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

Author's name: [1]P. Meenachi, [2]R.K. Jeshika, [3]D. Aruna and [4]T.Kumaran
[1,2,3 Department] *of Nutrition and Dietetics, Muslim Arts College, Kanyakumari 629174, Tamilnadu, India.*
[4]PG & Research Department of Zoology, Muslim Arts College, Affiliated to ManonmaniamSundaranar University, Thiruvithancode, Kanyakumari 629174, Tamilnadu, India.
E-mail:kumaranmac@gmail.com

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 Hemidesmusindicus,Avicennia marina and Ulvalactuca 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, Aeromonashydrophilaand Aeromonashydrophila; 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 demostrated by the plant extract for curing various diseases and leads to the isolation of new and novel compounds.*

Keywords *midesmusindicus*, *Avicennia marina*, *Ulvalactuca*, 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 & Citarasu2015). 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

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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 (VinodPrabhu 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 *Ulvalactuca* 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 *Hemidesmusindicus*,*Avicennia marina*and *Ulvalactuca*.

MATERIALS AND METHODS

Selection and Collection of samples

a. *Hemidesmusindicus*

Hemidesmusindicus, 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 tumoranti fungal, and anti ulcer properties (VinodPrabhu and Guruvayoorappan, 2012).

c. *Ulvalactuca*

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Green algae (*Ulvalactuca*) 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 Ulvalactuca 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).*Hemidesmusindicus*,*Avicennia marina*and *Ulvalactuca* were collected from Kulasekharammugaliadi hill and Manakudy costal 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 triterpenesanalysed 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 *Hemidesmusindicus*,*Avicennia marina*and *Ulvalactuca*. 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 35ºC 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, Aeromonashydrophila*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 37ºC 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).

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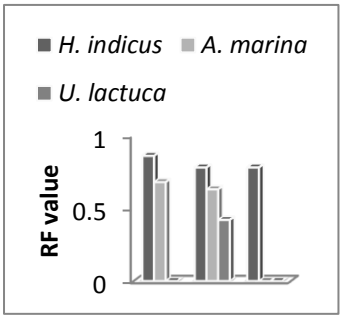
Table 1:Phytochemical Analysis of Hemidesmusindicus,Avicennia marina and Ulvalactuca extracts.

S.No.	Phytochemical group	*Hemidesmusindicus*	*Avicennia marina*	*Ulvalactuca*
1.	Steroids	+	+	+
2.	Terpenoides	+	+	-
3.	Titerpenoides	+	-	-
4.	Anthraquinones	-	-	-
5.	Cardiac glycosides	+	+	+
6.	Alkaloids	+	+	-
7.	Saponins	+	+	+
8.	Flavonoids	+	+	-
9.	Tannins	+	+	-

Note: + = Present; + = Absent

TLC STUDIES ON PLANT EXTRACTS

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



BIOAUTOGRAPHY

Bio autography method was used to detect active components by its zone formation. The maximum zone of inhibition is measured in *Avicennia marina*extract6.1 mm in dm against *P.aeruginosa*. The minimum zone of inhibition for the fraction of *Ulvalactuca*is 1.4 mm against *A.hydrophila* (Table 2).

*Table 2: Bioautography of the Hemidesmusindicus,Avicennia marina and Ulvalactuca against some pathogenic bacteri***a**

S.NO	Extracts	Zone of Inhibition(mm)		
		E. coli	***A.hydrophila***	***P. aeruginosa***
1	***H. indicus***	4.2mm	3.8mm	4.5mm
2	***A. marina***	5.9mm	5.4mm	6.1mm
3	***U. lactuca***	2.7mm	1.4mm	1.8mm

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, Aeromonashydrophila*and *Pseudomonas aeruginosa*the test organisms. Extract of *U. lactuca*was active against all the test organisms except *Aeromonashydrophila*. On the other hand, it was found that the methanol extract of *H. indicus*exhibited high activity against *Escherichia coli,Aeromonashydrophila* 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).

Table 3: Summary of the results of antibacterial activities of the aqueous extracts of Hemidesmusindicus,Avicennia marina and Ulvalactuca

Test organisms	Inhibition of zone (mm)				
	H. indicus **(100 mg/ml)**	***A. marina*** **(100 mg/ml)**	***U. lactuca*** **(100 mg/ml)**	**Ampicillin (100 mg/ml)**	**Distilled water**
E. coli	19	17	16	19	0.0
A. hydrophila	18	16	5	19	0.0
P. aeruginosa	15	16	15	19	0.0

DISCUSSION

Plants are the storehouses and rich sources of safer and cheaper chemical compounds. These

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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, Aeromonashydrophila*and *Pseudomonas aeruginosa*(Table. 3). Qualitative and quantitative standards for the identification of *Hemidesmusindicus*roots and the Pharmacognostical and phytochemical studies on *Hemidesmusindicus*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 *Ulvalactuca* 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 *UlvaLactuca*ossess 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.

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