ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL ANALYSIS OF *Opilia amentaceae* MEDICINAL PLANT EXTRACTS AGAINST BACTERIA CAUSING DIARRHOEA

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ABSTRACT

The study of medicinal plants as antimicrobial agents should be focused in part on ascertaining specific information about the plant's antimicrobial activity In the present investigation Phytochemical, antimicrobial activity were screened using a plant namely *Opilia amentaceaea* extract against diarrhoeal pathogens. In the phytochemical analysis sequential extractions was carried out different solvent extracts. Various phytochemical compounds was screened such as Tannins, Saponins, Flavonoids, Catachins and Sugar were screened in medicinal plants. Anthroquinones present only in *Anogeissus latifolia*. All crude extracts of those medicinal plants were tested against standard reference strains including *Escherichia coli ATCC 25922, Salmonella enterica typhimurium, Shigella dysentriae , Klebseilla pneumonia.* The results suggest that opilia amentaceae is scientifically validate the use of this plant in the traditional medicine for isolation and characterization of active role in future exploitation in medical microbiology. The purpose this study was to investicate the use of the selected plant extract was *opilia amentacea* in the treatment of diarrhoea which may lead to the discovery of attractive form of treatment.

Key words: *Opilia amentaceae*, phytochemical, antimicrobial activity, diarrhoeal pathogens.

INTRODUCTION

Plants have played a crucial role in maintaining human health and improving the quality of human life for thousands of years. Medicinal plants sector has traditionally occupied an important position in the socio cultural, spiritual and medicinal area of rural and tribal lives of India. Medicinal plants continue to be an important therapeutic aid for alleviating the ailments of humankind. Natural products are known to play an important role in both drug discovery and chemical biology. The acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to the available antibiotics has led researches to investigate the antimicrobial activity of medicinal plants. India is rich in medicinal plant diversity. All known types of agro-climatic, ecologic and adaphic conditions. Human use of plants as medicines could be dated back to the Middle Paleolithic Age, which is about 60 000 years ago, (Palombo, 2006).

Medicinal plants (otherwise referred to as herbs, herbal medicines, pharmacologically active plants or phyto medicinal) remain the dominant form of medicine in most countries. (Rao *et al*., 2004).

Many plants used as traditional medicines are now being validated through scientific research by isolation of bioactive compounds for direct use in medicines. For instance, drug discovery from medicinal plants led to the isolation of early drugs such as morphine from opium, cocaine, codeine, digitoxin and quinine, some of which are still in use
(Kokwaro, 2009)

In developing countries, diarrhoea often results from food and water contaminated by Salmonella typhi, Campylobacter jejuni and Shiga toxin-producing Escherichia coli (STEC), and water sources contaminated by *Giardia intestinalis* and *Cryptosporidium parvum* (Mathabe *et al*. 2006; Aboutaleb *et al*., 2014). *Shigella* spp., *Salmonella* spp., Campylobacter jejuni and the protozoan *Entamoeba histolytica* are notably responsible for acute bloody diarrhoea (Fajimi *et al*., 2000). Diarrhoea is also a leading cause of morbidity and mortality in HIV-infected children. HIV-infected children admitted with diarrhoea are more likely to have prolonged diarrhoea and malnutrition and require a longer hospital stay. They also have a higher frequency of recurrent diarrhoea and recurrent hospital admissions (Chhagan and Kauchali 2006). A major consequence of diarrhoea in young children is the need for medical and parental care, clinic visits, hospitalisation, and loss of work by the parents or the caregiver.

Antibiotics such as amoxicillin/clavulanate and co-trimoxazole, sometimes used in the treatment of infectious diarrhoea of bacterial origin, may paradoxically cause hepatotoxic reactions. Other side effects of antibiotics include cutaneous reactions, gastrointestinal disorders and yeast overgrowth (Anwar *et al*., 2009). At present, the continued development of new antimicrobials, other than antibiotic ones, particularly those used for the treatment of diarrhoea in children, is critically important.

Phytochemical screening of plants has revealed the presence of numerous chemicals including alkaloids, tannins, flavonoids, steroids, glycosides, saponins etc. Many plant extracts and phytochemicals show antioxidant/free radical scavenging properties. Secondary metabolites of plants serve as defense mechanisms against predation by many microorganisms, insects and herbivores.

Plant products have been part of phytomedicines since time immemorial. These can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc i.e. any part of the plant may contain active components.

Opilia amentacea is evergreen shrub that branches from the base, with stems usually up to 15 metres long. The plant is harvested from the wild for local use as a food, medicine and source of wood. It is found in a wide range of environments from coastal bushland, through Acacia woodland to dry forest, often in riverine forest; also in upland rainforest, at elevations from sea level to 1,900 metre.

The root is diuretic and purgative. A decoction or infusion is drunk for the treatment of fevers, mental illness, headache, influenza and stomach problem. The pounded root is mixed with sodium bicarbonate and the mixture licked to treat coughs. An extract of the leaves containing sodium bicarbonate is used as an anthelmintic.A cold-water extract is drunk to treat oedema and a decoction is used to relieve toothache. The bark is pounded and soaked and used to treat malaria. Fragrat opilia is found in Peninsular India through Srilanka to Tropical Australia and Africa. In the Present study antibacterial activity of *Opilia amentaceae Robx* was screened against human pathogens.

MATERIALS AND METHODS

Collection of plant samples

Opilia amentaceae Roxb.leaves were collected in the month of January 2018 from Siriya kalvarayan hills Eastern Ghats, Villupuram district. Samples were shade dried and pulverised under the room temperature for 48 hrs.

Preparation of the extract

The dried powders of leaf of *Opilia amentaceae* Roxb. were defatted with methanol (60-80ºc) in a Soxhlet Apparatus by continuous hot- percolation. The solvent was removed by kept into the incubator for 24 hrs. The resultant dried extracts were used for further study.

Microorganisms

Three bacterial species used were, *Escherichia coli ATCC 25922 (E.coli), Salmonella enterica typhimurium* (*S.enterica),* and *Shigella dysentriae, Klebseilla pneumoniae*. The cultures were obtained from K.A.P. Viwsanathan Govt. Medical College, Trichy, India. Bacterial species were subcultured on nutrient broth and incubated at 37°C for 18-24 h. The bacterial strains were cultured on nutrient agar slants. The cultures were maintained by sub culturing periodically and preserved at 4° C until further use.

Phytochemical analysis

The extracts were then tested for their respective phyto chemical compositions, as per the method of Brindha *et al.*1981. (Table 1).

Table 1 : Preliminary Qualitative Analysis by (*Brinda et al., 1981*) method.

S.no	Test	Observation	Inference
1.	2ml of test solution and a minimum amount of chloroform, 3 to 4 drops of acetic anhydride, followed by one drop of conc. H2so4 was added.	Purple colour changes to blue or green.	Steroid present.
2.	2ml of test solution was reacted with a piece of tin and two drops of thionyl chloride.	Violet or purple colour developed.	Triterpenoids present.
3.	2ml of test solution was mixed with a very small quantity of anthrone reagent and a few drops of conc. H2so4 and heated.	Green or purple colour developed.	Sugar present.
4.	2ml of test solution was mixed with 2ml of Fehlings reagent and 3 ml of water.	Red-orange colour formed.	Presence of reducing sugar.
5.	2 ml of the test solution was taken with 2N HCl, and the aqueous layer formed was decanted, to which, one to few drops of mayers reagent was added.	Formation of white precipitate or turbidity.	Alkaloids present.
6.	2 ml of test solution alcohol was taken along with a drop of neutral ferric chloride (5%) solution.	Intense blue colour developed	Phenolic compound present.
7.	2 ml of the solution in alcohol was mixed with a bit of magnesium and one or two drops of concentrated HCl and then heated.	Red or orange-red colour formed.	Presence of flavonoid.
8.	2 ml of test solution in alcohol was taken along with a few drops of ehrlichs reagent and a few drops of conc. HCl.	Pink colour formed.	Presence of catachins
9.	2 ml of test solution was mixed with water shaken well.	Foamy lather formed.	Saponins present.
10.	2 ml of test solution was mixed with water and then with lead acetate solution.	White precipitate was developed	Tannins present.
11.	2 ml of test solution was mixed with magnesium acetate solution.	Pink colour developed.	Anthroquinones present.
12.	2 ml of test solution was mixed with 1% ninhydrin in alcohol.	Blue or violet colour obtained.	Presence of aminoacid.

Determination of antibacterial activity using standard antibiotics by disc diffusion method

The disc diffusion method was used with few modifications to evaluate anti-microbial activities. Sterile Disc (Whatman, 6 mm) were impregnated with 50 µl of reconstituted crude extracts (1 mg mL-1) in the respective solvent used for extraction (ethanol), and placed on the surface of Muller-Hinton agar dispersion plates inoculated with bacterial cultures. Each extract was tested in triplicate. Control disc containing 50 µl DMSO (100 %) was used as negative control. Standard antibiotics such as vancomycin, gentamicin, bacitracin and amoxicillin-clavulanate (HIMEDIA) were used as reference or positive control. Agar plates containing bacteria were incubated at 37°C for 24 h. Inhibition zones were recorded as the diameter of growth-free zones, including the diameter of the disc in mm, at the end of the incubation period.

Determination of antibacterial activity by agar well diffusion method

Antimicrobial activity was determined by the well diffusion method according to National Committee for Clinical Laboratory Standards (NCCLS) (National Committee for Clinical Laboratory Standards, 1993). Petri plates containing 20 ml of, Nutrient (for bacteria) Agar medium were seeded with 1-3 day cultures of microbial inoculums (standardized inoculums 1-2 X 107 cfu/ml 0.5 Mcfarland standard). Wells (6 mm in diameter) were cut off into agar and 50 µl of plant extracts were tested in a concentration of 100 mg/ml and incubated at 37ºC (bacterial strains) and at 25ºC (fungal strains) for 24-48 h. The assessment of antimicrobial activity was based on measurement of the diameter of the inhibition zone formed around the well.

RESULTS AND DISCUSSION

The present study was conducted to investigate antibacterial properties of *Opilia amentaceaea* Powdered samples of plant leaves were extracted in different solvents.

Phytochemical analysis

The plant material was subjected to soxhlation and the dried paste was weighed and dissolved appropriately in the original solvent used for extraction. This mode of reconstitution of the plant material allows for convenient dissolution of the material, without precipitation. The yields of extraction from originally used 15-20 g of material were around 2 g. Before proceeding with ethanol as the solvent of choice for soxhlation, different solvents by quickly taking 2g of the plant powder and grinding it with a mortar and pestle, which was then, filtered using a Whatman no.1 filter paper. The, the extracts were assessed qualitatively for phytochemical composition. Based on the abundance of the phytochemicals present (in terms of qualitative analysis), we decided that most of the phytochemical classes were found in the ethanolic extract. We used different solvents such as water, methanol, acetone, chloroform, ethylacetate and hexane (in the order of decreasing polarities).

In the phytochemical analysis different solvent extracts were used. Tannins, Saponins, Flavonoids, Catachins and Sugar were present in the *Opilia amentaceaea.* yielded positive results for Anthroquinones, Tannins, Saponins, Flavonoids, Catachins and Sugar. Anthroquinones were completely absent in tested solvents. The results were tabulated.

Among the different extracts used, we identified that acetone extract tested negative for all the phytochemical classes. Water extract of *O. amentaceae* tested positive for tannins, saponins, flavonoids and catachins; however, it did not contain sugars or anthraquinones. Among the several solvents used, only the EtOH extract contained almost all the classes of phytochemicals (save, tannins). EtOH often extracts tannins,

polyphenols, polyacetylenes, flavonols, terpenoids, sterols and alkaloids. In this case, we did not obtain tannins presumably because tannins may be entirely absent in the plant. The results obtained during phytochemical analyses were photographed (eg. colour change/effervescence) immediately (Figure 1, 2 and 3; Table 2&3).

Figure 1: Photograph of the various solvent extracts of A. latifolia obtained by grinding 5g powder with mortar and pestle

Table 2. Preliminary phytochemical analysis of *Opilia amentaceae* (Roxb.)

S.NO	*TEST*	*Distilled water*	*Ethanol*	*Methanol*	*Chloroform*	*Hexane*
1.	**Anthroquinone**	-	-	-	-	-
2.	**Tannins**	-	+	-	-	-
3.	**Saponins**	+	+	+	-	+
4.	**Flavonoids**	-	+	+	+	-
5.	**Catachins**	-	+	-	-	-
6.	**Sugar**	-	+	-	-	+

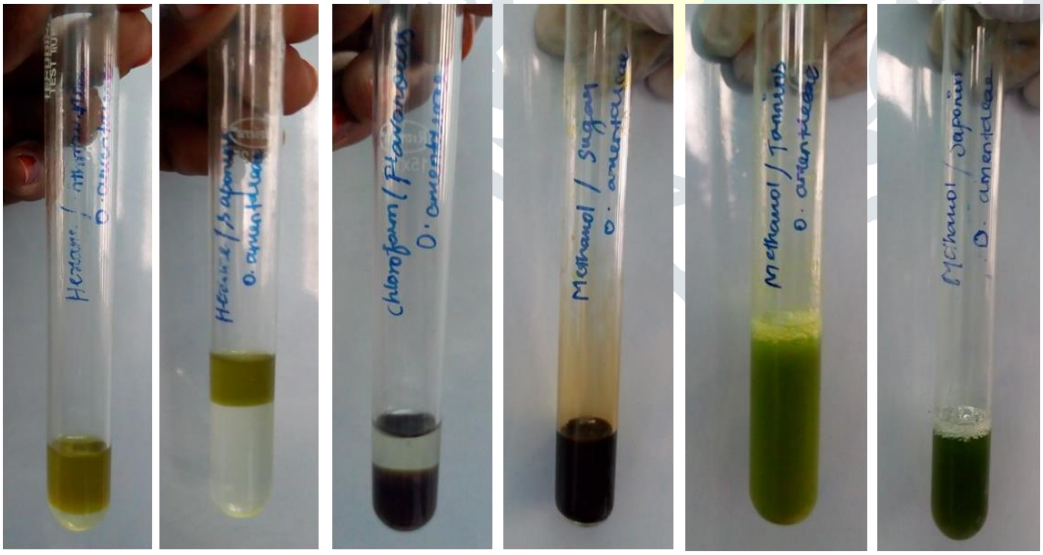

***Figure 2: Phytochemical analysis of Opilia amentaceae* (Roxb.).** *From left to right: Catechins,sugars, tannins, anthraquinones, flavonoids and saponin tests.*

Determination of antibacterial activity by agar well diffusion method

Antibacterial activity of ethanolic extract of *O.amentaceae* were tested against *E.coli, S.typhi* and *S.dysentriae.* Maximum zone of inhibition was observed in 0.2mg concentration against *E.coli* and *S.dysentriae. S.typhi* inhibited by 0.15mg concentration. Gentamicin, Bacitracin, Amoxyclav and Vancomycin disc were used as controls (Fig 4,5 and 6 and Table 4).

Figure 4: Well diffusion assay to measure the activity of the ethanolic extract of O. amentaceae against Escherichia coli ATCC25922.

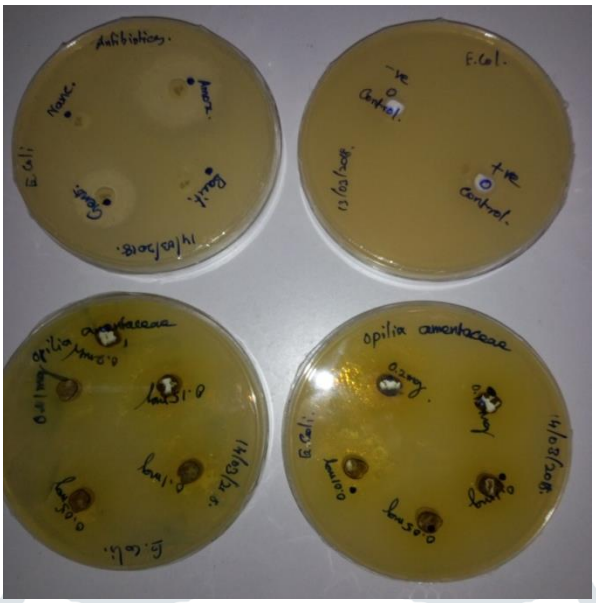

Figure 5 Well diffusion assay to measure the activity of the ethanolic extract of O. amentaceae against Shigella dysentriae.

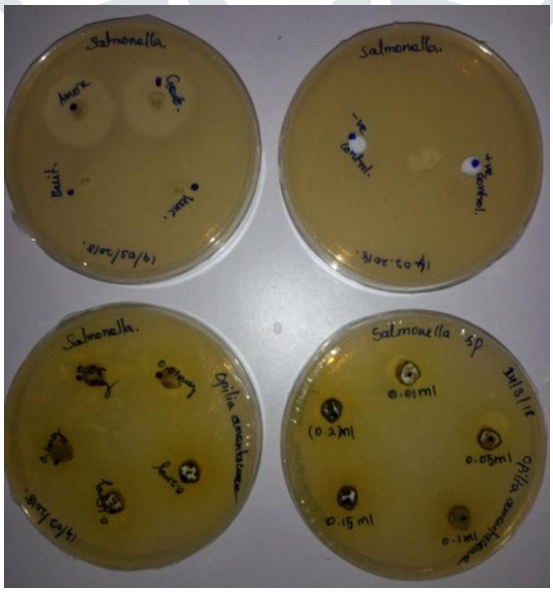

Figure 6: Well diffusion assay to measure the activity of the ethanolic extract of O. amentaceae against Salmonella typhi.

Table 4: Zones of inhibition for Ethanolic extract of *O. amentaceae.*

Organism	*0.01 mg*	*0.05 mg*	*0.1 mg*	*0.15 mg*	*0.2 mg*	*Gentamicin (10 µg)*	*Bacitracin (10 µg)*	*Amoxyclav (30 µg)*	*Vancomycin (10 µg)*
E. coli	0.0	0.0	0.0	0.3	0.5	2.0 cm	1.5 cm	2.7 cm	1.8 cm
S. typhi	0.1	0.3	0.4	0.7	0.6	2.9 cm	None	2.5 cm	None
S. dysentriae	0.0	0.0	0.0	0.4	0.5	2.5 cm	None	2.5 cm	None

The antibacterial effects of different concentrations of the Methanolic extract of *O. amentaceae*

The data for antibacterial activity which was acquired in duplicates has been plotted in the graph presented above. The most potent concentrations of the methanolic extract against the test organisms. For *E. coli*, the most potent concentration was 0.1 mg. For *S. dysentriae*, it was 0.15 mg. For *S. aureus*, it was 0.2 mg and for *S. typhi*, the most potent concentration was 0.15 mg (Fig 7).

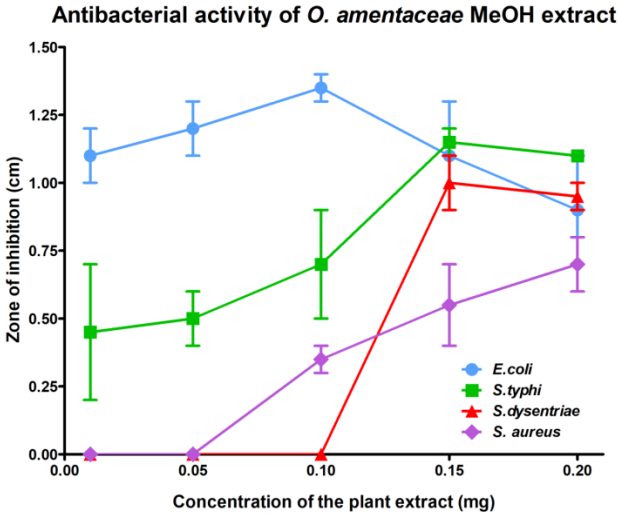

Figure 7: Summary of the antibacterial effects of different concentrations of the extract of O. amentaceae against clinically relevant human pathogens

DISCUSSION

In this study Phytochemical analysis of active extract demonstrated the presence of common phytoconstituents like tannins, glycosides, saponins, flavonoids and alkaloids. These are believed to be responsible for the observed antibacterial effects. Some studies have also attributed to their observed antimicrobial effect of plant extracts to the presence of these secondary plant metabolites (Nweze *et al.*, 2004). The presence of tannins suggests the ability of this plant to play a major role as anti diarrhoec and anti haemorrhagic agent (Price *et al.*, 1987). Presence of saponins revealed immense significance as anti hyper cholesterol, hypotensive and cardiac depressant properties (Asquith and Butler 1986). The presence of cardiac glycosides have been used for over two centuries as stimulants in cases of cardiac failure (Sood *et al.*, 2005).This perhaps justifies the already locally established function of the plant in the treatment and management of hypertension. The presence of these photochemical bases in *Opilia amentaceae* (Roxb.) accounts for its usefulness as a medicinal plant.The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds (Edeoga *et al.*, 2005). Secondary metabolites, which are loaded with diverse kinds of chemical compounds, may aid in either bacteriostasis or bactericidal killing of the pathogenic bacteria used as test species in our study.

In the present investigation *Opilia amentaceae* (Roxb.) inhibit the growth of variety of human pathogens due to the presence of Anthraquinones, Tannins, Saponins, Flavonoids, Catachins and sugar.

CONCLUSION

Further study of phyto chemicals present in these medicinal plants may reveal some new antibacterial compounds. The antibacterial activity and properties that support its use in folk medicine are also important future prospects. The plant extract needs to be characterized using GC-MS and the isolated compounds obtained through other methods such as preparative HPLC need to be used for antibacterial assays. With that information and with molecular docking and simulation studies (combined with drug target identification in microbes), we can obtain newer antimicrobial compounds that can rival antibiotics.

Herbal medicines are an essential and growing part of the international pharmacopeia. Knowledge of their medicinal properties is growing as a result of research and testing, which will make them an increasingly safe alternative or a preferred option to allopathic medicine. Today, there is a renewed interest in traditional medicine and an increasing demand for more drugs from plant sources. This revival of interest in plant-derived drugs is mainly due to the current widespread belief that “green medicine” is safe and more dependable than the costly synthetic drugs, many of which have adverse side effects. There is a growing interest in correlating phytochemical constituents of a plant with its pharmacological activity. Scientists have even started correlating the botanical properties of plants with their pharmacological activity. In future, more co-ordinated multidimensional research aimed at correlating botanical and phytochemical properties to specific pharmacological activities is expected. Thus, determining the biological activities of plants used in traditional medicine is helpful to the rural communities and informal settlements. Several studies are currently being undertaken to isolate the active compound(s) by bioassay-guided fractionation from the species that show high biological activity during screening. With the availability of primary information, further studies can be carried out like standardization of the extracts, identification and isolation of active principles and pharmacological studies of isolated compound. Therefore, these scientific investigations may be utilized to develop drugs for diseases. Further research is desired to isolate the compounds responsible for the observed biological activity.

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