India. Family Matters deepens Mistry's exploration of the way of life of the Parsi community. Yezad Chenoy, Nariman's son-in-law, is a member of the Parsi community, who weaves his way between family, friends, community, work and India itself. The present paper focuses on the Parsi religion and rituals as portrayed by the Parsi writer Mistry in his novel Family Matters.

Key Words: Parsi, Rituals, Fire, Temples, Zoroastrianism, Zarathustra, Kusti.

According to the Parsis, fire represents the presence of God and there are two distinct differences for the types of fire for the different temples. The first type of temple is the Atash Behram, which is the highest level of fire. The fire is prepared for an entire year before it can be installed and once it is, it is cared for to the highest possible degree. There are only eight such temples in India. The second type of fire temple is called Dar-I Mihr and the preparation process is not as intense as for the first type of fire. There are about 160 of these located throughout India ("Incredible India").

Unlike other communities, the Parsi community has striven hard to preserve, sustain and enhance its culture. Their presence in Indian writing in English is remarkable and their voices are audible within and outside India. The fact cannot be denied that there is something unique and quintessential about the Parsi culture. Their contribution to English literature needs to be acknowledged.

The Parsis came to India from Persia. Their prophet Zarathustra lived two hundred years before Moses. This religion is older than Judaism, Christianity and Islam. The Parsis have the practice of offering their early morning orisons to Ahura Mazda, facing the east. They recite their 'kusti' prayers at times set apart for that. In Zoroastrianism, a person is not

initiated as an infant like christening. Normally, a child is initiated into the faith when he or she is old enough to choose to enter into the faith. The initiation begins with a ritual bath and after a spiritual cleansing prayer, the child wears white pyjamas, a shawl and a small cap. Following the introductory prayers, the child is given the sacred items that are associated with Zoroastrianism: a sacred shirt and cord, sudre and kusti. The child then faces the main priest and fire is brought in to represent God. Once the priest finishes with the prayers, the child's initiation is finished and he or she is now a part of the community and religion.

In *Family Matters*, Coomy, a representative of the Parsi community, is a pious person. She performs all the religious rituals perfectly. She goes to the fire temple regularly. She offers her prayers every day and her spirituality is obviously evident, when she says, "My coals are ready for loban, the sun has gone down" (24). On the other hand, Yezad feels uncomfortable when Coomy brings the silver thurible, with loban smoke. He is unable to hold his laughter and mocks at Coomy saying, "Your aunty is a very pious woman" (25).

An important trait of the Parsis is that, they never marry a non-Parsi. In *Family Matters,* Nariman wants to marry Lucy, but cannot, because, she is not a Parsi. It is obvious that the Parsis crack cruder jokes about the non-Parsis and this is clearly seen, when Mr. Soli says "something insulting about ferangis who wiped their arses with paper instead of washing hygienically" (15).

The Parsis are the kings of banking in those days and Indira Gandhi's nationalizing the banks spoiled the prospects of the Parsis. In the novel, Dr. Fitter makes a pessimistic remark about the Parsis. He says, "Parsi men of today were useless, dithering idiots, the race had deteriorated" (51).When Nariman's ankle is hurt; both Jal and Coomy are unable to make a simple decision about taking him to the hospital for an X- ray. Instead, they take him to Dr. Fitter, who "has closed his practice" (50).

The Parsis bless the others by placing vermilion dot on the forehead, garlanding, sprinkling rice, presenting a coconut, putting a lump of sugar in the mouth, hugging and murmuring blessings in the ear. They have great respect for their priest Dustoorji Barya. When Nariman returns home from the hospital, he expects Coomy waiting by the door, with a tray of flowers, vermilion and a husked coconut. But, there is no one to perform the "aachhu-michhu" (61).

Funding trusts like Parsi Panchayat Education are there for the welfare of the Parsis. Parsi Dairy Farm supplies both creamy and affordable milk. Their newspaper is Jam-e-

Jamshed. The mother tongue of the Parsis is Gujarati. They address their God as "Dada Ormuzd" (391).

The pollution that is associated with death has to be handled carefully. A separate part of the home is designed to house the corpse for funeral proceedings before being taken away. The priest comes to say prayers that are for the cleansing of sins and to affirm the faith of the deceased. Fire is brought to the room and prayers are begun. The body is washed and placed in a clean sudre and kusti. The ceremony then begins, and a circle is drawn around the body into which only the bearers may enter.

As the funeral procession proceeds to the ceremony, they walk in pairs and are connected by a white fabric. A dog is essential in the funeral process because it is able to see death. The body is taken to the Tower of Silence where the vultures take care of it. Once the bones are bleached by the sun, they are pushed into a circular opening in the centre. The mourning process is four days long, and rather than creating graves for the dead, charities are established in honour of the person.

Coomy's dead body is clad in white and laid on the marble slab in the prayer hall. Before saying the 'kusti', the members of the family wash their hands and faces. The traditional method is to sponge the corpse with bull's urine. It is said that the funeral must not exceed beyond twenty-four hours from the time of death. If it gets delayed, it is undesirable within Zoroastrian rites.

The philosophy of Zoroastrian religion encourages material and spiritual successes. During times of prayer, they wear a black velvet prayer cap. In *Family Matters*, there is a reference to Wadiaji fire-temple. Yezad, who is irreligious, turns to be a pious person, after facing many troubles in life. He goes to the Wadiaji fire-temple. There is no rule that one has to be religious to enter the fire- temple. The sign board at the temple says, "Admittance For Parsis Only" (338) and Yezad is one and is entitled to go inside.

There are also references to the dwindling birth rate of the Parsis, their men and women marrying non-Parsis and the heavy migration to the West. The Parsis have been a small community right from the beginning but they have survived and prospered. Demography says that, they will be extinct in fifty years and Dr. Fitter makes a mocking comment, "Extinct, like dinosaurs …" (412). The Parsis are stern in maintaining their dignity. According to them, they are the only people in India, who follow the family planning message and the "rest of the country is breeding like rabbits" (413).

Yezad is a pure Parsi in everything. Though he seems to be an atheist in the beginning, he turns to be a religious person towards the end of the novel. He is so adamant that even while buying refrigerator, he demands it to be a Godrej, a venerable Parsi product. When his family moves to the Chateau Felicity after the death of Coomy, Yezad sets up on the cabinet, framed pictures of Zarathustra, the Udvada fire-temple, fire altars and so on.

Murad, the elder son of Yezad, is a rebellious youth. Often he mocks at the religious practices, performed by Yezad. So, frequently there is a quarrel between Yezad and Murad. The Parsis do not tolerate people entering the prayer space in an impure state. When Murad enters the prayer space without a shower after having his haircut, Yezad grits his teeth saying, "You are in the prayer space in your impure state" (462). Murad even dares to call such religious practices as mere "nonsense" (462). Yezad's faith in religion is a mere subject of ridicule for him.

Similar to the Parsi Panchayat Education, there is the League of Orthodox Parsis and the Association for Zarathustrian Education, which meet once a week to discuss the problems of the Parsis. There is a reference to a Parsi bigamist, who married a non-Parsi in Calcutta and a Parsi in Bombay. He was excommunicated by the panchayat, for his crime. This incident proves that, the Parsis marrying people outside their religion is strictly unacceptable. The Parsi men and women, who have illegal relations with non-Parsis, in or out of marriage, are punished by throwing shoes, if they repent publicly.

According to Yezad, the Parsi people are well-behaved. They do not misbehave in public places. When Yezad finds Murad kissing a non-Parsi girl, it chagrins him. He says that mixed marriages will destroy the pure Persian race, which is a unique contribution to the earth. Yezad cannot accept Murad having friendship with a Maharashtrian girl. After seeing the unpleasant behaviour of Murad with the Maharashtrian girl, Yezad says, "A Parsi girl would never behave in such a way" (483).

Another important feature of the Parsis is that they keep tokens of every religion. But, this practice is said to be traditional. For instance, Jal shows a stack of holy pictures to Yezad which include Sai Baba, Virgin Mary, a Crucifixion, Haji Malang, several Zarathustras, Our Lady of Fatima and Buddha. Yezad, after consulting his Orthodox League friends, decides to offer those non-Parsi pictures to the sea. So, he wraps the pictures in brown paper and loops a garland around the packet and throws it into the sea.

The Parsis follow the Zoroastrian calendar for prayers and religious ceremonies. In addition to milk and milk products, the Parsi Dairy Farm also affords sweets and sweetmeats. Roxana approaches the Parsi Dairy Farm to order sweetmeats for Murad's birthday.

During days of menstruation, women are not allowed to enter the drawing room at all. They have to sleep in the spare bedroom on those days and avoid the kitchen. In *Family Matters,* Roxana is not allowed to enter the drawing room, bed room and the kitchen during her periods. The servant comes to do the house-hold chores. When the servant gets her periods, Roxana monitors the domestic works.

The Parsis consider fish as auspicious creatures. This is obvious when Roxana decorates the floor with white chalk powder, using a fish motif stencil, for Murad's birthday. The birthday celebration of the Parsis is remarkable. On his birthday, Murad is asked to step into the chalk patterns with his right foot.

Roxana slips a garland of roses, lilies and jasmine round Murad's neck. Then, she places betel leaves, betel nuts, dates, flowers and a coconut in his hand. She applies vermilion to his fore head and sprinkles some rice over him. She then whispers blessings in his ear. Then, it is Yezad's turn to bless Murad. After this ceremony, the birthday boy is presented with gifts from his parents, which is followed by a sumptuous meal.

The religious components of identity are particularly important for the Parsi characters in the novel. However, the main concern for this vulnerable community in *Family Matters* centres on the issues of numerical decline and the merits or otherwise of traditional notions of ethnic purity. Towards the end of the novel, Dr. Fitter and Inspector Masalavala discuss the shrinking Parsi community and what should be done to halt the diminution. They enumerate the main features accounting for decreasing numbers: a dwindling birth rate, marrying outside the community and migration to the west.

Westernization and western ideas, once seen as the lifeline of the community are now identified as part of the problem. The sense of loss indicative of contemporary Parsi culture in India is articulated by the inspector: "To think that we Parsis were the ones who built this beautiful city and made it prosper. And in a few more years there won't be any of us left to tell the tale" (416). He also sighs saying, "But it will be a loss to the whole world. When a culture vanishes, humanity is the loser" (415).

Works Cited

"Incredible India." *Wikipedia.* n.p.n.d. Web. 8 Apr. 2018.

Mistry, Rohinton. *Family Matters*. London: Faber and Faber Ltd., 2002. Print.

Voice of the Voiceless: A Study of Arundhati Roy's "Operation Green Hunt's Urban Avatar"

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A voiceless group in a society emerges out when a person lacks the ability to stand out and demand human dignity on his own like the privileged class. In an era where issues related to human rights are under critical focus, literary depictions of such voiceless groups have acquired great importance. These literary depictions start giving an outlet for the tears, anguish and anger that reside inside the mute society. The centre or the ruling group can subdue and suppress the lower class but their voice can never be silenced forever. It is a fact that the voice which resists exploitation becomes aware of both their strength and dignity in the society. Through the writings of dedicated social activists, the voiceless are able to speak and write.

Writers concentrate on the aspect of rebuilding society on values which promote honour, dignity, justice and equality. After centuries of silence, the Dalit writers turn inward and talk about their own experiences. The words of the critic Archana proves how, "The time has come for Dalit writers not only to lament their subjugation but also to simultaneously celebrate with pride to the dauntless spirit of the Dalit Women" (245). This kind of literature has to shoulder an immense responsibility. It must be a literature of commitment with a powerful and pungent language of resistance. The voices of such writers focus on the marginalized and the oppressed and empower them to question and contest in the existing power structure of society. The article "Literature Should Become the Voice of Voiceless" states that, "Literature doesn't become literature unless it becomes the voice of the voiceless. We have a great responsibility of bringing forth the voices of marginalised and deprived community".

The writer and activist, Arundhati Roy is one such powerful person who has concentrated on the voiceless groups in society through her essays. "Operation Green Hunt's Urban Avatar" is an essay on Roy's feelings for the Maoist organisation. Her voice always supports the voiceless victims and she stands firm in her decision. Roy's views on the Maoists are quoted in the web essay "Operation Green Hunt's Urban Avatar", "Arundhati backs Maoists, dares authorities to arrest her. I am on this side of line. I do not care . . . pick me up put me in jail, she asserted". The report on Roy asserts that she saluted the people of

Dantewada after 76 CRPF and the police personnel were killed in a deadly attack by the Maoists. But Roy's own words in the web essay "Operation Green Hunt's Urban Avatar" prove her status like this, "The suggestion that I saluted 'the people of Dantewada' after the Maoists killed 76 Central Reserve Police Force (CRPF) is a piece of criminal defamation". Roy finds the death of the 76 CRPF men as something tragic. The fact is that all these men are pawns in the war of the rich against the poor.

At a meeting in Mumbai, Roy has expressed the fact that she is not in favour of the killing of ordinary people. In his net article "Scattered Truths, Bitter Seeds," Vasanth Kannabiran opines, "Operation Green Hunt, the anti-Naxal squads with a license to kill' and 'answerable to none' will set out to annihilate the Maoists. And since the Maoists are not tattooed or branded, only the number of dead will be a decisive indicator". The dead people can be the adivasis, the Maoists or the police personnel. The reason why Roy supports the resistance of the Maoists is that, the people have suffered a lot in the hands of the government. In her web essay "Operation Green Hunt's Urban Avatar," Roy asks "What the CRPF was doing with 27 AK-47s, 38 INSAS, 7 SLRS, 6 light machine guns, one stengun and a two-inch mortar in tribal villages".

Roy has a close link with the Maoists as she has passed through their forests during a visit to meet them. This experience is written by Roy in her famous non-fictional piece "Walking with the Comrades". In this essay, she calls the Maoists as a band of outlaws who are found in and around West Bengal, Bihar and Odisha border. These people live in the jungles and fought against the British Sponsored East India Company using their guerilla style attacks. They are referred to as cowboys, and are more or less compared to the Robin Hood of the fables. The Maoists fight for the welfare of their villagers. They help the poor by giving them money taken from the rich. Hence, the poor people look upon them as saviours. The web article by Kannabiran shows how the Maoists carry the burden of the people. Despite all the confusion, violence, mistakes and the problems, the ordinary people still place their hope for liberation from poverty, dispossession and exclusion on the shoulders of the Maoists because they help them practically.

After the freedom struggle, the Maoists do not receive any kind of support from the government. Their original decision to develop as a police force is not given any importance. The police and the soldiers are selected only from the rural populations and not from the Maoist Community. When the police force becomes corrupt, the Maoists decide to take law in their hands, join the naxal groups and fight for the rights of the people.

As days go on, the Maoists are projected as a national problem or threat to India. In order to solve the mystery behind the Maoists who are branded as terrorists, Roy undertakes a journey to their homeland. This journey prompts her to write a lot about the history of the Maoists. Roy, in her net essay "Operation Green Hunt's Urban Avatar," says that people's resistance against the corporate land grab consisted of a band width of movements with different ideologies, of which the Maoists are the most militant end. The government however is labelling every resistance and every activist movement 'Maoist' in order to justify dealing with them in a repressive, military fashion.

Roy understands that the various resistance movements including the Narmada Bachao Andolan are fighting against a common enemy. In a fight like this, she favours the Maoists. Her words in the web essay "Operation Green Hunt's Urban Avatar" go on thus, "I think it is much more interesting to interrogate the resistant to which we belong, I am on this side of the line. . . . But on this side of the line, we must turn around and ask our comrades questions". The Maoists are labelled as terrorists on the basis of media reports. Their information available on the press or the TV has never been analysed by the people or the government. Any activist trying to bring out the truths is immediately given a warning that if she or he is not with the government, she or he is with the Maoists. The voiceless victims are always the target of the government. Even the activist who speaks for the poor is also threatened, beaten and thrown in jail. At present, the Operation Green Hunt has started knocking the doors of the writers and the analysts. So, in India, free speech has also come under the threat of being suppressed and censored.

In this world, many are left voiceless especially, the poor, the weak and the vulnerable. It is not easy or simple to patch up their problems in the society. The first step will be to prevent these problems that affect them much. Hence, the words of Desmond Tutu quoted in the article "Being a Voice for the Voiceless in Politics" are apt quoting, "As Christians, we need to not just be pulling the drowning bodies out of the river. We need to be going upstream to find out who is pushing them in".

There are many ways in which people can work for the well-being of others. They can write to the MPs on the issues of concern and also get themselves into the political system by joining a party and seeking to work for change from the base level itself. It is only through politics that laws can be changed, policies can be reworked and voices can be heard. Alison Hill rightly points out in her article, "Being a Voice for the Voiceless in Politics," "Let us speak for the voiceless from within the political system and help make a difference in a world so scarred by injustice".

Works Cited

Archana, J.V.R. "Dalit Literature vis-à-vis African-American Literature: An Overview." *Dalit Movement and Literature*. Ed. B. Krishnaiah. New Delhi: Prestige, 2011. 241-51. Print.

Hill, Alison. "Being a Voice for the Voiceless in Politics." *resistanceandrenewal*.net. 13 Feb. 2016. Web. 12 Apr. 2017.

Kannabiran, Vasanth. "Scattered Truths, Bitter Seeds." *india-seminar.com*. n.d. Web. 13 Feb. 2016.

"Literature Should Become the Voice of Voiceless." *business-standard.com*. 14 Apr. 2015. Web. 12 Apr. 2017.

Roy, Arundhati. "Operation Green Hunt's Urban Avatar." *dawn.com*. 2016. Web. 13 Feb. 2016.

Ideal Woman versus Modern Woman in Virginia Woolf's Novel *To the Light House*

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ABSTRACT

Woolf raises issues on gender roles, and challenges the role of the Victorian woman, both in her novels as well as in her essays. The thoughts and feelings of women, their role within the family and outside the family as well as their identity are skyrocketed in Woolf's novel To the Light House. Woolf was prepared to try anything, thus her attitude is that of an innovator and experimenter. She is able to enter the inner life of a character. Women's suffering is focussed through her and is more effective when visualized. She breathes into her characters and they come to life. Woolf is an artist who believed in perfection and finish. Her words and sentences mean more than what they say. Her images too are suggestive, thus giving a vision of the true happenings in the society. Throughout the novel, the readers can see her style which is not only poetic and figurative but also natural, simple and spontaneous. Woolf dedicated many of her works to the feminist cause.

Women who belonged to the Victorian era were supposed to accept the conventions of society. Woolf made the Victorian women rebel against convention through her novels that indirectly voiced women's ambitions for individuality and power. *To the Light House* was written by Woolf when her mother died. She wrote in order to reconstruct and conserve her memories. The novel opens the door of the past. Mrs.Ramsay is modelled upon her mother and Mr.Ramsay is none other than the role taken by her father. The publication of Virginia Woolf's *To the Light house* was a landmark for both the author and the development of the novel in England. It won her the Prix Femina the following year, and gained her a reputation as one of Britain's most important living authors. David Daiches spotlights that in *To the Light House*, "there is a careful weaving together of characters' consciousness, author's comments, and one character's view of another" (qtd. in Tilak 55). The novel was not only a critical success but also very popular, selling in large quantities to a readership that encompassed a broad spectrum of social classes.

To the Light House is largely traditional in its structure. It is seen in three parts. Part I titled as *The Window* throws light on a house party on the island of Skye, where Professor Ramsay and his wife are on holiday with their children and some friends. Part II, *Time Passed* describes how during the long years of wars, the house is left to dust and silence as well as loneliness. Part III *The Light House* describes the visit to the lighthouse after the passing of the years. Mrs. Ramsay through her quite efficient and thoughtful personality survives even after her death. The readers are made aware of the slow passing of the time. It also tells about the painter Lily Briscoe who is introduced to the readers only in appearance and by what she

is doing. She is seen through Mrs. Ramsay's eyes. She is a painter, not by profession but by hobby and is also a philosopher. She proves her independence and fulfils the challenge owed to Charles Tansley that women can indeed paint. Therefore she is such a character who is prepared to be converted and prepared to experience as well as face life without giving a second thought to its consequences.

Woolf was very much concerned about women's life. Through Lily Briscoe, Woolf strives to create a meaningful space for her artwork in an increasingly critical and unkind world. In the beginning Woolf brings the description of Lily who is painting a scene by looking at the picture of Mrs. Ramsay sitting by the window with James. Whenever she feels that her painting has not come out well she recalls Mr. Tansley's words that "women can't paint or write"(*To the Light House* 66). This statement urges in her a strong desire to prove her skill. When James is told by his father that the weather will not be fine for the expedition, it is Mrs. Ramsay who being a mother more warm-hearted and sympathetic, at once assures him that the wind might change and they might still be able to go on the expedition. The vision of a mother consoling her son is really appealing to the eyes. Woolf shows her persona to the readers as a vision of a typical lady whose aim is to establish satisfactory human relationships and this fact makes her a good mother, a good wife, and a good hostess. Mrs. Ramsay tries to please everybody and forget her own sorrows. Mrs. Ramsay feels that women should respect men but this is unacceptable according to Lily as she wants to succeed in her goal as an artist. Thus after Mrs. Ramsay's death, her desire to complete her picture becomes more life-or-death. She wants to win Mrs. Ramsay. Unfortunately, the step where she used to sit is empty as she is gone forever. She has quit the battle but Lily does not want to leave the battle like a coward. She wants to fight the race of the living and the dead.

Michaela Mudure says that "The culmination of the bond between Mrs. Ramsay and Lily is finding the balance and being able to put the right touch exactly there where it should be, namely in the middle of the painting. It is the epiphany created by this very special female bond that makes Lily have her vision: the vision of a balanced future between the two sexes" (65-66).Woolf through the novel *To the Light House* brings the vision of two female minds. According to Mrs. Ramsay all the people on the earth should get married. Even after seeing the flaws in her own marriage, she is happy to live such a life. She never tries to argue with her husband and is such a personality that she in spite of her caged life tries to support her husband in public.

Lily feels triumphant as she is not equal to Mrs. Ramsay in her old fashioned values. She need not abide by the rules of any man. She can decide life for her own. Finally when

Lily finishes her painting the readers visualize that the painting matches her vision. There is a vision of how Lily is affected by Mr. Tansley's words and of how his offensive words get deeply carved in her heart and ring in her ears. This urges her to win and prove that women can indeed paint. Lily Briscoe wishes that she might create "the kind of art that would stand in the future as a testimony to the creative powers of her maligned gender" (qtd. in Asher 52). Lily gives a vision of how women must move forward leaving behind the scars of pain caused by men. Though Lily's painting of Mrs. Ramsay is completed after ten years, she feels that women must struggle to achieve their goal though sometimes it may take years to obtain it as success is sure to come. Lily is guided by sensitivity and intuition, which is a kind of hallmark of Woolf's women. Woolf gives a vision of gender in her novels. The readers are able to distinguish the roles of each personality. In order to live in the societal world, women follow the track of their ancestors. Mrs. Ramsay is held up as an ideal of womanhood .Woolf in order to create vibrancy and boldness in feminine traits introduces Lily because she feels that by showing the conflict between traditional and modern women, she can back up the spirits of suppressed women.

Lily is also very much a product of society, yet she has new ideas for the role of women and produces an answer to the problems of gender power. Lily sticks on to her aim and finds strength within her artistry. She rejects the traditional "mother-women" image and chooses on an identity that is unique in her society. She is labelled as a feminist because she rejects the traditional role of a submissive wife, irrationality and chaos. She being a lady of the Victorian era changes the thoughts of other women. During the Victorian Era, women were called 'angel in the house' in a position of being discriminated. Women had no freedom in substance and spirit and were bound by the male society. They were supposed to meet the social conventions they were given and were excluded from the many rights that the male had. Lily however is a sort of path finder for them.

Works Cited

Asher, E. W. "The Fragility of the Self in Virginia Woolf's *To The Light House* and Christa Wolf's Nachdenken Uber Christa T."*Journal of Comparative Literature and Aesthetics* 15.1-2 (2002): 49-65. Print.

Mudure, Michaela. "Women Bonding." *The Atlantic Literary Review Quarterly* 7.3 (July-Sept. 2006): 64-68. Print.

Tilak, Raghukul. *To the Light House*: *A Critical Study*. New Delhi: Nirman Press, 1976. Print.

Woolf, Virginia. *To the Light House*. New Delhi: Peacock Books, 1927. Print.

A New Historical Reading of Amitav Ghosh's *The Hungry Tide*

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ABSTRACT

New historicist approach concerns itself not only with the big and paramount national matters like partition and communal frenzy but also with political matters and international events of the past. The inscrutable and transcendental issues like the indivisible sanity, religion and alienation, themes of detachment and isolation become part of it. The search for freedom, passion for social justice and deep concern for the individual liberty in an increasingly collectivized society are very well represented in such works.

Key words : New Historicism, Partition, Morichjhapi, Refugees.

Ghosh's novel *The Hungry Tide* presents the eviction of thousands of Bengali refugees who had settled on the island by the government of West Bengal in 1978-79. These refugees are affected by partition. They are from Bangladesh. The government assured them that once in power they will allow them to settle in West Bengal but ironically once in power the government completely lost interest in the poor people. So the refugees settled themselves in the Sunderbans. The government claimed that the area is a reserved area for the preservation of tigers and thus the refugees remain unwanted all over. Thus Ghosh focus on how politics interfere in the life of the refugees.

The novel highlights the plight of the subalterns of Sundarbans in West Bengal. In this place the socio-political turmoil also extracts as the ravaging tides. The Sunderbans is infested with snakes, crocodiles, Bengal tiger and dolphins. The waves are treacherous in this place. The island is often subjected to floods, storms and famine. The tides reach the island and everyday thousands of mangroves disappear and re-appear hours later. No one dares to make home there but the dispossessed, displaced and the unwanted settle there because they have nowhere else to go. The people settled there are mostly fishermen. They depend on river for fish and crabs for sustenance. Each day of their life is unpredictable. Survival for them on those islands is precarious for them.

In the novel, Nirmal's diary contains stories of the refugee's lives and their struggles in Morichjhapi. Nirmal's diary depicts individuals' pathetic condition. Nirmal's diary is instrumental in retrieving the forgotten history of Morichihapi. This history stands till today as one of the darkest spots in the history of mankind. This event is " widely discussed in the Calcutta press, English as well as Bengali" (402). This is deliberately erased from the public

memory and history. The reference to this gruesome incident is now available in an article by Ross Mallick (3).

> Thus Sundarbans bears a name deceptive. At no moment can human beings have any doubt of the terrain's utter hostility to their presence, of its cunning and resourcefulness, of its determination to destroy or expel them. (8)

The narrative reveals historical truths that have been buried and forgotten. What Nirmal records in his diary is the struggle of the refugees. He records it not only as an observer but also as an active participant in the fight against the government. Nirmal brings out the painful struggle of these poor people. They are fallen as a prey to the unfulfilled promises of the government. The refugee's lamentable resistance against the government has been created by Ghosh with a definite purpose in the novel.

In Marchjhapi the settlers tried to build a utopia. In a few months, they cleared the forests and laid the roads. Huts were built in rows. They even invested so much care in creating organisations and institutions. The settlers had set up their own government and taken a census. The island had been equally divided into five zones and each family had been given five acres of land. They developed their life in Sundarbans by creating salt pans, planting tube wells, damming water for the rearing of fish, setting up bakeries, workshops, pottery and ironsmith shops.

The government of west Bengal viewed them as squatters, thugs, and land grabbers and so they ordered them to vacate. This incident resulted in a confrontation between the poor refugees and the government. It was alleged that the left front government used its party cadres and goons along with its police to disband the settlements.

The government made announcement to the settlers to leave the island. The moment in and around Morichjhapi was banned under the section 144. The police forced the people of island to go back. But the people denied leaving the place of their dream. The police had destroyed the tube wells, there was no water to drink, nothing to eat, settlers ate grass and drank water from puddles and ponds; and so cholera had broken out.

One of the settlers informed to the newspaper. High court ruled that barricading the settlers was illegal. They feel it as a notable victory for them. But still police cordon forced them to abandon their homes. Kusum's condition becomes terrible, she says:

> This island has to be saved for its trees, it has to be saved for its animals,and it is a part for by people from all around the world. Every day, sitting here, with hunger growing at our bellies we would listen to these words, over and over again. Who are these people? I wondered who love animals so much that they are willing to kill us for them...(261-262)

This shows the heartbreaking condition of these Marginalized people. Kusum was killed by the police cordon. Thousands of people were killed in war. Kusum faced the brutality of the authorities evicted on her. She refused to leave the land. But she revolted against the war thrust on the people of Morichjhapi.

Ghosh's deep and painstaking observation touching every literary and non-literary source is splendid and appreciable. Though not intentionally, but unknowingly he satisfied the basic ideas of New Historicism. So, there is no doubt to bring his novels into the category of few writers who effectively used the principles of New Historicism.

Works Cited

Carr, *E.H. What is History?*,Penguin Books: U.K., 2000. Print

Ghosh, Amitav. *The Hungry Tide*, New Delhi: Harper Collins Publication, 2004.

Kesavan, Mukul. *'The Written World',review of The Imam and the Indian.* The Hindu Literary Review: 2002. Print

Kirpal, Viney. *The New Indian Novel in English: A Study of the 1980's,* Allied publishers: New Delhi, 1984. Print

Women's Sacrifice during the Chola Period

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ABSTRACT

In the history of Tamil country particularly in the Chola period we have a number of evidences to illustrate the system of sacrificing the life of women. As women were subordinate to men, they had no voice and their will and wishes were also buried by the society. At this juncture they were forced to sacrifice their lives too for others in the form of Sati or Self immolation and Devadasi system by the Chola society. This paper analyses some of the aspects of the system of sacrificing the life of women that prevailed in the Chola period.

Sati or Self Immolation and Devadasi system were the most inhuman system which prevailed during the period of the Cholas. Sati was done by women on the funeral pyre of their husbands. Similarly in devadasi system women were compelled to dedicate their life for Deva or God. In short both systems were the kinds of sacrificing the lives of women for others. These two pathetic and horrible systems prevailed in the Chola period in Tamil Nadu. This paper illustrates the details of the above mentioned systems.

♦ To Sacrifice the life for their Husbands

Self immolation of woman on the funeral pyre of her husband was a common affair in the Chola period. When her husband was dead she should remain a widow or she should commit sati. Performance of sati was considered as a virtuous act of the Cholas. Though women were separated by the men folk in all aspects and so their aspirations were buried within the four walls of kitchen. At this juncture they were forced to sacrifice their precious lives after the death of their husbands.

An inscription found at Allur mentions that sati was committed by Gangama Deviyar, the wife of Virasola Ilango Velar. Likewise the wife of the chief Virasola Ilangovel alias Parantaka Kunjara Mallan committed sati. The Tiruvalangadu inscription of Rajaraja I refers to the sati committed by his grandmother Vanavan Mahadevi. Moreover the Tiruvalangadu plates and the Tirukkovalur inscription of Rajaraja I mentions that when Sundara Chola died in 970 A.D his wife Vanavanmahadevi, mother of Rajaraja I committed sati. An inscription of Rajadhiraja I dated in the 26th year from Brahmadesam in North Arcot district mentions that the queen Vira Mahadeviyar committed sati on the death of her husband Rajendra Chola in 1044 AD. Like that a feudatory queen Vamu committed sati after the death of

Tennattaraiyan of Malaiyur, a vassal under Kulottunga Chola I (1070 A.D.- 1120 A.D.). These events show that the denial of rights of living to women.

The woman who lives without committing sati after the death of her husband has to face torture from the relatives of the deceased husband. These widows suffered because of severe restrictions imposed on them. They were insisted to wear pure white cloth and not allowed to attend any social functions. Thus the life of the widows was worse than that of an ascetic. For instance an inscription found at the North wall of Gramchantheswara temple at Thirukoliyur in South Arcot district elaborates the tortured life of a widow. She says that she was treated as a slave of her family and was denied the right to attend all the social gatherings. By fearing the inhuman custom of Chola society and knowing the cruelty in widowhood life, they preferred sati rather than widowhood. In short here we can see that the women sacrificed her priceless lives for others.

♦ To Sacrifice the life for Divine Power

Another horrible system of sacrificing the life of women practised by the Chola society was the Devadasi system. Devadasies who dedicated themselves to the service of God or Deva were employed in the temples. They were treated as the chosen servants of God and in the Chola period they were known as the Devaradiyar. The girl to be admitted to the rank of Devadasi should not have attained puberty and they were offered for God at the age of 6 or 9. In short their lives were sacrificed by the society even without their permission.

Devadasies were considered to be the daughters of the deity (Devanar Magal). Fanning God with a fly whisk, offering kumbhadipa on special occasions, performing dances before the deity and singing formed the essential part in their daily worship. During festival times, they exhibited their artistic ability. Proficient dancers were conferred the title Talaikkoli. These dancing girls were also called kuttapillai signifying their important profession of dancing. In their old age, the devaradiyar were known as Kaliyuga Lakshmis. Thus right from childhood to ripe age they were forced to live in the temple campus for the divine power.

The institution of Devadasi prevailed earlier than King Parantaka I and continued after him. For instance the images of the dancing girls were also kept preserved in the temples. Their images were in certain important temples of Chidambaram, Tribuvanam, Tanjore etc. One of the gopurams of Chidambaram temple

contained the images of the dancing girls in 108 poses. This becomes the valuable and imperishable source about the existence of the Devadasi system in the Chola society.

The Tanjore inscription indicates the appointments of 400 Devadasies at the Brahadeswar temple of Tanjore by Rajendra Chola I. In 1119 A.D. some women were dedicated to the temple at Tiruvallam. They were also transferred from one place to another by the order of the king. For instance 400 dancing girls were transferred to the Tanjore temple from other temples. The temple also had the habit of purchasing Devadasies. In 1119 A.D., 4 women were purchased for 700 kasu to the temple at Tiruvalangadu. It happened during the reign of Rajadhiraja II. These purchased girls were known as Matha-Adimaigal and they were treated as slaves. In due course they were utilized by the men folk to quench their sexual thirst. Anyhow majority lost their colourful dreams on earth. Though it's a part of socio- religious customs, it is really a system of sacrificing the life of women in the name of divine power.

Thus from these epigraphical and literary references we know that the socio-religious customs of self- immolation and devadasi system prevailed in the Tamil country during the period of the Cholas. In short in the name of socio- religious customs from womb to tomb, women and their aspirations were buried within themselves and its zenith was the system of sacrificing their lives too for the sake of others

Reference

1. South Indian Inscription, Vol. III, No: 205, Verses 65-66.
2. South Indian Inscription, Vol, VII, No: 863.
3. Annual Report of Epigraphy, No: 260 of 1915.
4. Annual Report of Epigraphy, No: 162 of 1932-33.
5. Manoranjithamoni. C, *History of Tamil Nadu Up to 1536 A.D.*, Dave- Berly Publications, Tirunelveli, 2012.
6. Ramaswamy. A , *History of Tamil Nadu (Tamil),* New Century Book House, Chennai, 2017.
7. Ramachandran. C. E, & Raman. K. V, *Aspects of Indian History and Culture*, Books and Books Publishers and Distributers, New Delhi, 1984.

8. Selvaraj. C, *Women and Social Changes in the Chola Period*, Kanyakumari Academy of Arts and Science (KASS), Proceedings of the Research Papers of KASS's National Seminar, Vol. II, ISBN 978-81-909710-8-9.

9. Swaminathan. A, *The Cultural History of Tamil Nadu (Tamil),* Deepa Pathipagam, Chennai, 1998.

10. Sethuraman. G, *The Social and Cultural History of Tamil Nadu (Tamil)*, J.J. Publications, Madurai, 2001.

11. Ramakrishnan. A, *The Cultural History of Tamil Nadu (Tamil),* Sarvodaya Illakkiya Pannai Publications, Madurai, 2004.

12. Devanesan. A, *History of Tamil Nadu Up to 2000 A.D.,* Renu Publications, Marthandam, 2004.

13. P. Gomathinayagam, *The Socio-Cultural History of Tamil Nadu (Tamil),* Tensy Publications, Sivakasi,1991.

14. Devanesan. A, *History of Tamil Nadu Up to 2000 A.D.,* Renu Publications, Marthandam, 2004.

PXRD, Raman and PL Studies on NiO and Ni_9S_8 nanoparticles synthesized by Co-precipitation method

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ABSTRACT

Nickel oxide (NiO) and nickel sulphide (Ni_9S_8) nanoparticles (NPs) have been synthesized by co-precipitation method. The prepared nanoparticles are nanocrystallineNiO (17 nm)with rhombohedral lattice and Ni_9S_8 (42 nm) with orthorhombic lattice as confirmed through the JCPDS standards 89-3080 and 78-1886 respectively. Raman spectrum exhibited first order transverse (492 cm^{-1} in NiONPs and 470 cm^{-1}in Ni_9S_8NPs)and longitudinal (1049 cm^{-1} in NiO NPs and 996 cm^{-1}in Ni_9S_8NPs)optical phonon mode. In both the samples, the peak at 2436 cm^{-1} could be assigned to two phonon modes. In Ni_9S_8 nanoparticles, the peak at 1608 cm^{-1} corresponds to a two magnon band.On excitation with 325 nm wavelength, NiO and Ni_9S_8 nanoparticles released photoluminescence emission in the entire 350-600 nm visible region of the electromagnetic spectrum.

Keywords: NiO and Ni_9S_8 nanoparticles, grain size, phonon modes, blue-green emission

1. INTRODUCTION

In the recent years, transition metal oxides and sulphides are found to have unique optical, catalytic, electronic, electrical and magnetic properties. Due to their special structures and properties, these nanomaterials are widely used in photoelectric, recording materials, catalysts, sensors, ceramic materials etc.[1]. The bulk nickel oxide (NiO), due to its electronic structure and chemical bonding is a Mottinsulator and shows up an easy-plane antiferromagnetic (AFM) ordering of type-II; In the paramagnetic phase above the Neel temperature T_N= 523 K, NiO has a cubic rock-salt crystal structure (Fm-3m). Below T_N, the magnetic ordering results in that the spins of the Ni^{2+} ions order ferromagnetically in {111} planes and the structure of NiO undergoes a week cubic-to-rhombohedral distortion (R-3m) due to the magnetostriction effect [2]. The nickel oxide nanoparticles are used as an electrode material for lithium ion batteries and have shown notable catalytic, optical and electrochemical properties[3-4]. Nickel sulphidehas showed exceptional electrochemical properties and good electrical conductivity. Nickel sulphide can assume various thermodynamically stable crystal structures and stoichiometric forms including NiS, NiS_2, Ni_3S_2, Ni_3S_4, Ni_4S_3, Ni_7S_6 and Ni_9S_8 [1]. The synthetic conditions including temperature, pressure and reactant composition determine the crystal phase of nanocrystals. Therefore, it is important to develop phase-controlled synthesis methods to obtain desired materials for specific applications [5-12]. Numerous routes like thermal decomposition, microwave

pyrolysis, solvothermal, sonochemical, precipitation-calcination and microemulsion have previously been investigated for production of sulphide and oxide nanoparticles. Most of these methods have their own advantages and limitations [3]. The chemical co-precipitation method was selected for its simplicity, convenience, reproducibility and low calcination temperature. It is an excellent choice when higher purity and better stoichiometric control are required [7]. In this paper, a simple, cost-effective method of preparing NiO and Ni_9S_8 nanoparticles by co-precipitation is reported and its structural and optical properties are analyzed by powder X-ray diffraction (PXRD) method, Raman and photoluminescence (PL) spectroscopy.

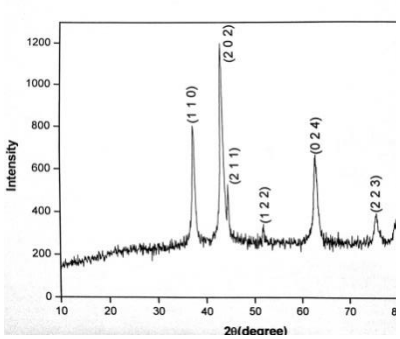
2. EXPERIMENTAL PROCEDURE

Synthesis: Co-precipitation method is used to prepare both nickel oxide and nickel sulphide nanoparticles. All the chemicals are of analytical purity. 0.5M of nickel acetate is dissolved in 100 ml distilled water separately and allowed to stir well for 30 minutes. Then 0.5M of urea is dissolved in 100 ml distilled water separately. Then both the nickel acetate and urea solutions are mixed together and 16M of ammonium hydroxide is added to the above mixed solution drop by drop to attain a pH of 12 and the solution is allowed for further stirring for 30 minutes. The precipitate is kept in hot air oven at 100^{o}C for drying. The obtained sample is green in colour. This powdered nickel oxide is kept in the muffle furnace at 600^{o}C for two hours. After calcination, the sample is black in colour. The same procedure is followed to synthesize nickel sulphide nanoparticles; but the precursors used are nickel acetate and sodium sulphide.

Characterization: The powder X-Ray Diffraction patterns of nickel oxide and nickel sulphide nanoparticles were recorded using XPERT-PRO Diffraction system with CuK_α radiation of wavelength 1.54056 Å. Raman studies were done using Micro-Laser Raman (Seiki, Japan) spectrometer. Photoluminescence (PL) studies for the synthesized nanoparticles were carried out using a photoluminescence spectrophotometer (Varian Cary Eclipse) and the emission spectra were recorded at a scan rate of 600 nm/min using an excitation wavelength of 325 nm.

3. RESULT AND DISCUSSION

The characteristic powder X-Ray diffraction patterns of nickel oxide and nickel sulphide nanoparticles are shown in Fig.1 and Fig.2.



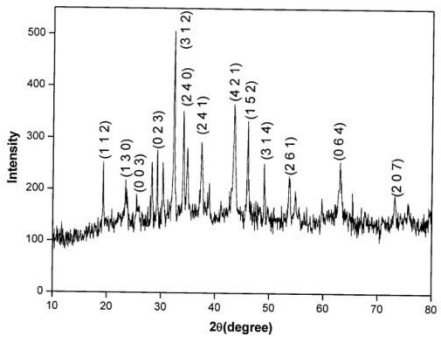
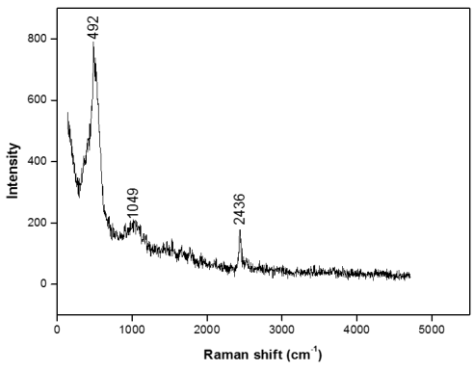


Fig.1 PXRD pattern of NiO nanoparticlesFig.2 PXRD pattern of Ni_9S_8 nanoparticles

The d-spacing values given in the powder X-Ray diffraction pattern of the synthesized NiO nanoparticles matched well with that of the rhomb-centered rhombohedral structure NiO as confirmed from the JCPDS File No. 89-3080.[a=b=5.910Å; c=7.225Å; cell volume=218.63(Å)^3 [2]. The crystal structure of the synthesized nickel sulphide nanoparticles is identified from the JCPDS File No. 78-1886. [a= 9.335 Å; b=11.21 Å; c=9.430Å; cell volume=987.65 (Å)^3(Ni_9S_8) as orthorhombic with an end centered lattice [1]. Hence the formation of nickel oxide and nickel sulphide nanoparticles is confirmed through PXRD studies. The average grain size of the synthesized nanoparticles is found out from the powder XRD pattern using Debye Scherrer's formula

$$D=0.9\lambda/\beta\cos\theta \text{ (nm)}$$

where λ is the wavelength of incident X-ray, β is the full width half maximum, θ is the Bragg's angle for the peak. The average grain size of NiO and Ni_9S_8 nanoparticles are found to be about 17 nm and 42 nm respectively.



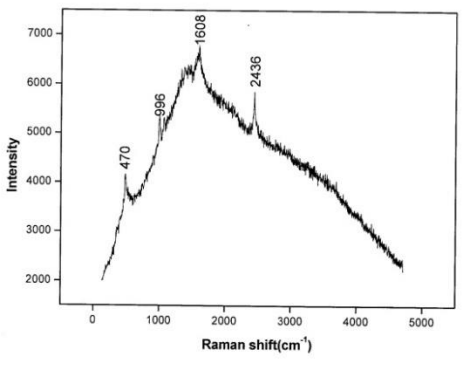
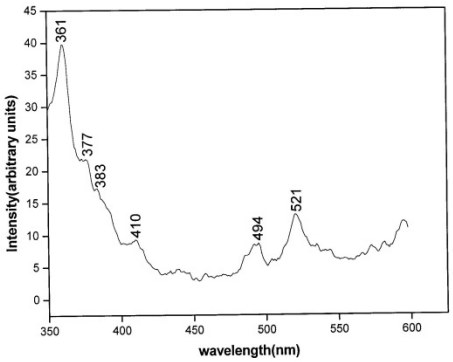


Fig.3. Raman spectra of NiOnanoparticles Fig.4. Raman spectra of Ni_9S_8 nanoparticles

Raman spectrum exhibited first order transverse optical (1TO) phonon modeat 492 cm^{-1} in NiO nanoparticles and at 470 cm^{-1}in Ni_9S_8 nanoparticles and longitudinal optical (1LO) phonon mode at 1049 cm^{-1} in NiO nanoparticles and at 996 cm^{-1}in Ni_9S_8 nanoparticles as shown in Fig.3 and Fig.4. [2]. In both the samples, the peak at 2436 cm^{-1} could be assigned to two phonon (2P) modes. In Fig.4, the peak at 1608 cm^{-1} corresponds to a two-magnon (2M) band of Ni_9S_8 nanomaterial [1].



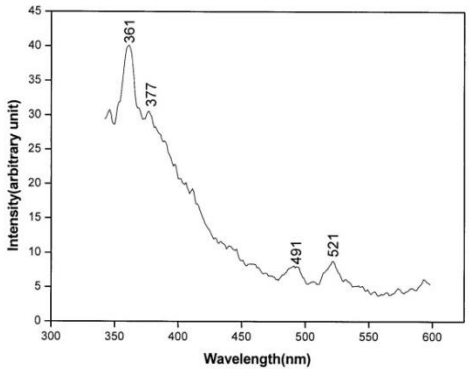


Fig.5.PL emission spectra of NiO nanoparticles Fig.6.PL emission spectra of Ni_9S_8 nanoparticles

In both the samples, the PL emission peaks (Fig.5 and Fig.6) have been observed covering the whole 350-600 nm visible region of the electromagnetic spectrum, when 325 nm wavelength is used for excitation [1]. The blue and green luminescence demonstrates the good quality of the prepared nanoparticles. The density of surface states in the nanocrystals would increase with a decrease in the size of crystallites of the prepared nanocrystals, due to the increased surface - to -volume ratio having smaller crystallites. This would reduce the probability of excitonic emission via non - radiative surface combination [6].

4. CONCLUSION

Nickel oxide and nickel sulphide nanoparticles are successfully synthesized by co-precipitation method. The preparation process has advantage of simple technology, good reactivity between components, nano-size particles, good yield and short preparation cycle. The formation of NiO nanoparticles in rhombohedral form and Ni_9S_8 nanoparticles in orthorhombic form is confirmed through PXRD studies with the average grain sizes found to be about 17 nm and 42 nm respectively.Raman spectra exhibited phonon modes of vibration of the nanopowder samples. The blue and green luminescence of the PL spectra demonstrates

the good quality of the prepared nanoparticles. Thus the synthesized NiO and Ni_9S_8 nanoparticles can be used for photocatalysis and antimicrobial applications.

REFERENCES

1. MuhamedShajudheen, V., Senthil Kumar, V., Anitha Rani, K.,, Uma Maheswari, A andSaravana Kumar, S., (2016). Structural and Optical Properties of NiS Nanoparticles Synthesized by Chemical Precipitation Method. International Journal of Innovative Research in Science, Engineering and Technology, 5(8), 15099-15103.
2. Mironova-Ulmane, N., Kuzmi, A., Grabis, J., Sildos, I., Voronin, V.I.,Berger, I.F. and Kazantsev, V.A., (2011). Structural and Magnetic Properties of Nickel Oxide Nanopowders, Solid State Phenomena, 168-169, 341-344.
3. Sunny S. Tarve, Swapnil G. Prabhulkar and Raju M. Patil., (2016). Nickel Oxide and Nickel Sulphide Nanomaterials: Promising Catalysts for the Decomposition of Hazardous Dye, Indian Journal of Research, 5(8), 195-199.
4. Angel Gutierrez, FelisaPerpinan, M., Ana E. Sanchez, and Carmen Torralba, M., (2012). Solvothermal Synthesis of NiO, Ni and NiS Nanoparticles Journal of Nanoscience and Nanotechnology, 12, 1–6.
5. Chencheng Sun, Mingze Ma, Jun Yang, Yufei Zhang, Peng Chen, Wei Huang and Xiaochen Dong, (2014). Phase-controlled synthesis of a-NiS nanoparticles confined in carbon nanorods for High Performance Supercapacitors, Scientific Reports4:7054, 1-6.
6. Kumphakar,P., Snigh,D., Tiwary,C.K., and Mitr, A.K., (2008). Chemical synthesis and visible photoluminescence Emission from Monodispersed ZnO nanoparticles, Chalcogenide letter, 5,387-394.
7. Wilson SacchiPeternele, Victoria Monge Fuentes, Maria LuizaFascineli, Jaqueline Rodrigues da Silva, Renata Carvalho Silva, Carolina Madeira Lucci and Ricardo Bentes de Azevedo, (2014). Experimental Investigation of the Coprecipitation Method: An Approach to Obtain Magnetite and Maghemite Nanoparticles with Improved Properties, Journal of Nanomaterials, 2014-682985, 1-10.
8. Diao, Y., Myerson, A. S., Hatton, T. A. and Trout, B. L.,(2011). Surface design for controlled crystallization: The role of surface chemistry and nanoscale pores inheterogeneous nucleation. Langmuir 27, 5324–5334.

9. Yuan, C., Wu, H. B., Xie, Y. and Lou, X. W. D., (2014). Mixed Transition-Metal Oxides: Design, Synthesis, and Energy-Related Applications. Angew. Chem. Int. Ed. 53, 1488–1504.
10. Lai, C.-H., Lu, M.-Y. and Chen, L.-J., (2012). Metal sulfide nanostructures: synthesis, properties and applications in energy conversion and storage. J. Mater. Chem. 22, 19–30.
11. Du, J. M. and Kang, D. J., (2007). A shape-controlled method to functionalize multiwalled carbon nanotubes with Ni_3S_2. Inorg. Chem. 46, 10307–10311.
12. Ghezelbash, A., Sigman, M. B. and Korgel, B. A., (2004).Solventless synthesis of nickel sulfide nanorods and triangular nanoprisms. Nano Lett. 4, 537–542.

Synthesis of Magnesium Ferrite by Co-Precipitation Method

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ABSTRACT

Magnesium ferrite was synthesized by a simple co-precipitation method. The crystal structure and the metal-oxide phase formation were investigated by powder X-ray diffraction (PXRD) and Fourier Transform Infrared Spectroscopy (FTIR). Lattice parameter, volume of the unit cell and X-ray density were determined from PXRD pattern and the lattice parameter values were verified by Bradley-Jay and Nelson-Riley plots. Average crystallite size was estimated from the PXRD pattern using Scherrer equation. The FTIR spectra showed two principal absorption bands in the range of 400cm^{-1} to 1000 cm^{-1}. The values of force constant for tetrahedral (K_T) and octahedral (K_O) sites were calculated.

Keywords: *$MgFe_2O_4$, PXRD, FTIR, lattice parameter, X-ray density, force constant.*

1. INTRODUCTION

Ferrites are the ferromagnetic oxides containing iron oxide and another metal oxide in proper proportion. They have the general formula of MFe_2O_4 (where M is a divalent metal ion, eg: Fe, Co, Ni, Mg, etc.). In a spinel structure, there are 56 ions, 32 oxygen and 24 metal ions in a unit cell. In most ferrite materials, the substituents play an important role in determining the variation of the physical properties, the magnetic and electric transport properties are affected by the substituents. A general formula of ferrite structure is denoted as $(M_{1-x}Fe_x)[M_xFe_{2-x}]O_4$, in which M shows cations that occupy tetrahedron sites and x is degree of inversion. Among the ferro-spinels, the inverse type is particularly interesting due to its high magneto crystalline anisotropy, high saturation magnetization and unique magnetic structure. Magnesium ferrite ($MgFe_2O_4$) possesses cubic structure and has a normal spinel structure. At this structure, Mg^{2+} ions occupy octahedron B site and Fe^{3+} ions occupy both tetrahedron A and octahedron B-sites. Thus, the compound can be represented by the formula $(Fe^{3+}{}_{1.0})[Mg^{2+}{}_{1.0}Fe^{3+}{}_{1.0}]O^{2-}$,where the round and the square brackets represent A and B sites, respectively. There are various preparation methods such as solid state reaction, sol-gel, chemical co-precipitation, hydrothermal etc. Among them co-precipitation method is employed as it is simple, easy and economic, also it is a proper technique for making small size and monodisperse nanoparticles [1].

2. EXPERIMENTAL PROCEDURE

2.1 Materials

Ferric nitrate nonahydrate ($Fe(NO_3)_3.9H_2O$), magnesium nitrate hexahydrate ($Mg(NO_3)_2.6H_2O$) are used as precursors; Sodium hydroxide (NaOH) and acetone of analytical reagent grade were used.

2.2 Sample Preparation

Magnesium ferrite nanoparticles ($MgFe_2O_4$) have been prepared by using co-precipitation method. Ferric nitrate nonahydrate and magnesium nitrate hexahydrate are used as precursors. 2M $Fe(NO_3)_3.9H_2O$ and 1M $Mg(NO_3)_2.6H_2O$ are dissolved in 40 ml of double distilled water and subjected to magnetic stirring. The pH of the solution is adjusted to 10 by adding drop wise 0.1M NaOH solution. Obtained brown precipitate was filtered and washed repeatedly with double distilled water and ethanol until a pH of 7 was achieved. The precipitate is dried at 100^0C for several hours and subsequently calcined at 400^0C and 600^0C for 3 hours to get magnesium ferrite nanoparticles as end product [2].Once again the final product is finely powdered. The synthesized sample was characterized by PXRD and FTIR. The synthesized nanoparticles are found to be dark brown in color.

3. RESULTS AND DISCUSSION

3.1. Powder X-ray Diffraction Analysis

Characterization of the final product was performed by Powder X-ray powder diffraction analysis. The PXRD pattern is shown in Fig.1 (a). It exhibits typical reflections (311), (220), (400), and (440) planes that are indications of the presence of the cubic spinel structure. This diffraction lines provide clear evidence on the formation of $MgFe_2O_4$. The entire diffraction peaks match well with the standard values (JCPDS file No: 89-3084) and are indexed. No secondary phase was detected in PXRD, ensuring the phase purity of the final product. The lattice parameter of $MgFe_2O_4$was obtained using UNITCELL software as a=8.3779 **Å**. The precise value of lattice parameter of pure $MgFe_2O_4$ was calculated using Bradley-Jay and Nelson-Riley extrapolation methods that minimize the influence of systematic errors. Extrapolation against $\cos^2\theta$ is called Bradley-Jay method whereas extrapolation against $\{(\cos^2\theta/\sin\theta) + (\cos^2\theta/\theta)\}$ is called Nelson-Riley plot. The lattice parameters calculated by different methods are tabulated in Table 1.

The particle size of magnesium ferrite nanoparticles was determined from the full width at half maximum (FWHM) of the XRD patterns using the Scherer formula **d = 0.9λ/βcosθ,** where d is the crystallite size (nm), β is the full width of the diffraction line at half the maximum intensity measured in radians, λ is the X-ray wavelength and θ is the Bragg angle. The crystallite sizes estimated using the Scherer formula was ranging from 7 to 21 nm. The actual (X-ray) density of $MgFe_2O_4$ nanoparticles is calculated using the formula $\mathbf{P_x = 8M/Na^3}$, Where *M* is the molecular weight of the sample, *N* the Avogadro's number and *a* lattice constant [3] and it is found as 5.313g/cc.

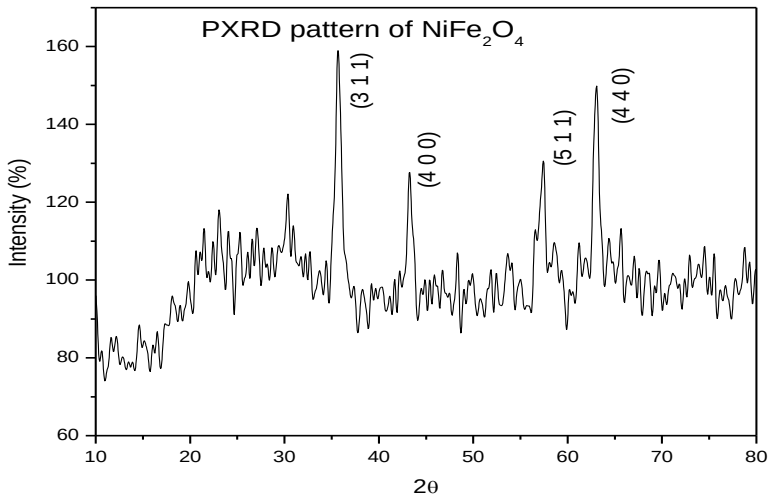
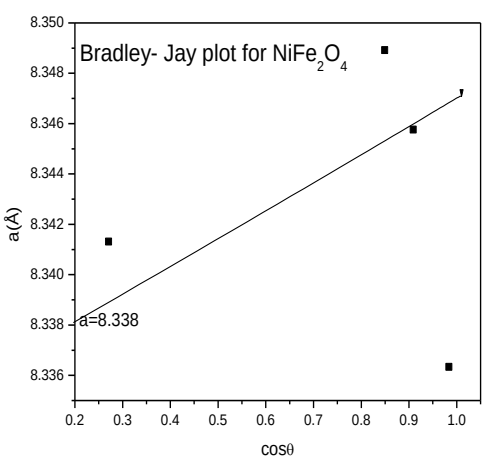


Fig. 1: (a) PXRD pattern of $MgFe_2O_4$nanoparticles



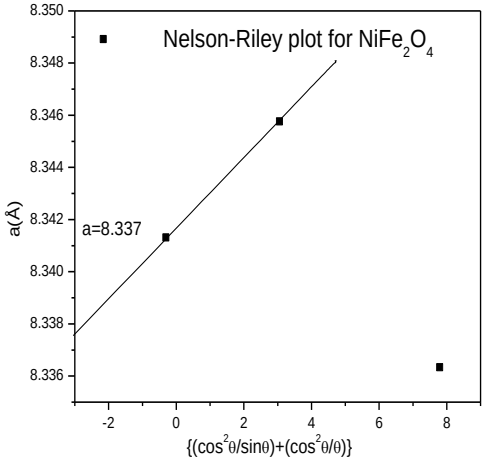


Fig. 1: (b) Bradley- Jay plot for $MgFe_2O_4$Fig. 1: (c) Nelson-Riley plot for $MgFe_2O_4$

Table 1: Lattice parameter 'a' by different methods

Method	Lattice parameter 'a' (Å)
JCPDS	8.3779
UNIT CELL	8.3779
By using formulaa $=d/(h^2+k^2+l^2)^{1/2}$	8.342
Bradley - Jay plot	8.3449
Nelson - Riley plot	8.3755

3.2. FTIR Studies

FTIR spectral analysis helps to confirm the formation of spinel structure in ferrite samples. In the FTIR spectrum two main broad metal–oxygen bands are observed in the $MgFe_2O_4$ spinels. The higher one (ν_1) observed in the wave number range 600–550 cm^{-1}, is caused by the stretching vibrations of the tetrahedral metal–oxygen bond. The lower band (ν_2) observed in the range 450–385 cm^{-1}[4].

The ν_1 and ν_2 for the as prepared $MgFe_2O_4$ sample was found to be 534cm^{-1} and 420cm^{-1}. The values of the force constants (K_T and K_O) for the band Fe^{3+}- O^{2-}at tetrahedral and octahedral sites were calculated using the relation $\mathbf{K = 4\pi^2\upsilon^2c^2m}$, where c is the speed of light, ϑ is the band wave number in cm^{-1} and m is the reduced mass for Fe^{3+} ions and O^{2-} ions ($2.061x10^{-23}$ g). The values of force constant for tetrahedral (K_T) and octahedral (K_O) sites were calculated as 2.086 $x10^5$dyne.$cm^{-1}$and 1.29 x 10^5dyne.cm^{-1} for the as prepared sample. The band observed at 1382$cm^{-1}$was due to the surface co-ordinated O- H bond in ferrite [5].

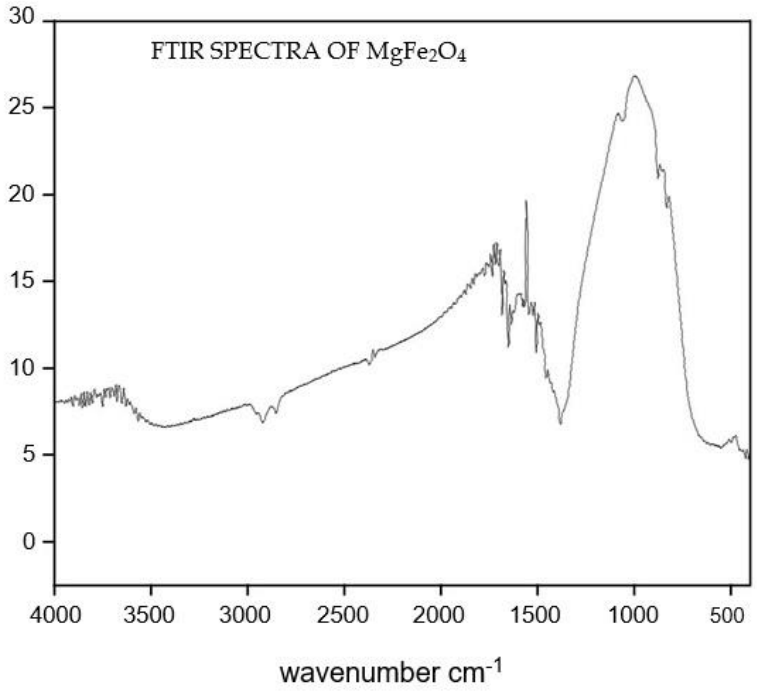


Fig. 2: FTIR spectrum of $MgFe_2O_4$nanoparticles

CONCLUSION

Magnesium ferrite nanoparticles (MgFe2O4) have been prepared using co-precipitation method. There is close agreement among the lattice parameter calculated from the JCPDS, XRD data through UNITCELL software, Bradley-Jay and Nelson-Riley extrapolation methods. The metal – oxide phase formation was confirmed through the FTIR band assignments. Force constant for the Fe – O bonds in the tetrahedral and octahedral sites of $MgFe_2O_4$was estimated.

REFERENCES

1. Aliahmad, M., Noori, M., Hatefi Kargan, N. and Sargazi, M., *"Synthesis of simple ferrite nanoparticles by co-precipitation chemical method"*, International Journal of Physical Sciences, Vol. 8, pp. 854-858, (2013).

2. Navneet Kaur, Manpreet Kaur, *"Comparative studies on impact of synthesis methods on structural and magnetic properties of magnesium ferrite nanoparticles"*, Processing and Application of Ceramics, Vol. 8,pp. 137–143, (2014).

3. Shrikant S.Jagtap, Sopan M.Rathod, *"Synthesis and characterization of Mg-Ni ferrite prepared by sol-gel auto-combustion method"*, Applied Research and Development Institute Journal, Vol. 8, pp 1-6(2013).
4. Angel Ezhilarasi A, Judith Vijaya J, John Kennedy L, Vasanth M, *"Green synthesis of Mg doped zinc oxide nanoparticles using aloe vera plant extract and its characterization"* Journal of Chemical and Pharmaceutical Sciences, Volume 9, Issue 3, pp 1450, (2016).
5. Kalyan Raju. M., *"FT-IR Studies of Cu Substituted Mg-Zn Ferrites for Structural and Vibrational Investigations"*, Chemical Science Transactions, Vol 4, pp. 137-142, (2015).

Pectinolyticactivity of micro-organisms during retting of coconut husk

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ABSTRACT

Traditional retting of coconut husk has caused serious threats to the coastal eco system. Coir-retting initiated by micro-organisms is being sustained in the retting medium for a continuous period of time. Bacteria, fungi and yeast play a vital role in the decomposition of the pectic substances and polyphenols, the known fibre-binding materials. Pectinolytic activity of micro-organisms like bacteria, fungi and yeast releases large amount of pectin, polyphenols and tannin which account for the formation of high levels of sulphide, phenol and ammonia, apart from nitrate and nitrite in the medium. Presence of Desulpho vibrio, Micrococcus, Pseudomonas, Clostridium, Bacillus and Aspergillus niger has confirmed the microbial interference in the pectin degradation. The sulphates formed through the action of Aspergillus niger from the released compounds are also reduced further to sulphide through Desulpho vibrio sp. Bacterial species like Pseudomonas and Micrococcus are associated with the leaching of poly phenols.

Key Words: Conventional; Retting; Pectinolytic; Leaching; Tannin

Introduction

Retting of coconut husk for the production of fibre is widespread throughout the coastal areas of Cape Comorin, the southern tip of peninsular India, in view of their economic importance. Bacteria, fungi and yeast, play a vital role in the decomposition of the pectic substances and polyphenols, the known fibre-binding materials. These microorganisms play a substantial role in the decomposition of husk through lignolytic, pectinolytic, cellulolytic and hemicellulolytic activities with the liberation of cellulose, poly phenols, CO_2, CH_4, NH_3, H_2S and other organic compounds (Immanuel *et al*., 2014). The release of organic acids like poly phenols and tannin into the retting effluents make the entire medium acidic (Cholarajan *et al*.,2011). Sulphate-reduction and nitrification too contribute to acidity and thus the water quality of the nearby resources has deteriorated with the accumulation of toxic organic compounds.

The anoxic conditions in the retting zones are due to restricted circulatory process and heavy breathing by the organic matter in the medium (Kadeeja Beevi *et al*., 2004) and during this time, oxidation- reduction potential falls in the effluent and the sediments. Bacterial sulphate- reduction is a potential source of high sulphide concentration in sediment (Dan *et al*.,2007) and the rate of sulphate- reduction is actively controlled by the supply of organic matter.

$SO4^{2-} + 8e^- + 10H^+ \rightarrow H_2S + 4H_2O \quad E^0 = -221mv$

The sulfurous odour is a marker for the presence of sulfate-reducing bacteria (*Dexter et al.*,2003) and the organic carbon used by these bacteria are limited to a few single organic molecules such as lactate and pyruvate which are partly oxidised to acetate (Kolmert,1999). In the anoxic sediments, hydrogen sulphide precipitates black metal sulphides affecting soil redox, metal concentration and oxygen availability (Holmer *et al*., 2011). The presence of sulphide in the water sources near the retting zones may be due to the intrusion of sulphide from the retting zones due to the microbial decomposition of organic load (Manoj *et al.*,2014).

Materials and Methods

The study area lies between 8^0 2' and 8^0 4' N latitudes and 77^0 26' and 77^0 30'E longitudes along the South-West Coast of peninsular India, encompassing Cape Comorin which receives heavy rain during South West and North East monsoons. Coconut husks are normally allowed to ferment in retting ponds adjoining the estuaries and allied water bodies. The present study was undertaken during summer.

The effluent samples collected in sterile plastic containers were subjected to serial dilution method in the laboratory and the bacterial, fungal and yeast populations developed were isolated by pour plate method and the isolated colonies were identified using biochemical tests. Nutrient agar is used for the isolation of aerobic bacteria, TSA agar and TSC agar were used for the isolation of anaerobic bacteria (Orth, 1977). Potato agar and yeast malt agar were used for the isolation of fungi and yeast. The bacteria were identified based on colony characteristics, gram staining methods, and various biochemical tests which include indole, catalase, coagulase, oxidase, sugar fermentation, citrate and urease tests (Bergey's Manual,1994). For fungi, the cultures were separated into groups based on their morphological characteristics including growth pattern, colony texture, pigmentation, and growth rate of the colonies (Promputtha *et al.*, 2005). Identification of each isolate of yeast up to species level was carried on the basis of standard morphological and physiological/ biochemical tests (Barnett *et al*., 2000; Kurtzman & Fell, 2006).

Results and discussion

The important groups of bacteria involved in the retting activity are heterotrophic, phosphate-solubilizing, aerobic nitrogen-fixing, sulphate-reducing and denitrifying in character. These microorganisms play substantial role in the decomposition of husk through lignolytic, pectinolytic, cellulolytic and hemicellulolytic activities with the liberation of

cellulose, poly phenols, CO_2, CH_4, NH_3, H_2S and other organic compounds. Methanogenic bacteria generate methane by breaking down organic matter anaerobically, releasing carbon dioxide and methane and methanotrophic bacteria oxidize methane to carbon dioxide. Pectinolytic activity of microorganisms like bacteria, fungi and yeast releases large amount of pectin, polyphenols and tannin which account for the formation of high levels of sulphide, phenol and ammonia, apart from nitrate and nitrite in the medium. Bacteria such as *Pseudomonas, Micrococcus, Bacillus, Staphylococcus* (Fig.1) *Clostridium* (Fig.2) and *Desulphovibrio* (Fig.3), fungi such as *Aspergillus niger, Aspergillus flavour, Pencillium chrysogenum, Pencillium erythromellis* (Fig.4) and yeast such as *Candida albicans* were isolated and identified in the retting effluents have been confirmed through a series of biochemical tests (Table : 1). Presence of Desulphovibrio, Micrococcus, *Pseudomonas, Clostridium, Bacillus* and *Aspergillus niger* has confirmed the microbial interference in the pectin degradation. This in turn favours the formation of hydrogen sulphide from the decomposition of organic compounds containing sulphur. The sulphates formed through the action of *Aspergillus niger* from the released compounds are also reduced further to sulphide through *Desulphovibrio* sp.

$$(CHO)_{106}(NH_3)_{16}H_3PO_4+53SO_4^{2-} \xrightarrow[\text{Sulphate reduction}]{\text{Desulphovibrio}} 106CO_2+106H_2O + 16NH_3 + H_3PO_4 + 53S^{2-}.$$

Bacterial species like *Pseudomonas* and *Micrococcus* are associated with the leaching of poly phenols. Due to the de-nitrification process which is active during summer, under a near-anoxic condition ammonia is also released from the retting medium under the influence of *Bacillus* and *Pseudomonas*. Based on the study, a possible mechanism is also suggested to explain the role of these microbial agents (Fig. 5).

Table 1 Identification of Micro organisms

Biochemical Test	**Microorganisms**				
	***Bacillus* sp.**	***Pseudomonas* sp.**	***Clostridium* sp.**	***Micrococcus* sp.**	***Desulfovibrio* sp.**
Gram Staining	+	-	+	+	-
Nitrate	+	Denitrifying	+	-	-
Indole	-	-	-	-	-
MR	-	-	-	+	-
VP	+	-	-	+	-
Citrate	+	+	+	+	-
Urease	-	+	+	+	-
TSI	K/K	K/K	A/A	K/NC	A/A
Glucose	+	-	+	-	+
Sucrose	+	-	+	-	+
Lactose	-	-	+	-	+
Amylase	+	-	+	-	+
Gelatin	+	+	+	+	-

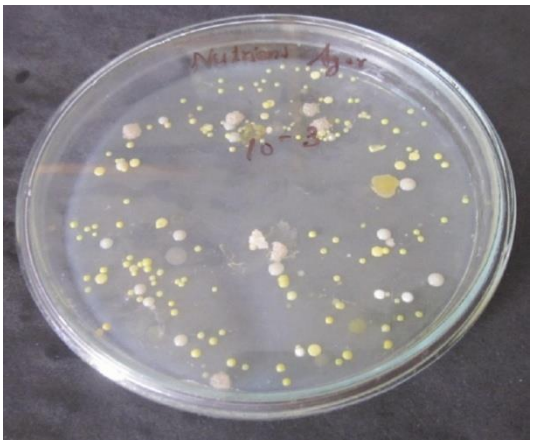
K - Alkaline

A - Acid

NC - No Colouration

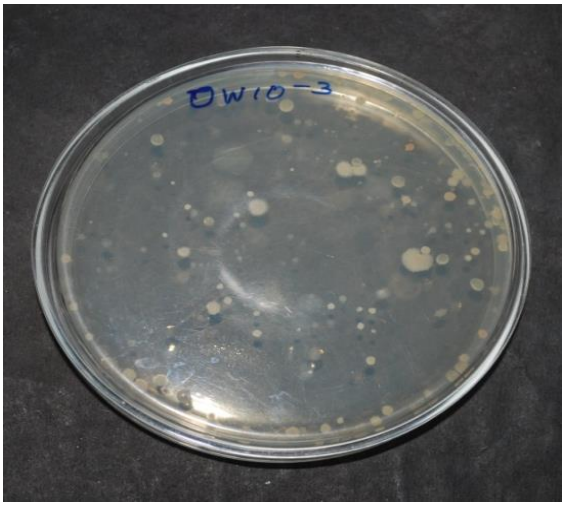
VP - Voges–Proskauer

MR - Methyl red



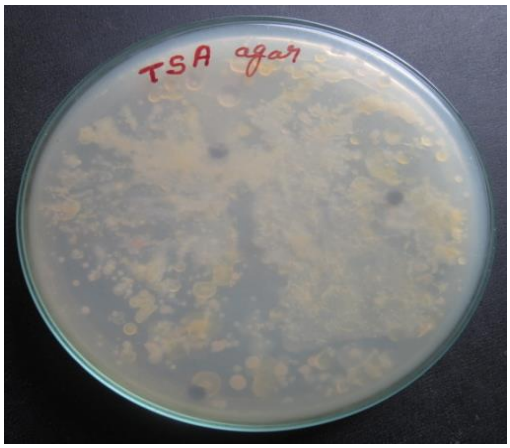
- *Staphylococcus* sp.
- *Micrococcus* sp.
- *Bacillus* sp.
- *Pseudomonas* sp.

Fig.1 Bacteria in the retting zones



- *Clostridium* sp.

Fig.2 Bacterial colonies along with *clostridium* sp.



- *Desulphovibrio*sp.

Fig. 3 Bacterial colonies along with *Desulpho vibrio* sp.

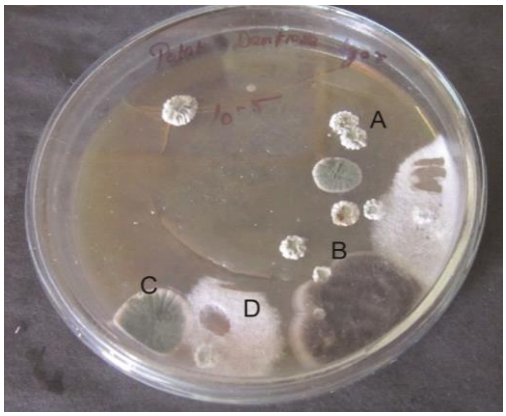


Fig. 4 A,C,D - *Pencillium* sp. B – *Aspergillus niger*

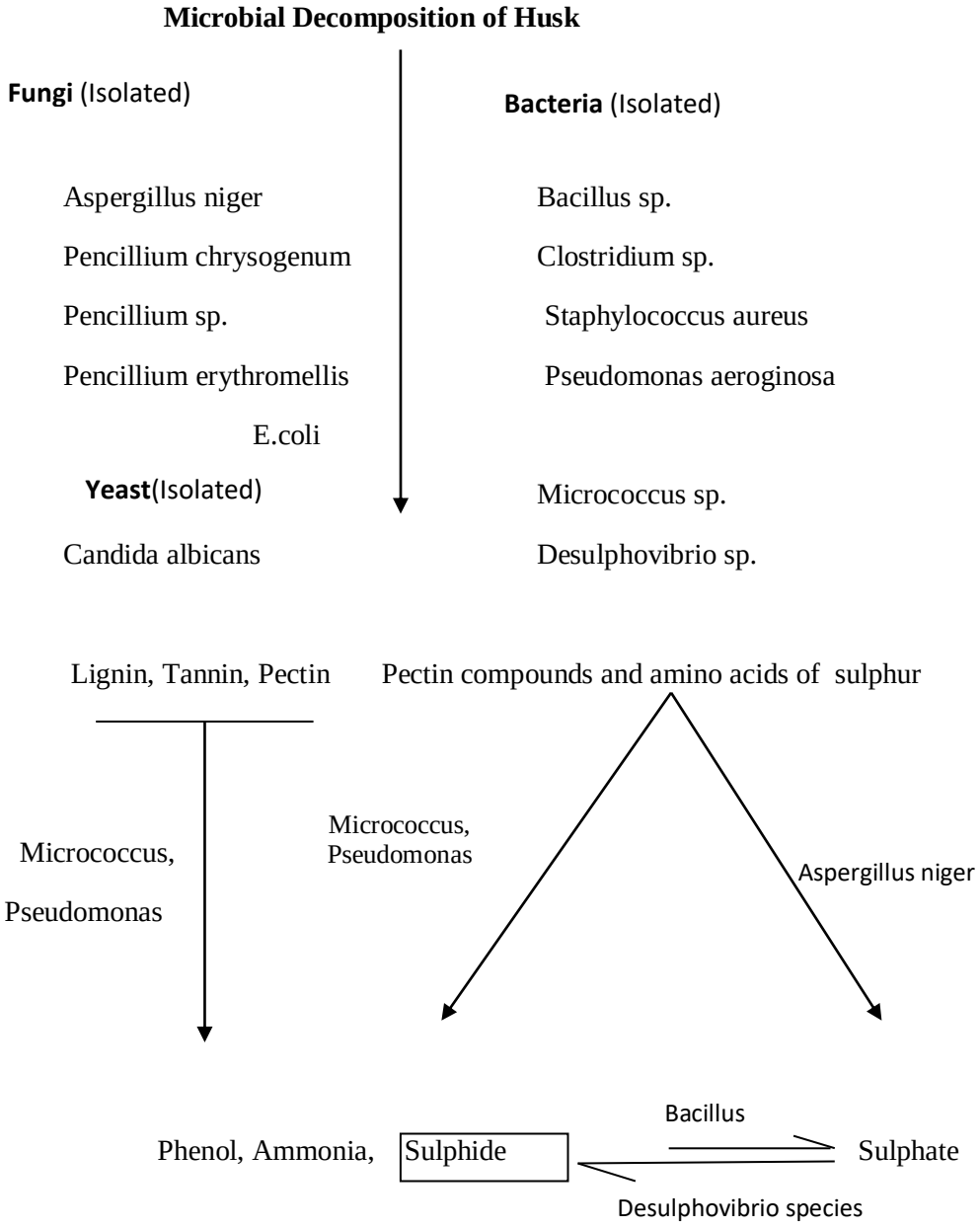


Fig. 5 Mechanism of Fermentation of Coconut husk

Conclusion

In the retting zones, the sulphates formed through the action of *Aspergillus niger* from the released compounds are also reduced further to sulphide through *Desulpho vibrio* sp. Bacterial species like *Pseudomonas* and *Micrococcus* are associated with the leaching of poly phenols. *Bacillus* and *Pseudomonas* play an important role in de-nitrification process active during summer resulting in the liberation of ammonia. Due to the microbial interference, toxic compounds are liberated into the environment thereby causing ecological degradation.

References

1. Barnett, I.A., Payee, P.W. and Yarrow, D. (2000) Yeasts: Characterization and Identification, 3rd ed. Cambridge University Press, Cambridge.
2. Bergey, D.H., John, G. Holt. (1994) Bergey's manual of determinative bacteriology.
3. Cholarajan, A., Kasthuri, J., Vijayakumari, R. and Muthukumaran, P. (2011) Physico-chemical and Microbial Analysis of Coir Industry Effluent, Asian J. Res. Pharm. Sci., V. 1, No.2, pp. 44-46.
4. Das, M.R., Paulmurugan, R., Thomas Sabu, F. and Sandhya, C. (2004) Impact of the physico-chemical parameters on the microbial population and its nature in a major retting zone of Kerala, Inter. J. Environ. Stud, V. 61, pp. 571-578.
5. *Dexter Dyer and Betsey, (2003). A Field Guide to Bacteria. Comstock Publishing Associates, Cornell University Press.*
6. Holmer, M. and Bondgaar, E.J. (2011) Photosynthetic and Growth response of eelgrass to low oxygen and high sulphide concentrations during hypoxic events, Aquatic Botany, V. 70, pp. 29-38.
7. KadeejaBeevi, M. Sree Kumar, S. and BijoyNandan, S. (2004) Environmental Hazards of retting zones in Kayamkulam backwaters, Kerala, Proceed.Indian National Congress, pp. 233-238.
8. Kolmert, A. (1999) Sulfate-reducing bacteria in bioremediation processes. Licentiate thesis, Lund University, Sweden.
9. Kurtzman, C.P. and Fell, J.W. (2006) "Yeast Systematics and Phylogeny Implications of Molecular Identification Methods for Studies in: Ecology", Biodiversity and Ecophysiology of Yeasts, The Yeast Handbook, Springer.
10. Manoj, K., Suraj, M.A., Shidhi, P.R., Hidesh, H., Sheeja, K., Pradeep, P. and Jisha, P. (2014)Study on Environemntal Degradation by Retting Activities in Backwater of Kodunganallur, Thrissur District, Kerala State, South India, Journ.ofEnv. Sci., Computer Sci. Eng. and Tech., V. 3, No.1, pp. 156-163.

11. Orth, D.S.(1977) Comparison of sulfite-polymyxin-sulfadiazine medium and tryptose-sulfite-cycloserine medium without egg yolk for recovering Clostridium perfringens, Appl. Envir. Microbiol., V. 33, pp. 986-988.
12. Promputha, I., Lumyong, S., Lumyong, P., McKenzie, E.H.C., Hyde, K.D. (2005) A new species of Anthostomella on Mag-nolialilifera from Northern Thailand,Mycotaxon, V. 91, pp. 413–418.

Binding of tris (bathophenanthrolinedisulphonate)ruthenium(II) cation with polyphenols in aqueous medium

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ABSTRACT

Polyphenols constitute one of the most common and widespread group of substances in flowering plants, occurring in all vegetative organs and fruits. Polyphenols containing gallol (gallic acid) and catechol (quercetin) groups have very different activities, depending on the metal ion. The binding of $[Ru(bpds)_3]^{2+}$ (bpds = bathophenanthrolinedisulphonate) complex with polyphenols (gallic acid and quercetin) in aqueous medium at pH 12.5 has been studied by absorption spectral techniques. The complex shows a ligand centred (LC) absorption peak at 278 nm and a metal to ligand charge transfer (MLCT) absorption peak at 463 nm in aqueous medium. The binding constant (K_b) for this reaction is determined from the Benesi-Hildebrand plot using absorption intensity data. The K_b of gallic acid with $[Ru(bpds)_3]^{2+}$ complex at 463 nm is 2.90 x 10^2 M^{-1} whereas for quercetin is 4.73 x 10^3 M^{-1} respectively.The K_b indicates that quercetin undergoes strong binding with the $[Ru(bpds)_3]^{2+}$ complex in the ground state than that of gallic acid. Structural effect seems to play a vital role on the binding of the gallic acid and quercetin with the complex.

Keywords: $[Ru(bpds)_3]^{2+}$ complex; Gallic acid; Quercetin; Benesi-Hildebrand equation; Binding constant

1. Introduction

Transition metal complexes have been found useful in pharmaceuticals since the discovery of cis-platin [1]. Several water soluble metal complexes attracted owing to their good anticancer or antibacterial properties. Among the metal complexes reported, the ruthenium complexes have certain advantages because of its solubility in water as well as low toxicity. Although there are several reports on the synthesis and medicinal properties of ruthenium complexes, the DNA targeted ruthenium complexes with intercalating ligands may be important anticancer agent. Ruthenium complexes with tris-(phenanthroline) (phen) derivatives are also extensively studied due to their interesting physico-chemical and biological properties [2]. Many complexes with phenanthroline ligand have been known for their anticancer property. Such complexes are namely useful in elucidating chemical principles which govern the recognition of nucleic acids, in developing photochemical reagents as new diagnostic tools, in the design of novel chemotherapeutics and in electron transfer mediated by the DNA double helix [3]. The photochemistry and photophysics of

Ruthenium-phenanthroline complexes have attracted the chemists in the design of light-driven water splitting photoanodes [4], molecular probes [5], construction of solar cells [6], sensors [7], molecular machine devices [8] and organic light emitting diodes [9]. This is due to the combination of excellent photophysical and electrochemical properties such as luminescence in solution at room temperature, moderate quantum yield and excited state lifetime, spectroscopically distinguishable metal redox states, tunable electronic properties, ability to undergo energy and electron transfer processes and chemical stability [10].

Polyphenols have considerable interest in the field of food chemistry, pharmacy and medicine due to a wide range of favourable biological effects including antioxidant properties. Polyphenols are antioxidants, which are known to influence bio availability of the metal in the body. The antioxidant property of polyphenols is mainly due to their redox properties. Some organic molecules binding to nucleic acids are of great interest in modern medicine because they constitute a significant portion of the anti-cancer drugs. Binding studies of flavonoids with DNA are useful for the understanding of the reaction mechanism and providing guidance for the application and design of new and more efficient drugs targeted to DNA.

Polyphenols with gallol and catechol groups are generally the most potent antioxidants, primarily because of the large iron-binding stability constants for these groups. Polyphenols containing gallol (gallic acid) and catechol (quercetin) groups have very different activities, depending on the metal ion. Gallic acid and quercetin binds with DNA, proteins and human serum albumim. Ruthenium(II)-phenanthroline complexes also bind with DNA. In order to understand the role of antioxidants with ruthenium(II)-phenanthroline complexes, the present work focuses on the ground state binding of $[Ru(bpds)_3]^{2+}$ (bpds = bathophenanthrolinedisulphonate) complex with gallic acid and quercetin in aqueous medium at pH 12.5.

2. Experimental Section

$RuCl_3.3H_2O$, bpds ligand, gallic acid and quercetinwere purchased from Sigma-Aldrich. The solvents for the synthesis of the complex were procured from Merck. The double distilled deionized water was used as a solvent for the binding studies.

2.1 Synthesis of ($[Ru(bpds)_3]^{2+}$) Complex

$RuCl_3.3H_2O$ (1 mmol) and bpds (3 mmol) were treated with 25 ml of water and refluxed under nitrogen atmosphere for 12 h. The resultant red solution was filtered hot and

evaporated to dryness to give a red-brown solid. This was recrystallized from ethanol-water mixture. The resulting product was characterized by UV-visible spectroscopy.

2.2 Absorption spectral measurement

Sample solutions of the $[Ru(bpds)_3]^{2+}$complex and the quenchers (gallic acid and quercetin) were freshly prepared for each measurements. The absorption spectral measurements were carried out using SHIMADZU UV-1800 spectrophotometer. The binding of $[Ru(bpds)_3]^{2+}$ complex with various concentrations of gallic acid and quercetin (5.8×10^{-5} - 3.5×10^{-4} M) in aqueous medium at pH 12.5 has been studied by absorption spectral technique. Phenolate ions of the gallic acid and quercetin for the binding studies were prepared by mixing the corresponding polyphenols with NaOH and the pH of the solution was maintained at 12.5 to confirm that the quenchers were present as phenolate ions. The binding constant (K_b) of the $[Ru(bpds)_3]^{2+}$ complex with the quenchers in aqueous medium were evaluated with the aid of Bensi-Hildebrand equation.

$$1/\Delta A = 1/K_b\Delta\varepsilon\,[H] + 1/\Delta\varepsilon\,[G]$$

where, [H] is the concentration of the host (complex), [G] is the concentration of the guest (quencher), ΔA is the change in the absorbance of the [H] on the addition of [G]. $\Delta\varepsilon$ is the difference in the molar extinction coefficient between the free [H] and [H]-[G] complex. The plot of $1/\Delta A$ vs 1/[G] gives a straight line. The K_b can be obtained from the ratio of Y-intercept to the slope of the straight line.

3. Results and Discussion

The structure of the $[Ru(bpds)_3]^{2+}$ complex and the polyphenols used in the present study are shown in **Fig.1** and **Fig.2**. The absorption spectrumof $[Ru(bpds)_3]^{2+}$ complex is carried out in aqueous medium at pH 12.5 (**Fig. 3**). The complex $[Ru(bpds)_3]^{2+}$complex shows the LC peak at 278 nm and the metal to ligand charge transition (MLCT) peak at 463 nm. The MLCT transition involves electronic excitation from the metal orbital [dπ (Ru)] to the ligand centered acceptor π* orbitals (ligand).

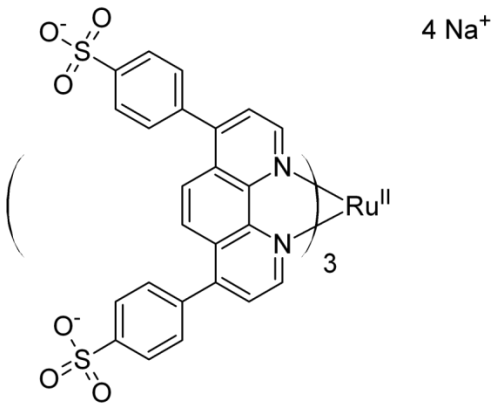

Fig. 1 Structure of $[Ru(bpds)_3]^{2+}$ complex

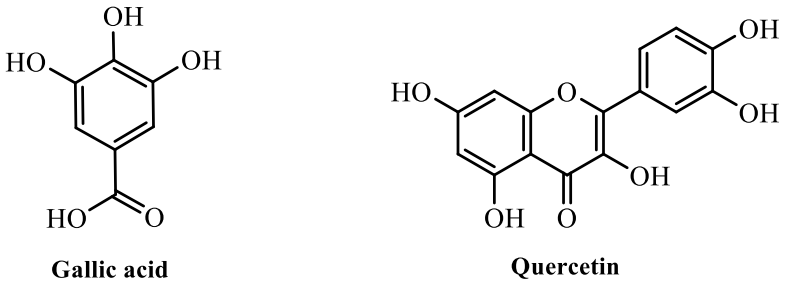

Fig. 2 Structure of polyphenols

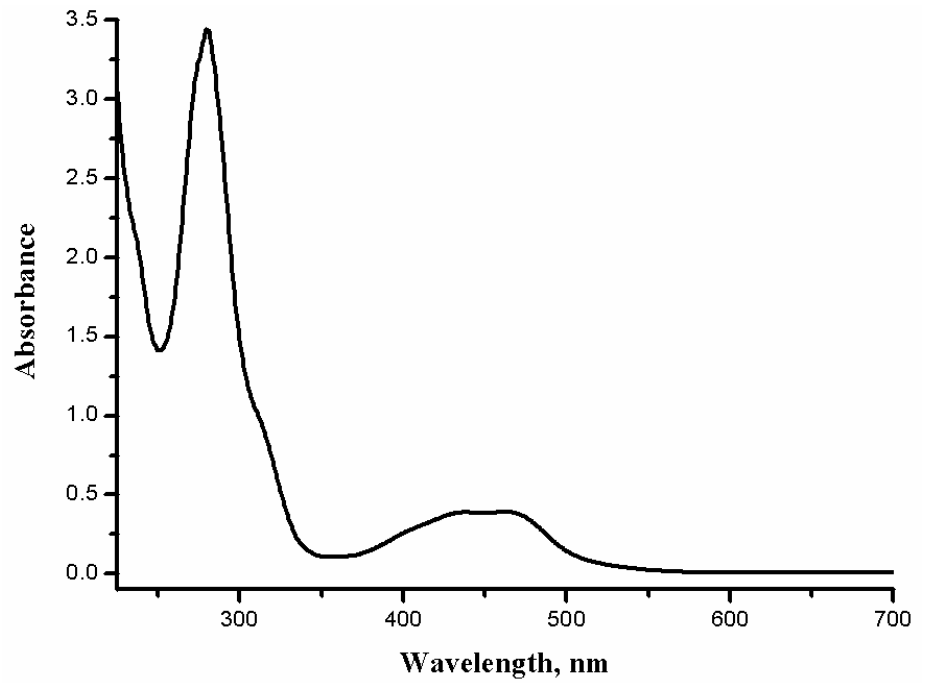

Fig. 3 Absorption spectrum of $[Ru(bpds)_3]^{2+}$ complex in aqueous medium

The absorption spectral studies of $[Ru(bpds)_3]^{2+}$ complex with the incremental addition of gallic acid and quercetin show an increase in the MLCT absorption maximum, this indicates the formation of ground state complex (**Fig. 4**). Gallic acid and quercetin have weak absorption at 454 and 426 nm [11]. Gallic acid and quercetin binds with $[Ru(bpds)_3]^{2+}$ complex in aqueous medium at pH 12.5 since, gallic acid and quercetin have weak absorption close to the region where Ru(bpds) complex have strong MLCT absorption. The K_b of this complex with gallic acid and quercetin are determined from the Benesi-Hildebrand plot (**Fig. 5**). The K_b of gallic acid with $[Ru(bpds)_3]^{2+}$ complex at 463 nm is 2.90 x 10^2 M^{-1} whereas for quercetin is 4.73 x 10^3 M^{-1} respectively.

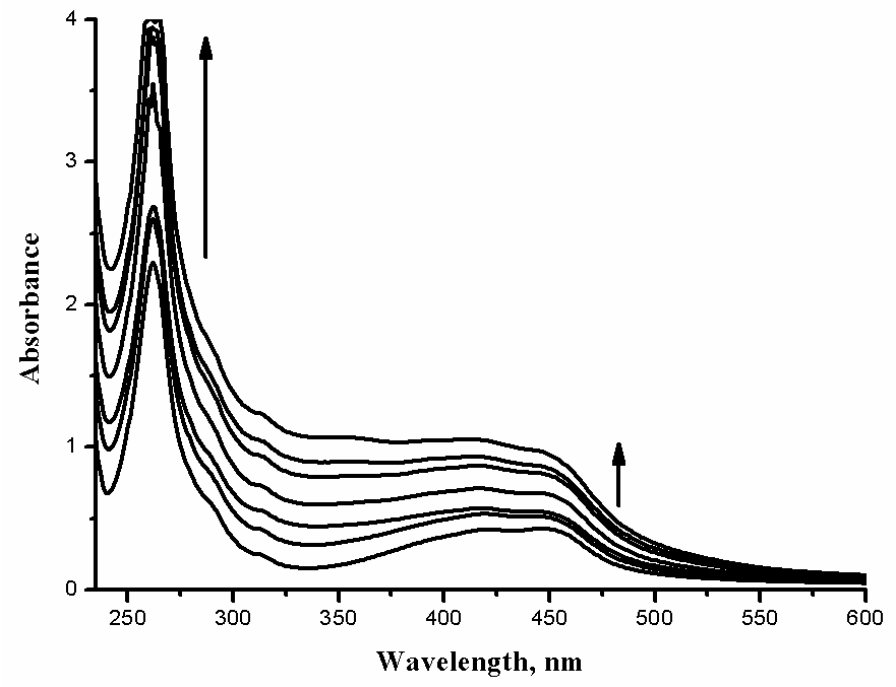

Fig. 4 Absorption spectra of $[Ru(bpds)_3]^{2+}$ complex with incremental addition of gallic acid in aqueous medium at pH 12.5

The ground-state interactions between polyphenols and the phenanthroline rings of $[Ru(bpds)_3]^{2+}$ complexes are hydrophobic or π - stacking in nature [12]. To the extent that π - π stacking interactions exist between the ligands of Ru(II) complex and the quencher, the binding becomes stronger. The binding takes place in the MLCT absorption maximum of the complex in the ground state. The K_b calculated for gallic acid and quercetin from MLCT absorption data shows that quercetin undergoes strong binding with the $[Ru(bpds)_3]^{2+}$ complex than that of gallic acid.

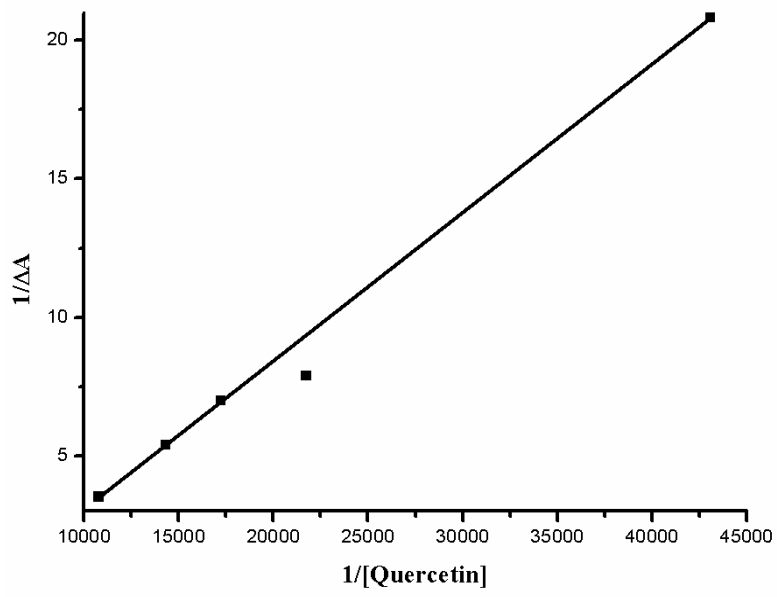

Fig. 5 Benesi-Hildebrand plot on MLCT absorption of $[Ru(bpds)_3]^{2+}$ complex with incremental addition of quecetin in aqueous medium at pH 12.5

Gallic acid consists of 3 phenolic–OH groups whereas quercetin consist of 4 phenolic–OH groups and at pH 12.5 all the phenolic–OH are converted into phenolate ions. The binding constant depends on the number of phenolic–OH groups. As the number of phenolic–OH groups increases the binding constant also increases. Hence quercetin shows a higher binding constant than that of gallic acid with $[Ru(bpds)_3]^{2+}$ complex. Thus, the K_b depends on the substituent present in the polyphenols.

Conclusion

The binding of gallic acid and quercetin with $[Ru(bpds)_3]^{2+}$ complex in aqueous medium at pH 12.5 has been studied by absorption spectral techniques. The K_b of the $[Ru(bpds)_3]^{2+}$ complex with gallic acid and quercetin are determined from the Benesi-Hildebrand plot. The K_b depends on number of phenolic–OH groups of the polyphenols. As the number of phenolic–OH groups increases the binding constant also increases. Quercetin shows higher binding constant than that of gallic acid due to the presence of more number of phenolic–OH groups. This study confirms the structural effect on the binding of biologically important phenolate ions with $[Ru(bpds)_3]^{2+}$ complex.

References

1. Iida, J., Bell-Loncella, E.T., Purazo, M.L., Lu, Y., Dorchak, J., Clancy, R., Slavik, J., Cutler, M.L. and Shriver, C.D., (2016). Inhibition of cancer cell growth by ruthenium complexes.J. Transl Med.,doi: 10.1186/s12967-0160797-9.
2. Turei, I., A. and Kljun, P., (2015). New synthetic routes for ruthenium-1,10-phenanthroline complexes, Tests of cytotoxic and antibacterial activity of selected ruthenium complexes.ActaChim. Slov., 62: 337 – 345.
3. Bugarcic, T., Habtemariam, A., Deeth, R.J., Fabbinani, F.P., Parsons, S. and Sadler, P.J., (2009). Ruthenium(II)-arene anticancer complexes with redox-active diamine ligands.Inorg Chem., 48: 9444 – 9453.
4. Young, K.J., Martini, L.A., Milot, R.L., Snoeberger, R.C., Batista, V.S., Schumuttenmear, C.A., Crabtree, R.H. and Brudvig, G.W., (2012). Light driven water oxidation for solar fuels.Coord. Chem. Rev., 256: 2503 – 2520.
5. Tan, L.F., Xie, L.J. and Sun, X.N., (2013). Binding properties of Ru(II)-polypyridyl complexes with poly (U) poly(A)* poly(U) triplex: the ancillary ligand effect on third-strand stabilization.J. Biol. Inorg. Chem., 18: 71 – 80.
6. Sannino, D., Vaiano, V., Ciambelli, P., Zama, I. and Gorni, G., (2013). Evaluation of N719 amount in TiO_2 films for DDSC by thermo gravimetric analysis. J. Therm. Anal. Calorim., 111: 453 – 458.
7. Schimittel, M. and Lin, H., (2008). Electropolymerisableruthenium(II)- phenanthroline carrying azo crown ether receptors: metal ion recognition in thin film redox sensors. J. Mater. Chem., 18: 333 – 343.
8. Balzani, V., Credi, A. and Venturi, M., (2009). Light powered molecular machines. Chem. Soc. Rev., 38: 1542 – 1550.
9. Chi. Y. and Chou, P.T., (2010). Transition- metal phosphorous with cyclometalating ligands: Fundamentals and applications. Chem. Soc. Rev., 39: 638 – 655.
10. Sibert, R., Winter, A., Schubert, U.S., Dietzek, B. and Popp, J., (2011). Molecular mechanism of duel emission in terpyridine transition metal complexes- Ultrafast investigations of photo induced dynamics. Phys. Chem. Chem. Phys., 13: 1606 – 1617.
11. Ramešová, S., Sokolová, R., Degano, I., Bulíčková, J., Žabka, J. and Gál, M., (2012). On the stability of the bioactive flavonoids quercetin and luteolin under oxygen-free conditions.Anal. Bioanal. Chem., 402: 975 – 982.
12. Li, C. and Hoffman, M.Z., (1999). One-electron redox potentials of phenols in aqueous solution.J. Phys. Chem. B, 103: 6653 – 6656.

Direct and indirect shoot multiplication from petiole explants of *Jatropha maheshwarii* Subr & Nayar

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ABSTRACT

A simple and reliable protocol for plant regeneration from petioles was developed. Highest shoot regeneration (85%) was recorded for l.0 mg/L BAP in which an average of 7.4 ± 2.3 shoots per culture was produced. The petiole produced highest percentage (85) of calli on Murashige and Skoog (MS) medium supplemented with 2, 4-D 2.5mg/L. Among the various concentrations and combination tested, BAP 1.0 mg/L with IAA 0.2 mg/L was found to be the best combination for maximum 9.5±0.84 shoot multiplication. BAP and IAA was the optimal combination for shoot elongation. The best rooting was obtained on medium incorporated with 2.0 mg/L IBA which induced 5.1±0.4 cm roots without intervening callus. Regenerated plantlets were successfully transplanted to soil with 80% survival.

Key words: *Jatropha maheshwarii, regeneration, shoot multiplication, callus.*

Introduction

Conservation of plant diversity assumes greater importance as the world is facing unprecedented loss of biological diversity. The conservation and sustainable use of biodiversity need to be central to all developmental activities in developing countries such as India as our economy is largely dependent on agriculture, forestry and bio-industrial products.

Realizing the threat of extinction, earlier workers felt the need to develop conservation strategies and quick propagation protocol such as *in vitro* techniques. Several conservation strategies were developed mainly in terms of *in situ* and *ex situ* conservation in the past decade [1, 2, 3].

Tissue culture techniques have been successfully used for the conservation of biological diversity by multiplication of plant species that have extremely poor populations, for species with restricted reproductive capabilities and for recovery and reintroduction [4].

Jatropha curcas primarily propagated through seeds, and significant variations in seed yield and oil content have been observed in plants raised through seeds [5, 6, 7]. Thus, the conventional propagation through seeds is not reliable and vegetative propagation by stem cuttings is inadequate to meet the demand [8, 9].

Jatropha maheshwarii Subr. and Nayar of the family Euphorbiaceae is a rare and endemic medicinal plant distributed in the coastal sandy areas of Tirunelveli and Kanyakumari districts of Tamil Nadu, India[10].

Materials and Methods

Explants *J. maheshwarii* had been collected from the natural populations at Kallikulam and Kanyakumari. Young twigs were collected from the mother plants and brought to the lab for tissue culture experiments. Petioles were excised separately from the leaf and used for *in vitro* propagation. The explants were trimmed into appropriate size and washed well in running tap water for 10-15 minutes. They were then treated with 0.2% Bavistin (fungicide) for 5 minutes followed by thorough rinse with distilled water. Further, they were surface sterilized with Tween-20 for 5 minutes and rinsed well with double distilled water. The explants were then taken to the transfer room and the rest of the sterilization process was carried out under Laminar Air Flow Chamber.

Explants were taken in wash bottles and treated with 0.1% mercuric chloride ($HgCl_2$) solutions for 3 minutes followed by four or five rinses (each rinse for 2 minutes duration) with sterile double distilled water. Finally the explants were placed on sterile petridish and the size of the explant was reduced to 1.0-2.0cm, by using sterile surgical blade. Then, the explants were inoculated on MS medium fortified with different concentrations and combinations of plant growth regulators (BAP, NAA, IAA and TDZ) for the induction of multiple shoots. Care was taken during the inoculation of explants. The culture tubes and vessels, inoculated with explants were incubated in the culture room at 25±2^{o}C and the relative humidity was maintained around 90%. The cultures were illuminated with white fluorescence lamps at 3000 lux intensity for a photo period of 12-15 hrs per day. The results of the cultures were observed periodically and recorded. The percentage of response, number of shoots per explants and length of the shoot were recorded.

Similarly for indirect shoot multiplication, the explants petiole was inoculated on MS medium supplemented with different concentrations of 2, 4-D. Initially for the induction of callus the cultures were incubated at 25±2^{o}C and illuminated with white fluorescence lamp at 3000 lux intensity for 12-15 hrs per day. After 20 -30 days, the maximum callus formation was observed from the explants. Well developed callus derived from the explants was also used for shoot multiplication separately. Calli were excised into 0.5cm^2 mass under Laminar Air Flow Chamber and inoculated on MS medium fortified with different concentrations and combinations of plant growth regulators viz BAP, NAA, IAA and TDZ for the induction of multiple shoots. The cultures were incubated under culture room conditions for the induction of multiple shoots. The results were observed and recorded at regular time intervals.

Results

Direct shoot induction from petiole explants

Culture of petiole explants inoculated on MS medium enriched with different plant growth regulators induced multiple shoot production (Table-1; Plate-1 a-c). The first shoot formation was observed after 8 days of culture. The best response was obtained on MS medium supplemented with BAP (1.0 mg/L) in which an average of 7.4±2.3 shoots per culture was produced with 85% of response. Higher concentration of BAP resulted in reduction in the number of shoot buds. Incorporation of BAP (0.5 mg/L) in combination with IAA (0.2 mg/L) stimulated the production of 3.5±1.9 shoots per explant. Effect of TDZ (2.0 mg/L) fortified MS medium showed the result of 3.6±1.5 shoots per culture with 65% of response. BAP in combination with NAA was less effective than all other hormones and combinations, in inducing shoots. Shoots failed to elongate on MS medium supplemented with either BAP or TDZ or BAP in combination with NAA . Transfer of shoots to MS medium containing BAP (0.5 mg/L) in combination with IAA (0.2 mg/L) resulted in elongation of shoots (6.3±0.81 cm). In this study the effect of BAP (1.0 mg/L) showed maximum 7.4±2.3 shoots. Though the percentage (55-75) of response was moderate in all other hormones, maximum numbers shoots produced per culture ranged from 1-3.

Callus induction from petiole explants

The petiole explants cultured on MS medium incorporated with different concentrations of 2, 4-D induced callus after 7 days. All the concentrations of 2, 4-D used, produced callus with variable responses (Table-2). In concentrations of 0.5, 1.0, 1.5, 3.0 and 4.0 mg/L there was no significant variation found on the percentage (70-75) of response. Well profused green, compact callus was produced on explants cultured on MS medium supplemented with 2, 4-D 2.5 mg/L. The highest percentage (85) of response was initiated on this concentration of 2.5 mg/L. In lower concentrations 0.5 mg/L and 1.0 mg/L and in higher concentration of 4.0 mg/L friable calli were formed in comparison to those produced at 1.5 mg/L 3.0 mg/L of 2,4-D.

Indirect shoot induction from callus of petiole

The result of the green compact calli subcultured on MS medium supplemented with BAP/ TDZ alone or BAP in combination with IAA/ NAA is recorded in table-3. Among the various concentrations and combination tested, BAP (1.0 mg/L) with IAA

(0.2 mg/L) was found to be the best combination for maximum (9.5±0.84) shoots multiplication. After 12 days of inoculation, many shoots emerged from the petiole. Elongation of multiple shoot (7.7±0.6 cm) was obtained on the medium containing concentration of BAP (1.5 mg/L) and IAA (0.2 mg/L). This was the optimal concentration for shoot elongation of multiple shoot buds (Plate-1, d-i). The combination of BAP with NAA produced less number of multiple shoots per callus mass as the shoot elongation was minimum. Addition of BAP (0.5 mg/L) alone on MS medium enhanced 60% of shooting response with 6.7±1.3 shoots. The average length of the shoot obtained on this concentration was 4.4±0.4 cm. On medium supplemented with 1.5 mg/L TDZ showed 75% of shooting response with an average number of 7.3±1.5 shoots per callus. However the number of shoots decreased compared to BAP and IAA combination. Hence the additions of IAA enhanced the average shoot length.

Discussion and conclusion

In this study highest shoot regeneration (85%) was recorded at l.0 mg/L BAP in which an average of 7.4±2.3 shoots per culture was produced. Earlier study reported similar effects on nodal explants of *J. curcas* responded better in BA augmented medium than KIN supplemented medium[11] .

In the present study MS medium fortified with 2, 4-D induced profused calli. Similar observation had been reported in *J. curcas* [12,13] . It is in contrary to the present study where IAA enhanced callus formation and shoot organogenesis in J. curcas petiole [11,14,9,15].

The current report is in corroborated with the earlier report of *J.curcas* [16] in producing multiple shoots and elongation per callus supplemented with 1.0 mg L^{-1} BAP + 0.5 mg L^{-1} IAA while the study on the plantlet regeneration from petiole segments of African violet by [17] showed maximum shoot multiplication on naphthaleneacetic acid (NAA) and 6-benzylaminopurine (BA).

Auxins are considered to be causative agents for root differentiation. Among the different auxins used IBA showed better results in root induction. The well developed shoots cultured on ½ MS medium fortified with 2.0 mg/L IBA produced 3-5 roots per shoot. Similar results were reported in *J. curcas* where ½ MS medium supplemented with IBA 3.0 mg/L produced 4-6 roots [18,19] .

Conclusion

In the present study an efficient protocol is developed to regenerate *Jatropha maheshwarii* a rare and endemic species through tissue culture. From the study it is clear that BAP and BAP in combination with IAA showed high production of adventitious shoot regeneration from the petiole explant. Hence the study is of prime importance for *in vitro* propagation of rare and endemic plant species without destroying the mother plant and subsequently the biodiversity. This is the novel method of conserving the natural populations of medicinal plants reduce the risk of extinction.

Table-1: Effect of PGRs on shoot multiplication from petiole explants.

Plant Growth Regulators (mg/l)				Shooting response (%)	Average number of Shoots per callus mass	Mean length of shoot (cm)
BAP	IAA	NAA	TDZ			
0.1				70	6.5 ± 1.17^{bc}	4.6 ± 1.62^{cde}
0.5				75	6.7 ± 1.33^{b}	4.3 ± 0.61^{cde}
1.0				85	7.4 ± 2.36^{a}	3.5 ± 0.43^{efgh}
1.5				80	5.3 ± 1.95^{c}	2.6 ± 0.72^{gh}
2.0				70	3.1 ± 1.69^{de}	2.5 ± 0.48^{h}
2.5				65	1.3 ± 1.15^{jk}	3.6 ± 0.72^{efgh}
0.1	0.2			70	2.6 ± 2.79^{e}	5.0 ± 0.66^{bc}
0.5	0.2			70	3.5 ± 1.95^{d}	6.3 ± 0.81^{a}
1.0	0.2			70	3.3 ± 3.30^{de}	6.0 ± 0.62^{ab}
1.5	0.2			65	2.6 ± 1.9^{ef}	5.5 ± 0.63^{ab}
2.0	0.2			65	2.0 ± 1.69^{gh}	4.8 ± 0.82^{bcd}
2.5	0.2			60	2.0 ± 1.95^{gh}	4.6 ± 0.46^{cde}
0.1		0.2		50	2.5 ± 1.19^{efg}	2.5 ± 0.30^{h}
0.5		0.2		55	2.0 ± 1.66^{gh}	2.6 ± 0.44^{gh}
1.0		0.2		60	2.0 ± 1.95^{gh}	3.5 ± 0.38^{efgh}
1.5		0.2		60	1.3 ± 1.19^{jk}	2.8 ± 0.96^{fgh}
2.0		0.2		55	1.0 ± 1.45^{k}	2.5 ± 0.37^{h}
2.5		0.2		50	1.0 ± 1.40^{k}	3.1 ± 0.36^{fgh}
			0.1	55	1.7 ± 9.49^{ij}	3.7 ± 0.61^{defg}
			0.5	60	1.9 ± 1.52^{i}	3.5 ± 0.96^{efgh}
			1.0	60	2.3 ± 0.96^{fg}	4.0 ± 0.30^{cdef}
			1.5	65	2.1 ± 1.10^{fg}	4.0 ± 0.40^{cdef}
			2.0	65	3.6 ± 1.57^{d}	3.6 ± 0.42^{efgh}
			2.5	60	3.5 ± 3.05^{ef}	2.5 ± 1.2^{h}

Mean value within the same column followed by the same superscript(s) are not significantly different ($p \leq 0.05$) according to ANOVA and LSD multiple range test.

Table-2: Effect of 2, 4-D on callogenesis of petiole explants.

Concentration of 2,4-D	Number of explants inoculated	Percentage of response	Texture of the calli	Color of the calli
0.5	20	70	Friable	white
1.0	20	70	Semi compact	white
1.5	20	75	Semi compact	Greenish white
2.0	20	80	compact	Greenish white
2.5	20	85	compact	Green
3.0	20	75	compact	GreenishYellow
4.0	20	70	Friable	White

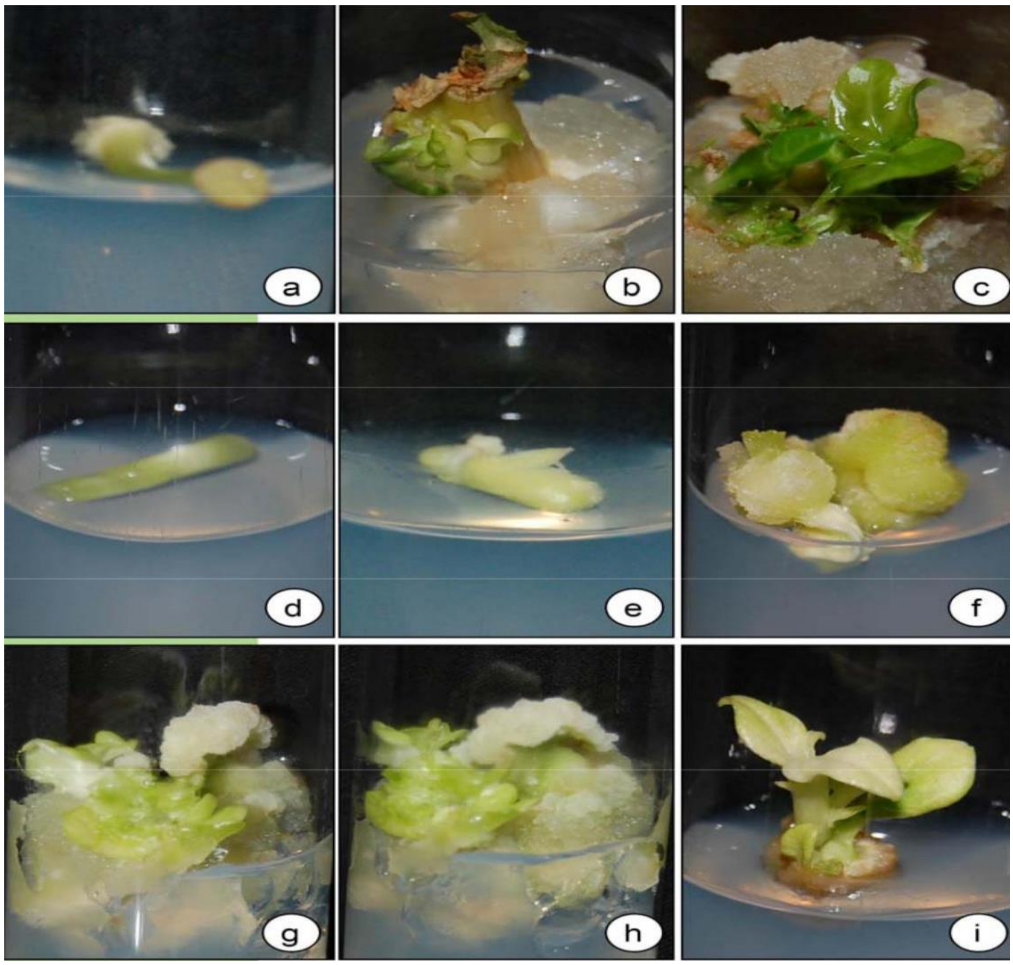
Mean value within the same column followed by the same superscript(s) are not significantly different ($p \leq 0.05$) according to ANOVA and LSD multiple range test.

Table-3: Effect of PGRs on shoot multiplication of the callus derived from petiole explants

Plant Growth Regulators (mg/l)				Shooting response (%)	Average number of Shoots per callus mass	Mean length of shoot (cm)
BAP	IAA	NAA	TDZ			
0.1				60	5.9 ± 1.19^{defg}	4.3 ± 0.31^{h}
0.5				60	6.7 ± 1.33^{cd}	4.4 ± 0.40^{gh}
1.0				65	5.3 ± 1.26^{fghi}	5.0 ± 0.51^{e}
1.5				70	5.8 ± 0.78^{defg}	4.7 ± 0.10^{f}
2.0				65	4.7 ± 1.41^{hijk}	4.7 ± 0.32^{f}
2.5				65	3.8 ± 1.22^{kl}	4.7 ± 0.22^{f}
0.1	0.2			75	8.0 ± 1.31^{b}	6.1 ± 0.44^{d}
0.5	0.2			80	9.0 ± 0.96^{ab}	6.5 ± 0.65^{c}
1.0	0.2			90	9.5 ± 0.84^{a}	6.7 ± 0.75^{b}
1.5	0.2			60	7.9 ± 1.66^{b}	7.7 ± 0.62^{a}
2.0	0.2			60	7.4 ± 1.44^{c}	6.7 ± 0.32^{b}
2.5	0.2			60	6.5 ± 1.31^{cde}	6.4 ± 0.30^{c}
0.1		0.2		40	3.7 ± 0.94^{l}	3.9 ± 0.31^{i}
0.5		0.2		50	4.0 ± 1.82^{jkl}	3.5 ± 0.71^{k}
1.0		0.2		55	4.8 ± 1.47^{hij}	3.7 ± 0.59^{j}
1.5		0.2		50	4.6 ± 1.07^{ijkl}	4.7 ± 0.53^{f}
2.0		0.2		50	4.8 ± 1.03^{hij}	4.3 ± 0.42^{h}
2.5		0.2		40	4.2 ± 0.22^{jkl}	3.9 ± 0.20^{i}
			0.1	50	4.8 ± 1.78^{hij}	2.7 ± 0.30^{n}
			0.5	70	5.2 ± 1.31^{hi}	2.9 ± 0.10^{m}
			1.0	75	6.0 ± 1.31^{defg}	3.7 ± 0.31^{j}
			1.5	75	7.3 ± 1.56^{c}	4.5 ± 0.80^{g}
			2.0	60	6.2 ± 1.03^{def}	3.3 ± 0.10^{l}
			2.5	60	5.6 ± 1.15^{efgh}	2.9 ± 0.25^{m}

Mean value within the same column followed by the same superscript(s) are not significantly different ($p\leq0.05$) according to ANOVA and LSD multiple range test.

Plate - 1



References

1. Ramsay M M, Jacskon A D and Porley R D 2000 *A pilot study for ex situ conservation of UK bryophytes.* In: *BGCI*, ed. EuroGard 2000 – EBGC, Canary Islands, Spain, Las Palmas de Gran Canaria: 52-57
2. Engelmann F and Engels J M M 2002 Technologies and strategies for *ex situ* conservation. In managing Plant Genetic Diversity. Ramanatha Rao V, Brown A H D and Jackson M T (eds) Wallingford and Rome; *CAB International and IPGRI* 89-104.
3. Paunescu A 2006 - *Strategii de conservare a biodiversităţii.* In Biodiversitatea de la Concepte Fundamentale la Aplicaţii Biotehnologice, *Ed. Acad. Rom., Bucureşti* 120-126 *Regulation* **47** 83-90.

4. Bramwell D 1990 The role of *in vitro* cultivation in the conservation of endangered species; In Herna´ndez Bermejo J E, Clemente M, Heywood V, (Eds) Proc.

5. Kumar N, and Reddy M P (2010). Plant regeneration through the direct induction of shoot buds from petiole explants of *Jatropha curcas*: a biofuel plant. Ann. Appl. Biol. 156: 367-375.
6. Pant K. S., Khosla V., Kumar D., Gairola S. (2006) Seed oil content variation in *Jatropha curcas* L. in different altitudinal ranges and site conditions in H.P. India. Lyonia, 11, 31-34.
7. Jha, T. B., Mukhejee, P., Datta, M. M., 2007. Somatic embryogenesis in *Jatropha curcas* Linn., an important biofuel plant. Plant Biotechnol. Rep. 1, 135-140.
8. Heller, J., 1996. Physic Nut, *Jatropha curcas*. Promoting the conservation and use of underutilized and neglected crops. Rome, Italy: International Plant Genetic Resources Institute (IPGRI).
9. Openshaw, K., 2000. A review of *Jatropha curcas*: an oil plant of unfulfilled promise. Biomass & Bioenergy 19: 1-15.
10. Subramanyan and Nayar M P 1964 A new species of *Jatropha* from Madras state *Bull. Bot. Surv. India* **6** 331-332.
11. Datta MM, Mukherjee P, Ghosh B, Jha TB (2007) In vitro clonal propagation of biodiesel plant (Jatropha curcas L.). Current Science, 93, 1438–1442.
12. Soomro R and Memon R A 2007 Establishment of callus and suspension culture in *Jatropha curcas*.; *Pak. J. Bot.* **39** 2431-2441
13. Rakkimuthu R Nithya Priya S and Aravinthan K M 2011 *In vitro* propagation of Jatropha curcas Linn- A valuable multipurpose Crop. *Advanced Biotech* **11(2)** 24-25
14. Sujatha M, Makkar H P S and Becker K 2005 Shoot bud proliferation from axillary nodes and leaf sections of non-toxic *Jatropha curcas* L.; *Plant Growth*.
15. Wei Q, Lu WD, Liao Y, Pan SL, Xu Y, Tang L, Chen F (2004). Plant regeneration from epicotyl explants of Jatropha curcas. J. Plant Physiol. Mol. Biol. 30(4):475-478.
16. Shamsiah, A., Awal, A., Nurathrah, S., Khairul-Azmir, M., Norrizah J, S., 2011. Effects of BAP concentration on *in vitro* shoot multiplication and callus induction of *Jatropha curcas*. ISHS Acta Horticulturae 923: 30
17. Bilkey, P.C., B.H. Mc Cown, and A.C. Hildebrandf. 1978. Micropropagation of African violet from petiole cross section Hort Science 13: 37-38.
18. Rajore S and Batra A 2007 An alternative source for regenerable organogenic callus induction in *Jatropha curcas*.; *Ind. J. of Biotech*., **6** 545-548
19. Shrivastava S and Banerjee M 2008 *In vitro* clonal propagation of physic nut (*Jatropha curcas* L): Influence of additives.; *Int. J. Integrative Biol.* **3** 73-79

Detection and physico-chemical depiction of an agglutinin from the diving grasshopper *Bermiella acuta* (Serville)

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ABSTRACT

The whole body extract of the diving grasshopper Bermiella acuta extracted using cold physiological saline was analyzed for the presence of naturally occurring agglutinin using hemagglutination assay. The extract showed the presence of agglutinin by way of specifically agglutinating rabbit and rat erythrocytes with high avidity. Physico-chemical characterization of the agglutinin of the grasshopper Bermiella acuta revealed that the agglutinating activity was optimum at pH 7.5 and temperature 30^{o}C. Addition of both calcium and magnesium although increased the agglutinability up to 10 mM concentration, optimum HA titer was observed only with the addition of 10 mM calcium suggesting the presence of a C type lectin. Calcium dependency was further affirmed by the drastic reduction in HA titer with the addition of very low concentrations of di- and tetra sodium EDTA. The extract, when pre-adsorbed with rabbit and rat erythrocytes, lost its ability to agglutinate both the erythrocytes suggesting the presence of a single agglutinin. Hemagglutinability was inhibited by the sialoglyco proteins lactoferrin>fetuin> porcine stomach mucin = porcine thyroglobulin > bovine thyroglobulin and sugars N acetyl galactosamine>fucose> maltose > N-acetyl glucosamine = N -acetyl mannosamine. However, the exact nature of the agglutinin can be established only after purification.

Keywords: Grasshopper, agglutinin, hemagglutination, hemagglutination inhibition. erythrocytes, cross adsorption

1. Introduction

Lectins and hemagglutinins are proteins/glycoproteins, which have at least one non-catalytic domain that exhibits reversible binding to specific monosaccharides or oligosaccharides. Theyare proteins found in a diversity of organisms and react with sugars in glycolipids, glycoproteins or oligosaccharides and agglutinate erythrocytes via cell surface glycoproteins and glycolipids [1,2]. Lectins can agglutinate cells and precipitate polysaccharides, glycoproteins and glycolipids [3,4]. Their specificity is usually defined in terms of a monosaccharide(s) or simple oligosaccharides that inhibit lectin-induced agglutination [5,6]. An agglutinin may recognize a part of a sugar [7], a whole sugar [8], their glycosidic linkages [9] or a sequence of sugars [10,11]. These properties enable lectins to mediate different biological processes such as cell-cell interactions [12], induction of apoptosis [13,14], cytotoxic activity [15], antibacterial and antiviral activity [16,17], antiproliferative activity for cancer cells [18], mitogenic activity [18, 19] and antitumor activity [20]. Isolation and characterization of lectins are of paramount importance for elucidation of the basic properties and biological functions of these proteins. Hence in the present work, an attempt is made to purify a lectin from the extract of the whole body of the diving grasshopper *Bermiella acuta.*

2. Material and Methods

2.1. Collection and maintenance of animals

The diving grasshopper *Bermiella acuta*, used in this investigation were collected from the side slope of Patanamkal canal at Vellicode, Kanniyakumari District, Tamil Nadu, India. They were brought to the laboratory 7 days before the experiment and were maintained in a plastic container containing a bowl of water and wet soil. They were fed with blades of fresh grass in the morning and evening.

2.2. Preparation of tissue extract

The extract of the whole body was prepared by homogenizing 100 mg of the grasshopper after removing the wings in 1 ml of cold 0.7% saline using a tissue homogenizer. The extract was centrifuged at 4000 x g for 10 minutes at 4ºC and the supernatant was used for hemagglutination assay.

2.3. Collection and preparation of erythrocytes

Blood from different mammals was collected by venipuncture of the ear (rabbit), cardiac puncture (rat), from the blood bank (Human A, B, O) and from the slaughter house (cow, goat and chicken) directly in modified Alsevier's medium (pH 6.1) containing sodium citrate (30 mM), sodium chloride (77 mM), glucose (114 mM), neomycin sulphate (100 µg/ml) and chloramphenicol (330 µg/ml) at a ratio of 2:8. Erythrocytes were suspended and washed three times by centrifugation at 4000 g for 5 minutes with ten volumes of Tris-Buffered Saline (TBS) pH 6.5 (Tris-HCl: 50 mM, NaCl: 100 mM; $CaCl_2$: 10 mM) and resuspended in the same as 1.5% suspension.

2.4. Hemagglutination (HA) Assay

The HA activity of the agglutinin in the extract of the whole body was assayed by measuring its ability to agglutinate erythrocytes. HA assays are performed at 30°C by serial dilution of the extract (25 µl) with TBS (25 µl) and mixing with 25 µl of 1.5% erythrocyte suspension. HA titer was determined by the visual estimation of erythrocyte agglutination on microtiter plates 60 minutes after adding the cells. The HA titer (the units of agglutinin activity) is the reciprocal of the highest dilution of the sample that gave agglutination.

2.5. Effect of pH, temperature, cations and chelators on HA titer

To develop strategies for affinity purification, HA assay was also performed with high agglutinating rabbit erythrocytes at different pH, temperature and using buffer with

different concentrations of cations Calcium, Magnesium and Manganese and chelators, di and tetra sodium EDTA.

To study the effect of pH on HA titer, the extract of the whole body of the diving grasshopper *Bermiella acuta* was mixed and serially diluted with equal volume of TBS at specific pH (5-10) and incubated for 1 hour before adding erythrocyte suspension.

To study the effect of temperature on HA titer, the extract of the whole body of the diving grasshopper *Bermiella acuta* was incubated for 1 hour at specific temperature (10-60°C) and used for HA assay.

To study the effect of cations and chelators on HA titer, the extract of the whole bodyof the diving grasshopper *Bermiella acuta* was mixed and serially diluted with equal volume of TBS containing specific concentration (0.01,0.1,1.0,10 and 100 mM) of cations (Calcium and Magnesium) and chelators (di and tetra sodium EDTA) incubated for 1 hour before adding erythrocyte suspension.

2.6. Cross-adsorption Assay

Packed erythrocytes (rabbit/rat) were prepared by repeated washing of erythrocytes in 0.9% saline by centrifugation at 4000 g for 5 minutes until we get a clear pellet. Extract of the whole body was mixed with equal volume of packed rabbit/rat erythrocytes and incubated for 18 hours at 4°C with occasional shaking. After centrifugation, the supernatant was analyzed for HA.

2.7 Hemagglutination inhibition (HAI) Assay

The extract of the whole body of the diving grasshopper *Bermiella acuta* was diluted to sub agglutination concentration (dilution at which hemolymph was able to provide 2 wells HA) was added to each well containing 25 µl of known concentration of serially diluted inhibitors (sugars and glycoprotein). After incubation for 1 hour, 25 µl of 1.5% rabbit erythrocyte suspension was added. The HAI titer is reported as the reciprocal of the highest dilution of inhibitors giving complete inhibition of agglutination after 60 minutes.

3. Results

3.1. HA activity of hemolymph: The extract of the whole body of the grasshopper *Bermiella acuta*, agglutinated rabbit and rat erythrocytes with diverse specificity. The HA titer with rabbit erythrocytes was found to be higher than the other red blood cells tested (Table 1).

3.2. HA activity after adsorption with different erythrocytes: When the extract of the whole body of the grasshopper *Bermiella acuta* adsorbed to rabbit/rat erythrocytes was used for HA assay it failed to agglutinate the erythrocytes of any other species (Table 2).

Table 1. Hemagglutinins in the extract of thewhole body of the diving grasshopper *Bermiella acuta*

Erythrocytes (n=10)	HA Titer
Human A	0
Human B	0
Human O	0
Rabbit	**2048**
Rat	128
Goat	0
Cow	0
Dog	0

Table 2. Cross adsorption assay of the extract of the whole body of the diving grasshopper *Bermiell aacuta*

Erythrocytes Adsorbed (n=5)	HA titer	
	Rabbit	Rat
None	2048	128
Rabbit	0	0
Rat	0	0

3.3. Effect of pH, temperature, cations and chelators: The extract of the whole body of the grasshopper *Bermiella acuta* had maximum agglutinin activity at pH 7.5 and temperature 30^{o}C (Table 3). Inclusion of increasing concentrations of cations Calcium/Magnesium in the buffer showed a gradual augmentation in the HA titer up to 10 mM concentration. HA got reduced with the addition of increasing concentrations of di and tetra sodium EDTA (Table 4).

Table 3. Effect of pH and temperature on hemagglutination assay of the extract of the whole body of the diving grasshopper *Bermiella acuta*

pH (n=10)	HA Titer	Temperature (n=10)	HA Titer
5.0	8	10	8
5.5	8 -16	15	8
6.0	64	20	16
6.5	512	25	512
7.0	1024	**30**	**2048**
7.5	**2048**	35	1024
8.0	512	40	128
8.5	256	45	64
9.0	64	50	16
9.5	16	55	8
10.0	8	60	8

Table 4. Effect of cations and chelators on hemagglutination assay of the extract of the whole body of the diving grasshopper *Bermiella acuta*

Concentration (mM) (n=5) (n=10)	HA Titer			
	Ca^{2+}	Mg^{2+}	Disodium EDTA	Tetra sodium EDTA
0.01	8	8	64	32
0.1	128	16	32	16
1.0	512	128	16	8
10.0	**2048**	256	8	4
20.0	512	64	4	2
30.0	128	32	2	2
40.0	32	16	2	0
50.0	8	4	0	0

3.4. Inhibitors of HA: N-Acetyl galactosamine (GalNAc), lactoferrin, fetuin and porcine thyroglobulin inhibited the agglutinin activity with great potency compared to the other sugars/glycoproteins tested for inhibition. Sugars such as lactose and trehalose and

glycoproteins such as Porcine stomach mucin, Bovine submaxillarymucin and α acid glycoprotein did not inhibit HA at concentration 50 mM and 2.5 mg/ml respectively (Table 5).

Table 5. Hemagglutinin inhibition titer of the extract of the whole body of the diving grasshopper *Bermiell aacuta*

Sugars/glycoprotein inhibitors (n=5)	HAI Titer	Minimum concentration required for inhibition (mM/µg/ml)	Inhibitory potency (%)
N-acetyl galactosamine	64	1.562	100
Fucose	16	6.25	25
Maltose	4	25	6.25
N-acetyl glucosamine	2	50	3.125
N-acetyl mannosamine	2	50	3.125
Lactose	0	-	-
Glucosamine	0	-	-
Lactoferrin	1024	4.88	100
Fetuin	256	19.58	25
Porcine thyroglobulin	16	312.5	1.562
Porcine stomach mucin	16	312.5	1.562
Bovine thyroglobulin	4	1250	0.39
Bovine submaxillary mucin	0	-	-
α-acid glycoprotein	0	-	-

4. Discussion

The extract of the whole body of the grasshopper *Bermiella acuta*, recognized rabbit and rat erythrocytes with diverse specificity. It was reported that different animal species have characteristic receptor determinants on their erythrocyte surface [21] and intraspecies variations are also found [22]. The ability of the grasshopper agglutinin to agglutinate rabbit and rat erythrocytes with high HA titer argues for the specific recognition of the sugars constituting the glycocalyx of these erythrocytes, which serve as receptors to ligands as in the eukaryotic cells [23]. Invertebrates do not possess an adaptive immune system based on a multitude of highly specific antibodies and antigen receptors equivalent to that of vertebrates. However, the great success of those primitive organisms, particularly arthropods, must

certainly rely on efficient immune defenses capable of protecting these animals against the multiple invading microorganisms, which continuously threaten their survival. Due to the fact that lectins have the ability to bind carbohydrate and promote the agglutination of different cells, such as bacteria and other invading pathogens, it is reasonable to assume that these molecules may be regarded as having a potential role in invertebrate non-self recognition reactions. They can agglutinate microorganisms and enhance their phagocytosis by mediating binding between the hemocyte surface and a foreign body-opsonic role, and are apparently synthesized by invertebrate immune cells, the hemocytes[24]. Since maximum HA titer was observed with rabbit erythrocytes, further experiments were restricted to rabbit erythrocytes.

The removal of HA following adsorption of the extract to rabbit/rat erythrocytes suggest the presence of a single hemagglutinin as reported in *Thyropygus descriptus*[25], *C. antennarius* [26], *Scylla serrata* [27,28] and *Paratelphusa jaquemontii*[29]. The extract when adsorbed to rabbit and rat erythrocytes, the agglutinin would have totally bound to the erythrocytes leaving no remnance of agglutinability capable of recognizing these erythrocytes after first adsorption. Though the serological studies show that activity to one type of erythrocyte can be adsorbed by that type of erythrocytes, leaving residual agglutinating activity to other type of erythrocytes [30], the extract of *Bermiella acuta* was fully adsorbed by both rabbit and rat erythrocytes with a single adsorption.

The extract of *Bermiella acuta* was also sensitive to pH and temperature. Maximum hemagglutinability was observed at pH 7.0 and temperature 30^{o}C. Conformational changes occur due to the change/dissociation of the binding sites of the agglutinin when there is decrease/increase in pH and temperature which may suppress/accelerate the hemagglutination activity. The loss of agglutinating activity of the whole body extract of the grasshopper with increased temperature may be due to destabilization of sporadic weak interactions of tertiary structure responsible for native conformation of lectin [31].

The extract of *Bermiella acuta* showed maximum activity with the addition of 10 mM $CaCl_2$ in the buffer (TBS). Cations are involved in stabilizing the primary structure of agglutinins. C-type lectins containing single / poly C-type carbohydrate recognition domains (C-type CRDs) can bind to carbohydrate residues on the cell surfaces in a calcium dependent manner [32,33]. Probably, the divalent cations may trigger/suppress the hemagglutination activity depending on their concentration. The calcium ion may act as a bridge between the protein and the sugar through direct interactions with the hydroxyl groups of sugar. HA titer got drastically reduced with the addition of 0.1 to 10 mM of di/tetrasodium EDTA in the buffer. Tetra sodium EDTA, the strongest inhibitor may presumably exert its effect through

chelation of divalent metals structurally associated with the agglutinin. So, it can be stated that the midgut gland agglutinin depends on cations for its activity.

The agglutinability was inhibited by lactoferrin and fetuin, the glycoproteins rich in N-acetyl neuraminic acid and N-glycolyl neuraminic acid respectively. The glycoproteins differ not only in their sialic acid content but also with respect to the distribution of the carbohydrate chains and their linkages to protein. So, the exact binding ability of the agglutinin can be illustrated only after purification. Among the methods of purification, affinity chromatography is considered to be a the best method of purification as it yielded higher quantity of lectin with great purity. So, this lectin can be purified by affinity chromatography using lactoferrin as ligand in the cyanogen bromide activated Sepharose 4B.

Conclusion

The presence of calcium dependent sugar binding agglutinins in arthropods such as the diving grasshopper *Bermiella acuta* suggest that these agglutinins may be involved in the innate immunity of these organisms. The sialic acid specificity of the agglutinin proposes for its possible application in microbiology and oncology following purification.

5. References

1. Stromberg P, Nyholmt G, Paschert I, Normark S (1991). Saccharide orientation at the cell surface affects glycolipid receptor function. Proc. Natl. Acad. Sci. USA 88: 9340-9344.
2. Sharon N (2008). Lectins: past, present and future. Biochem. Soci. Trans. 36: 1457-1460.
3. Lis H, Sharon N (1998) Lectins: Carbohydrate specific proteins that mediate Cellular recognition. Chemical Reviews, 98(2):637-674.
4. Zhang, Y., Qiu, L., Song, L., Zhang, H., Zhao, J., Wang, L., et al., (2009). Cloning and characterization of a novel C-type lectin gene from shrimp *Litopenaeus vannamei*. Fish Shellfish Immunol. 26, 183–192.
5. Sharon N, Lis H (1972). Cell-agglutinating and sugar-specific proteins. Science 177: 949-959.
6. Goldstein IJ, Hughes RC, Monsigny M, Osawa T, Sharon N (1980) What should be called a lectin? Nature 285:66.
7. Shimizu S, Ito M, Niwa M (1977) Lectins in the hemolymph of Japanese horseshoe crab, *Tachypleus tridentatus*. Biochem. Biophys. Acta 500: 71-79.
8. Bretting H, Kabat, EA. (1976) Purification and characterization of agglutinins from the sponge *Axinella polypoides* and a study of their combining sites. Biochemistry 15: 3228-3236.

9. Koch OM, Lee CK, Uhlenbruck G (1982). Cerianthin lectins: a new group of agglutinins from *Cerianthus membranaceus*. Immunobiology 163: 53-62.
10. Kobiler D, Mirelman D (1980). Lectin activity in *Entamoeba histolytica* trophozoites. Infect. Immun. 29: 221-225.
11. Mauchamp B (1982). Purification of an N-acetyl-D-glucosamine specific lectin (P.B.A.) from epidermal cell membranes of *Pieris brassicae*. L. Biochimie 64: 1001-1008.
12. Gabor, F. Bogner, E. Weissenboeck, A. Wirth, M. (2004). The lectin–cell interaction and its implications to intestinal lectin-mediated drug delivery. Advanced Drug Delivery Reviews, 56:459–480.
13. Perillo NL, Pace KE, Seilhamer JJ, Baum LG (1995). Apoptosis of T cells Mediatedby galectin-1.Nature, 378:736–9.
14. Vervecken W, Kleff S, Pfuller U, Bussin A (2000). Induction of apoptosis by Mistletoe lectin I and its subunits. No evidence for cytotoxic effects caused by isolated A- and B chains. The International Journal of Biochemistry & Cell Biology, 32:317-326.
15. Kawsar SMA, Mamun SMA, Rahman MS, Yasumitsu H, Ozeki Y (2010). Growthinhibitory effects on microorganisms by a D-galactose-binding lectin purified from the sea hare (*Aplysia kurodai*) eggs: An *in vitro* study. Nature and Science, 8(2):82-89.
16. Araújo, R.M.S. Ferreira, R.S. Napoleão, T.H.; Carneiro-da-Cunha, M.G.; Coelho, L.C.B.B. Correia, M.T.S. Oliva, M.L.V.; Paiva, P.M.G. (2012). *Cratae vatapia* bark lectin is an affinity adsorbent and insecticidal agent. *Plant Science (Limerick)*, 183: 20-26.
17. Napoleão TH, Pontual EV, Lima TA, Santos NDL, Sá RA, Coelho LCBB, Navarro DAMF, Paiva PMG (2012). Effect of *Myracrodruonurundeuva* leaf lectin on survival and digestive enzymes of *Aedesaegypti* larvae. Parasitology Research110:609-616.
18. Bah CS, Fang EF, Ng TB, Mros S, McConnell M, Bekhit AD (2011). Purification and characterization of a rhamnose-binding chinook salmon roe lectin Withantiproliferative activity towards tumor cells and nitric oxide-inducing activity towards murine macrophages. Journal of Agriculture and Food Chemistry, 59(10):5720-5728.
19. Maciel EVM, Araújo-Filho VS, Nakazawa M, Gomes YM, Coelho LCBB, Correia MTS (2004). Mitogenic activity of *Cratylia mollis* lectin on human lymphocytes. Biologicals 32:57–60.
20. Andrade CA, Correia MTS, Coelho LCBB, Nascimento SC, Santos-Magalhães NS (2004). Antitumor activity of *Cratylia mollis* lectin encapsulated into liposomes. International Journal of Pharmaceutics, 278:435–445.

21. Yamakawa T, Suzuki S (1953) The chemistry of lipids of post hemolytic residue or stroma of erythrocytes. IV. Distribution of lipid hexamine and lipid hemataminic acid in red blood corpuscles of various species of animals. J. Biochem. 40: 7-10.
22. Yasue S, Honda S, Miyagawa S, Inoue J, Hasegava A, Yamakava T (1978) Difference in the form of sialic acid in red blood cells glycolipids of different breeds of dogs. J. Biochem. 83: 1101-1107.
23. Hakomori SI (1973). Glycolipids of tumor cell membrane. Adv. Cancer. Res. 18: 265-315.
24. Marques, M.R.F. and Barracco, M.A. (2000). Lectins, as non-self-recognition factorsin crustaceans. Aquaculture 191 . 23–44.
25. MR Basil-Rose, MH Ravindranath, Sr PD Mercy (2014). Physico-chemical characterization of a natural agglutinin from the hemolymph of amillipede *Thyropygusdescriptus* ISJ 11: 331-336.
26. Ravindranath MH, Higa HH, Cooper EL, Paulson JC (1985a). Purification and characterization of an O-acetyl sialic acid specific lectin from a marine crab *Cancer antennarius*. J. Biol. Chem. 260: 8850-8856.
27. Mercy Sr. PD, Ravindranath MH (1992). An agglutinin with unique specificity for N-glycolylsialic acid residues of thyroglobulin in the hemolymph of a marine crab *Scylla serrata* (Forskal).Experientia 48: 498-500.
28. Mercy Sr. PD, Ravindranath MH (1993). Purificationand characterization of N-glycolylneuraminic acid-specific lectin from *Scylla serrata*.Eur. J. Biochem. 215: 697-704.
29. Maghil D, Mercy PD, Bai RN, Suriya JS (2003). Purification and characterization of a sialic acid specific lectin from the hemolymph of the freshwater crab *Paratelphusa jaquemontii*.Eur. J. Biochem. 270: 4348-4355.
30. Noguchi H (1903). On the multiplicity of the serum hemagglutinin of cold blooded animals. Zentr.Bakt. Abt. 34: 2865.
31. Singh AP, Saxena KD (2013). Effect of temperature, pH and denaturing agents on biological activity of MCJ lectin. Chem. Sci. Trans 2:1508-1512.
32. Ghazarian H, Idoni B, Oppenheimer SB (2011). A glycobiology review: carbohydrates, lectins and implications in cancer therapeutics. Acta Histo chem.; 113(3):236-47.
33. Kilpatrick DC (2002). Animal lectins: a historical introduction and overview Biochim Biophys Acta. 1572(2-3):187-97.

Antifungal activity of centipede *Rhysida longipes longipes*

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ABSTRACT

Centipedes which are considered venomous arthropods are rarely investigated for its antifungal efficacy. Hence, in this study it was attempted to analyse the antifungal activity of the whole body extract, hemolymph and the N-Acetyl Glucosamine (GluNAc) specific lectin purified from the hemolymph of the centipede Rhysida longipes longipes. The samples were tested against three fungi namely Candida albicans, Aspergillus flavus and Aspergillus niger. The results indicated that the hemolymph treated with butanol exhibited antifungal activity against the Aspergillus species and methanol treated hemolymph against C. albicans. However, this study revealed R. longipes longipes lectin as an antifungal agent against C. albicans with amaximum zone of inhibition of 10 mm.

Keywords: *Aspergillus flavus, Aspergillus niger,* Antifungal activity, *Candida albicans,* Lectin

1. Introduction

An elaborated internal defense system is necessary to resist infection of parasitic organism (e.g bacteria, fungi or protozoan) and viruses. Bacteria and fungi which are very frequent in the habitat of terrestrial arthropods may invade the hemocoel through wounds, multiply and lead to lethal infections if there is no effective immunological response which can destroy the pathogens. Such an internal defense system seems to be an indispensable pre-requisite for survival of a species when taking into account the high possibility of getting wounds in natural populations of various chilopods [1] which normally will lead to infections.

Antimicrobial resistance continues to grow quickly among key bacterial pathogens all over the world [2, 3]. Development of new antimicrobials is therefore imperative and there is a need for potent antimicrobial agent against pathogenic organisms. Extracts from centipedes and millipedes are used to treat many diseases in China and other parts of the world [4].

Antimicrobial peptides (AMPs) are important components of innate immunity and their distribution is widespread, including bacteria, fungi, plants and animals. Scolopendrin I, scolopin 1 and 2 were the few AMPs isolated from the centipede *Scolopendra subspinipes mutilans* [5, 6, 7]. Scolopin 1 and 2 exerted cytotoxicity through hemolytic activities against both human and rabbit erythrocytes. Chaparro *et al.*[8] reported antimicrobial activities in Chilopoda body extracts. An antimicrobial peptide, lacrain, was isolated by them from the

body extract of the Brazilian Chilopoda *Scolopendra viridicornis.* Lacrain has a sequence composed of eight amino acid residues and a molecular mass of 925.5 Da. Lacrain represents a novel molecule with powerful antibacterial activity that could be used as a new template for the development of drugs against clinically resistant bacterial strains. In this study, hemolymph, whole body extract and GluNAc specific lectin isolated from the hemolymph of the centipede *Rhysida longipes longipes* were assessed for their antifungal activity.

2. Materials and methods

2.1. Experimental Animal

Centipedes *Rhysida longipes longipes* were collected from Holy Cross College Campus, Kanyakumari District, Tamilnadu, India and brought to the laboratory.

Rhysida longipes longipes

2.2. Sample collection

2.2.1. Hemolymph: Hemolymph was collected from the centipede as per the procedure described by Xylander and Nevermann [9] and was lyophilized.

2.2.2. Whole body extract: Healthy centipede was weighed and cold saline was added in the proportion 100 mg/ml and ground to a fine paste. The extract was centrifuged, filtered and the supernatant was then lyophilized.

2.2.3. GluNAc specific lectin: GluNAc specific hemagglutinin present in the hemolymph of the centipede *R. longipes longipes* was purified following the method of Nowak and Barondes [10] and Vinoliya [11].

2.3. Fungal strain

Aspergillus flavus, *Aspergillus niger* and *Candida albicans* were obtained from INBIOTICS laboratory, Nagercoil, Kanyakumari District, India.

2.4. Antifungal assay

Antifungal susceptibility tests were determined by disc diffusion method [12]. Test samples were prepared in the ratio 1:1 and the discs were impregnated with 50 µl of each

sample i.e. methanol and butanol extract of hemolymph and whole body, and aqueous extract of GluNAc specific lectin respectively and placed in the medium. Discs with standard antibiotics (Fluconazole) as positive control and solvents as negative control were also placed in the medium to compare the test results (Fig. 1).

3. Results

Antifungal activity of methanol and butanol extract of hemolymph, whole body and aqueous extract of GluNAc specific lectin of *R. longipes longipes* were tested against three pathogenic fungi *Aspergillus flavus, Aspergillus niger* and *Candida albicans*. Butanol extract of hemolymph inhibited the growth of *A. flavus* and *A. niger* with a zone of 7 and 9 mm respectively while *C. albicans* was best inhibited by the methanol treated hemolymph and the purified lectin (10 mm) (Fig. 2 and Table 1).

4. Discussion

Centipedes have been used in traditional medicine for it's pharmacological efficacy in treating diseases [13]. Centipede venoms have been found to be an excellent source of proteins/peptides that has antibacterial, antifungal and anticancerous property [14, 15, 16]. However, reports on the antifungal activity of hemolymph, whole body and lectin of centipede are limited. In the present study the anti-fungal activities of solvent extracts of *R. longipes longipes* against three pathogenic fungi *C. albicans, A. flavus* and *A. niger* were studied. *Aspergillus* species were found to be sensitive to the butanol extract of hemolymph suggesting the presence of a bioactive compound which could be either an antimicrobial peptide [17] or a lectin [18] or even hemocyanin [19] as reported in other arthropods. *Candida albicans* was sensitive to the centipede samples and the purified lectin suggesting the possibility of specific receptors on the cell surface of the fungus that could bind to the bioactive molecule thereby inhibiting the growth of the fungi. Potential modes of action may include fatal depolarization of the normally polarized membrane, formation of physical pores, scrambling of the usual distribution of lipids between the leaflets of the bilayer, and damage to critical intracellular targets or cell death by leakage of cellular contents and disturbance of membrane functions [20]. Similar antifungal activity has been observed with a novel lactoferrin B antifungal peptide from the *Scolopendra subspinipus mutilans* [14, 16] and *Rhysida nuda nuda* [21].

Fungal infections caused by *Candida* have been known since the fourth century [22] and are responsible for the majority of fungal infections currently diagnosed that leads to disease [23, 24]. Currently available antifungal agents are either toxic or act in a fungi static

manner, and resistance to these agents is already an emerging problem. Hence identifying an antifungal agent from centipede which is widely used in traditional medicine would be of immense help in the pharmaceutical industry.

Fig. 1: Antifungal activity of positive and negative controls against tested fungi

Fig. 2: Antifungal activity of hemolymph, lectin and whole body of *R. longipes longipes* against *A. flavus, A. niger* and *C. albicans*

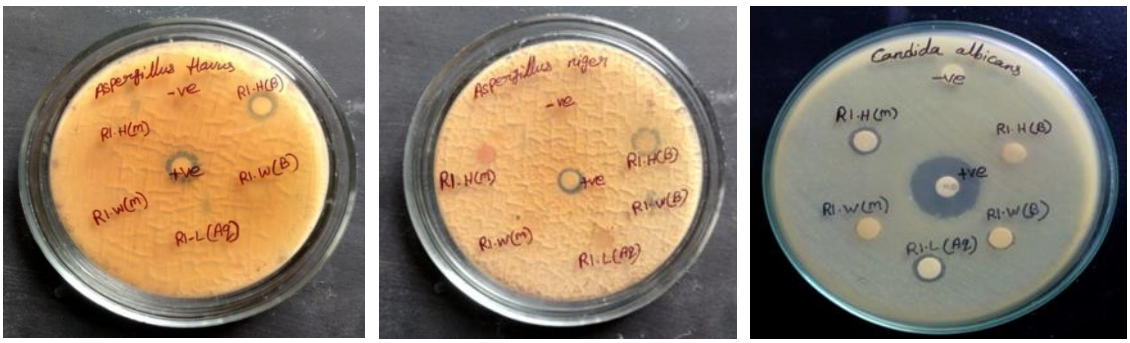

Table 1: Antifungal activity of *R. longipes longipes*samples against tested fungi

Samples	*Aspergillus flavus*	*Aspergillus niger*	*Candida albicans*
	Zone of inhibition (mm)		
Rl. H (M)	-	-	9
Rl. H (B)	7	9	-
Rl. W (M)	-	-	-
Rl. W (B)	-	-	-
Rl. L (Aq)	-	-	10
+ ve control(Flucanozole)	21	10	21
-ve control(Solvents)	-	-	-

Rl- *Rhysida longipes*; H - Hemolymph; W - Whole body; L - lectin;
M - Methanol; B - Butanol; Aq - Aqueous

5. Reference

1. Frund, H.C. (1990). The occurrence and frequency of Scarsin centipede.Veroff.Univ. Innsbruck 177. Abstracts 8th International Congress on Myriapodology, 65: 597-599.
2. Bax, R., Mullan, N. and Verhoef, F. (2000). The millennium bugs - the need for and development of new antibacterials. International Journal of Antimicrobial Agents, 16(1): 51-59.
3. Bhavani, S.M. and Ballow, C.H. (2000). New agents for Gram-Positive bacteria. Current Opinion in Microbiology, 3(5): 528-537.
4. Barceloux, D.G. (2008). Medical toxicology of natural substances.Foods, fungi, medical herbs, plants and venomous animals.Hoboken, New Jersey, John Wiley and Sons, INC.
5. Peng, K., Kong, Y., Zhai, L., Wu, X., Jia, P., Liu, J. and Yu, H. (2010). Two novel antimicrobial peptides from centipede venoms. International Journal of Toxicology, 55(2-3): 274-279.
6. Malta, M.B., Lira, M.S., Soares, S.L., Rocha, G.C., Knysak, I., Guizzze, S.P., Santoro, M.L. and Barbaro, K.C. (2008). Toxic activities of Brazilian centipede venoms. Toxicon, 52: 255-263.
7. Wenhua, R., Shuangquan, Z., Daxiang, S., Kaiya, Z. and Guang, Y. (2006). Induction purification and characterization of a peptide Scolopendrin I from the venom of centipede *Scolopendra subspinipes mutilans*. Indian journal of Biochemistry and Biophysics, 43: 88-93.
8. Chaparro, E., da Silva, P.I Junior. (2016). Lacrain: the first antimicrobial peptide from the body extract of the Brazilian centipede *Scolopendra viridicornis*, 48(3): 277-85.
9. Xylander, W.E.R. and Nevermann, L. (1990). Antibacterial activity in the hemolymph of Myriapoda (Arthropoda). Journal of Invertebrate Pathology, 56: 206-214.
10. Nowak, T.P. and Barondes, S.H. (1975). Agglutinin from *Limulus polyphemus*: purification with formalinized horse erythrocytes as the affinity adsorbent. Biochimica et Biophysica Acta (BBA) – Protein Structure, 393 (1): 115-123.
11. Vinoliya, J. (2006). Centipede hemolymph lectin: Nature, purification and possible functions. Ph. D Thesis, Manonmaniam Sundaranar University, Tirunelveli.
12. Bauer, A.W., Kirby, W.M.M., Serris, J.C. and Turck, M. (1966). Antibiotic susceptibility testing by a standardized disc method. American Journal of Clinical Pathology, 45: 493-496.
13. Pemberton, R.W. (1999). Insects and other arthropods used as drugs in Korean traditional medicine. Journal of Ethno Pharmacology, 65(3): 207-216.

14. Choi, H., Hwang, J.S. and Lee, D.G. (2013). Antifungal effect and pore-forming action of lactoferricin B like peptide derived from centipede *Scolopendra subspinipes mutilans.* Biochimica et Biophysica Acta, 1828: 2745-2750.
15. Kwon, Y.N., Lee, J.H., Kim, I.W., Kim, S.H., Yun, E.Y., Nam, S.H., Ahn, M.Y., Jeong, M., Kang, D.C., Lee, I.H. and Hwang, J.S. (2013). Antimicrobial activity of the synthetic peptide Scolopendrasin II from the centipede *Scolopendra subspinipes mutilans*. Journal of Microbiology and Biotechnology, 23(10): 1381-1385.
16. Lee, J.H., Kim, I.W., Kim, H.S., Kim, M.I, Yun, E.Y., Nam, S.H., Ahn, M.Y., Kang, D. and Hwang, J.S. (2015). Anticancer activity of the antimicrobial peptide Scolopendrasin VII derived from the centipede, *Scolopendra subspinipes mutilans*. Journal of Microbiology and Biotechnology, 25(8): 1275-1280.
17. Scott, M. and Hancock, R.E. (2000). Cationic antimicrobial peptides and their multifunctional role in the immune system. Critical Reviews in Immunology, 20: 407-431.
18. Elayabharathi, T., Vinoliya Josephine Mary, J., Mary Mettilda Bai, S. (2017). Partial purification and characterization of hemolymph lectin of marine crab Atergatis ocyroe by adsorption on formalinized erythrocytes. International Journal of Biology Research. 2(4): 115-119.
19. Petit, V.W., Rolland, J.L., Blond, A., Cazevielle, C., Djediat, C., Peduzzi, J., Goulard, C., Bachere, E., Dupont, J., Destoumieux-Garzon, D. and Rebuffat, S. (2016). A hemocyanin-derived antimicrobial peptide from the penaeid shrimp adopts an alpha-helical structure that specifically permeabilizes fungal membranes. Biochimica et Biophysica Acta, 1860(3): 557-568.
20. Micheal A Zasloff (2002). Antimicrobial peptides of multicellular organisms. Nature, 415(6870): 389-395.
21. Vargila, F. (2017). Defense system of centipede *Rhysida nuda nuda* and its biomedical application.M. Phil Thesis, Holy Cross College (Autonomous), Nagercoil, India.
22. Calderone, R. A. (2002). *Candida* and candidiasis. ASM Press, Washington, D.C
23. Calderone, R., Diamond, R., Senet, J.M., Warmington, J., Filler, S. and Edwards, J. E. (1994). Host cell-fungal interactions. Journal of Medical Veterinary Mycology, 32**:** 151-168.
24. Cutler, J. E. (1991). Putative virulence factors of *Candida albicans.* Annual Review of Microbiology, 45**:** 187-218.

Morphological identification and phylogenetic analysis of a grapsid crab, *Grapsus albolineatus* (Lamarck, 1818) (Decapoda, Brachyura, Grapsidae)

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ABSTRACT

The study envisioned the identification of selected crab viamorpho-taxonomy using ninety two morphological characters. Crabs of Grapsus albolineatus were collected from the Kanniyakumari coastal areas. They were morphologically identified through description of colors, dentations of the carapace and shapes of chelipeds. The crab resembles genus of Grapsus, distinguished by the form of the male thoracic sternum, III maxilliped and the structure of the male gonopods. T*he adult morphology-based phylogeny of G. albolineatus documented a paraphyletic taxon, shared with other grapsids with high bootstrap value claiming it taxonomic position super familyas Grapsoidea and family - Grapsidae.*

Keywords: crustacean, taxonomy, phylogeny, parsimony, *Grapsus albolineatus*

Introduction

Crabs of the infra-order Brachyura are one of the most diverse groups of crustaceans with approximately 7,000 described species, 1271 genera in 98 families and 38 super families occurring in marine, freshwater, and terrestrial habitats [1]. The relationships among the brachyuran families are poorly understood owing to the high morphological intricacy of the group.Studies associated to taxonomy and systematics of crabs isinadequate, neverthelessseveral works are being carried out in various other characteristics. The greater degree of adaptation of brachyuran crabs to varied environments and their pattern of distribution results individual variability within the species in their morphology, polymorphisms and sexual dimorphisms eventually leads misperception and difficulty in identification of brachyuran crabs [2]. Researchers distinguished brachyuran crabs grounded on minor morphological characters on carapace such as spination – its presence or absence, orientation and arrangement or the number, transverse ridges, specific markings first male pleopods or the third maxillipeds etc. [3,2]

The family Grapsidae is presently divided into four subfamilies, Grapsinae, Plagusiinae, Sesarminae and Varuninae, based on the adult morphology[4]. However, the sub-familial arrangement of some genera within the Grapsidae has been questioned by recent contributions based on larval morphology [5,6] and molecular data using the 16S rRNA

[7].Keeping this in perspective, the present study analysis the adult morphology and morphology-based phylogenyin thegrapsid crab*G. albolineatus*based upon 92 adult morphological characters. This study also provides an extensive systematic account of the brachyuran crabs employing the difference in first male pleopods, shape of the male abdomen and the third maxillipeds.

Materials and methods

Collection and rearing of the specimens

The animals were collected from Kadiapattanam (1058'N; 7831'E.) and Muttom (983'N; 7671'E), Kanyakumari district, Tamilnadu, India, by hand picking and 'lift net' with bait along the intertidal and sub tidal zones. Adult male crabs were categorized and maintained in the laboratory in near natural conditioned and fed with egg white.

Morphological examination and taxonomy

The candidate crab is not endangered / protected species and were morphologically identified at species level using the keys followed by [8,1] and by other taxonomic experts and subsequently matched with the online databases of World Register of Marine Species (WoRMS).Drawings are made by using compound microscope and dissection microscope, each equipped with a camera lucida.

Morphological coding and matrix construction[9,10]

The morphological coding matrix was constructed using 92 morphological characters(69 binary and 23 multi-state)comprising additional brachyuran crabsgathered from previous reports.

Phylogenetic tree construction and evaluation

Phylogenetic analysis was performed under the PAUP* version 4.0 [11].Phylogenetic tree was constructed byNeighbour Joining (NJ)[12] algorithms and Maximum Parsimony (MP)[13]. The inferred phylogeny was statistically evaluated through skewedness tests[14].

RESULTS

The crab was recognized as*Grapsus albolineatus* (Lamarck, 1818)as per the World Register of Marine Species.They arecommonly called as Mottled sally- light- foot.

Scientific synonyms (www.species-identification.org)

Cancer strigosus (Herbst 1799); *Grapsus albo-lineatus (*Lamarck 1818); *Grapsus (Goniopsis) strigosus* (not Linnaeus, 1761) deHaan(1835); *Grapsus (Goniopsis)*

flavipes (MacLeay 1838); *Grapsus albolineatus* (Banerjee 1960; Michel 1964; Crosnier 1965;Sakai1976a; Takeda andNunomura 1976; Hwang and Yu 1980; Miyake 1983; Suzuki 1985;Dai et al. 1986; Fukui et al. 1989; Dai and Yang 1991; Chang and Chen 1992; Tzeng and Chen 1992; Wang and Liu 1996a; Yu et al. 1996; Ho and Hung 1997; Jeng 1997; Jeng et al. 1997; Lai et al. 1997; Wang and Liu 1998; Muraoka 1998; Ng 1998c; Sakai 1999; Shokita et al. 2000).

Holotype

The morphological characters (92) were categorized into eight groups: carapace, eyes, antennae and antennules, mouth parts, abdomen, thoracic sternum, gonopods, cheliped and walking legs.

Carapace appears olive green with white transverse markings dorsal and the ventral side staysrelatively whitish. The legs are reddish to brownish with pale yellow patches and spots. The palm of the cheliped remains white or yellowish white externally and the fingertips are bluish violet (Fig. 1, 2). Carapace round in shape and slightly depressed in the centre (Fig. 3). Frontal teeth are absent. Lateral margins are strongly arched with numerous oblique striate. Antero-lateral margins rounded, each with one tooth, however, the postero-lateral margin appears smooth. The protogastric, mesogastric regions and branchio-cardiac groove are not discernible. The intestinal region is longitudinally bloated.

Eyes are enclosed in orbits, which are ovate and deep. Both supra- and infra-orbital margins are smooth and prominent. The antenna is absent; nevertheless the antennule is short and invisible dorsally (Fig. 4). The buccalcavernis bordered on both sides by the pterygostomial regions, rooted by the epistome and inside by the endostome. The epistome shorter and it has two ridges which are curved and touch the upper border of the buccal cavern (Fig. 5). Two smaller feeding appendages are situated below the three pairs of maxillipeds; I and II maxilla (Fig. 6). A distinct rhombidal gap existsbetween the III maxillipeds. The mouth is bordered by a pair of well- calcified, jaw-like and highly modified appendages, the mandibles.

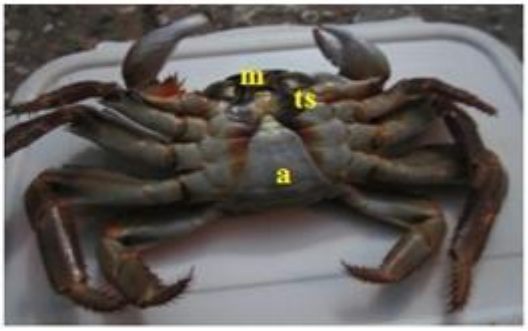
Adult male and female crabs are distinguished by the shape of their abdomen: triangular (broadly T-shaped) in males (Fig. 7) and dome-shaped in females. In males, the 6^{th} abdominal segment with lateral margin converges distally and remainsshorter than the 5^{th} segment. Thoracic sternum is wide (Fig. 8); 1-4 are distinct andthe 8^{th} sternum bears small spines, however, the suture is indistinct and poorly defined. A median groove and the button are absent in the sternite.The cristiform margin remainsvague in *G. albolineatus*. Males

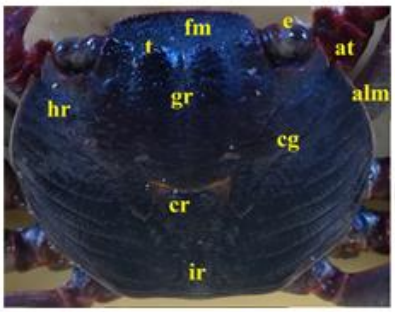
possess 2 pairs of outward direct gonopods; stout and slightly bent G1 and broad G2 with narrow distal end facing outward (Fig. 9).

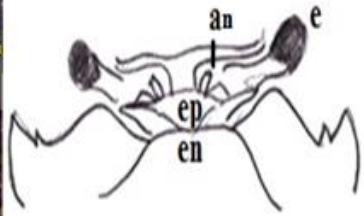
Chelipeds are sub-equal (Fig. 10). The inner border of the ischium and the merus has numerous spines and tubercles. Merus consists of 3-4 spine-shaped teeth on the anterior margin. The inner surface of the carpus possesses a large sword-shaped tooth. The palm shows four ridges on external surface. The dactylus is narrowing and pectinated with a small spine on the lateral side. The upper dactylus is broad and curved at the tip, while the lower is spoon-shaped and immovable finger. The tooth in the chelipede is 10 in number: 5 upper and 6 lower. The transverse ridges on the merus of the walking legs (Fig. 11) are distinct withonediverging ridge on the outer surface of the carpus. The dactylus of each walking leg is claw-like, slender and bears two rows of spines, one at each margin.

Fig 1 Dorsal View

Fig 2 Ventral View







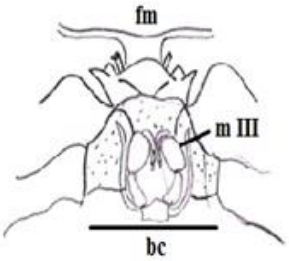


Fig. 3. Carapace **Fig. 4. Anterior view of eye, antennae** **Fig. 5. Mouth parts**

c - carapace; e - eye; fm - frontal margin; ch - cheliped; alm - anterolateral margin; w1,2,3,4 - walking leg 1-4; m - mouth parts; a - abdomen; ts – telson; hr - hepatic region; t - tubercles; fm - frontal margin; gr - gastric region; at - anterolateral tooth; cg - cervical groove; cr - cardiac region; ir - intestinal region; an - antenna; ep - epistome; en – endostome; mIII - maxilliped III; bc - buccal cavern; fm – frontal margin

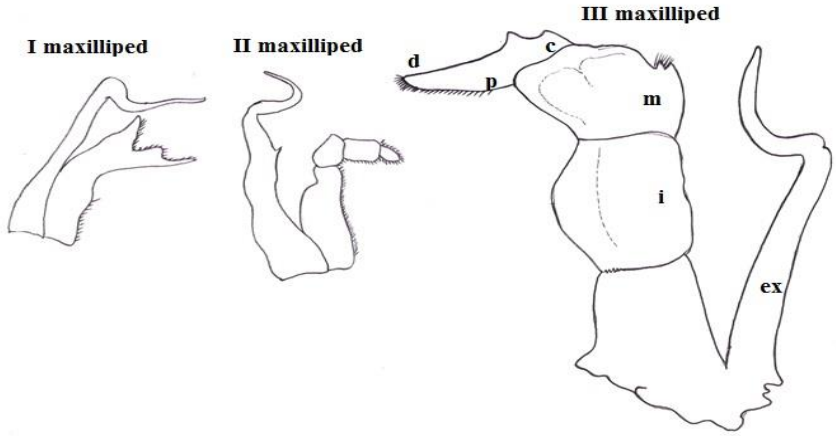


Fig. 6. Mouth parts of I, II and III maxilliped.

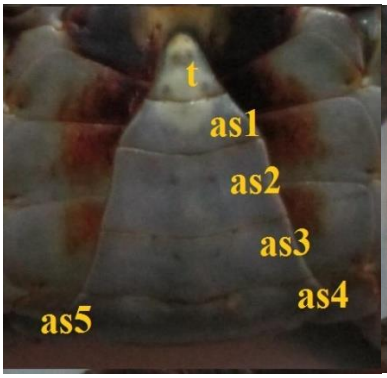


Fig. 7. Abdomen

Fig. 8. Thoracic sternum

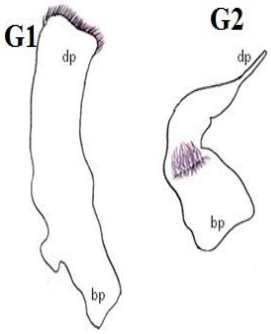


Fig. 9. Gonopods

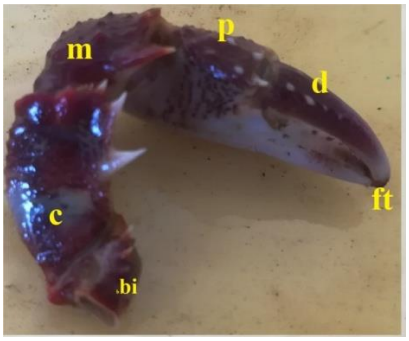


Fig. 10. Cheliped

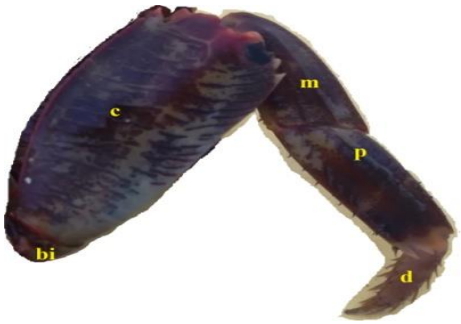


Fig. 11. Walking leg

m III – third maxilliped d – dactylus; p – propodus; c – carpus; m – merus; i – ischium; ex – exopod; t - telson; as1 to 5 - abdominal somites; s1, s2, s3, s4, s5, s6 – sternites 1 - 6; ab - abdomen; G1- gonopod1; G2- gonopod2; dp- distal part; bp- basal part; ft – finger teeth

Character coding

Table 1 represent the data matrix of the 92 morphological characters for 11 specimens and further used for distance based tree inference (NJ) and character based bootstrapping (MP).

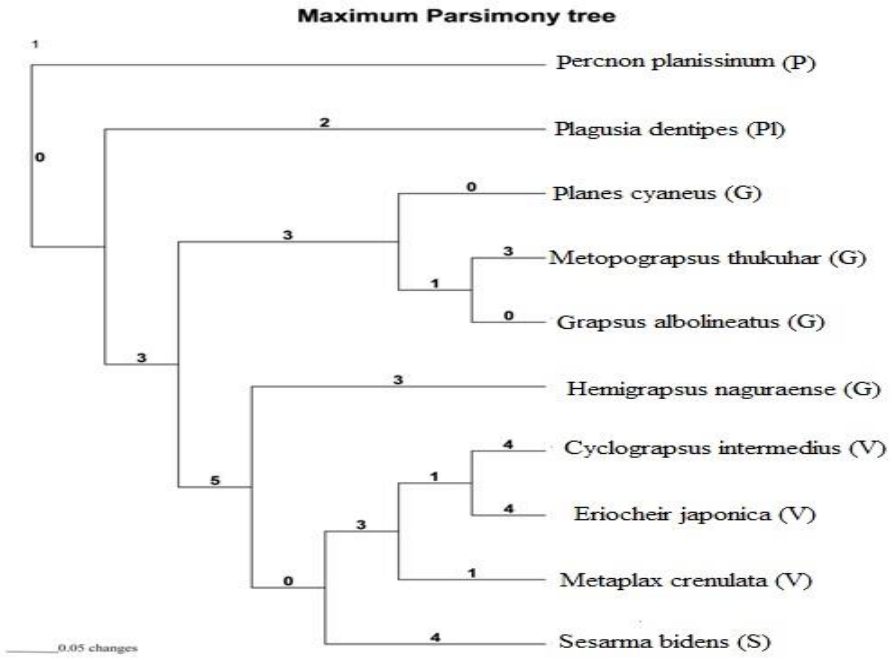
Maximum Parsimony (MP) tree

The topology of the morphological character analysis of the data set of 10 taxa (Grapsidea, Sesarmidae, Varunidae, Percnidae, Plagusiidea,). The phylogram with 100 bootstrap replicates was divided into 2 main branches. Branch I (Branch Length (BL) - 2) including a species showing *Percnon planissinum* while Branch II (BL - 2) was subdivided into 4 separated internal node including : Internal node 1(BL - 2), Internal node 3 (BL - 3) Internal node 4(Fig. 12)

The MP search for maximum 100 trees resulted in a score of best tree = 62 and number of trees retained = 3. The other details are as follows majority-rule consensus tree length (TL) - 63, consistency index (CI) - 0.6032, retention index (RI) - 0.6154, rescaled consistency index (RC) - 0.3712, homoplasy index (HI) -0.3968 with branch support values are presented in Fig. 13. Two main lineages; first lineage is *P. dentipes* and the second lineage comprising *S. bidens, P. cyaneus, G. albolineatus, M. thukuhar, E. japonica*, C .*intermedius*, *M. crenulata, H. sanguinensis*. The candidate crab *G. albolineatus* was paraphyletic and sister to *M. thukuhar* with 89% of high BS value.

Table. 1 Data matrix

Name of taxa	character coding (1-92
Metopograpsus_thukuhar	01113002100000012100000000000000111011100011101201111110000011001020001100110310010010010
Planes_cyaneus	20113002100001010210000000000000011101100001111011011110000001100102000110011031001001001
Cyclograpsus_intermedius	00013002100001110210000000000001211011111010110120111111101101200202000110011031001001001
Metaplax_crenulata	00013002101001110210000100000001211011110101101201111110010120020200011001103100100100100
Sesarma_bidens	10013002100001110211000000000001211011110111101201111110101011002020001100111310110010010
Hemigrapsus_sanguinensis	00013002101001110210000100000001101011100010110120111111010012002020001100110310110010010
Percnon_planissinum	00013002101001110210000100010000220101010000111011111111000000110020200011001103100100100110
Plagusia_dentipes	00013002101001110210000100010000020101010000111011011110000001100102000110011031001001001 10
Eriocheir_japonica	12013002101001110210000000000000010101100001111012011111101001200202000110011031001001001
Grapsus_albolineatus	00303002110001010210110011010001101011111000111001101111100000102000111211021131001001001



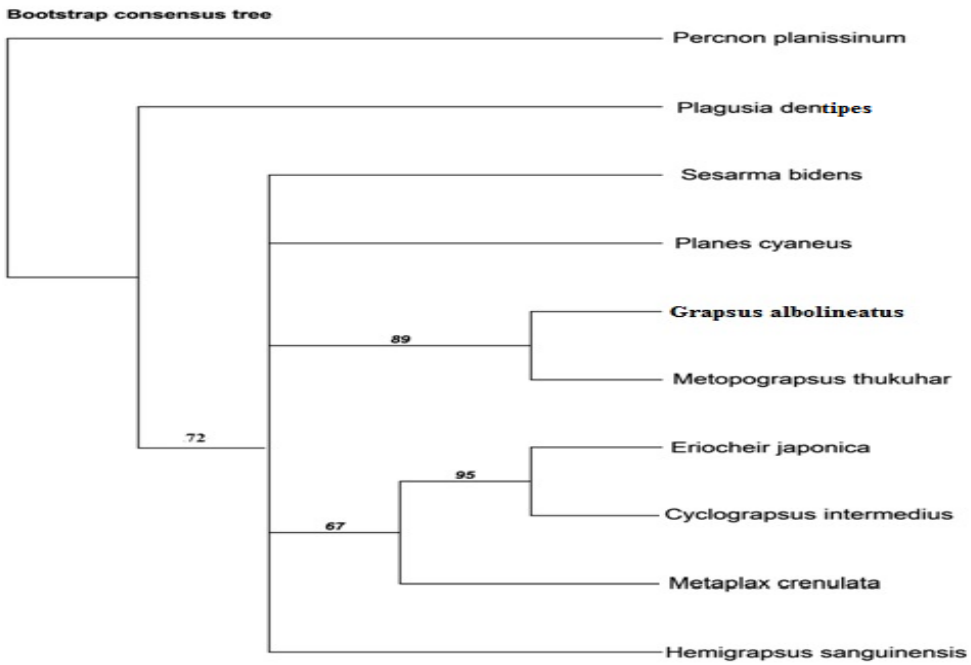


Fig. 12.Maximum Parsimony tree of morphology of *G. albolineatus*Numbers indicate the branch length

Fig. 13.Bootstrap 50% majority rule consensus tree of *G. albolineatus*

Neighbour joining tree

Neighbour joining (NJ) tree was constructed from the coding matrix (Table. 1). The topological confidence levels were evaluated with 100 replicates of the species character to provide estimates of robustness at each node. The score of *G. albolineatus* is 0. 023, which is neighbour to *M. thukuhar* (NJ score - 0.028) and *P. cyaneus* (NJ score - 0.026) as shown in Fig. 14.

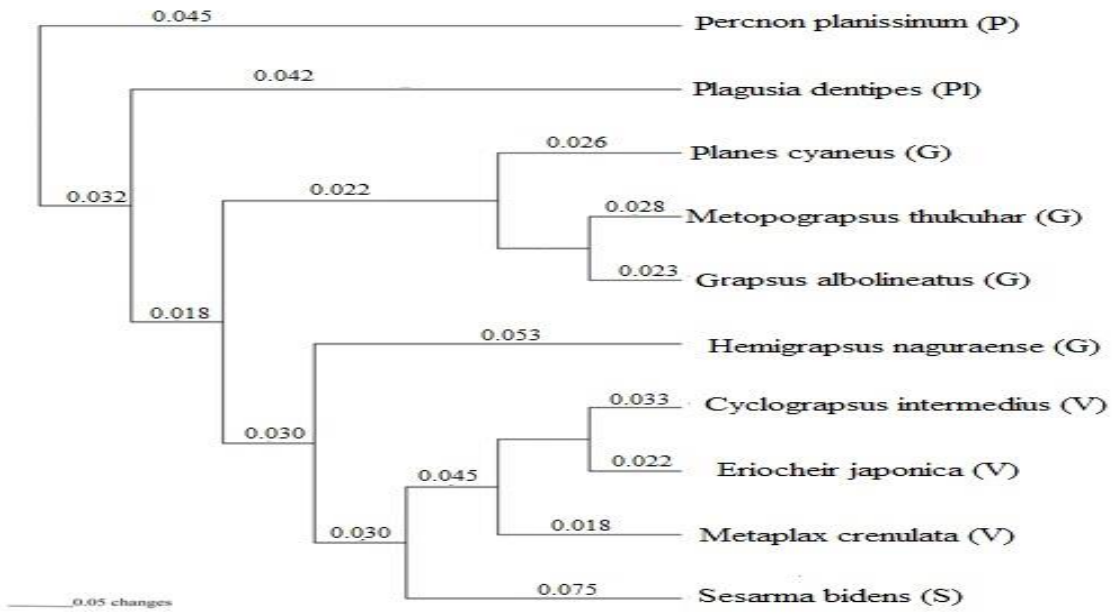


Fig. 14. Phylogram derived by Neighbour-joining cluster analysis for *G. albolineatus.*

Evaluating random trees (Skewness Test)

The distribution of 10 to 1 million random trees for 10 taxa and 92 characters was used for evaluation. The validity of estimating the skewness of a 10,000 random trees was generated from each data set to analyze the skewness of tree length frequency distributions. The G1 values indicated a strongly significant phylogenetic signal (g1: - 0.756, $P < 0.05$) in morphological data set (Fig. 15).

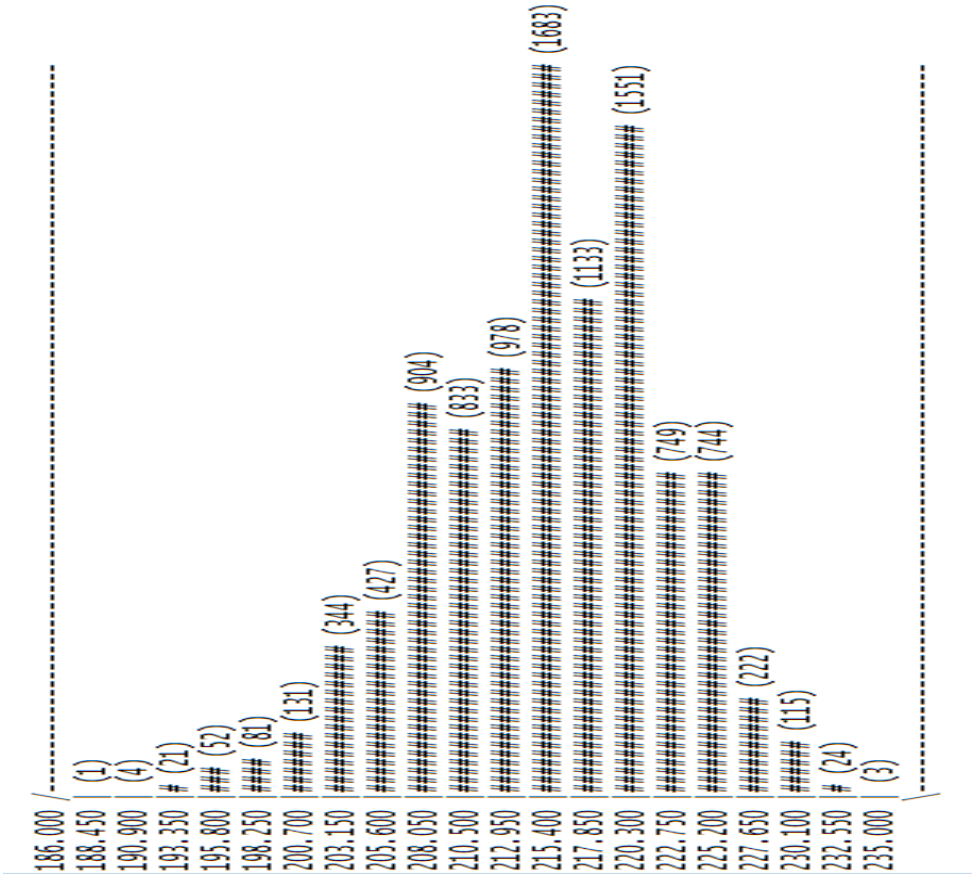


Fig.15. Skewed frequency distribution of 1 million random trees for 11 taxa and 92 characters; all unordered and un-scaled (1,00,000)

Discussion

Brachyurans are the most successful of all decapod groups, both in terms of taxonomic diversityand in the variety of their lifestyles. Grapsidae, the higher taxa in Decapods is subdivided into four subfamilies Grapsinae, Varuninae, Sesarminae, and Plagusiinae [15, 4, 16]. Recently, . Serrano-Sánchez [17] followed the classification of Ng [1], who stated that Grapsoidea have been subdivided into eight families: Gecarcinidae, Glyptograpsidae, Grapsidae, Plagusiidae, Sesarmidae, Varunidae and Xenograpsidae.

The carapace of *G. albolineatus*is rounded and slightly depressed as reported by [18, 8]while square-shaped [19], discoidal [18] and subcircular [20] in few crabs. Carapace of brachyuran crab has four regions: gastric, cardiac, intestinal and branchial [21] with marked difference among the subfamilies of Grapsidae that are essential for adult comparison [18,22, 23]. In the test crab, these four regions are well prominent with transverse ridges similar to othergrapsid crabs [24]and sesarmid crabs [25, 26].

The eye stalks of grapsids are stout and short [4] as represented in the candidate crab. Such crabs generally appear to be non-predatory and burrowing in flat areas of soft sediments [27, 28]. The antennules are well known as being sensory [29] that vary in morphology and distribution. The small antennules are present and visible in ventral side as reported inthe other Grapsidae [30, 31]. The most remarkable specialization of the pterygostome is exhibited by inter-tidal grapsoids (Sesarmidae, and Cyclograpsinae of Varunidae) that live predominantly in mangrove and inter-tidal regions [33, 32]stands well advanced in *G. albolineatus.* The rhomboidal gap and slightly curved and narrow merus of III maxillipede remains a typical grapsoid character as reported inother crabs [22, 4].The shapes and relative proportions of the articles of the III maxillipeds are some of the most commonly used characters in brachyuran taxonomy [34, 32].

The narrow male abdomen consists of six unfused abdominal somites and a telsonin grapsines, plagusiines, sesarmines and varunines [3, 4, 20, 24] similar to grapsid crabs under study, but in most species, the third to fifth abdominal segments of grapsid crab are freely movable [8]. Guinot [36] mentioned that in the Thoracotremata deep sterno-abdominal cavity was often anteriorly delimited by a cristiform margin standsin thegrapsid crab of our interest. The male gonopores of *G. albolineatus* is located laterally on sternite 8, a condition reported in plagusiines, grapsines and the sesarmines, however, in varunines and in few sesarmines it is internal [26, 4, 24]. Ng [38} and Davie [32] reported that the tip of the G1 varies from acute to truncate in Grapsidae, Sesarmidae, Ocypodidae, and Gecarcinidae), twisted in Plagusia and linear in sesarmines and varunines. The second gonopod (G2) also varies a great deal in form, from the whip-like to sigmoidal(very small, comma-shaped) in grapsinae and sesarminae [4, 1] akin to what has been observed in the candidate crab.

G. albolineatus is considered to be grapsids owing to the presence of the following morphological characters of Grapsidae: almost depressed sub-circular carapace with strongly deflexed widefront, infra-orbital margin facing down the buccal cavity, scattered setae on pterygostome, slender third maxillipeds, rhomboidal gap between the ischium and merus,and distinct sternal sutures. These characters are also akin to the morphological studies carried out by [1, 20, 32]. Morphological studies of the abdominal retaining structures of representatives of the grapsidae show, however, clear differences in the structure of the sternum compared with all other Grapsoidea. A phylogenetic analysis of sperm (Thanamalini and Suganthi, unpublished) and other morphological characters also suggest a closer relationship of the grapsinae to the grapsidae a result which is supported by present studies.

The phylogenetic trees inferred from the morphological characters are strongly supported, hypotheses of relationship among subfamily crabs. Several researchers have concluded that the accuracy of trees inferred from morphological data may be improved by the inclusion of characters that are presumably less subject to the selective pressures that may lead to convergence, such as carapace shape, antennae form, and gonopod structure [35]. Construction of the phylogenetic tree clearly shows that the orders Varunidae and Sesarmidae are sister groups of Grapsidea. To conclude, the adult morphology-based phylogeny of *G. albolineatus* documented a paraphyletic taxon, shared with other grapsids with high bootstrap value belongs to the super family Grapsoidea and the family Grapsidae.

References

1. Ng, PKL, Guinot, D & Davis, PJF 2008, 'Systema Brahyurorum. Part I. An annotated checklist of extant brachyuran crabs of the world', Raffles Bulletin of Zoology Supplements, vol.17, pp.1-268.
2. Sethuramalingam, S & Ajmal Khan, S 1991, 'Brachyuran Crabs of Parangipettai Coast', CAS in Marine Biology Publication, Annamalai University, India, pp. 92.
3. Chhapgar, BF 1957, 'Marine crabs of Bombay State, Contribution No.1, Taraporevala', Marine Biological station. pp. 1-129.
4. Karasawa, H & Kato, H 2001, 'The systematic status of the genus Miosesarma Karasawa, 1989 with a phylogenetic analysis within the family Grapsidae and a review of fossil records (Crustacea: Decapoda: Brachyura)', Palaeontology Research, vol. 5, no.4, pp. 259–275.
5. Pereyra Lago, R 1993, 'Larval development of *Sesarma guttatum* (Decapoda, Brachyura, Grapsidae) reared in the laboratory with comments on larval generic and familial characters. Journal of Crustacean Biology', vol. 13, pp. 745-762.
6. Schubart, CD & Cuesta, JA 1998, 'The first zoeal stages of four Sesarma species from Panama, with identification keys and remarks on the American Sesarminae (Crustacea: Brachyura: Grapsidae)', Journal of Plankton Research, vol. 20, pp. 61-84.
7. Schubart, CD, Cuesta, JA, Diesel, R & Felder, DL 2000, 'Molecular phylogeny, taxonomy, and evolution of non-marine lineages within the American grapsoid crabs (Crustacea: Brachyura)', Molecular Phylogenetetic and Evolution, vol. 15, pp. 179–190.
8. Ng, PKL 1998, 'Crabs. In: carpenter K. E. and V. H. Niem. Fao species identification guide for fishery purposes. cephalopods, crustaceans, holothurians and sharks', The living marine resources of the Western Central Pacific, FAO, Rome, vol. 2, pp. 1054–1155.

9. Sternberg Von, R & Cumberlidge, N 2001,'On the heterotreme-thoracotreme distinction in the Eubrachyura de Saint Laurent, 1980 (Decapoda, Brachyura), Crustaceana, vol. 74, pp. 321-338.
10. Karasawa, H & Schweitzer, CE 2006, 'A new classification of the *Xanthoidea sensu lato* (Crustacea: Decapoda: Brachyura) based on phylogenetic analysis and traditional systematic and evaluation of all fossil *Xanthoidea sensu lato'*, Contributions to Zoology, vol. 75, pp. 23-73.
11. Swofford, DL 2002, 'PAUP. Phylogenetic Analysis Using Parsimony (*and other methods)' Version 4.0b10. Sinauer Associates, Sunderland, Massachusetts.
12. Saitou, N & Nei, M 1987, 'The neighbour-joining method: a new method for reconstructing phylogenetic trees', Molecular Biology and Evolution, vol. 4, no. 4, pp. 406-425.
13. Fitch, W 1971, 'Toward defining the course of evolution: minimumchange for a specified tree topology', Systematic Zoology,vol. 20, pp. 406-416.
14. Hillis, DM 1991, 'Discriminating between phylogenetic signal and random noise in DNA sequences. In: M. M. Miyamoto and J. Cracraft [eds.], Phylogenetic Analysis of DNA Sequences' , Oxford University Press, New York, N.Y. pp. 278-294.
15. Niem, VH 1993,'Phylogenetic and systematic position of *Sesarma rubripes* Rathbun, 1897 (Brachyura: Grapsidae)', Zoologische Mededeligen,vol. 67,pp. 185-195.
16. Schubart, CD, Cannicci, S, Vannini,M & Fratini,S 2006,'Molecular phylogeny of grapsoid crabs (Decapoda, Brachyura) and allies based on two mitochondrial genes and a proposal for refraining from current super-family classification', Journal of Zoological Systematics and Evolutionary Research, vol. 44, pp. 193-199.
17. Serrano-Sánchez, ML, Hegna, TA, Schaaf, P, Centeno-García, E, Pérez, L& Vega, FJ 2015, 'The aquatic and semiaquatic biota in Miocene amber from the Campo La Granja mine (Chiapas, Mexico): paleoenvironmental implications', Journal of South American Earth Sciences, vol. 62, pp. 243-256.
18. Banerjee, SK 1960,'Biological results of the Snellius Expedition. XVIII. The genera Grapsus, Geograpsus and Metopograpsus (Crustacea Brachyura)', Temminckia vol.10,pp. 132-199.
19. Al-Ghais, SM & Cooper, RT 1996, 'Brachyura (Grapsidae, Ocypodidae, Portunidae, Xanthidae and Leucosiidae) of Umm Al Quwain mangal, United Arab Emirates' , Tropical Zoology, vol. 9, pp. 409–430.

20. Naderloo, R 2011,'Grapsoid crabs (Decapoda: Brachyura: Thoracotremata) of the Persian Gulf and the Gulf of Oman', Zootaxa, vol. 3048, pp. 1–43.
21. Ng, PKL & Sivasothi, N 1999 (editors), A Guide to the Mangroves of Singapore. II. Animal Diversity. Singapore Science Centre, pp. 168.
22. Crosnier, A 1965, 'Crustacés Décapodes Grapsidae et Ocypodidae', In: Faune de Madagascar, vol.18, pp. 1–143.
23. Cuesta, JA & Schubart, CD 1999, 'First zoeal stages of *Geograpsus lividus* and *Goniopsispulchra* from Panama confirm consistent larval characters for the subfamily Grapsinae (Crustacea: Brachyura: Grapsidae)', Ophelia Vol. 51, pp. 163-176.
24. Poupin, J, Davie, PJF & Cexus, JC 2005, 'A revision of the genus Pachygrapsus Randall, 1840 (Crustacea: Decapoda: Brachyura, Grapsidae), with special reference to the Southwest Pacific species', Zootaxa, Vol. 1015, pp.1-66.
25. Abele, LG, Campanella, PJ & Salmon, M, 1986, 'Natural history and social organization of the semi-terrestrial grapsid crab *Pachygrapsus transversus* (Gibbes)', Biology and Ecology, Vol. 104, No. 1-3, pp. 153-170.
26. Yeo, DCJ & Ng, PKL 1999,'The state of freshwater crab taxonomy in Indochina (Decapoda, Brachyura). In: Schram, F.R. and J.C. Von Vaupel Klein (eds.) Crustaceans and the biodiversity crisis', Proceedings of the Fourth International Crustacean Congress, Amsterdam, The Netherlands, July 20–24, 1998. Vol. 1. Leiden: Brill. pp. 637–646.
27. Barnes, RSK 1968, 'Relative carapace and chela proportions in some Ocypodid crabs Brachyura, Ocypodidae)', Crutaceana, vol. 14, no. 2, pp. 131-136.
28. Zeil, J & Hemmi, JM 2006, 'The visual ecology of fiddler crabs', Journal of Computational Physiology, series.A, vol. 192, pp. 1 – 25.
29. Horner, AJ, Weissburg, MJ & Derby, CD 2004,'Dual antennular chemosensory pathways can mediate orientation by Caribbean spiny lobsters in naturalistic flow conditions', Journal of Experimental Biology,vol. 207, no. 21, pp. 3785-3796.
30. Silas, EG & Sankarankutty, C 1960, '*On the castle building habit of the crab Cardisoma carnifex (Herbst) (Family Geocarcinidae), of the Andaman Islands'* , Journal of the Marine Biological Association of India, vol. 2, no. 2, pp. 237-240.
31. Dev Roy, MK & Das, AK 2000,'Taxonomy, ecobiology and distribution pattern of the Brachyuran Crabs of mangrove ecosystem in Andaman Island$', Recordings of zoological Survey of India, Occ. Paper No. ISS pp. 1-211.
32. Davie, PJF, Guinot, D & Ng, PK 2015,'Systematics and classification of Brachyura. In: P. Castro; PJF Davie; D. Guinot; FR Schram, and JC von Vaupel Klein (eds), Treatise on

Zoology – Anatomy, Taxonomy, Biology', The Crustacea, Leiden, Brill, vol. 9, pp. 1049–1130.

33. Abele, LG 1982, Systematics, the Fossil Record, and Biogeography. Vol. 1 of The Biology of Crustacea (D. E. Bliss, editor-in-chief). Academic Press, NewYork.
34. Sternberg von, R & Cumberlidge, N 1998, 'Taxic relationships within the Grapsidae MacLeay, 1838 (Crustacea: Decapoda: Eubrachyura)', Journal of Computational Biology, vol. 3, no. 2, pp. 115–136.
35. Abele, LG 1992,'A review on the grapsid crabs genus *Sesarma*(Crustacea:Decapoda: Grapsidae) in America with the description of a new genus', Smithsonian contributions to Zoology,vol. 527, pp. 1-60.
36. Guinot, D & Bouchard, JM 1998, 'Evolution of the abdominal holding systems of brachyuran crabs (Crustacea, Decapoda, Brachyura)', Zoosystema, vol. 20, pp. 613–694.
37. Davie, PJF & Ng, PKL 2007, 'A new genus for cave-dwelling crabs previously assigned to Sesarmoides (Crustacea: Decapoda: Brachyura: Sesarmidae)', Raffles Bulletin of Zoology Supplement, vol.16, pp. 227–231.
38. Ng, PKL, Wang, CH, Ho, PH& Shih, HT2001, 'An annotated checlist of brachyuran crabs from Taiwan (Crustacea: Decapoda)', National Taiwan Museum Special Publication Series, no.11, pp. 86.

Antibacterial and antifungal activity of the tissue extracts of marine crab *Atergatis ocyroe* (Herbst, 1801)

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ABSTRACT

Antimicrobial activity of the different solvent (butanol, ethyl acetate and chloroform) extracts of haemolymph, muscle, gill, carapace and hepatopancreas of Atergatis ocyroe was investigated. Among the different tissues tested, muscle, gills, carapace, and hepatopancreas exhibited better antimicrobial activity against the clinical pathogens tested when compared to hemolymph. Among the different pathogens (Staphylococcus aureus, Streptococcus mutans, Escherichia coli, Proteus vulgaris, Aspergillus flavus, Aspergillus niger) tested, S. aureus, S. mutans, E. coli and P. vulgaris were highly sensitive to the butanol extracts of muscle, gills, carapace and hepatopancreas. Ethyl acetate extracts inhibited the growth of the pathogens and a maximum zone of inhibition (21 mm) was observed with P. vulgaris. Thus from our studies it can be concluded that Atergatis ocyroe possesses antimicrobial components which can be isolated and used as a drug in future.

Key words: Antimicrobial activity, *Atergatis ocyroe*, solvent extracts, tissues, zone of inhibition

1. Introduction

Marine environment is a reservoir of bioactive products and numerous pharmacological substances of marine origin have been developed [1]. Among the different natural products obtained from the marine organisms, crabs are identified as potential sources of new antibiotics that have found extensive applications in the treatment of infectious diseases [2]. Crab metabolites found in different tissues and organs were known to possess antibacterial, antifungal, antiviral, anticancer and anti-inflammatory properties that make it promising in the pharmaceutical industry [3, 4]. Different bioactive compounds have been isolated and tested for its medicinal value. For example, glucosamine from the carapace for treatment of osteoarthritis [5], astaxanthin a keto-carotenoid as an antioxidant [6] and hemocyanin as an antibacterial agent [7] has been reported from crabs.

Antibacterial activity of haemolymph extracts from marine crabs was investigated against different bacterial strains and the results demonstrated antimicrobial potential against pathogenic bacteria [8]. Similarly tissues of marine crab *Atergatis integerrimus* inhibited the growth of clinical pathogens [9, 10] and whole body extract of *Portunus pelagicus* and *S. serrata* documented antibacterial activity against fish pathogenic bacteria [4].

Screening of marine crabs for bioactive compounds, understanding of chemical structure and biological activity will lead to the formulation of novel drugs with specific

actions. Hence the present investigation was taken up to study the antibacterial and antifungal activity of tissue extracts of the marine crab *A. ocyroe.*

2. Materials and methods

2.1. Experimental animal and sample collection

The marine crab *A. ocyroe* obtained from the fishing nets of fishermen of Kanyakumari were maintained in sea water and transported to the laboratory. Hemolymph was collected by inserting a sterile 1.0 ml syringe with 22 gauge needle into the arthrodial membrane at the base of third walking leg. Gills, muscles, carapace and hepatopancreas were carefully dissected.

2.2. Preparation of extracts for antimicrobial assay

1 g each of gills, carapace, muscle, and hepatopancreas were homogenized and extracted with 10 ml of 70 % chloroform, butanol and ethyl acetate and kept for three days at room temperature. The extracts were filtered through Whatman No 1 filter paper, concentrated by evaporating at room temperature and used for the antimicrobial assay. Hemolymph treated with the above mentioned solvents was kept at room temperature for three hours before subjecting it to antimicrobial assay.

2.3. Test microorganisms

Human pathogenic bacteria (*Staphylococcus aureus, Streptococcus mutans, Escherichia coli, Proteus vulgaris*) and human pathogenic fungi (*Aspergillus flavus* and *Aspergillus niger*) used in this study were procured from Inbiotics Laboratory, Nagercoil.

2.4. Antimicrobial assay

In vitro antimicrobial assay was carried out by disc diffusion technique [11]. Sterile discs were impregnated separately with 25 µl samples (extracts of gills, muscle, carapace, hepatopancreas and hemolymph) of *Atergatis ocyroe* and were placed in Muller Hinton Agar plates/ Sabouraud's Dextrose Agar plates seeded with test microbial culture. Streptomycin 25 µl for antibacterial activity and fluconazole 25 µl for antifungal activity in the form of a standard antibiotic disc as positive control and solvent dipped and evaporated disc as negative control was used. After incubation at 37°C for 24 hours, antimicrobial activity was determined. Antimicrobial activity was expressed in terms of diameter of zone of inhibition in mm.

3. Results

Antimicrobial activity of the different solvent (butanol, ethyl acetate and chloroform) extracts of muscle, gills, carapace, hepatopancreas and haemolymph were studied. Solvent extracts of tissues of *Atergatis ocyroe* showed a wide array of antimicrobial activity. Butanol extract was found to be effective against all the tested pathogens. Butanol extract of gills showed a maximum zone of inhibition against *S. aureus* (16 mm), *E. coli* (16 mm) and *P. vulgaris* (14 mm). Pathogenic bacteria *S. aureus* (13 mm), *E. coli* (11 mm) and *P. vulgaris* (12 mm) were also sensitive to the butanol extracts of muscle. Maximum inhibitory zone of 17 mm was observed with the butanol extract of carapace and 15 mm with hemolymph against *S. mutans*. Butanol extract of hepatopancreas elicited maximum zone of inhibition towards all the tested pathogens except the *A. flavus* (Table - 1, Plate - 1).

Ethyl acetate extracts of the crab tissues showed antimicrobial activity against human pathogenic bacteria and fungi tested. Muscle extract showed a maximum zone of inhibition of 21 mm against *P. vulgaris*, 10 and 7 mm against *A. flavus* and *A. niger* respectively and carapace 18 mm against *E. coli*. Hemolymph recorded a maximum inhibitory zone of 18 mm against *S. mutans* (Table - 2, Plate - 2).

Chloroform treated tissue extracts failed to inhibit the growth of the all bacteria and fungi tested (Table - 3).

Table 1: Antimicrobial activity of butanol extracts of *A. ocyroe* on pathogenic microbes

Pathogens	Zone of inhibition (mm)						
	Gills	**Muscle**	**Carapace**	**Hepatopancreas**	**Heamolymph**	**Positive control**	**Negative control**
S. aureus	16	13	12	15	-	24	-
S. mutans	9	-	17	16	15	14	-
E. coli	16	11	13	13	-	14	-
P. vulgaris	14	12	10	15	-	23	-
A. flavus	-	-	9	-	-	13	-
A. niger	8	-	-	10	-	20	-

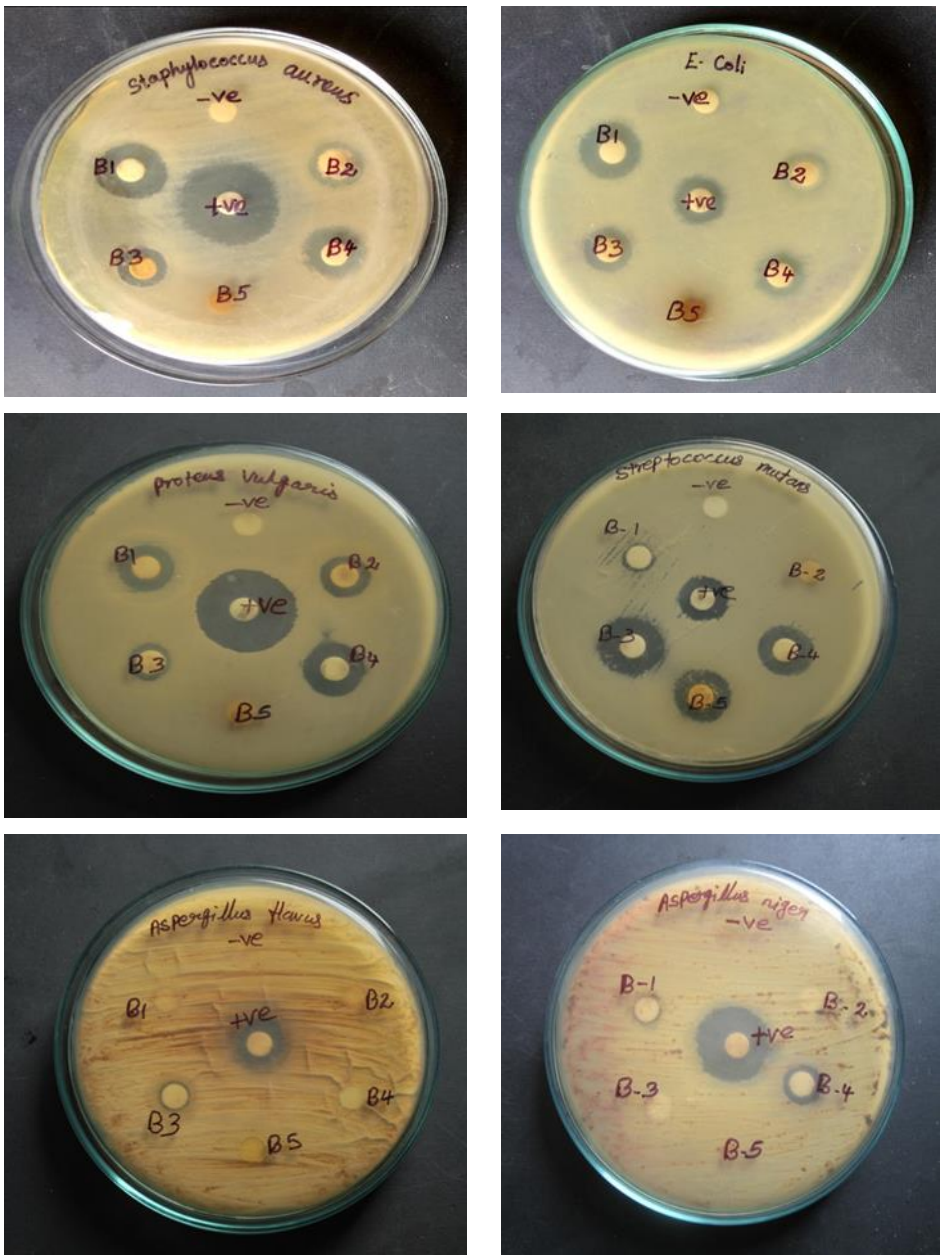
Table 2: Antimicrobial activity of ethyl acetate extracts of *A. ocyroe* on pathogenic microbes

Pathogens	Zone of inhibition (mm)						
	Gills	Muscle	Carapace	Hepatopancreas	Heamolymph	Positive control	Negative control
S. aureus	-	-	8	-	11	24	-
S. mutans	-	-	-	-	18	15	-
E. coli	8	-	18	-	-	16	-
P. vulgaris	-	21	-	9	-	23	-
A. flavus	-	10	-	-	-	19	-
A. niger	-	7	-	-	-	10	-

Table 3: Antimicrobial activity of chloroform extracts of *A. ocyroe* on pathogenic microbes

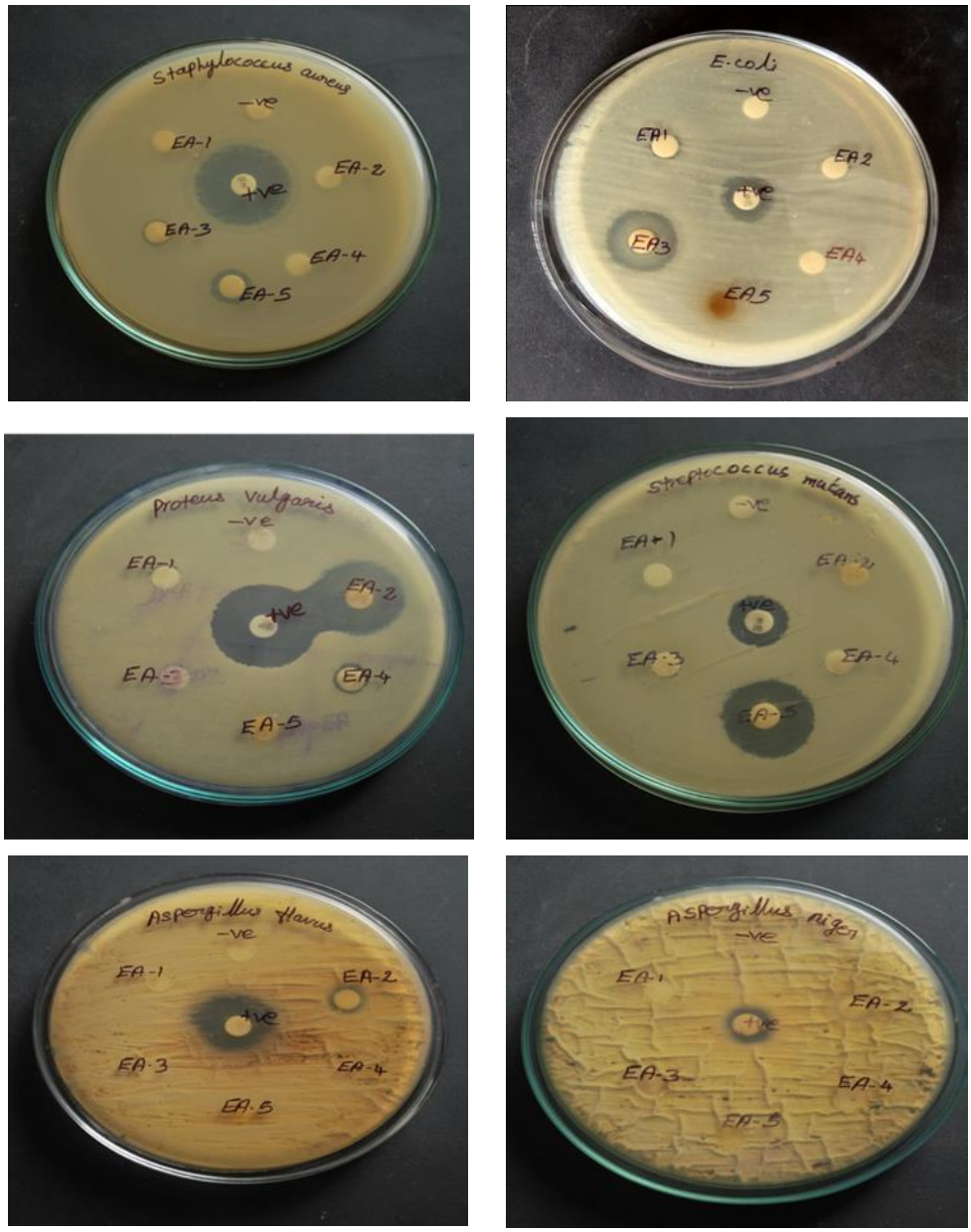
Pathogens	Zone of inhibition (mm)						
	Gills	Muscle	Carapace	Hepatopancreas	Heamolymph	Positive control	Negative control
S. aureus	-	-	-	-	-	23	-
S. mutans	-	-	-	-	-	13	-
E. coli	-	-	-	-	-	14	-
P. vulgaris	-	-	-	-	-	17	-
A. flavus	-	-	-	-	-	10	-
A. niger	-	-	-	-	-	20	-

Plate 1: Antimicrobial activity of butanol extracts of tissues of marine crab *A. ocyroe*



B – Butanol, 1- gills, 2- muscle, 3- carapace, 4- hepatopancreas, 5- hemolymph

Plate 2: Antimicrobial activity of Ethyl acetate extracts of tissues of marine crab *A. ocyroe*



EA- Ethyl acetate, 1- gills, 2- muscle, 3- carapace, 4- hepatopancreas, 5- hemolymph

4. Discussion

The present study demonstrated the presence of antimicrobial compounds in tissues, such as gills, muscle, carapace, hepatopancreas and hemolymph of crab *A. ocyroe*. Maximum activity was mainly located in the gills, muscle, carapace and hepatopancreas which may be due to the anti-lipopolysaccharide factor (ALF) or antimicrobial protein (AMP) which are expressed in the tissues to defend against the bacterial pathogens present in the environment [12, 13]. The crab haemolymph showed antimicrobial activity against few pathogenic strains of gram-positive bacteria. Among the different extracts tested the butanol and ethyl acetate

extracts of tissues were more effective in extracting the bioactive compound and the chloroform extract failed to inhibit any of the microbes tested.

Antibacterial activity has been observed in the haemolymph of some mangrove crabs against clinical pathogens [8]. Haug *et al.,* [14] detected antibacterial activity in different body-parts and hemolymph of *Pandalus borealis* (northern shrimp), *Pagurus bernhardus* (hermit crab), *Hyas araneus* (spider crab) and *Paralithodes camtschatica* (king crab) against *Escherichia coli*, *Vibrio anguillarum*, *Corynebacterium glutamicum* and *Staphylococcus aureus.* Different solvent extracts of heamolymph of *O. macrocera* showed anti-microbial activity against both gram positive and negative pathogenic bacteria strains [15]. Bharathi *et al.* [10] reported antimicrobial activity of solvent extract of whole body, carapace, hepatopancreas, gills, testes, mandible, muscle, egg and hemolymph of *Atergatis integerrimus* against human and fish pathogenic bacteria and human pathogenic fungi. Antibacterial activity has been reported in the haemolymph of the *C. sapidus, S. serrata* and *Ocypode macrocera*. These findings indicate that antibacterial factors are present both in crustacean tissues and haemolymph/haemocytes.

The antibacterial activity might be due to antimicrobial proteins [16], lectins [17], of the innate immune system. The exoskeleton of crustaceans is composed mainly of chitin, a polymer of *N*-acetyl-glucosamine covalently bound to protein. Biologically, a deacetylase transforms chitin to chitosan by hydrolysing the acetamido groups of *N*-acetyl-glucosamine [18]. It has been reported that both chitin and chitosan from crustaceans possess antimicrobial activity [19]. The detection of antibacterial activity in the exoskeleton suggests that this activity is important in the defence against micro-organisms present in the marine environment.

Antifungal activity against *A. flavus* and *A. niger* was also observed with the tissue extracts as reported by Zodape, [9] with different tissues of *A. integerrimus*. *Aspergillus* species possesses chitin and glycans on the cell wall [20] and expresses saccharide moieties on the cell surface [21]. The bioactive compounds of the tissue extracts of the crab, *A. ocyroe* may specifically recognize these sugars and may either cause cell wall degradation or inhibit the cell cycle [22].

In the present study, the tissue extracts of test crab showed antimicrobial activity against a range of different pathogenic strains. The results suggest the presence of antimicrobial substances in the crab to combat microbial infection. Thus from our study it is

evident that the marine crab *A. ocyroe* possesses bioactive compounds that can be targeted against clinical pathogens.

5. Reference

1. Rameshkumar, G., Ravichandran, S. and Aravindhan, T. (2009). Antimicrobial proteins from the Crab *Charybdis lucifera* (Fabricius, 1798). Middle East Journal of Scientific Research, 4: 40-43.
2. Ming, L., Xiaoling, P., Yan, L., Lili, W., Qi, W., Xiyong, Y., Boyao. W. and Ning, H. (2007). Purification of antimicrobial factors from human cervical mucus. Human Reproduction, 22 (7): 1810-1815.
3. Chalupnik, A., Waszczuk, K. Halubek-Gluchowska, K., Piasecki, T. Gotszalk, T. and Rybka. J. (2014). Bacterial cells by monitoring of Limulus amebocyte lysate coagulation. Biosensors and Bioelectronics, 58: 132-137.
4. Laith, A. A., Ambak, K., Abol-Munafi, A.B., Nurhafizah, W.W.I. and Najiah, M. (2017). Metabolomic analysis of marine and mud crabs based on antibacterial activity. Aquaculture Reports, 7: 7-15.
5. Pham, T., Cornea, A., Blick, K.E, Jenkins, A. and Scofield, R.H. (2007). Oral glucosamine in doses used to treat osteoarthritis worsens insulin resistance. The American Journal of the Medical Sciences, 6: 333-339
6. Suganya, V. and Asheeba, S. T. (2015). Antioxidant and antimicrobial activity of astaxanthin isolated from three varieties of crabs International Journal of Recent Scientific Research, 6 (10): 6753-6758.
7. Velayutham, M., Kamanuri, S. K., Saravanan, K. and Munusamy, A. (2016). Cation metals specific hemocyanin exhibits differential antibacterial property in mud crab, *Scylla serrata*. Biologia, 71: 176-183.
8. Veeruraj, A., Ravichandran, S. and Rameshkumar, G. (2008). Antibacterial activity of crab haemolymph on clinical pathogens. Trends in Applied Sciences Research, 3: 174-181.
9. Zodape, G.V. (2014). Studies on the antibacterial and antifungal activities of bioactive compounds of intertidal crab *Atergatis integerrimus* (Lamarck) of west coast of Mumbai. Bionano Frontier, 7 (2): 278-283.
10. Bharathi, T., Jilian, V.P. and Vinoliya, J.M. (2016). Antimicrobial activity of tissue extracts of the marine crab *Atergatis integerrimus* (Lamarck, 1801). Proceedings of the Kanyakumari Academy of Arts and Science, 3: 1-5.

11. Bauer, A.W., Kirby, W.M., Sherris, J.C. and Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disc method. American Journal of Clinical Pathology, 45 (4): 493-496.
12. Yedery R. D. and Reddy K. V. R. (2009). Purification and characterization of antibacterial proteins from granular hemocytes of Indian mud crab, *Scylla serrata*. Acta Biochimica Polonica, 56 (1): 71-82.
13. Imjongjirak, C. Amparyup, P. and Tassanakajon, A. (2011). Two novel antimicrobial peptides, arasin-likeSp and GRPSp, from the mud crab *Scylla paramamosain*, exhibit the activity against some crustacean pathogenic bacteria. Fish and Shellfish Immunology, 30 (2): 706-712.
14. Haug, T., Kjuul, A.K., Stensvag K., Sandsdalen, E. and Styrvold, O.B. (2002). Antibacterial activity in four marine crustacean decapods. Fish and Shellfish Immunology, 12 (5): 371-385.
15. Ravichandran, S., Sivasubramaninan, K. and Anbuchezhian, R.M. (2010). Antimicrobial activity from haemolymph of the crab *Ocypode macrocera* (H.Milne-Edwards1852). World Applied Sciences Journal, 11(5): 578-581.
16. Hikima, S., Hikima, J. Rojtinnakorn, J., Hirono, I. and Aoki T. (2003). Characterization and function of kuruma shrimp lysozyme possessing lytic activity against Vibrio species. Gene, 316: 187-95.
17. Krishnamoorthi, A., Selvakumar, S. and Shanthi, G. (2016). Antimicrobial activity of lectins isolated from the haemolymph of marine crab *Scylla serrata* (Forskal 1775). International Journal of Modern Research and Reviews, 4 (11): 1350-1352.
18. Koide, S.S. (1998). Chitin-chitosan: Properties, benefits and risks. Nutrition Research, 18: 1091-1101.
19. Tsai, G.J. and Su, W.H. (1999). Antibacterial activity of shrimp chitosan against *Escherichia coli*. Journal of Food Protection, 62 (3): 239-243.
20. Pinto, M.R., Barreto-Bergter, E. and Taborda, C.P. (2008). Glycoconjugates and polysaccharides of fungal cell wall and activation of immune system. Brazilian Journal of Microbiology, 39 (2): 195-208.
21. Leal, A.F.G., Lopes, N.E.P., Clark, A.T.R., Filho, N.T.P., Beltrao, E.I.C. and Neves, R.P. (2012). Carbohydrate proliferating of fungal cell wall surface glycoconjugates of *Aspergillus* species in brain and lung tissues using lectin histochemistry. Medical Mycology, 50 (7): 756-759.
22. Selitrennikoff, C.P. (2001). Antifungal proteins. Applied and Environmental Microbiology, 67 (7): 2883-2874.

Antimicrobial activity of the tissue extracts of estuarine crab, *Metopograpsus messor* (Forskal, 1775)

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ABSTRACT

The search for antimicrobial agents has taken a definite direction and marine crabs have been found to possess antimicrobial activity against pathogens like bacteria, fungi and viruses. The present investigation was taken up to study the antimicrobial potential of different solvent extracts of carapace, hepatopancreas, gills, testes and haemolymph of Metopograpsus messor. Five human pathogenic bacteria such as Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Proteus mirabilis, Klebsiella pneumonia and two fungi Aspergillus niger and Candida albicans were used for antimicrobial studies. E. coli and P. mirabilis were highly sensitive to the chloroform extracts and maximum zone of inhibition of 30 mm was observed with the chloroform extracts of carapace, testes, hepatopancreas, and hemolymph. Acetone and chloroform extracts of the tissues recorded moderate activity against K. pneumoniae. Thus the results revealed that Metopograpsus messor has potential antimicrobial compounds.

Keywords: antimicrobial activity, bioactive compounds, *Metopograpsus messor*, pathogens.

1. Introduction

The marine environment has proven to be a source of many bioactive metabolites with great potential for pharmaceutical and other applications [1]. Invertebrates which contribute about 95 % of the extant species in the animal kingdom [2] have developed unique defense mechanisms/ modalities to detect microbial surface 'antigens' like lipopolysaccharides (LPS), lipoteichoic acids, lipoproteins, peptidoglycans (PGN), 1,3-β-D-glycans, toll like receptors mediated antibacterial peptides [3] and respond through haemolymph coagulation [4], melanin formation [5], agglutinin/lectin mediated complement activation [6], generation of reactive oxygen intermediates (ROIs), nitric oxide (NO) [7] and phagocytic system, encapsulation and nodule formation which cooperate with humoral immune reactors to kill invading pathogens [8]. These mechanisms, which together compose the innate immune system, defend invertebrates from invading pathogens like bacteria, fungi and viruses [9].

Antimicrobial peptides/proteins are a major component of the innate immune defense system in marine invertebrates. These molecules are the first line of host defense in various species and the knowledge acquired over the last two decades on the identification and characterization of antimicrobial peptides/proteins in crustaceans has revealed their essential role in the immune response and also in the capacity of these animals to survive infection. Antibacterial peptides or proteins have been most extensively studied [10] due to the

following reasons: (1) they occur in the haemolymph/serum of almost every species examined and (2) interact directly with foreign materials, particularly the potential microbial pathogens and there by appear to serve as humoral recognition function in second line of defense.

Brachyuran crabs have shown pronounced activities and may be useful in the biomedical area. The potential of marine crabs as a source of biologically active products is largely unexplored. A broad, based screening of marine crabs for bioactive compound is necessary. Hence the present study aimed at analyzing the antimicrobial activity of the different tissue of the crab *Metopograpsus messor*.

2. Materials and methods

2.1: Experimental animal and sample collection: *Metopograpsus messor*were collected from Manakudy estuary, Kanyakumari Districtin the month of December 2017. Haemolymph was collected by cutting walking legs of the crab with a fine sterile scissor. The haemolymph collected was centrifuged at 2000 rpm for 15 minutes at 4ºC to remove haemocytes from the haemolymph. Supernatant was collected and stored at 4ºC until use. Gills, carapace, testes and hepatopancreas were carefully dissected and stored at 20ºC prior to extraction for antimicrobial work.

2.2 Preparation of extracts for antimicrobial activity: Crab tissue extracts were prepared following the method of Karthikeyan *et al.* [11]with slight modification. 1 g each of gills, carapace, testes and hepatopancreas were homogenized and extracted with 10 volumes (v/w) of acetone, chloroform and aqueous and kept for three days at room temperature. The extracts were filtered through Whatman No 1 filter paper, concentrated by evaporating in room temperature to give a dark gummy mass and used for the antimicrobial agar disc diffusion assay. Haemolymph was treated with all the above mentioned solvents (1:1) and used for antimicrobial studies.

2.3 Microbial strains: Antimicrobial activity of crab haemolymph was determined against five bacterial strains viz., *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumoniae* and two fungi *Aspergillus niger* and *Candida albicans*.These pathogens strains were obtained from the Scudder Laboratory, Nagercoil.

2.4 Assay of antimicrobial activity using disc diffusion method [12]**:** 20 ml of sterilized Muller Hinton Agar / Sabouraud's Dextrose Agar was poured into sterile petri-plates. After solidification 100 µl of fresh culture of pathogenic bacteria (*E. coli, P. aeruginosa, S. aureus, P. mirabilis, K. pneumoniae*) were swabbed on the respective Muller Hinton Agar plates and 100 µl of fresh culture of pathogenic fungi (*A. niger* and *C. albicans*) were swabbed on the

respective Sabouraud's Dextrose Agar plates. The discs impregnated with 50 µl of samples were kept over the agar plates using sterile forceps. Positive controls used were ampicillin (100 mg) for antibacterial assay and fluconazole (10 mg) for antifungal assay. The plates were incubated for 24 hours at 37°C. After incubation the diameter of inhibitory zones formed around each discs were measured (mm) and recorded.

3. Results

3.1. Antimicrobial activity of haemolymph: The acetone haemolymph extract of *M. messor* showed high antibacterial activity on *P. mirabilis* (18 mm). The acetone haemolymph extract did not inhibit the growth of *S. aureus* and *K. pneumoniae*. The chloroform haemolymph extract showed high antibacterial activity on *P. mirabilis* (30 mm) followed by *E. coli* (26 mm) and *P. aeruginosa* (14 mm). The aqueous haemolymph extract failed to inhibit the growth of all tested pathogenic bacteriabut showed minimum activity against *A. niger*. All the other extracts did not inhibit the growth of the tested fungal species (Table 1, Plate 1).

3.2. Antimicrobial activity of gills: The acetone gills extract of *M. messor* found to be significantly active against *E. coli* (23 mm) followed by *S. aureus* (20 mm) and *P. mirabilis* (18 mm). However they did not show inhibitory activity against *K. pneumoniae* and *P. aeruginosa*. The chloroform extract ofgills strongly active against *E. coli* (21 mm) followed by *P. aeruginosa* (20 mm), *P. mirabilis* (19 mm) and *S. aureus* (15 mm) while the aqueous extract of the gills inhibited the growth of only *E. coli* (10 mm). The acetone extract of gills showed activity against *A. niger* (14 mm) but all other extracts did not inhibit the growth of the tested fungal species (Table 2, Plate 2).

3.3. Antimicrobial activity of carapace: The acetone extract of carapace was found to be significantly active against *K. pneumonia* (23 mm), followed by *P. mirabilis* (20 mm), *P. aeruginosa* (18 mm). The chloroform extract showed maximum activity against *E. coli* (30 mm) followed by *K. pneumoniae* (25 mm). Aqueous extract of carapace failed to inhibit the growth of tested bacterial species. The acetone extract of carapace inhibited the growth of *A. niger* (20 mm) while the chloroform carapace extract showed high antifungal activity on *A. niger* (18 mm). Aqueous extract of carapace failed to inhibit the growth of tested fungal species (Table 3 and Plate 4).

3.4. Antimicrobial activity of testes: Acetonic extract of testes showed strong antibacterial activity on *E. coli* (28 mm) followed by *K. pneumoniae* (20 mm) and *P. aeruginosa* (19 mm). Chloroform extract of testes exhibited maximum activity against *E. coli* (30 mm) and

K. pneumoniae (27 mm). While the aqueous extract of the testes inhibited the growth of *P. mirabilis* (10 mm) and showed moderate activity against *C. albicans* (9 mm), other extracts of testes did not inhibit the growth of the tested fungal species (Table 4, Plate 4).

3.5. Antimicrobial activity of hepatopancreas: Acetoneextract of hepatopancreas showed the strong antibacterial activity against *E. coli* (27 mm) followed by *P. mirabilis* (24 mm). Chloroform extract of hepatopancreas showed maximum activity against *E. coli* (30 mm), followed by *K. pneumoniae* (22 mm), and *P. aeruginosa* (21 mm). Aqueous extract of the hepatopancreas inhibited the growth of *S. aureus* (10 mm) only. The acetone extract of hepatopancreas showed maximum antifungal activity against *C. albicans* (25 mm) and did not inhibit the growth of *A. niger,w*hile the chloroform extract inhibited the growth of *C. albicans* (30 mm). However the aqueous extracts did not inhibit the tested fungal species (Table 5, Plate 5).

Table 1: Antimicrobial activity of haemolymph of *M. messor* against tested pathogens

Pathogen	Strain of pathogen	Zone of inhibition (mm)			
		Acetone	Chloroform	Aqueous	Positive control
Bacteria	*Escherichia coli*	17	26	-	24
	Pseudomonas aeruginosa	9	14	-	10
	Staphylococcus aureus	-	8	-	12
	Proteus mirabilis	18	30	-	15
	Klebsiella pneumoniae	-	-	-	12
Fungi	*Aspergillus niger*	-	-	15	34
	Candida albicans	-	-	-	15

Table 2: Antimicrobial activity of gills of *M. messor* against tested pathogens

Pathogen	Strain of pathogen	Zone of inhibition (mm)			
		Acetone	Chloroform	Aqueous	Positive control
Bacteria	*Escherichia coli*	23	21	10	22
	Pseudomonas aeruginosa	-	20	-	15
	Staphylococcus aureus	20	15	-	11
	Proteus mirabilis	18	19	-	15
	Klebsiella pneumoniae	-	-	-	7
Fungi	*Aspergillus niger*	14	-	-	8
	Candida albicans	-	-	-	15

Table 3: Antimicrobial activity of carapace of *M. messor* against tested pathogens

Pathogen	Strain of pathogen	Zone of inhibition (mm)			
		Acetone	Chloroform	Aqueous	Positive control
Bacteria	*Escherichia coli*	14	30	-	22
	Pseudomonas aeruginosa	18	-	-	12
	Staphylococcus aureus	15	15	-	12
	Proteus mirabilis	20	15	-	10
	Klebsiella pneumoniae	23	25	-	27
Fungi	*Aspergillus niger*	20	18	-	11
	Candida albicans	-	-	-	9

Table 4: Antimicrobial activity of testes of *M. messor* against tested pathogens

Pathogen	Strain of pathogen	Zone of inhibition (mm)			
		Acetone	Chloroform	Aqueous	Positive control
Bacteria	*Escherichia coli*	28	30	-	20
	Pseudomonas aeruginosa	19	18	-	12
	Staphylococcus aureus	11	10	-	15
	Proteus mirabilis	15	9	10	11
	Klebsiella pneumoniae	20	27	-	25
Fungi	*Aspergillus niger*	-	-	-	9
	Candida albicans	-	-	9	11

Table 5: Antimicrobial activity of hepatopancreas of *M. messor* against tested pathogens

Pathogen	Strain of pathogen	Zone of inhibition (mm)			
		Acetone	Chloroform	Aqueous	Positive control
Bacteria	*Escherichia coli*	27	30	-	20
	Pseudomonas aeruginosa	-	21	-	14
	Staphylococcus aureus	-	-	10	13
	Proteus mirabilis	24	-	-	16
	Klebsiella pneumoniae	16	22	-	34
Fungi	*Aspergillus niger*	-	-	-	9
	Candida albicans	25	35	-	11

4. Discussion

In recent years, great attention has been paid to study the bioactivity of natural products due to their potential pharmacological utilization. The present research investigation highlights the antimicrobial peptides of haemolymph and other tissues of *Metopograpsus messor*, an estuarine crab collected from the Manakudy estuary, Kanyakumari district. The results revealed that the haemolymph, hepatopancreas, carapace, gills and testes of the crab had antimicrobial activity against different range of bacterial strains and fungal strains. Previous work shows that decapod crustaceans contain factors with antibacterial activity in

the haemolymph and different body parts [13, 14]. The influence of crab haemolymph against the wide range of clinical pathogens proves that crustaceans are very good source of antimicrobial potency [15]. Antibacterial peptides can also be induced in response to wounding or infection in the cuticles [16] and these are secreted into the haemolymph of which some are lysozyme [17] and andropin [18]. These proteins show strong resistant to the microbial growth.

From the present study it was observed that *Metopograpsus messor* has potential antimicrobial components which are evident from the high zone of inhibition recorded with the solvent extracts of haemolymph, gills, carapace, testes and hepatopancreas against human pathogens. The chloroform extracts showed better results when compared to acetone, aqueous suggesting chloroform solvent as efficient in eluting the bioactive compounds. Comparison of antibacterial activity with antifungal revealed that the tissues extracts were more effective against fungi when compared to bacteria. Our results are in confirmation with Zodape [19] who studied the antibacterial and antifungal activity of bioactive compounds of *A. integerrimus*.

The haemolymph of crustaceans possess defence molecules that help to fight against the pathogens in their environment. The results of the present study showed that the haemolymph and tissue extracts of the crab *Metopograpsus messor* inhibited the growth of bacteria and fungi to a greater extent indicating the presence of antibiotics that has the potential to act against a wide range of clinical pathogens thereby making them reliable candidate for very good source of antimicrobial potency.Thus the antimicrobial assay did in this study serve as a base line data for further studies that may confirm the hypothesis that brachyuran crabs are indeed potential sources of novel compounds with biomedical applications. Further purification of the active compounds is necessary in order to identify their chemical nature and to evaluate their potency as a novel drug.

5. References

1. Munro, N.J., Snow, K., Kant, J.A and Landers, L.P. (1999). Molecular diagnostics on micro fabricated electrometric devices: from slab gel-to capillary-to microchip-based assays for T-and b- cell lympho proliferative disorders. Clinical Chemistry, 45: 1906-1917.
2. Smith, V.J. (1991). A question of pain in invertebrates, ILAR Journal, 33 (1-2): 25-31.
3. Underhill, D.M. and Ozinsky, A. (2002). Toll-like receptors: key mediators of microbe detection. Current Opinion in Immunology, 14: 103-110.

4. Iwanaga, S., Morita, T., Harada, T., Nakamura, Niwa, M., Takada, K., Kimra, T. and Sakakibara, S. (1978). Chromogenic substrates for horse shoe crab clotting enzyme, its application for the assay of bacterial endotoxin, Homeostasis, 7: 183-188.
5. Sugumaran, M. (2002). Comparative biochemistry of eumelanogenesis and the protective roles of phenoloxidase and melanin in insects.Pigment Cell Research, 15: 2-9.
6. Fujita, T. (2002). Evolution of the lectin-complement pathway and its role in innate immunity.Nature Reviews. Immunology, 2: 346-353.
7. Raman, T., Arumugam, M. and Mullainadhan, P. (2008). Agglutinin-mediated phagocytosis associated generation of superoxide anion and nitric oxide by the haemocytes of the giant freshwater prawn *Macrobrachiumrosenbergii*. Fish Shellfish Immunology, 24: 37-45.
8. Bogdan, C., Rollinghoff, M. and Diefenbach, A. (2000). Reactive oxygen and reactive nitrogen intermediates in innate and specific immunity. Current Opinion in Immunology, 12: 64-66.
9. Iwanaga, S and Lee, B. (2005). Recent advances in the innate immunity of invertebrate animals. Journal of Biochemistry and Molecular Biology, 38: 128-150.
10. Smith, V.J and Chisholm, J.R. (2001). Antimicrobial proteins in crustaceans.Advances in Experimental Medicine and Biology, 484: 95-112.
11. Karthikeyan, L A., Shanthi, V.A. and Nagasathaya (1951). Preliminary phytochemical and antibacterial screening of crude extract of the leaf of *Adhatodavasica*. International Journal of Green Pharmacy, 10: 78-80.
12. Bauer, A.W., Kirby, W.M.M., Sherri, M.J.C. and M. Turick (1996). Antibiotic susceptibility testing by a standardized single disc method. American Journal of Clinical Pathology, 45: 493-496.
13. Haug, T.A.K., Kjuul, E., and Sandsdalenvand, O.B. (2002). Antibacterial activity in four marine crustacean decapods. Fish Shell Immunology, 12 (5): 371-85.
14. Rameshkumar, G., Ravichandran, S., Kaliyavarathan, G. and Ajithkumar, T.T. (2009). Antimicrobial peptide from the crab, *Thalamittacrenata* (Latreille, 1829). World Journal of Fish and Marine Science, 1(2): 74-79.
15. Anbuchezhian, R.M., Ravichandran, S., Rameshkumar, G. and Ajithkumar, T.T. (2009). Influence of crab haemolymph on clinical pathogens, Advances in Biological Research, 3 (3-4): 104-109.
16. Hoq, M.I., Seraj, M.U. and Chowdhury, S. (2003). Isolation and characterization of antimicrobial peptides from the mud crab, *Scylla serrata*. Pakistan Journal of Biological Sciences, 6: 2345-1353.

17. Lee, W. J. and Brey, P.T. (1995). Isolation and characterization of the lysozyme-encoding gene from the silkworm *Bombyx mori*. Gene, 161 (2): 199-203.
18. Samakovilis, C.P., Kylsten, D.A., Kimbrell, A., Engstrom, A. and Hultmark, D. (1991). Theadropin gene and its product, a male specific antibacterial peptide in *Drosophila melanogaster*.The EMBO Journal, 10: 163-169.
19. Zodape, G.V. (2014). Studies on the antimicrobial and antifungal activities of bioactive compounds of intertidal crab *Atergatis integerrimus* (Lamarck) of West Coast of Mumbai. Bionano Frontier, 7 (2): 278-283.