PHARMACOLOGICAL INVESTIGATION AND ANTIBACTERIAL STUDIES ON THE EXTRACTS OF HEMIDESMUS INDICUS, AVICENNIA MARINA AND ULVA LACTUCA

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Abstract
Natural products as pure compounds provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. Medicinal plants are the richest bio resource of drugs of traditional systems of medicine, modern medicines, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. Phytochemical analysis of Hemidesmus indicus, Avicennia marina and Ulva lactuca extracts revealed that numerous compounds in plants traditionally used for medicinal purposes have many therapeutical properties. The result of the phytochemical studies revealed the presence of Saponins, Tannins and Alkaloids. The concentrations of the extracts used were 25 mg/ml, 50 mg/ml and 100mg/ml respectively. At these concentrations, the extract inhibited the growth of Escherichia coli, Aeromonas hydrophila and Aeromonas hydrophila; and produced percentage inhibition ranging between 72.4% to 86.5%. The may due to the presence of the phytochemicals present in the plant. The results suggest that the phytochemical properties and anti-bacterial activity demonstrated by the plant extract for curing various diseases and leads to the isolation of new and novel compounds.

Keywords
Hemidesmus indicus, Avicennia marina, Ulva lactuca, Phytochemical, Antibacterial.

INTRODUCTION
Human health problems, allergy and hypersensitivity are increasing day by day which are directly related to the growing populations of immune compromised individuals. In developing countries, more than 5 billion people live in extreme poverty and a large number among them are suffering and trying to get safe water and basic medicines (WHO, 1995). In these peculiar environments, the population has no other alternative than to rely on traditional medical practitioners and medicinal plants for primary health care and more than 80% of the rural population depends on plants. Today, there is no doubt that complimentary medicines have wide acceptability and are attracting attention from scientific community world-wide (Afzal et al., 2011). There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious disease.

Phytochemical constituents such as alkaloids, flavonoids, tannins, saponins, and several other aromatic compounds are secondary metabolites of plants that serve a defense mechanism against prediction by many microorganisms. In India, different regions have specific features according to the climatic conditions (Kumaran & Citarasu 2015). These plants including medicinal plants are also used as a feeding for animals. They are indirectly shown by their effects by which animals do not suffer by any types of diseases. Growing plants are one of the cheapest sources of feeding for animals having crude proteins of 14-25% (Babu Shankar et al., 2011). Many plants have been investigated in recent times and found to contain active substances that are medically useful, whereas many more are yet to be scientifically investigated. Herbal medicinal products are virtually known to contain phytochemicals. Plants
are important source of potentially useful structures for the development of new chemotherapeutic agents. Mangrove plants are widely used folk remedies and ethno botanical literatures have described the usage of plant extracts to treat human diseases since time immemorial. Avicennia marina (A. marina) commonly known as grey mangrove has been used as traditional medicine for decades with multifunctional biological activity. Methanol extract of A. marina (whole plant parts) contains variety of medicinal compounds that have wide range of biological properties such as anti-oxidant, anti-inflammatory, anti-microbial, anti-ageing, and antiviral activities. The identified compounds of A. marina extract could be used as herbal medicine for treating variety of biological dysfunctions with minimum risk of adverse effects with maximum potency for better quality of life during treatment (Vinod Prabhu and Guruvayoorappan, 2012). Seaweeds are a valuable food source as they contain protein, lipids, vitamins and minerals (Marinho-Soriano et al., 2006). The nutritional properties of seaweeds are poorly known and normally are evaluated from the chemical composition. Seaweeds are not only a useful food source to humans, whole plants and seaweed mixes have been used in animal nutrition (Ventura and Castanon, 1998). The different extracts and fractions of Ulva lactuca show moderate to significant antimicrobial activity against the strains used. Active constituents were carbohydrates, steroids and glycosides found in the extracts of n-hexane, chloroform, and water: ethanol may be responsible for antimicrobial activity (Alang et al., 2009). The present study was designed to evaluate the fundamental phytochemical constituents and antimicrobial activities of Hemidesmus indicus, Avicennia marina and Ulva lactuca.

MATERIALS AND METHODS
Selection and Collection of samples

a. Hemidesmus indicus

Hemidesmus indicus, Indian sarsaparilla is a species of plant that is found in South Asia. Roots are woody and aromatic. The stem is numerous, slender, terete, thickened at the nodes. The plant is well known for its antioxidant, antimicrobial and anti-inflammatory activity. Other uses of the plant are against syphilis, leucorrhoea, bronchitis, chronic rheumatism, urinary diseases, leprosy, leucoderma and as purgative, diaphoretic, diuretic, antipyretic and antidiarrheal. The major chemical constituents are coumarin, hemidesmine, hemidine, hemidesine and rutin (Mohammed Moideen et al., 2011).

b. Avicennia marina

Avicennia marina grows as a shrub or tree to a height of three to ten metres, or up to 14 metres in tropical regions. The grey mangrove species can tolerate high salinity by excreting salts through its leaves. The methanol and ethyl acetate extracts of Avicennia marina were more potent in their antibacterial activity. Preliminary phytochemical screening was also done for the leaves extract of Avicennia marina and the studies were done about the antiparasitic, antifungal and antibacterial activity of Avicennia marina. They provide a rich source of steroids, triterpenes, saponins, flavonoids, alkaloids and tannins. A. marina commonly known as grey mangrove belongs to the family Acanthaceae (Avicenniaceae). A. marina has been used as traditional medicine for years with variety of biological activity such as anti oxidant, anti bacterial, anti tumor anti fungal, and anti ulcer properties (Vinod Prabhu and Guruvayoorappan, 2012).
c. Ulva lactuca

Green algae (Ulva lactuca) have been extensively under research for the last two decades. Since number of active constituents have been extracted from different species of green algae (e.g. biologically active steroids. The green algae Ulva lactuca Linnaeus have been found to have antimicrobial, antibacterial, preservative, anticoagulant, antiperoxidative, antihyperlipidemic, hepatoprotective, anti-inflammatory, antiprotozoal, antiviral, leishmanicidal activities and also employed as dietary fibers (Alang et al., 2009). Hemidesmus indicus, Avicennia marina and Ulva lactuca were collected from Kulasekharam mugaliadi hill and Manakudy coastal region, kanayakumari district, Tamilnadu, India.

EXTRACT PREPARATION
Shadow dried root powder plant materials were boiled at above 100°C with two hour. After filtered the extracts, the supernatant was collected and the residue were discarded. The supernatant was condensed in the water bath and the condensate was extracted again by methanol. The methanolic extract was concentrated in rotatory evaporator under reduced pressure at the room temperature of 45°C to 50°C in order to avoid the evaporation of plant materials. Aqueous extract was concentrated using Lyophilizer and stored at 4°C.

PHYTOCHEMICAL SCREENING
Phytochemical active constituents such as alkaloids, anthraquinones, cardiac glycosides, flavonoids, terpenoids, tannins, Saponins, Sterols and triterpenes analysed based on (Sofowora, 1993; Trease, 1989). This screening was carried out with the methanolic extracts using chemical methods and thin-layer chromatography (TLC) according to the methodology given in Wagner and Bladt 1996.

AUTOBIOGRAPHY
A TLC Bioautographic method was used to detect active components. After application of the extract on a silica gel plate, thin layer chromatography (TLC) was developed using ethylacetate: methanol (9:1) as the eluent system for Hemidesmus indicus, Avicennia marina and Ulva lactuca. Observe the bands; the TLC plates were dried for complete removal of solvents. Then the fractions of TLC were spotted on already swabbed agar plates by bioautography method to evaluate the activity of the different essential compounds, and the plates were incubated at 35°C for 24 hours. The activity of compound can detect by its zone formation.

ANTIBACTERIAL ACTIVITY
The test organisms were standard laboratory strains of Escherichia coli, Aeromonas hydrophila and Pseudomonas aeruginosa. Muller-Hinton agar media were poured on to sterile Petri plates. When the media solidified, 0.1ml of inoculums with 0.5 OD was poured over feeder layer and spread evenly with a sterile spreader. A well of 6 mm diameter was made by using a sterile cork borer. Each well-received the extract was tested in a different concentration (25 mg/ml, 50 mg/ml and 100 mg/ml. Distilled water was used as negative control while ampicillin was used as positive control. And the commercial antibiotics like as Ampicillin and Tetracycline tested against pathogens. They were incubated at 37°C for 24 hours. After incubation, the diameter of the inhibition zone was measured.

RESULTS
Phytochemical Screening
The phytochemical screening of methanolic extracts showed the presence of different types of active constituents, namely alkaloids, cardiac glycosides, flavonoids, terpenoids, tannins, Sterols and Saponins. These compounds were present in almost all the plants extracts. The details were
given in the (Table 1).

Table 1: Phytochemical Analysis of Hemidesmus indicus, Avicennia marina and Ulva lactuca extracts.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Phytochemical group</th>
<th>Hemidesmus indicus</th>
<th>Avicennia marina</th>
<th>Ulva lactuca</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Steroids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Terpenoids</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Triterpenoids</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Anthraquinones</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Cardiac glycosides</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Saponins</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>Tannins</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: + = Present; - = Absent

TLC STUDIES ON PLANT EXTRACTS

On TLC analysis for the hot water extract Hemidesmus indicus, Avicennia marina and Ulva lactuca was revealed that, the single spot were obtained, and it observed under UV-illuminator. The Hemidesmus indicus fraction obtained having the Rf values of 0.86 and 0.68. The Avicennia marina fraction obtained having the Rf values of 0.78, 0.63 and 0.42. The Ulva lactuca fraction obtained having the Rf values of 0.78. And it shows on Fig (1).

ANTIMICROBIAL ACTIVITY

The antimicrobial activities of the plant extracts against the three bacteria strains examined were assessed by the presence or absence of inhibition zones. The aqueous extract of A. marina exhibited moderate level antimicrobial activity against Escherichia coli, Aeromonas hydrophila and Pseudomonas aeruginosa the test organisms. Extract of U. lactuca was active against all the test organisms except Aeromonas hydrophila. On the other hand, it was found that the methanol extract of H. indicus exhibited high activity against Escherichia coli, Aeromonas hydrophila and Pseudomonas aeruginosa. To screen the antibacterial activity against tested organisms, ampicillin were used as a standard. It was found that tetracycline (100 mg/ml) standard showed moderate activity than plant extracts against tested microorganisms (Table 3).

DISCUSSION

Plants are the storehouses and rich sources of safer and cheaper chemical compounds. These
natural plant products have been reported to have various activities like anti stress, growth promoters, appetizer, tonic, immune stimulants and antimicrobials (Citarasu et al., 2002). Moreover, the substances are obtained from natural sources, besides possessing other interesting properties like non-toxic, biodegradable and biocompatible (Citarasu et al., 2003).

The results of our studies have shown that Asparagus racemosus contains Saponins, Tannins, Flavonoids, Steroids, Alkaloids and Cardiac glycosides. The plant extract also showed antibacterial activity at concentrations of 25 mg/ml, 50 mg/ml and 100mg/ml respectively. At these concentrations, the extract inhibited the growth of E.coli, A.hydrophila and P. aeruginosa and produced percentage inhibition ranging between 72.4% to 86.5%. Therefore, the ethnomedical application of the plant in the treatment of bacterial infections is justified.

The antibacterial activity of aqueous extract (100 mg/ml) showed that the extract has activity against Escherichia coli, Aeromonas hydrophila and Pseudomonas aeruginosa (Table. 3). Qualitative and quantitative standards for the identification of Hemidesmus indicus roots and the Pharmacognostical and phytochemical studies on Hemidesmus indicus roots will be highly useful (Rajan et al., 2011). Mangrove and mangrove associates contain biologically active antiviral, antibacterial and antifungal compounds. Extracts from different mangrove plants are active against human and plant pathogens. A. marina leaves were used as test plants due to the presence of much evidence that prove their therapeutic value against microbial infections (Chandrasekaran et al., 2009). Our results proved that different plant extracts of A. marina in different solvents showed antibacterial activity against the tested bacterial species.

Green alga Ulva lactuca was successively extracted with n-hexane, chloroform and ethanol: water (1:1) by maceration. The phytochemical analysis revealed the presence of carbohydrates, steroids and glycosides. The extracts were subjected for study of antimicrobial activity. Green algae Ulva Lactuca possess antimicrobial activity (Alang et al.,2009). In the view of this, an effort was made to check the isolated compounds from the algae for their anti-microbial activity. The inhibition of microorganisms under standardized conditions was utilized to demonstrate microbial action of the compounds. Similar results were reported Kumaran & Citarasu 2015. A.racemosus contain potential antimicrobial components that may be of great use for the development of pharmaceutical industries as a therapy against various diseases. In conclusion, the antibacterial activity exhibited by this plant extract may be due to the presence tannins, saponins and flavonoids in plant which have been reported to have antibacterial properties.

REFERENCES