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Phytochemical screening and comparative analysis of antimicrobial activity of root and leaf extracts of *Tinospora coridifolia*, *Phyllanthus niruri* and *Abrus precatorious*, important medicinal plants

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Medicinal plants have been a basic source of antibiotics against a variety of diseases over the years. This study was carried out to investigate the antibacterial and antifungal activities of the ethanolic extracts of *Tinospora cordifolia* Miers. *Phyllanthus niruri* L. and *Abrus precatorious* L. against the pathogenic *Escherichia coli* and fungi *Aspergillus niger* and *Epidermophyton floccosum* by the filter paper disc method. Among the three plant extracts tested, *T. cordifolia* leaf extract showed maximum zone of inhibition of 24.0 mm against the bacteria *E. coli* at the concentration of 100 mg/ml. Extract of *P. niruri* exhibited similar inhibition zone against the bacterium, that is, 22.0 mm. *A. precatorius* leaf extract showed no inhibition. Increase in the concentration of plant extract increased the zone of inhibition. The root extract of *A. precatorius* showed significant inhibition against the fungi in comparison with other plant extracts. This study validates the use of tested medicinal plants in the treatment of various diseases.

Key words: Antibacterial and antifungal activities, ethanolic extraction, inhibition zone, *Tinospora cordifolia, Phyllanthus niruri, Abrus precatorius*.

INTRODUCTION

Many of the plant materials used in traditional medicine are readily available in rural areas at relatively cheaper rates than modern medicines (Mann et al., 2008). Medicinal plants represent a rich sources of secondary metabolites, many of which are antimicrobial agents (Mahesh and Satish, 2008). Medicinal plants have been used since a long time as a source of medicine to combat various ailments including infectious diseases. According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs (WHO, 1999). In developing countries, medicinal plants are used in traditional medicine. Such plants have been investigated for better understanding of their medicinal properties. Number of medicinal plants has been used for their antifungal and antibacterial properties (Adamu et al., 2005) and in the treatment of a wide range of infections (Mongalo et al., 2013). *Abrus precatorius* Linn. family Fabaceae is commonly known as Jequerity, crab's eye, Rosary pea. The plant is native to Indonesia and grows in tropical and subtropical areas of the world. This plant is

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introduced to India and distributed throughout India. A deciduous, wiry climber with tough branches, leaves abruptly pinnate with many pairs of leaflets, the rachis ending in a spine, the leaflets oblong rounded at both ends. Fruits are short pods containing hard shiny scarlet and black seeds. Their seeds are often used as beads and in percussion instruments. The seed contains the toxic poison abrin analogous to ricin. The seeds paste is applied locally against skin diseases. Leaves are used as substitutes for liquorice considered useful in biliousness and in leucoderma, itching and other skin diseases. Roots are used as diuretics and also in preparations prescribed for gonorrhea, jaundice and haemoglobinuea bile.

Tinospora cordifolia Miers. is one of the most versatile rejuvenative herbs belonging to the family Menispermaceae. It is also called as amrita or nectar of life, as it is extremely useful in strengthening the immune system of the body and keeping the functions of its various organs in harmony (Desai et al., 2002). The extract of the plant contains several bitter principles, glucosides, alkaloids, a glycoside-giloin, a non-glucoside-gilenin, gilosterol, alkakoid tinosporin, tinosporic acid, tinosporol, berberine, tinosporidine, sitosterol isolated, cordifol, heptacosanol, octacosonal and a new furanoid diterpene-tinosporide (Singh et al., 2003). The stem of *T. cordifolia* is used as an ingredient in Ayurvedic preparations used in general debility, dyspepsia, fevers and urinary diseases. The root is a powerful emetic and is used for treating visceral obstruction; its watery extract is used in leprosy. Administration of the polysaccharide fraction from *T. cordifolia* was found to be very effective in reducing the metastatic potential of B16F-10 melanoma cells (Leyon and Kuttan, 2004). *T. cordifolia* is reported to have immunostimulatory property (Mathew and Kuttan, 1999). It is considered as a general tonic in Ayurveda.

The positive effect of *T. cordifolia* on leucocytes suggests its use as an adjuvant in cancer therapy. Activation of macrophage by *T. cordifolia* leads to increased colony forming units, leading to leucocytosis and improvement in neutrophil function. It was found that the herbal mixture containing this plant was effective in treatment of advanced malignancies. In addition it helped in diseases like raktapitta, anaemia, cardiac debility, diabetes, sexual debility and spleen disorders (Mathew and Kuttan, 1999). *Phyllanthus niruri* is an herb commonly known as stonebreaker under family Euphorbiaceae. *P. niruri* contains a number of compounds such as phyllanthin, hypophyllanthin, lignansniranthin, nirtetralin, quercetin and phyltetralin (Amir et al., 2003). The whole plant and its aerial parts have been used for many traditional remedies, mostly for biliary and urinary tract ailments. It has also been proven effective in liver diseases like jaundice and liver cancer. The extract of the plant has been used for bacterial infections such as cystitis, prostatitis, and venereal diseases. It also assists in reducing anemia symptoms, diabetes and hypertension and showed diuretic, analgesic, stomachic, antispasmodic, febrifugal, and cell protective properties (Naik and Juvekar, 2003). The plant extract was found to decrease the amount of hepatitis B virus found in the blood stream (Venkateswaran et al., 1987). The plant extract have been reported to block DNA polymerase, the enzyme needed for the hepatitis B virus to reproduce (Rajeshkumar et al., 2002). The present investigation was carried out to screen root and leaf extracts of *A. precatorius*, *T. cordifolia* and *P. niruri* for antibacterial and antifungal activities.

MATERIALS AND METHODS

Plant and extraction

The plants of *A. precatorius*, *T. cordifolia* and *P. niruri* were collected in May, 2011 from the Botanical garden of Orissa University of Agriculture and Technology, College of Agriculture, Bhubaneswar. Fresh roots and leaves of the plants were washed and dried in shade. The dried leaves and root sample powdered (powdered plant material, 5 g) was loaded in the inner tube of the of Soxhlet apparatus and then fitted into a round bottom flask containing ethanol. The solvent was boiled gently (60 to 80°C) over a heating mantle using the adjustable rheostat. The extraction was continued until complete extraction was effected (24 to 30 h) and the solvent was removed at the reduced pressure with the help of rotary vacuum evaporator to yield a viscous dark green or brown residue.

Biochemical and phytochemical analysis

Both biochemical (carbohydrates, tannins, protein) and phytochemical (alkaloids, saponins) content in the three medicinal plants were carried out using standard methods (Bharathi et al., 2012; Patel et al., 2011). 100 mg of the crude extracts were taken and mixed with 1 ml methanol. From this stock, different concentrations were prepared, namely, 6, 10, 30, 50 and 100 mg/ml. These concentrations were used for antimicrobial test. Various solvents such as ethanol and petroleum ether have been used for extraction of secondary metabolites.

Growth and maintenance of microorganism

Bacterial cultures of *Escherichia coli* and fungal cultures of *Aspergillus niger* and *Epidermophyton floccosum* were collected from the central repository of Department of Microbiology, Orissa University of Agriculture and Technology, Bhubaneswar for conducting the experiment on antimicrobial activity. The bacterial cultures were maintained on nutrient broth (NB) at 37°C and fungi were maintained on Potato Dextrose Agar (PDA) at 28°C.

Preparation of standard antibiotic and antifungal solutions

Cefotaxime at various concentrations (100, 50, 10, and 1 mg/ml) (M.P Biomedical limited, Mumbai, India) and Bavistin (100, 50, 10, and 1 mg/ml) were used for bacteria and fungus, respectively.

Antibacterial susceptibility tests using disc diffusion method

Plant extracts were tested for antibacterial activity by the disc diffusion method according to National Committee for Clinical

Table 1. Phytochemical analysis of the plant extract derived from *T. coridifolia, P. niruri* and *A. precatorious*,

Plant constituents	Test/Reagent	Observation		
		A. precatorius	***P. niruri***	***T. cordifolia***
Alkaloid	Mayer's test	+ve	+ve	+ve
Carbohydrates	Molish test	+ve	+ve	+ve
Proteins	Burette and Millions test	+ve	+ve	+ve
Tannins	Ferric chloride and Lead acetate	+ve	+ve	+ve
Saponins	Foam and NaOH	-ve	+ve	+ve
Amino acids	Ninhydrine	+ve	+ve	+ve

+ Indicate present; – Indicate absent.

Laboratory Standard guidelines (NCCLS, 2001). A single colony of the organism (*E. coli*) was aseptically transferred with an inoculating loop to a 50 ml of fresh sterile saline broth in a test tube which was vortexed thoroughly and incubated overnight at 37°C. The growth of bacteria was measured through spectrophotometrically. About 100 µl of the inoculums was aseptically transferred to autoclaved petri dishes containing 15 ml agar broth medium and spread thoroughly using sterile glass spreader. The sterile paper discs (5 mm) were soaked in the leaf extract of different concentrations, namely 6, 10, 30, 50, and 100 mg/ml for 2 h and gently placed individually on the seeded nutrient agar plates. The plates were incubated at 37°C for overnight in an inverted position. Zones of inhibition were measured in millimeters, including sterile paper disc. Cefotaxime was used as standard antibiotic. Negative controls were performed using paper discs loaded with methanol. Each experiment was repeated thrice.

Antifungal susceptibility testing using disc diffusion method

The leaf extracts were tested against fungi, that is, *A. niger* and *E. floccosum* using the paper disc diffusion method. The PDA plates were seeded with the fungal spores and were allowed to solidify. The sterile paper discs (5 mm) were soaked in the leaf extract of different concentrations, namely, 6, 10, 30, 50, and 100 mg/ml for 2 h. The paper discs containing the extracts were placed at different areas on the surface of each plate. The plates were incubated at 28°C for 24 h. Bavistin was used as standard antifungal agent. A disc soaked in methanol was used as negative control. Antimicrobial activity of the extract against the tested fungi was indicated by growth-free "zone of inhibition" near the respective disc. Each experiment was repeated thrice.

Minimum inhibitory concentration (MIC) using microdilution assay

The MIC of the extracts was determined according to Elizabeth et al. (1999). A final concentration of 0.5% (v/v) Tween-20 was used to enhance crude extract solubility. A series of two fold dilution of each extract, ranging from 0.2 to 100 mg/ml was prepared. After sterilization, the medium was inoculated with 3 µl aliquots of culture containing approximately 105 Colony Forming Unit (CFU)/ml of each organism of 24 h slant culture in aseptic condition and transferred into sterile 6 inch diameter petri dishes and allowed to set at room temperature for about 10 min and then kept in a refrigerator for 30 min. After the media solidified, a number 3-cup borer (6 mm) diameter was properly sterilized by flaming and used to make three to five uniform cups/wells in each petri dish. A drop of molten nutrient agar was used to seal the base of each cup. Different plant crude extracts ranging from 0.2 to 100 mg/ml were added to the cups/wells of each petri dish and the control plates were maintained without plant extracts. Inhibition of organism growth in the plates containing crude extracts was judged by comparison with growth in blank control plates. The MICs were determined as the lowest concentration of extracts inhibiting visible growth of each organism on the agar plate.

RESULTS AND DISCUSSION

Many of the infectious diseases are still a major challenge to health issues all over the world. The emergence of resistance to antibiotics has further compounded the problem (Alli et al., 2011). The need for new antimicrobial compounds has become imperative. The ethonobotanical importance of the tested medicinal plants has been highlighted and it is used for various diseases. The results of antimicrobial activity of leaf and roots extract of the *T. coridifolia, P. niruri* and *A. precatorious* are shown in Tables 1 and 2. The different concentrations of crude extracts were tested against the pathogenic bacteria, namely, *E. coli,* and fungus *A. niger* and *E. flocussom* (Figures 1 and 2). It was observed that *T. cordifolia* leaf extract showed maximum zone of inhibition of 24.0 mm against the bacterium *E. coli* at the concentration of 100 mg/ml (Figure 1). *P. niruri* showed similar zone of inhibition, that is, 22.0 mm, whereas *A. precatorius* leaf extract showed no inhibition. Increase in the concentration of plant extract increased the zones of inhibition. Root extract of *A. precatorius* showed more antifungal activity (Figure 2). No antifungal activity was recorded in *T. cordifolia* extracts. Parekh and Chand (2005) reported that the leaf extracts of *A. precatorius* showed no antibacterial activity. Although the root extracts generally showed higher activity as compared to the leaf extracts (Darabpour et al., 2011). The extracts possessed broad spectrum of activity against both the bacteria and the fungus as earlier observed by Yisa (2009) and Latha et al. (2006). The leaf crude extract of *T. cordifolia* possessed very good antibacterial activity whereas leaf extract from *A. precatorius* showed significant antifungal property. Results for MIC values of

Table 2. Antimicrobial activity at different concentrations of plant extract.

Plant species	Plant part used	Concentration (mg/ml)	Zone of inhibition (mm)		
			E. coli	Fungus	
				A. niger	*E. floccosum*
A. precatorius	Root	0	NA	NA	NA
		6	6 mm	-	-
		10	7 mm	-	-
		30	11 mm	-	-
		50	12 mm	-	-
		100	15 mm	30 mm	45 mm
	Leaf	6	NA	NA	NA
		10	NA	NA	NA
		30	NA	NA	NA
		50	NA	NA	NA
		100	NA	NA	NA
P. niruri	Leaf	0	NA	NA	NA
		6	14 mm	NA	NA
		10	18 mm	NA	NA
		30	19 mm	NA	NA
		50	20 mm	NA	NA
		100	22 mm	NA	NA
T. cordifolia	Root	0	NA	NA	NA
		6	NA	NA	NA
		10	NA	NA	NA
		30	NA	NA	NA
		50	NA	NA	NA
		100	NA	NA	NA
	Leaf	0	NA	NA	NA
		10	15 mm	NA	NA
		20	20 mm	NA	NA
		30	21 mm	NA	NA
		50	22 mm	NA	NA
		100	24 mm	NA	NA

NA: No activity.

root and leaf extracts are presented in Table 2 and ranged from 6.0 to 100 mg/ml. Methanolic extracts of the roots of *A. pecatorius* exhibited low minimal inhibitory activity against *E. coli* than the leaf extracts of *T. cordifolia* and *P. niruri*. Luseba et al. (2011) reported the MIC of 90% methanolic extract of *Jatropha zeyheri* root at 0.63 mg/l against *E. coli* and 2.5 mg/ml against both *Staphylococcus aureus* and *Pseudomonas aeruginosa*. To our knowledge, there are no reports on the antibacterial and antifungal effects of the leaf and root extracts of *T. cordifolia*, *A. precatorius* and *P. niruri.*

Conclusions

The results of the present investigation showed that leaf and roots extract of *T. cordifolia*, *A. precatorius* and *P. niruri* possesses antibacterial activity against *E. coli*.

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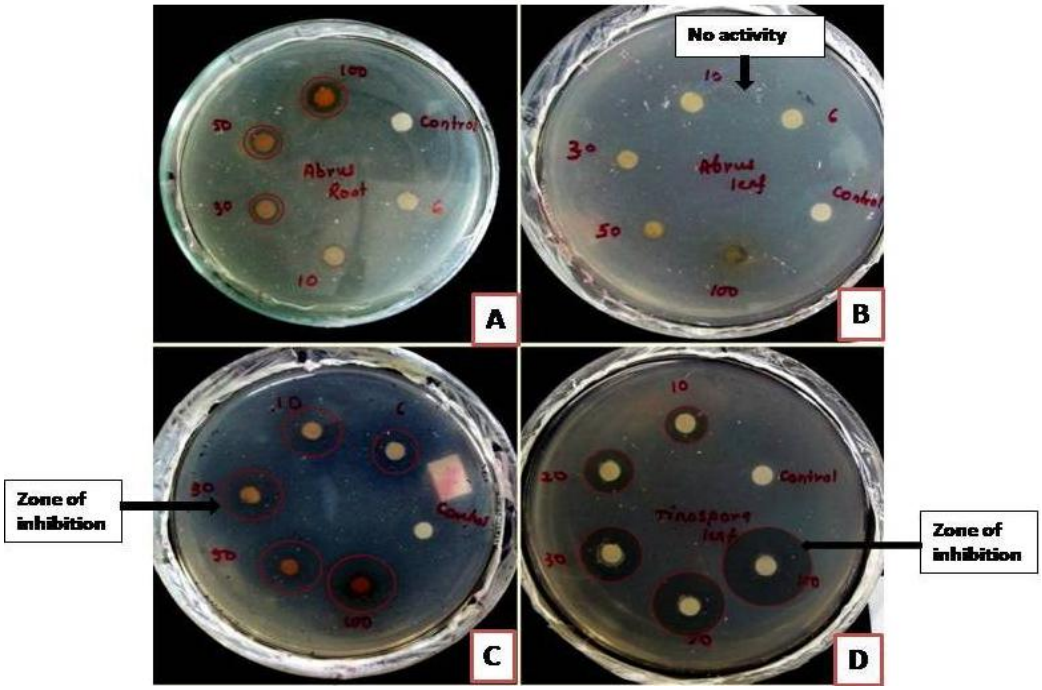

Figure 1. Antibacterial activity of plant extract for *E. coli*. (A) Root extract of *A. precatorius*, (B) leaf extract of *A. precatorius*, (C) leaf extract of *P. niruri*, (D) leaf extract of *T. cordifolia.*

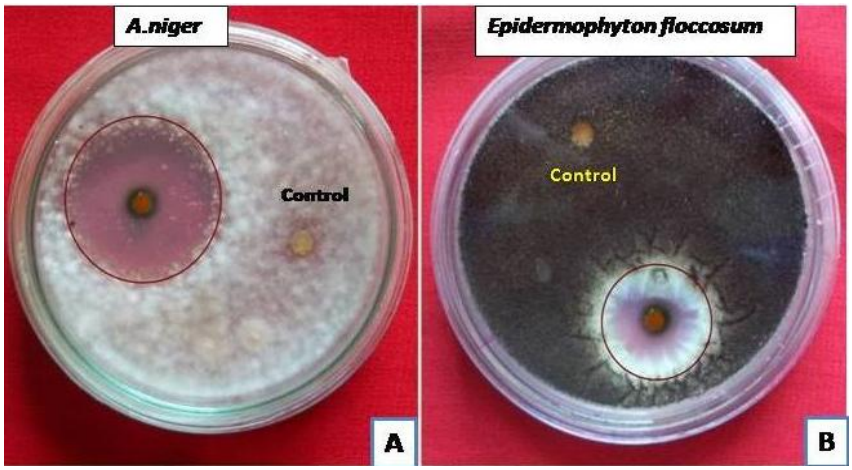

Figure 2. Antifungal activity of the root extract of *A. precatorius* (100 mg/ml). (A) *A. niger*, (B) *E. floccosum*.

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REFERENCES

Adamu HM, Abayeh OJ, Agho MO, Abdullahi AL, Uba A, Dukku HU, Kebede BM (2005). An ethnobotanical survey of Bauchi State herbal plants and their antimicrobial activity. J. Ethnopharmacol. 97(1):421-427.

Alli AI, Ehinmidu JO, Ibrahim YKE (2011). Preliminary phytochemical screening and antimicrobial activities of some medicinal plants used in Ebiraland. Bayero J. Pure Appl. Sci. 4:10-16.

Amir M, Kumar S, Singh SK (2003) Chemical and Biological Review Of *Phyllanthus Niruri.* India. J. Nat Prod. 19(4):3-13.

Bharathi V, Shanmuga PA, Janathul FS (2012). Antibacterial activity of stem extracts Of *Ocimum Basilicum*. J. Chem. Bio. Phy. Sci. 2(1):298-301.

Darabpour ED, Bavi AP, Motamedi H, Seyyed NSM (2011).

Antibacterial activity of different partsd of Peganum harmala L. Growing in Iran against multi-drug resistant bacteria. EXCLI J. 10:252-263.
Desai VR, Kamat JP, Sainis KB (2002). An Immunomodulator From *Tinospora Cordifolia* With Antioxidant Activity In Cell-Free Systems. Proc. Indian Acad. Sci. 114(6):713-719.
Elizabeth M, Adrien SJ, David WW (1999). Comparison of e-test and broth microdilution methods for antifungal drug susceptibility testing of molds J. Clin. Microbiol. 37(5):1480-1483.
Latha M, Ramkumar KM, Pari L, Damodaran PN, Rajeshkannam V, Suresh T (2006). Phytochemical and antimicrobial study of an antidiabetic plant: Scoparia dulcis. J. Med. Food. 9(3):391-399.
Leyon PV, Kuttan G (2004). Effect of *Tinospora Cordifolia* on the Cytokinin profile of angiogenesis-Induced Animals. Int. Immunopharmacol. 4(13):1569-1575.
Luseba D, Letsoalo ME, Katerere D (2011). A comparative study of antibacterial activities of wild and cultivated plants used in ethnoveterinary medicine. Afr. J. Biotechnol. 10(36):7058-7062.
Mahesh B, Satish S (2008). Antimicrobial activity of some important medicinal plant against plant and human pathogens. World J. Agric. Sci. 4:839-843.
Mann A, Banso A, Clifford LC (2008). An antifungal property of crude plant extracts from *Anogeissus Leiocarpus* and *Terminalia avicennioides*. Tanzania. J. Health Res. 10(1):34-38.
Mathew S, Kuttan G (1999). Immunomodulatory and antitumour activities of *Tinospora cordifolia*. Fitoterapia. 70(1):35-43.
Mongalo NI, Opoku AR, Zobolo AM (2013) Antibacterial activity of root and leaf extracts of *Jatropha zeyheri* Sond (Euporbiaceae). Afr. J. Biotechnol. 12(5):476-480.
Naik AD, Juvekar AR. (2003). Effects of alkaloidal Extract of *Phyllanthus Niruri* on HIV replication. Indian J. Med. Sci. 57(9):387-393.
NCCLS (2001). National Committee for Clinical Laboratory Standard guidelines. Performance standards for antimicrobial susceptibility testing: 11th informational supplement.
Parekh JD, Chanda S (2005). Efficacy of aqueous and methanolic extracts of some medicinal plants for potential antibacterial activity. Turk. J. Biol. 29:203-210.
Patel JP, Gami B, Patel K, Solanki R (2011). Antibacterial activity of methanolic and acetone extract of some medicinal plants used in Indian Folklore. Int. J. Phytomed. 3:261-269.
Rajeshkumar NV, Joy KL, Kuttan G, Ramsewak RS, Nair MG, Kuttan R (2002). Antitumour and anticarcinogenic activity of *Phyllanthus Amarus* Extract. J. Ethonopharmacol. 81(1):17-22.
Singh SS, Pandey SC, Srivastava S, Gupta VS, Patro T, Ghosh AC (2003) Chemistry and medicinal properties Of *Tinospora cordifolia* (Guduchi). India J. Pharmacol. 35:83-91.
Venkateswaran PS, Millman I, Blumberg BS (1987). Effects of an extract from *Phyllanthus Niruri* on Hepatitis B And Woodchuck Hepatitis Viruses: *In Vitro* and *In Vivo* studies. Proc. Natl. Acad. Sci. 84(1):274-278.
WHO (1999). WHO monographs on selected medicinal Plants. WHO,Geneva.
Yisa J (2009). Phytochemical analysis and antimicrobial activity of *Scoparia dulcis* and *Nympaea lotus*. Australian J. Basic Appl. Sci. 3(4):3975-3979.