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RESEARCH ARTICLE

STUDIES ON THE ANTIBACTERIAL ROLE OF SOME SELECTED PLANTS OCCURRING AT PURULIA DISTRICT OF WEST BENGAL, INDIA

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ABSTRACT

During the present study solvent extracts (water, ethanol, acetone, methanol and chloroform) of roots, bulb, aerial part, and leaves of various local plants viz. *Urginea indica* (Roxb.) Kunth. (Liliaceae), *Cotula anthemoides* L. (Asteraceae), *Curculigo orchioides* Gaertn. (Amaryllidaceae), *Annona squamosa* L. (Annonaceae) occurring at Purulia District of West Bengal were evaluated for phytochemical analysis and their antibacterial activity using agar well diffusion method. The significant results are obtained in case of *U.indica* and *C.anthemoides*. The phytochemical analysis revealed the presence of alkaloids, flavonoids, tannins, phlobatannins, terpenoids and saponins in the studied plant parts.

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INTRODUCTION

Medicinal plants are the richest bio-resources for the discovery of novel bioactive compounds. They are the gifts of nature to cure several diseases among human beings (Bushra *et al*., 2003). Their curative potentiality is due to the presence of a widearray of complex chemical compounds known as secondary metabolites *viz*., alkaloids, flavonoids, saponins and phenolic compounds present in different parts namely root, stem and leaf which are used for antimicrobial and therapeutic purposes (Jalalpure *et al*., 2004, Lewis 2006). There are several reports documenting the efficacy of plant extracts on microorganism by a number of researchers in different parts of the world (Bonjar *et al*., 2004 and Boer *et al*., 2005). Although many of the plant species have been tested for their antimicrobial efficacy still a vast majority has not been evaluated thus far (Tashikalange *et al*., 2005). It is, therefore, essential for the systematic evaluation and scientific validation of plants used in traditional medicine for several diseases. Hence, there is an urgent need to screen medicinal plants for exploring their promising biological activity. Herbal medicines are in great demand in the developed as well as developing countries due to their wide medicinal and biological applications. The healing potentiality of the plant continues to play significant role in the primary health care of about 80% of the world's population (Duraipandiyan *et al*., 2006). The uses of plant extracts as antimicrobials have become popular because the effective life span of antibiotic is limited and over prescription as well as misuse of antibiotics are causing antimicrobial resistance (Alam *et al*., 2009). The increased prevalence of multidrug resistance in pathogenic microorganisms and undesirable side effects of certain antibiotics have triggered immense interest in the search for new antimicrobial drugs of plant origin. Over the past twenty years, there has been a lot of interest in the investigation of natural materials as source of new antibacterial agents (Warner *et al*., 1999). Until natural Products have been approved as new antibacterial drugs, there is an urgent need to identify novel substances active towards highly resistant pathogens (Recio, 1989 and Alavijeh *et al*., 2012). According to WHO the traditional medicine are proven to be efficacious & safe and about 80 % of the world population is dependent on the traditional medicines and a major part of traditional therapies involves the use of plant extracts or their active constituents. Purulia is the good source of medicinal plants in west Bengal. About 85% of the rural Populations of Purulia depends on wild plants for the treatment of different diseases. In the present Study different plant extracts have been used to inhibit the growth of four pathogenic bacterial strains. Different solvents are used for this study to determine the effectiveness of plant

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extracts. From this study it is proved that out of 4 different plant extracts acetone extract of *Cotula anthemoides* root and ethanol extract of *Urginea indica* bulbis more effective against *Staphylococcus aureus*

MATERIALS AND METHODS

Selection of plants

Selection of plants based on ethno medicinal uses against different diseases in Purulia district,–four medicinal plants (Table 1) along with their scientific names, families, vernacular names and parts used for the treatment of different diseases have been presented in the Table 1 below. Plants-were identified with the help of authentic reference specimen, books, journals and floras (Bentham and Hooker 1862-1883, Prain 1903, Das *et al*., 2015) and documented in the herbarium of A.M. College, Jhalda.

Table 1. Plants used for the treatment of Curbuncle

S.No.	Scientific name	Family	Vernacular name	Parts used
1	*Cotula anthemoides*	Asteraceae	Pisainandi	Whole
2	*Urginea indica*	Liliaceae	Banpiyaj	Bulbs
3	*Curculigo orchioides*	Amaryllidaceae	Talmuli	Roots
4	*Annona squamosa*	Annonaceae	Atapata	Leaves

Collection of plant samples

Medicinal Plant samples were collected during rainy season, 2013 from Purulia district, West Bengal, India and it is available in any season of year.

Extraction

Plant material extraction process :

After collection of plant materials, they are cut in small pieces and they are dried under shade for 12 days. After drying infinally grinded in powder by grinder machine. Then thepowdered material of each plants was extracted with acetone, ethanol, petroleum benzene, chloroform, and distilled water using soxhlet Apparatus. About 10 grams of powder was loaded in soxhletextraction unit and exhaustively extracted using 100ml of solvents such as acetone, ethanol, petroleum benzene chloroform, and distilled water respectively at 60^0C for 18hours. Thereafter, it was filtered with the help of WhatmanNo.1 filter paper and used for various phytochemical and antibacterial tests.

Bacterial strain and culture conditions

Authentic pure cultures of pathogenic bacteria like *Escherichia coli (E.coli* MTCC 443), *Staphylococcus aureus (S. aureus* MTCC 3160) and *Salmonela typhi (S. typhi* MTCC 890) were provided by microbiological laboratory and clinical detection center Paschim Medinipur and *Bacillus subtilis* from Vidyasagar University Microbiology Department Paschim Medinipur, India. They were cultured in tryptone soybroth or agar (TSB or TSA) in aerobic condition at 37°C.

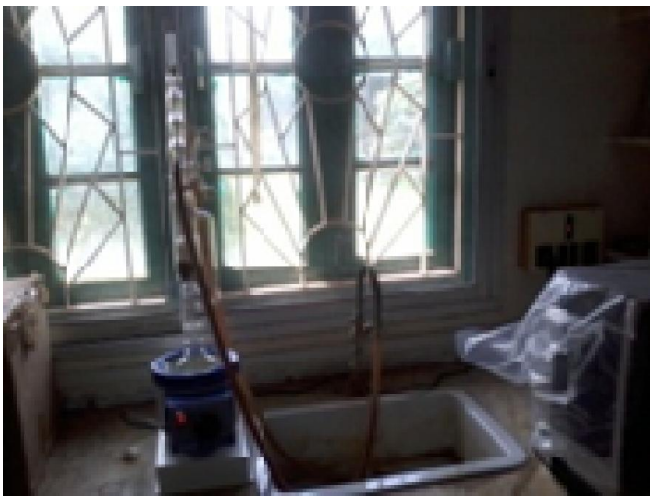

Fig. 1. Preparation of plant extract by using Soxhlet apparatus

Fig. 2. Different Plant Extracts

Antibacterial Activity Assay

Antibacterial assay was carried out by Agar well diffusion method. Fresh microbial cultures of 100µl was spread on Muller Hilton Agar plate with cotton swab. A well of 6mm diameter was punched off into agar medium with sterile cork borer and filled with 100µl of each (100µg/ml) ethanol, acetone, methanol, distilled water and chloroform extracts by using micropipette in each well in aseptic condition. The petriplates were then kept in a refrigerator to allow pre diffusion of extracts for 30 minutes and further incubated in an incubator at 37°c for 30 minutes for 24 hours extroverted position. The antibacterial screening was evaluated by measuring the zone of inhibition. An inhibition zone of 6mm or greater (incubating diameter of well) was considered antibacterial activity. The experiment was done in triplicate and the mean diameter of inhibition zone was calculated. The results showed that the remarkable inhibition of the bacterial growth was against the tested organism.

Phytochemical Screening

Phytochemical analysis of the test sample was carried out according to standard methods (Harbone 1998, Fransworth 996, Rangari 2002).

Test for Alkaloids

A fraction of extract was treated with 3-5 drops of Wagner's reagent [1.27 g of iodine and 2 g of potassium iodide in 100 ml of water] and observed for the formation of reddish brown precipitate (or coloration).

Tests for Carbohydrates

Few drops of Molisch's reagent were added to 2ml portion of the various extracts. This was followed by addition of 2ml of concentrated H_2SO4 down the side of the test tube. The mixture was then allowed to stand for two-three minutes. Formation of a red or dull violet color at the interphone of the two layers was a positive test.

Test for Flavonoids

2ml of extracts was treated with few drops of 20% sodiumhydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute hydrochloric acid, indicates the presence of flavonoids.

Test for Phenols

A fraction of the extracts was treated with aqueous 5% ferricchloride and observed for formation of deep blue or blackcolor.

Test for Phlobatannins

Deposition of a red precipitate when 2 ml of extract was boiled with 1ml of 1% aqueous hydrochloric acid was taken asevidence for the presence of phlobatannins.

Test for Saponins

To 2 ml of extract was added 6ml of water in a test tube. Themixture was shaken vigorously and observed for the formation of persistent foam that confirms the presence of saponins.

Test for Tannins

2mls of extract was treated with 10% alcoholic ferric chloride solution and observed for formation of blue or greenish colorsolution.

Test for Terpenoids

1ml of chloroform was added to 2ml of each extract followed by a few drops of concentrated sulphuric acid. A reddish brown precipitate produced immediately indicated the presence of terpenoids.

RESULTS

Preliminary phytochemical screening

The preliminary phytochemical analysis in different plant extracts showed the active compounds in presence of highconcentration, such as flavonoids, phenolic compounds, alkaloids, terpenoids. Also, the active compounds are absentor in low concentration, such as saponins, phlobatannins, alkaloids and carbohydrates shown in Table 2.

Antibacterial activity assay

The ethanol extracts of *Urginea indica* bulbs (IZ 15.50±0.25 mm) and acetone extract of *Cotula anthemoides* roots

Fig. 3. Picture showing inhibition zone using plant extract

Table 2. Phytochemical analysis of four different plant species by using different solvent extracts

S. No.	Plant species	Solevnt used	Phytochemicals							
			Alk	Car	Fla	Phe	Phlo	Sap	Tan	Ter
1	*Cotula anthemoides*	Chloroform	+	-	+	-	-	-	-	+
		Acetone	+	-	-	-	-	+	-	-
		Ethanol	+	+	+	+	-	+	+	+
		Methanol	-	+	+	-	-	+	+	+
		Water	-	+	-	-	-	+	+	-
2	*Urginea indica*	Chloroform	+	-	+	-	+	-	-	+
		Acetone	+	-	+	-	-	-	-	-
		Ethanol	-	+	-	+	-	+	+	+
		Methanol	-	+	+	-	+	+	+	+
		Water	-	+	-	-	-	+	+	+
3	*Annona squamosa*									
		Chloroform	+	-	+	-	-	-	-	+
		Acetone	-	-	+	+	-	-	-	+
		Ethanol	+	+	+	+	+	+	+	-
		Methanol	-	+	-	-	-	+	+	+
		Water	-	+	-	-	-	+	+	+
4	*Curculigo orchioides*	Chloroform	+	-	+	-	-	-	-	+
		Acetone	+	-	-	-	-	-	-	-
		Ethanol	+	+	+	+	+	+	+	+
		Methanol	-	+	+	-	-	-	+	-
		Water	-	+	-	-	-	+	+	+

Table 3. Antibacterial activity of plant extracts. Numbers indicate the mean diameters of inhibition of triplicate experiments ± standard deviation

S.No.	Plant species	Solevnt used	Diameter of inhibition zone(mm)			
			E coli	Saureus	Styphi	B subtilis
1	*Cotula anthemoides*	Chloroform	NM	8.5±.25	7±.5	-
		Acetone	9.5±.25	13.45±.20	12.5±.2	8.5±.5
		Ethanol	-	8.5±.5	11.5±.2	NM
		Methanol	10.5±.2	NM	8.5±.5	NM
		Water	7±.5	NM	9.5±.2	-
2	*Urginea indica*	Chloroform	-	NM	NM	NM
		Acetone	10±.2	8.5±.5	NM	11.5±.5
		Ethanol	NM	15.5±.25	9.5±.5	-
		Methanol	9.5±.7	NM	11.5±.2	-
		Water	-	7±.5	NM	NM
3	*Annona squamosa*	Chloroform	-	NM	NM	NM
		Acetone	7.5±.6	NM	-	NM
		Ethanol	7.5±.2	8.5±.5	-	NM
		Methanol	NM	-	-	-
		Water	NM	7±.2	NM	NM
4	*Curculigo orchioides*	Chloroform	-	-	-	-
		Acetone	-	-	8.5±.5	-
		Ethanol		7±.2	NM	-
		Methanol	7±.2	NM	NM	NM
		Water		7±.5	-	7±.2

'–' indicates no growthinhibition'.
'NM' indicates zone of inhibition not measured.

(IZ 13.45±0.20mm) were found to be more active against *S.aureus* compared to other solvent extracts

DISCUSSION

Medicinal plants were of great importance to the health of individuals and communities. Phytochemical analysis conducted on the plant extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities. Analysis of the plant extracts revealed the presence of phytochemicals, such as charbohydrates, phenols, tannins, flavonoids, saponins, phlobatannins, terpenoids and alkaloids. In this present study, preliminary phytochemical analysis revealed a large amount of flavonoids, phenolic compounds, terpenoids and cardiac glycosides present in different plant extracts. The results conclude that some of the extracts of the plant parts used in the present investigation showed significant antibacterial activities against disease causing pathogen. Thus ethanol extract of *Urginea indica* bulbs and acetone extract of *Cotula anthemoides* roots can be used in the treatment of different diseases caused by above mentioned pathogenic bacteria. Further phytochemical analysis and in vivo studies are necessary to corroborate the findings.

Conclusion

Phytochemicals present in different plant extracts indicates their potential as a source of principles that may supply novel medicines. Further studies are therefore suggested to ascertain their anti-microbial, antispasmodic and anti-helminthic activities. Furthermore, isolation purification and characterization of the phytochemicals found present will make interesting studies. The results of present investigation clearly indicate that the antibacterial activity vary with the plant parts used. The present investigation data on antibacterial potency of *U indica* bulbs and *C anthemoides* roots provide basis for synthesis of novel antibiotics. From our investigation of screening different plant parts, the result obtained confirm the therapeutic potency of plants used in traditional folkloric medicine and suggest that the plant extracts possess compound with antibacterial properties that can be used as antimicrobial agents in new drugs for the therapy of different diseases caused by *S aureus, E coli, S typhosa, B subtilis*. In addition these results form a good basis for selection of candidate plant parts for further phytochemical and pharmacological investigation. The most active extracts can be subjected to isolation of the therapeutic antimicrobials and undergo further pharmacological evaluation.

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