Study of Antimicrobial Activity of Ethnomedically Important Plant

Murraya paniculata

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Abstract

Murraya paniculata is a potent ethno medicinally important plant used both in traditional medicine as an analgesic and for wood and its activity against S. aureus established its traditional uses too. Selaginella is also used traditionally in wounds, postpartum, menstrual disease against microbes Lycopodium is used in Rheumatism and disease of lungs and kidneys and our study also revealed the scientific base behind its uses. The result of our experiments showed that in vitro antimicrobial screening of extracts and pure compounds of Murraya paniculata indicated positive activity against Staphylococcus aureus. They showed acetone extracts of the plants showed in inhibition zone of 13mm, methanol extract showed in inhibition zone of 9mm and ethanol extract showed in inhibition zone of 7mm but the distilled water extract showed no inhibition zone. We also performed work on selaginella and lycopodium but got no inhibition zone.
INTRODUCTION

Plants have been an integral part of human society, since the start of civilization. India is rich in its plants diversity, a number of plants have been documented for their medicinal potential which are in use by the traditional healers, herbals folklorists and in Indian system of medicine, viz: Ayurveda, Unani, Siddha apart from Homeopathy & Electropathy. These plant species play major role in the health care of the nation’s population. Natural products have been applied to human healthcare of thousands of years. Many drugs presently prescribed by physicians are either directly isolated from plants or are artificially modified versions of natural products (Wang, et.al. 2007). These medicines are safe and environment friendly. According to the WHO about 80 % of the world’s population relies on traditional medicine for their primary health care (Behera, 2006). Phytotherapy seems to be an alternate system of medicine for the villagers, presiding in the sub urban / rural areas (Nandankunjidam, 2006). A large no of plants are being used as medicinal agents all over the world (Chowdhury et al., 2005). Limitation of synthesized compounds in the treatment of chronic disease and potential of plant-based medicine as a more effective and cheaper alternative, was probably responsible for the fast growing industry of herbal medicine (Rojas et al., 1992). India is endowed with a rich wealth of medicinal plants of the 340 plant medicines mentioned in the Charak Samhita (1000 B.C.), The Indian Pharmacopoeia(1966) recognizes only 85 medicinal plants used in various pharmaceutical preparations. Most of these continue to be gathered from the wild to meet the demand. Thus, despite the rich heritage of knowledge on the use of plant drugs, little attention had been paid to grow them as field crops in the country till the latter part of the nineteenth century (http://www.krishiworl.com/html/medi_aroroplants1.html). To date plants continue to be a major source of commercially consumed drugs. Even most synthetic drugs have their origin from natural plant products (Sofowara, 1982). Man has used plants to treat common infectious diseases and some of the traditional medicines are still included as part of the habitual treatment of various maladies (Heinrich et al., 2005).

Plants produce a diverse range of bioactive molecules making them a rich source of different types of medicines (Stuffle and Douros, 1982). Higher plants Sources of medicinal compounds have continued to play a dominant role in the maintenance of human health since ancient times. Over 50% of all modern clinical drugs or of natural product origin and a natural product play and vital role in modern drug development in the pharmaceutical industry (Baker et al., 1995).

Medicinal plants are plants whose extracts can be used directly or indirectly for the treatment of different ailments. Therefore, the use of traditional medicine and medicinal plants in most developing countries, as a basis for the maintenance of good health, has been widely observed (Edward, 2001). Scientist throughout the world are trying to explore the precious assets of
medicinal plants to help the suffering humanity. Furthermore, in the world more than 30% of the pharmaceutical preparations are based on plants (Shinwari & Khan, 1998).

Scientific interest in the medicinal plant has burgeoned in recent times due to increased efficiency of new plant derived drugs and rising concerns about the side effects of modern medicine. The continuing emergence of drug resistant organism and the increasing evolutionary adaptations by pathogenic organisms to commonly used. Therefore, the search for new drug from novel sources such as plant continues to be necessary (Fransworth & Morris, 1976).

Infectious diseases are the leading cause of death worldwide. The clinical efficiency of many existing antibiotics is being threatened by the emergence of multidrug resistant pathogens (Bandow et al., 2003). Bacterial pathogens have evolved numerous defense mechanisms against antimicrobial agents and resistance to old and newly produced drug is on the rise. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to screening of several medicinal plants for their potential antimicrobial activity (Colombo and Bosisio, 1996; Scazzocchio et al., 2001). There are several reports in the literature regarding the antimicrobial activity of crude extracts prepared from plants (El-seedi et al., 2002; Rojas et al., 2003; Duraipandiyan et al., 2006; Parekh and Chanda, 2007a).

Plants with possible antimicrobial activity should be tested against an appropriate microbial model to confirm the activity and to ascertain the parameters associated with it. The effects of plant extract on bacteria have been studied by a very large no of researches in different parts of the world (Ates and Erdogrul, 2003). Much work has been done on ethnomedicinal plants in India (Negi et al., 1993). Interest in a large number of traditional natural products has increased (Taylor et al., 1996). It has been suggested that aqueous and ethanolic extract from plants used in allopathic medicine are potential source of antiviral, Anti tumoral and antimicrobial agents (Chung et al., 1995). The selection of the crude plants extracts for screening program has the potential of being more successful in initial steps than the screening of pure compounds isolated from natural products (Kusumoto et al., 1995).

History of studies on antimicrobial activity of plants can traced back to the works of Osborn (1943) who reported the occurrence of antimicrobial substance in green plants still then the work on screening newer plants for drug discoveries continued in great pace. In early 90’s, Benjamin (1998) investigated the use of Cassia alata in traditional Nigerian system of medication. Shelef (1983) studied the antimicrobial effects of spices. Unaeze (1986) studied the antimicrobial activity of certain medicinal plant used in traditional medicine in Nigeria. Zaika (1988) worked on the antimicrobial activity of species and herbs. Palacious (1991) studied the antimicrobial and antymycotic effects of Mimosa pudica in experimental animals. Rojas et al. (1992) screened for antimicrobial activity of crude drug extracts and pure natural products from

The histories of studies on the antimicrobial activity of Indian plants were incorporated in the work of Kumar et. al. (1987), Vedavathy et.al. (1991) etc. they were followed by, Samy et.al. (1998), Arora et.al. (1999), Srinivasan et.al. (2001), Ahmed et.al. (2001), Natarajan et.al. (2003), Shyamala et.al. (2003), Nair et. al. (2005) etc who all reported significant antimicrobial potential of the herbs and plants used traditionally. Amongst them, Vedavathy reported the antipyretic activity of six indigenous medicinal plants of Tirumala hills. Ahmed et.al. (2001) studied the antimicrobial and photochemical studies on 45 Indian medicinal plants against multidrug resistant human pathogens. Venkata Ratnam (2006) in his PhD thesis elucidated the medico-botanical, phytochemical and antimicrobial properties of certain rare and endemic medicinal plants from Gundlabrahmeswaram Wildlife Sanctuary, Andhra Pradesh, India. Prekh et.al. (2007) investigated the antimicrobial and phytochemical properties of twelve species of Indian medicinal plants.

In recent years, Venkatesan et.al. (2009) worked on the antimicrobial activity of *Aegle marmelos* against pathogenic organism compared with control drug and reported the successful inhibition of *Bacillus subtilis* followed by *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*. Anibijuwon et.al. (2009) worked on the antimicrobial activity of *Carica Papaya* (Pawpaw Leaf) on some pathogenic organisms of clinical origin form South–Western Nigeria and showed that the extracts were active against both Gram–negative and Gram-positive bacteria tested and might indicate a broad spectrum of activity. This observation is very significant because of the possibility of developing therapeutic substances that will be active against multidrug-resistant organisms. It also signified the traditional usage of Carica by the local community. Aswar et.al. (2009) studied the antibacterial and antifungal activity of *Vitex nigundo* (Verbenaceae). They used different extracts of leaves of *Vitex negundo* for its antimicrobial and antifungal activity on five bacterial species and three fungal species viz: *Staphylococcus aureus, Proteus vulgaris, Bacillus subtilis, E. coli, Pseudomonas aeruginosa* and *Aspergillus niger*,
Aspergillus flavon, Candida albicans respectively. They found that among all extracts, water-ethanol (50:50) extract showed maximum antimicrobial and water extract showed maximum antifungal activity against all tested species. Study of antimicrobial activity and phytochemical analysis of Coriander sativum against infectious diarrhea was carried out by Uma et.al. (2009). The results of antimicrobial activity revealed that methanol extract of the plant exhibit good activity compared to chloroform and aqueous extracts to E.coli, Solmonella sp. And Shigella sp. Gandhiraja et.al. (2009) worked on the phytochemical screening and antimicrobial activity of the plant extracts of Mimosa Pudica L. against selected microbes and reportd the antimicrobial activity of the plant extract against three potentially pathogenic microorganism Aspergillus fumifatus, Citrobacter diversens and Klebsiella pneumonia at different concentrations of the extract to understand the most effective activity. Hasim et.al. (2009) investigated the activity of four plants against seven standard pathogenic microbes and showed that the results were promising towards discovery of new based drugs useful to treat the various multidrug resistant microbes.

Preliminary phytochemical and antimicrobial properties of Pueraria tuberose (Willd.) DC: was done by Venkata Ratnam et.al. (2009) who showed that the different solvent extracts have significant antimicrobial activity and it might be due to the presence of different biochemicals like flavonoids in it. Duraiapandiyan et.al. (2009) showed that Sphaeranthes indicus L had antibacterial affects on Staphylococcus aureus, S. epidermidis and Candida albicans and the S. indicus flower n- hexane extract seems to be a promising antimicrobial agent.

Usha et.al. (2010) screened the antimicrobial activity of Morinda citrifolia L and revealed that plant extracts showed inhibitory activity against the tested organisms. Evaluation of antibacterial activity of flower extract of Cassia auriculata was undertaken by Maneemegalai et.al. (2010). They reported that Cassia auriculata was observed to have antibacterial activity of Garlic against three fungi namely Aspergillus flavus, Curvularia lunata and Fusarium moniliforme. Antimicrobial activity of ethanolic extracts of Syzygium aromaticum and Allium sativum against food associated Bacteria and Fungi. Ram Kumar Pundir et. al. (2010) evaluateed the in vitro antimicrobial activity of ethanolic extracts of Syzygium aromaticum (clove) and Allium sativum (garlic) against Gram-positive and Gram-negative food associated bacteria (Bacillus subtilis, B. megaterium, B.polymyxa, B. sphaericus, Staphylococcus aureus and Escherichia coli) and Molds (Penicillium oxalicum, Aspergillus flavus, A. luchuensis, Rhizopus stolonifer, Scopulariopsis sp. And Mucor sp.) from all the above citations it can be clearly understood that evaluations of herbs based new drugs have gathered a great pace in among modern researchers and it is high time to initiate the same in our region also.

In the present age of synthetic drug, tendency of utilization drugs of plant origin is gradually increasing and term “Herbal“ is getting popularity day by day. An analysis of modern medicine revealed that about one fourth of all the medicines are derived from plant
sources. Ethnomedicobotanical investigation is therefore, becoming significant, as it is regarded as the basic tool of herbal drug research. North East India comprising of seven sister states has a rich potentiality of medicinal plants. Peculiarity in topographical, geological and meteorological conditions resulted the growth of plants of great diversity in the regions. Considering the above facts a good no of investigations have worked out the ethno botanical aspects of different ethnic groups of region. Extensive use of modern medication systems has helped us to combat various dreaded diseases and has made life more secured on one hand, and on the other increased the average life span of human. But the entry of modern medication system has warded off the age old belief and traditional knowledge of the ethnic people. With the progression of time the lineage of the people had been tending towards modern system and traditional knowledge does not see a bright future. So it is the prime time to conserve and exploit the traditional knowledge of the ethnic people study them scientifically and ensure their application.

MATERIALS AND METHODS

EXTRACTION OF PLANT MATERIALS

The studied plant materials were collected from various localities of Karimganj districts like Kamalpur, Mahishan etc. in the month of August 2013. The plant samples were authenticated & the voucher specimens were prepared and deposited in the herbarium of the Deptt of Botany & Biotechnology, Karimganj College, Karimganj, Assam. The local usages of the plants were also recorded. Leaves & tender twigs of Gomphrena celosioides was used. Rhizomes was used in case of Z. zerumbet. Fresh plant materials were thoroughly washed under tap water, pat dried with blotting paper and then extracted in organic solvents like methanol and acetone. Extracts were also made in distilled water. The volume ratio was maintained to 1:1. The extracts were kept in petriplates for 24 hrs to evaporate weight to the organic solvents. After 24 hrs, the resultant extracts were filtered and concentrated to dryness. A little autoclaved distilled water was added to the dried extracts (1ml) before addition.

MICROBIAL STRAINS USED AND THEIR PREPARATIONS

Microbial strains viz: Staphylococcus aureus (gram positive) and Escheria coli (gram negative) was obtained from the Department and were grown on nutrient broth (Hi – media) at 37°C for 18 -24 hrs and were maintained on respective agar slant at 4°C.
PREPARATION OF INOCULUM

Bacteria were cultured over night at 37°C in nutrient agar (NA) media and used as inoculums.

ANTIMICROBIAL DISC DIFFUSION ASSAY

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Scientific Name</th>
<th>Family</th>
<th>Common Name(Bengali)</th>
<th>Parts Used</th>
<th>Uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Muraya paniculata</em></td>
<td>Rutaceae</td>
<td>Kamini</td>
<td>Leaves</td>
<td>It is used both in traditional medicine and analgesic for wood.</td>
</tr>
<tr>
<td>2</td>
<td><em>Selaginella</em></td>
<td>Selaginellaceae</td>
<td>Sajivani</td>
<td>Dried Plants</td>
<td>It is used in menstrual disorder, skin disease, headache, fever.</td>
</tr>
</tbody>
</table>
The dried extracts were dissolved in autoclaved distilled water to make a final concentration of 1 gram per ml. The standard disc – diffusion method was used to evaluate the antimicrobial activity (Murray et al. 1995, NCCLS, 1999). Using sterilized jhonson ear buds, the petridishes containing nutrient agar were seeded. Discs of 6 nm in diameter (Watman No-1 filter paper) were placed on seeded agar plates and impregnated with 10 micro litres of 0.5 gram /ml crude extracts of the studied samples. Gentamicin & Cephalexinl discs were used as positive control the solvents were used as negative control. Test plates were incubated at 37°C for 18 -24 hours. antimicrobial activity was evaluated by measuring the zone of inhibition against each organism. The experiments were conducted in triplicates and the data was tabulated.

ANTIMICROBIAL ASSAY

The antimicrobial disk diffusion assay using the ethno botanically important plants yielded quite interesting result. Many of the extracts, showed good inhibitory activity on both the bacteria tested. The result have been tabulated below, the table containing botanical name of the plant, extracts used and name of the bacteria used. The inhibitory zone has been measured in mm.
TABLE 2: Zone of inhibition of extracts

<table>
<thead>
<tr>
<th>Plants</th>
<th>Extracts</th>
<th>Zone of inhibition (mm) in <em>Staphylococcus aureus</em></th>
<th>Zone of inhibition (mm) in <em>Escherichia coli</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Muraya paniculata</em></td>
<td>Acetone</td>
<td>13</td>
<td>_</td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>7</td>
<td>_</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>9</td>
<td>_</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td><em>Selaginella</em></td>
<td>Acetone</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td><em>Lycopodium</em></td>
<td>Acetone</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>Standard antibiotic</td>
<td>Gentamicin</td>
<td>_</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Cephalexin</td>
<td>15</td>
<td>_</td>
</tr>
</tbody>
</table>
FIG- A view of zone of inhibition of extraction of murraya paniculata against Eschrichia coli

FIG- A view of zone of inhibition of extraction of murraya paniculata against S. aureus

FIG- A view of zone of inhibition of extraction of Selaginella against E.coli
FIG- A view of zone of inhibition of extraction of Selaginella against S.aureus

FIG- A view of zone of inhibition of extraction of Lycopodium against E.coli
DISCUSSIONS AND DATA INTERPRETATION

Herbal medicines are a valuable and reality available resources for primary health care and complementary health care system. Unfortunately many species of plants containing substances of medicinal value have yet to be discovered; though large numbers of plants are constantly being screened for their antimicrobial effects. It has been suggested that phytochemical extracts from plants hold promise to be used in allopathic medicine as they are potential sources of antiviral, antitumoral and antimicrobial agents (Nair et al, 2005).

For the experiment one gram positive bacteria viz-Staphylococcus aureus was collected from the department. It was seen that all the plants studied have significant inhibitory effects on S. aureus. Methanolic extracts of all the plants had antimicrobial activity. But distilled water extracts in most cases did not show significant results. It might be due to the fact that the active principles were distilled water insoluble.

The result of our experiments from the above table2 showed that in vitro antimicrobial screening of extracts and pure compounds of Murraya paniculata indicated positive activity against Staphylococcus aureus. They showed acetone extracts of the plants showed in inhibition zone of 13mm, methanol extract showed in inhibition zone of 9mm and ethanol extract showed in inhibition zone of 7mm but the distilled water extract showed no inhibition zone. We also performed work on selaginella and lycopodium but got no inhibition zone.

FIG- A view of zone of inhibition of extraction of Lycopodium against S.aureus
The differences in the inhibitory effect of various plant extracts may be due to qualitative and quantitative differences in the antibacterial principles or compounds present in them. In this investigation it becomes certain that most effective crude extract was acetone for which maximum zone of inhibition was recorded; followed by methanol that also inhibited the growth of bacteria. While ethanol extract showed minimal antibiotic activity. This action may be synergistic and not due to the efficiency of one single substance. The above results revealed that the plant extracts could be effective antibiotic in controlling gram positive pathogen.

*Murraya paniculata* is a potent ethno medicinally important plant used both in traditional medicine as an analgesic and for wood and its activity against *S. aureus* established its traditional uses too. *Selaginella* is also used traditionally in wounds, postpartum, menstrual disease against microbes *Lycopodium* is used in Rheumatism and disease of lungs and kidneys and our study also revealed the scientific base behind its uses. From the result it is clear that three ethno botanically important plants can be used to control the various human pathogens. The above results also revealed that plant extracts could be effective antibiotics.

It has been shown in various studies that polarity of antibacterial compound is crucial for their activity. Therefore it is obvious that extracts prepared using organic solvents were more active against bacterial species. Similar observations have been reported by Thongson et al. Thus it can also be inferred that phytochemical screening should be done to discover the bioactive principles of the studied plants. More over similar types of work can be instigated to unreveal the wealth of traditional knowledge to usher a new dawn of drug discovery and economic pursuit of the region.

**CONCLUSIONS**

From the previous results and discussion, it can be inferred and hence concluded that, intending towards the theme of the project and the consequent thrust on the subject matter under consideration, the traditional knowledge recorded in our survey and in vitro experiment using the test organisms and different extracts validated the data. Against *S. aureus* the most potent plant was found to be *Murraya paniculata*. In both cases all the solvent extracts worked significantly. This result can pave for discovering some new plant based drugs potent for controlling the pathogen as well as help us the emerging fear of multi drug Resistant pathogenic microbes. Also the side effects of the modern synthetic drugs can also be averted. Further works are needed to throw light upon the bioactive principles and analyzing the antimicrobial activity of those. The results as cited can provide a basis towards experiments and consequent novel drug
discovery to usher a new dawn of hope to our biodiversified region and intensify the employability of youth here.

**REFERENCES**


