http://dx.doi.org/10.4314/ajtcam.v10i3.20

ANTIMICROBIAL EFFICACY AND PHYTOCHEMICAL ANALYSIS OF *INDIGOFERA TRITA* LINN.

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Abstract

An *in vitro* antimicrobial activity and phytochemical analysis of various extracts of *Indigofera trita* L. *viz.* petroleum ether, chloroform, acetone, ethanol and aqueous extracts were carried out. A total of 21 microorganisms (19 bacteria and 2 fungal strains) were used for antimicrobial activity by disc diffusion method and a standard procedure was used to identify the phytochemical constituents. Petroleum ether extract showed moderate inhibitory activity against *Staphylococcus aureus* (14.40 mm), *S. epidermidis* (14.20 mm), *Salmonella paratyphi* A (12.80 mm), *Streptococcus mutans* (12.20 mm), *Escherichia coli*, *Proteus vulgaris*, *S. typhi* and *Burkholderia cepacia* (12.00 mm). The chloroform extract also showed antimicrobial activity against *S. epidermidis* (14.20 mm), *S. typhimurium* (12.60 mm), *S. paratyphi* A, *S. brunei* and *Yersinia enterocolitica* (12.00 mm). The acetone extract of *I. trita* showed considerable inhibitory activity against *S. epidermidis* (18.20 mm), *S. typhimurium* (14.60 mm), *S. infantis* (13.80 mm), *S. aureus* (13.40 mm), *Y. enterocolitica* (13.00 mm) and *Enterobacter aerogenes* (12.00 mm) were documented. Ethanol extract showed significant antimicrobial activity against *S. epidermidis* (18.60 mm), *S. paratyphi* A (14.60 mm), *Y. enterocolitica* (13.40 mm), *S. typhi* (12.40 mm), *S. aureus*, *E. aerogenes*, *S. typhimurium* and *S. infantis* (12.00 mm). Aqueous extract of *I. trita* considerably inhibited *S. epidermidis* (13.80 mm), *S. paratyphi* A and *Y. enterocolitica* (12.20 mm), *E. aerogenes* and *Haemophilus parahaemolyticus* (12.00 mm). All the five extracts showed a minimal antifungal activity when compared to antibacterial activity. The result revealed that the antimicrobial properties of *I. trita* might be associated with the presence of phenolic compounds, flavonoids, tannins, glycosides, saponins, phytosterols and alkaloids.

Key words: *Inidigofera trita*, Phytoconstituents, Antimicrobial activity, Antifungal activity, Disc diffusion method.

Introduction

Today's health care systems relay largely on plant based materials (Kumar et al., 2012). In India, Ayurvedic system of medicine has excited for over four thousand years and it was various parts of the plants that were used in siddha, ayurvedha and unani medicine for the diseases of human beings (Palaniswamy et al., 2010). Plants contain a large number of naturally occurring chemicals that have biological activity and also have a potential for producing new drug of great benefit to mankind (Dahikar et al., 2009; Parekh and Chandren, 2006). Modern medicine has evolved from folk medicines and traditional system only after through chemical and pharmaceutical screening (Boopathi and Sivakumar, 2011). In recent years, one of the more alarming trends in clinical microbiology has been the increasing incidence of resistance to antimicrobial agents among pathogens causing various diseases (Adwan et al., 2011). In addition, misuse of the antibiotics which can lead to the development of antibiotic resistance is also a major health concern (Al-Bari et al., 2007). Recently the application of traditional medicinal plants and their products as therapeutic agents has immensely increased throughout the globe (Vijayanand and Hemapriya, 2011). Medicinal plants have been important source of products in treating common infections and overcoming the problems of resistance and side effects of currently available antimicrobial agents (Hemaiswarya et al., 2008). Many plants have been employed because of their antimicrobial traits which are due to compounds synthesized during secondary metabolism and it was specifically targeted against the resistant microorganisms (Vijayanand and Hemapriya, 2011; Nostro et al., 2006). Moreover, the current costs of the chemotherapeutic agents are unaffordable to the public especially in developing countries. Therefore. attempts must be directed towards the development of effective natural, non-toxic drugs for treatment. Also their toxicity is very low when compared to the currently available commercial antibiotics (Sarala et al., 2010). However, several active principles of many medicinal plants have been isolated and introduced as valuable drugs in moderrn system of medicine. So, researchers are increasing and turning their attention to natural products looking for new leads to develop better drugs against microbial infection (Saravanan et al., 2011). The phytochemical research based on ethno-pharmacological information is generally considered to be an effective approach in the discovery of new anti infective agents from higher plants (Duraipandiyan et al., 2006).

Indigofera trita L. (Family: Fabaceae), an under-shrub, is widely distributed in India, Ceylon, South Africa and North Australia (Sanjappa, 1984). The plant is known as Kattuavuri and Punal murungai in Tamil Nadu. *Indiofera trita* has long been used by tribes and native medicinal practitioners to treat diseases such as rheumatism, arthritis, inflammation, tumor and liver

http://dx.doi.org/10.4314/ajtcam.v10i3.20

diseases (Nadkarni, 1996; Kirtikar and Basu, 1993). Literature review revealed that the plant *I. trita* is having antitumor (Senthilkumar et al., 2007), hepatoprotective, antioxidant, (Senthilkumar et al., 2008), anti-inflammatory and analgesic activities (Senthilkumar et al., 2009). Based on the above details, the present study is aimed to investigate the antimicrobial activity of various extracts of *I. trita* against a wide range of pathogenic microorganisms.

Materials and Methods

Collection of plant materials

The whole plant part of *I. trita* was collected from the foothill of Yercaud, Salem in the month of February 2008. The plant was then authenticated by Dr. G.V.S. Murthy, joint Director, Botanical Survey of India, Coimbatore, Tamil Nadu, India. (REF.BSI/SC/5/23/07-08/TECH-1384). A voucher specimen was preserved in our laboratory for future reference.

Extraction of Plant Materials

The plant materials were shade dried and pulverized. 250 g of powdered material was packed in Soxhlet apparatus and subjected to continuous hot percolation for 8 h using 450 ml of petroleum ether, chloroform, acetone, ethanol and water as solvent. All the extracts were concentrated under vacuum and dried in a dessicator.

Phytochemical Analysis

Various extracts of *I. trita* were analyzed for the phytochemical constituents *viz*., carbohydrates, glycosides, fixed oils, fats, proteins, amino acids, saponins, tannins, phytosterol, alkaloids, phenolic compounds, flavonoids, gum and mucilage using standard procedures described by Harbourne (1984), Hebert et al. (1984), Basset et al. (1985) and Kokate (1990).

Antimicrobial Screening
Source of microbial strains

Strains of human pathogenic microorganisms used in this study is as follows, three gram positive bacteria *Staphylococcus aureus* (MTCC 96), *Staphylococcus epidermidis* (MTCC 435) and *Streptococcus mutans* (MTCC 890); sixteen gram negative bacteria, *Escherichia coli* (MTCC 739), *Klebsiella pneumoniae* (MTCC 432), *Enterobacter aerogenes* (MTCC 111), *Proteus vulgaris* (MTCC 742), *Proteus mirabilis* (MTCC 425), *Salmonella typhi* (MTCC 733), *Salmonella paratyphi* A (MTCC 735), *Salmonella typhimurium* (MTCC 98), *Salmonella infantis* (MTCC 1167), *Salmonella enterica* (MTCC 660), *Salmonella brunei* (MTCC 1168), *Pseudomonas aeruginosa* (MTCC 424), *Burkholderia cepacia* (MTCC 1617), *Vibrio parahaemolyticus* (MTCC 451), *Haemophilus parahaemolyticus* (MTCC 1776) and *Yersinia enterocolitica* (MTCC 80); two fungus, *Candida albicans* (MTCC 183) and *Cryptococcus neoformans* (clinical isolate). The microorganisms were originally obtained from MICROBIAL TYPE CULTURE COLLECTION CENTRE (MTCC), INSTITUTE OF MICROBIAL TECHNOLOGY, CHANDIGARH, INDIA. Cultures were maintained as respective agar slants in screw-capped bottles and stored at 4˚C. All cultures were checked for viability and purity by regular plating.

Minimum Inhibitory Concentration (MIC) - Disc Diffusion Method (Determination of antimicrobial activity)

The antimicrobial activities of *I. trita* (petroleum ether, chloroform, acetone, ethanol and aqueous extract) were tested by disc diffusion method (Bauer- Kirby et al., 1966). The culture plates were prepared by pouring 20 ml of sterile Hi-sensitivity (HIMEDIA- M 486) agar medium. The depth of the medium was approximately 4 mm. Three to four similar colonies of pure cultures were inoculated with tryptone soy broth (HIMEDIA- M 323), further, it was incubated at 37˚C for 2-8 h and inoculum size was adjusted to yield uniform suspension containing 10^5-10^6 cells/ml (McFarland's standard). The agar surface of the plates was swabbed in three directions, turning the plates at 60˚ between each swabbing. Confluent growth is desirable for accurate results. The sterile discs (6 mm- HIMEDIA) were used for the loading plant extracts. Five different concentrations were prepared (250, 500, 750, 1000 and 1250 µg) and loaded in appropriate discs. The impregnated discs were incubated at 37˚C for an hour. The dried discs were placed over the surface of swabbed medium with equal distance to avoid the overlapping of the zones of inhibition. Then discs were pressed gently on the surface of the medium. The prepared plates were refrigerated for 30 min (Pre-diffusion time). The plates were incubated at 37˚C for 16-18 h during which the activity was evidenced by the presence of zones of inhibition surrounding the discs. Each experiment was done in triplicate. Specific standard antibiotic disc were used as control against each microbial strain.

Results

The extractive yield values of *I. trita* against different solvents were recorded in Table 1. Based on extractive yields, it

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Table 1: Data showing the extractive values of *Indigofera trita* L.

Plant name	Part used	Material of extraction	Yield in Percentage				
			Petroleum ether	Chloroform	Acetone	Ethanol	Aqueous
Indigofera trita Linn	Entire plant	Continuous hot percolation Method	2.2	2.43	3.34	3.9	4.8

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Table 2: Preliminary phytochemical Analysis results of various extracts of *Indigofera trita* L.

Name of the extracts	Carbohydrate	Glycosides	Fixed oils and Fats	Proteins and amino acids	Saponins	Tannins	Phytosterol	Alkaloids	Phenolic compounds	Flavonoids	Gums and mucilages
Petroleum ether	+	-	+	-	-	-	+	-	-	-	-
Chloroform	+	-	-	+	-	-	-	+	-	-	-
Acetone	+	+	-	+	+	+	-	-	+	+	-
Ethanol	+	+	-	+	+	+	-	+	+	+	-
Aqueous	+	+	-	+	+	+	-	-	+	+	-

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Table 3: Antimicrobial activity of *Indigofera trita* L. (petroleum ether, chloroform, acetone, ethanol and aqueous extracts)

Name of the organisms	Standard antibiotic mcg/disc	Zone in mm	Zone of inhibition in mm				
			Petroleum ether extract	Chloroform extract	Acetone extract	Ethanol extract	Aqueous extract
			Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
Staphylococcus aureus	Amoxycillin (30)	28	14.40 ± 1.16	11.00± 0.44	13.40± 0.67	12.00 ± 0.00	13.20 ± 0.96
Staphylococcus epidermidis	Cloxacillin (5)	28	14.20 ± 1.06	14.20± 0.80	18.20± 1.62	18.60 ± 0.87	13.80 ± 0.37
Streptococcus mutans	Amoxycillin (30)	28	12.20 ± 1.46	11.60 ± 0.24	10.40 ± 0.24	10.40 ± 0.67	11.00 ± 0.00
Escherichia coli	Gatifloxacin (5)	30	12.00 ± 0.00	11.20 ± 0.20	11.20 ± 0.37	11.60 ± 0.24	11.00 ± 0.31
Klebsiella pneumoniae	Gatifloxacin (5)	20	10.00 ± 0.00	10.8 0± 0.37	10.60 ± 0.40	11.40 ± 0.24	11.40 ± 0.24
Enterobacter aerogenes	Gatifloxacin (5)	22	11.00 ± 0.00	11.40 ± 0.40	12.00± 0.00	12.00 ± 0.00	12.00 ± 0.00
Proteus mirabilis	Levofloxacin (5)	19	11.20 ± 0.20	10.40 ± 0.24	10.40 ± 0.24	11.20 ± 0.37	10.20 ± 0.20
Proteus vulgaris	Levofloxacin (5)	21	12.00 ± 0.00	-	11.00 ± 0.44	09.40 ± 0.60	11.00 ± 0.31
Salmonella typhi	Chloramphenicol (30)	22	12.00± 0.00	11.40 ± 0.24	10.80 ± 0.37	12.40 ± 0.40	09.20 ± 0.48
Salmonella paratyphi A	Chloramphenicol (30)	23	12.80 ± 0.48	12.00 ± 0.00	15.40 ± 1.50	14.60 ± 0.60	12.20 ± 0.73
Salmonella typhimurium	Chloramphenicol (30)	24	11.60 ± 0.24	12.60 ± 0.24	14.60 ± 0.81	12.00 ± 0.00	11.20 ± 0.20
Salmonella infantis	Chloramphenicol (30)	19	11.2 0± 0.20	11.60 ± 0.24	13.80 ± 0.37	12.00 ± 0.00	11.20 ± 0.20
Salmonella enterica	Chloramphenicol (30)	20	10.20 ± 0.20	11.00 ± 0.00	12.00 ± 0.77	11.60 ± 0.24	11.00 ± 0.63
Salmonella brunei	Chloramphenicol (30)	21	11.80 ± 0.20	12.00 ± 0.00	10.80 ± 0.48	10.60 ± 0.40	08.20 ± 0.48
Pseudomonas aeruginosa	Amikacin (30)	20	11.00 ± 0.31	10.80 ± 0.37	10.80 ± 0.48	10.60 ± 0.24	10.60 ± 0.40
Burkholderia cepacia	Amikacin (30)	22	12.00 ± 0.00	10.80 ± 0.37	11.60 ±0.24	11.60 ± 0.24	10.80 ± 0.37
Vibrio parahaemolyticus	Tetracycline (30)	23	10.80 ± 0.20	10.00 ± 0.89	10.40 ± 0.24	08.80 ± 0.48	10.00 ± 0.63
Haemophilus parahaemolyticus	Tetracycline (30)	29	10.80 ± 0.37	10.80 ± 0.37	11.60 ± 0.24	11.60 ± 0.67	12.00 ± 0.31
Yersinia enterocolitica	Ticarcillin (75)	22	11.80 ± 0.20	12.00 ± 0.00	13.00 ± 0.44	13.40 ± 0.24	12.20 ± 0.20
Candida albicans	Nystatin (100)	20	11.00 ± 0.44	10.60 ± 0.24	10.80 ± 0.37	10.00 ± 0.00	10.60 ± 0.24
Cryptococcus neoformans	Ketoconazole (10)	21	10.20 ± 0.20	10.20 ± 0.24	10.60 ± 0.24	10.00 ± 0.00	10.40 ± 0.24

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was confirmed that the yield gradually increased depending on the polarity of solvents (petroleum ether - 2.2%, chloroform - 2.43%, acetone - 3.34%, ethanol - 3.9% and aqueous - 4.8%).

The screening of phytochemical analysis of *I. trita* revealed that the carbohydrates were present in all the extracts. Glycosides, saponins, tannins, phenolic compounds and flavonoids were present in high polarity solvents (acetone, ethanol and aqueous). Alkaloids were only present in chloroform and ethanolic extracts. Phytosterols, fixed oils and fats were found to be present only in petroleum ether extract. Proteins and amino acids were present in all extracts except petroleum ether and none of the extracts exhibited the presence of gums and mucilages (Table 2).

The antimicrobial activity of various extracts of *I. trita* (petroleum ether, chloroform, acetone, ethanol and aqueous) were determined against 21 microorganisms including two fungi (Table 3). Petroleum ether extract showed moderate antimicrobial activity against all tested organisms and the average zone of inhibition ranged from 10.20 ± 0.20 to 14.40 ± 1.16. *S. aureus* (14.40 mm) and *S. epidermidis* (14.20 mm) were inhibited considerably by petroleum ether extract. 20 microorganisms were moderately inhibited by chloroform extract, the mean zone of inhibition ranged from 10.20 ± 0.24 to 14.20 ± 0.80 and *S. epidermidis* alone considerably inhibited 14.20 mm. Acetone extract of *I. trita* was effective against all tested organisms, the mean zone of inhibition ranged from 10.40 ± 0.24 to 18.20 ± 1.62 and this extract showed considerable antimicrobial activity against *S. epidermidis* (18.20 mm), *S. paratyphi* A (15.40 mm), *S. typhimurium* (14.60 mm), *S. infantis* (13.80 mm) and *S. aureus* (13.40 mm). Ethanolic and aqueous extracts invariably inhibited all microorganisms. The ethanolic extract was effective against *S. epidermidis* (18.20 mm), *S. paratyphi* A (14.60 mm) and *Y. enterocolitica* (13.40 mm) and aqueous extract considerably inhibited *S. epidermidis* (13.20 mm). In overall assessment all the extracts were found to possess antimicrobial potentiality and it should be further established with isolated compounds.

Discussion

The potential of higher plants as source for many new drugs are still largely unexplored. The use of medicinal plants still plays a major role to establish the basic health needs in developing countries. Nearly 80% of the world population rely on traditional medicine for primary health care, most of which involves the use of natural products (Sandhya et al., 2006). Plant based antimicrobial compounds have enormous therapeutically potential value as they can serve the purpose without any side effects that are often associated with synthetic antimicrobials (Sukanya et al., 2009). Plants extracts are potential sources of novel antimicrobial compounds especially against microorganisms which is responsible for human infections (Joshi et al., 2011). The first step towards this aspect is the *in vitro* screening of antimicrobial activity and phytochemical analysis (Tona et al., 1998). Some of these observations have helped to identify the active principle which is associated with antimicrobial activities and in developing drug for the therapeutic use in human beings (Mahesh and Satish, 2008). In recent years, the antibiotics have lost their effectiveness due to the development of resistant strains of bacteria and which has mediated with resistant genes (Davies, 1994 and Service, 1995). The emergence of multidrug resistant strains is a serious threat and makes chemotherapy more difficult (Sarala et al., 2010). Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases (Berahou et al., 2007 and Salmao et al., 2008). The toxicity of new generation antibiotics discourages their use in treatment. Moreover, the current cost of most of the chemotherapeutic agents is unbearable to the society especially in developing countries like India (Sarala, 2010). Therefore attempts must be directed towards the development of effective natural, non-toxic drugs for treatment of human infections. Based on literature there was no extensive research report on antimicrobial activity of *I. trita*. A few reports only based on antitumor activity (Senthilkumar et al., 2007), antioxidant and hepatoprotective (Senthilkumar et al., 2008), anti-inflammatory and anti-analgesic (Senthilkumar et al., 2009) were reported. The present work is a pioneer attempt and has explored the antimicrobial properties *I. trita*. Preliminary phytochemical screening of *I. trita* consists of carbohydrates in all extracts, phenolic compounds, tannins, glycosides, saponins and flavonoids present in high polar solvents (acetone, ethanol and aqueous). Alkaloids were only present in chloroform and ethanolic extracts, phytosterol, fixed oils and fats were present in petroleum ether extract, protein and aminoacids were present in all extracts except petroleum ether. Gum and mucilage were not present in any of these extracts.

The antimicrobial activities of various extracts of *I. trita* revealed that all the extracts were effective against all tested microorganisms invariably. The petroleum ether extract inhibited all microorganisms moderately and effective against *S. aureus* and *S. epidermidis*, 20 microorganisms were moderately inhibited and effective against *S. epidermidis* with maximum zone of inhibition. Acetone extract showed good inhibitory effect against all tested organisms and highly effective with *S. epidermidis*, *S. paratyphi* A, *S. typhimurium*, *S. infantis* and *S. aureus*. Ethanolic and aqueous extracts also showed considerable antimicrobial activity, the ethanolic extract inhibited considerably *S. epidermidis*, *S. paratyphi* A and aqueous extract effective against *S. epidermidis*. In this present study antimicrobial properties explored with 21 microorganisms with five solvents and considerable results were also documented. A research report was similarly studied with *I. trita* L. F. SPP. *subulata* (vahl ex poir) by Vinoth et al. (2011) and ethanolic extract only showed moderate inhibitory activity against *P. aeruginosa* and *S. aureus*.

In overall assessments, the study result showed moderate inhibitory activity against almost all microorganisms and it is probably due to presence of phytochemicals in the respective extracts. Many substances may be antimicrobial, but only few of them will be potential therapeutic agents for the simple reasons that mammalian cells are more sensitive to chemical inhibitions than microbial cells (Sivakumar et al., 2006). The crude products obtained from cheaper sources are often associated with a large number of compounds that have discomforting abilities due to toxicity of drugs (Ramdas et al., 2006). Hence the herbal drugs have to be subjected to extensive toxicological and clinical tests to confirm the prescribed status. Thus the ethnobotanical

http://dx.doi.org/10.4314/ajtcam.v10i3.20

approach will be like a search for molecular diversity subjecting a wide variety of new molecules from plant sources and testing them with as many different tests as possible (Muhammed et al., 2005).

The present study has proven a spectrum of antimicrobial activities, which facilitates a support to some medicinal uses of a few medicinal plants. However further studies are certainly needed to isolate, characterize the phytoconstituents which will be accounted for the antimicrobial properties against human pathogens.

Acknowledgements

The authors are thankful to Prof. Dr. M. Karunanithi, Chairman and Secretary, Vivekanandha Educational Institutions, Elayampalayam, Tiruchengode, Namakkal District, Tamil Nadu for providing all the facilities for our research work.

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