Isolation and characterization of phytochemicals from the leaves of *Tridax procumbens L.* and *Tylophora indica* (Burm.f) Merill. as antibacterial on a few species of pathogenic bacteria

SYNOPSIS

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SYNOPSIS

Chapter	Contents	Contribution
1.0 INTRODUCTION	The Ethnobotanical records suggest that plants are the sleeping giants of pharmaceutical industry. Systematic screening of them may result in the discovery of novel effective compounds. The increasing prevalence of multidrug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raises the spectra of untreatable bacterial infections and adds urgency to the search for new infection fighting strategies. India throughout its long history, has accumulated a rich body of empirical knowledge of the use of medicinal plants for the treatment of various diseases.	Akinpelu and Onakoya 2006
1.1 Pharmocognasy	*Tridax procumbens* Linn. (*Tridax*) of the family Asteraceae (Compositae) commonly known as 'Ghamra' and in English popularly called 'coat buttons' because of appearance of flowers has been extensively used in Ayurvadic system of medicine for various ailments and is dispensed for "Bhringraj" by some of the practitioners of Ayurveda which is well known medicine for liver disorders. The plant is native of tropical America and naturalized in tropical Africa, Asia, Australia and India. It is a wild herb distributed throughout India having a length	

	of 12-24cm long with few simple leaves of 6-8cm long, with solitary peduncles and capitulum inflorescence. *Tylophora indica* (Burm. f.) Merill, commonly called Antamul or Indian ipecac, is an important medicinal plant belonging to the family Asclepiadaceae. It is a perennial, woody, climbing shrub and is found on plains, hilly slopes and the outskirts of the forests of eastern and southern India. The rhizomes are short about 3 to 4 mm thick, knotty with numerous fine roots. The leaves are egg-shaped, oblong to orbicular measuring 3-10 cm x 1.5 -7cm.Its stalk is 0.5-2 cm long. The flowers are few to many umbel like cyme. It is very popularly used for the treatment of asthma. In Ayurvedha, the plant has been used in treatment of asthma, dermatitis and rheumatism. The other reported activities include immunomodulatoy, anti-inflammatory, anticancer and antiamoebic.	Ganguli and Sainis, 2001 Gopalkishnan et al., 1980 Butani et al., 1987
1.2 Phytochemistry	The phytochemical screening of *Tridax procumbens* revealed the presence of alkaloids, carotenoids, flavonoids (catechins and flavones) and tannins. Leaf of *Tridax procumbens* mainly contains luteolin, glucoluteolin and quercetin.Also isoquercetin has been reported from its flowers. In addition fumaric acid, β-sitosterol and tannin have also been reported in the plant.	Salahdeen et al., 2004; Mahato&Chaudhary, 2005

1.3 Pharmacology	The phytochemical screening of this plant showed the presence of alkaloids, flavonoids and tannins. The pharmacological importance of *Tylophora indica* plant is mainly due to the presence of alkaloid such as tylophorine and tylophorenine. Besides, root contains a potential anti-tumor alkaloid tylophorinidine *Tridax procumbens* possesses significant antiinflammatory, hepatoprotective, wound healing, antidiabetic activity and antimicrobial activity against both gram positive and negative bacteria. The leaf extracts also have been used for bronchial catarrh, dysentery, diarrhoea and prevent falling of hair and promotes the growth of hair and as insect repellent. The roots and leaves of *Tylaphora indica* have long been used in the treatment of asthma, bronchitis, whooping cough, dysentery, rheumatic gouty pains and hydrophobia.	Mulchandani et al., 1971 Vilwanathan et al., 2005 Anonymous, 1976
2.0 REVIEW OF LITERATURE	The search for newer sources of antibiotics is a global challenge, preoccupying research institutions, pharmaceutical companies and academia, since many infectious agents are becoming resistant to synthetic drugs. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity. Plants have the	Latha and Kannabiran, 2006 Scazzocchio et. al., 2001 Van der Watt and Pretorius, 2001

2.1 ***Tridax procumbens***	major advantage of being the cheapest and most effective alternative sources of drugs. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug lead compound because of the unmatched availability of chemical diversity. Whole plant of *Tridax procumbens* has reported for its antimicrobial activity on various species of bacteria. Fresh plant juice is applied twice a day for 3-4 days to cure cuts and wounds. The extract of whole plant of *Tridax* showed antibacterial activity only against *Pseudomonas aeruginosa.* The disk diffusion method was used to test the antibacterial activity. Four strains of bacteria comprising two-gram positive *Bacillus subtilis, Staphylococus aureus* and two gram negative *Escherichia coli* and *Pseudomonas aeruginosa* were subjected to the above test. The ethylacetate fraction of the aerial parts of T.procumbens exhibited the highest phenolic content. Compounds SA-3 and SA-4 were isolated using silica gel CC from the ethyl acetate fraction. These compounds were identified as Kaempferol 3-0-a-c-rhamnopyranosyl-(316)-2-D-glycopyranoside and lupeol respectively	Cos et. al. 2006 Mahato and Chaudhary, 2005 Surendra et al., 2011

2.2 ***Tylophora indica***	*Tylophora indica* contains several active principles including phenanthroindolizidine alkaloids, tylophorine and tylophorenine. The roots and leaves of this plant have been used for the treatment of several illness including asthma, bronchitis, whooping cough, dysentery and diarrhoea. Various medicinal properties of the alkaloid tylophorine, tylophorinidine and tyloindicines, which is extracted from *T. indica*, had been well studied. Phytochemical screening of the methanolic leaf extract revealed the presence of alkaloids, flavonoids, tannins and saponins. Thin Layer Chromatography on the leaf extract showed the highest Retention Factor value. The antibacterial activity of ethylacetate and methanol extracts of *Tylaphora indica* was investigated by well-diffusion method against bacterial pathogens associated with HIV. The plant extracts showed better inhibitory activity against the tested organisms like *Pseudomonas, Klebsiella, Salmonella typhi* etc.	Gellert, 1982 Shivpuri et. al. 1972, Gellert, 1982 Bhutani et al.,1987 Ali et al.,2001 Bharathi Balasubramanian et al 2010

3.0 AIM AND OBJECTIVES

From the foregoing literature,it is apparent that the antibacterial properties of these plants have not been explored for their pharmaceutical implications. Further more the individual phytochemical constituents are tested for their antibacterial attributes.

3.1 Aim

- To isolate and characterize the phytochemicals of *Tridax procumbens* and *Tylophora indica* and to evaluate the antibacterial attributes.

3.2 Objectives

- To screen the phytochemicals of *Tridax procumbens* and *Tylophora indica* for their antimicrobial properties.
- To assay the bioactive phytochemicals of *Tridax procumbens* and *Tylophora indica* using TLC bioautography.
- To identify and characterize the antibacterial attributes of the phytochemicals obtained from the leaves of *Tridax procumbens* and *Tylophora indica*.
- To evaluate the efficacy of antibacterial compounds against the pathogenic bacteria such as *Escherichia coli, Proteus mirablis, Vibrio cholerae, Pseudomonas aeruginosa* and *Staphylococcus aureus* using disc diffusion, minimum inhibitory concentration and minimum bactericidal activity.
- To decipher the mode of action of

	phytochemicals as antibacterial in the light of leakage of membrane activity, enzymatic activity, membrane remodeling, DNA intercalating activity and DNA cleavage. ❖ To envisage and implicate the possibility of developing a novel antibacterial drug from these phytochemicals against the pathogenic bacteria.	
4.0 MATERIALS AND METHODS		
4.1 Pharmacognosy	4.1.1 Systematic validation and collection of Plant samples 4.1.2 Extract Preparation: 4.1.3 Standard Bacterial Smaples 4.1.4. Antibacterial activity of ten different medicinal plants tested against pathogenic bacteria. 4.1.5. Minimum inhibitory concentrations (MICs). 4.1.6. Minimum bactericidal concentrations (MBCs).	Bauer et al., 1996 Brantner and Grein, 1994 Hufford et al., 1975
4.2 Phytochemistry	4.2.1 Phytochemical analysis of the plant extract. 4.2.2. Different phytochemical extracts tested against pathogenic bacteria(alkaloid, terpenoid, flavonoid and glycosides)	Trease and Evans (1989) Harborne, 1998 Surya and John,2001 Macias et al,2002 Amal et al,2009

4.3 Pharmacology (in vitro studies)	4.2.3 TLC of plant extracts 4.2.4 TLC-bioautography 4.2.5 Compound Chracterization- General Experimental Methods 4.3.1 Assay about the effect of antibacterial compound on leakage of the membrane in pathogenic bacteria 4.3.2 Assay about the effect of antibacterial compound on enzymatic activity of respiratory chain dehydrogenases in pathogenic bacteria 4.3.3 Action of antibacterial compound on the membrane structure of pathogenic bacteria 4.3.4 Action of antibacterial compound on the membrane vesicles structure of pathogenic bacteria.	Gaw et. al. 2002; Srivastava et. al. 2004 Sawaya et al., 2004 Miller 1959; Bradford 1976 Iturriaga et al., 2001; Kim et al., 1994; Kim et al., 2009 SEM, Hitachi S-3000N Sapra et al. 2003
5.0 RESULTS **5.1 Pharmacognosy**	Methanolic leaf extracts of *Tridax procumbens* and *Tylophora indica* showed highest inhibition of both Gram positive and Gram negative bacteria. The antibacterial activity was determined by measuring the diameter of the zone of inhibition, i.e, the mean of triplicates + S.D of three replicates. Minimum inhibitory concentrations results revealed that the OD value was higher in the control because the bacteria caused turbidity. There was a gradual decrease in the optical density at higher dilution.	

5.2 Phytochemistry	Phytochemical screening of methonolic extract of *Tridax procumbens* and *Tylophora indica* showed the presence of alkaloids, terpenoids, flavonoids and glycosides. Different phytoconstituent extracts of the two plants tested against pathogenic bacteria revealed that terpenoid extract of *T. procumbens* and alkaloid extracts of *T. indica* have been found promising against pathogenic bacteria. The bioactive phytochemicals present in the extracts of *Tridax procumbens* (Rf 0.66) and *Tylophora indica* (Rf 0.72) were identified through bioautography of the TLC Plate. The compounds of *Tridax procumbens* with Rf value 0.66 and *Tylophora indica* with Rf value 0.72 were confirmed in view of IR , NMR and Mass spectrum as triterpenoids and alkaloids respectively.	
5.3 Pharmacology (in vitro studies)	Assaying on the phytochemistry disclosed the absence of reducing sugar and protein in the culture indicating no leakage of cell membrane in the control samples. However when the bacteria were treated with the compounds *Tridax procumbens* Rf 0.66 and *Tylophora indica* Rf 0.72, the presence of reducing sugar and protein were detected, which revealed the leakage of cell membrane.	

6.0 DISCUSSION

When the same compounds were tested on respiratory chain dehydrogenases of gram positive and negative bacteria showed, increased activity in bioactive compounds treated bacterial cultures and no change were observed in the negative control. Under SEM the higher concentrated compounds treated pathogenic bacteria showed more cell damage than the lower concentration of the compounds.

Similarly the compounds treated pathogenic bacteria showed break in the DNA by interfering with the coupling of DNA phosphodiester bond cleavage.

Among the ten selected medicinal plants (*Mukia maderaspatensis, Elephantopus scaber, Acalypha fruticosa, Justicia simplex, Lepidagathis cristata, Tridax procumbens, Rhinacanthus nasutus, Trichodesma* indicum, *Tylophora indica* and *Oldenlandia umbellate),* the methanolic leaf extracts of *Tridax procumbens* and *Tylophora indica* showed the highest inhibition on the pathogenic bacteria. Nevertheless, *Tylophora indica* was found to be more effective than *Tridax procumbens.*

	Phytochemical screening of methonolic extract of *Tridax procumbens and Tylophora indica* showed the presence of alkaloids, terpenoids, flavonoids and glycosides. The presence of terpenoids in *Tridax procumbens* falls in line with Muhammad Saiq Ali and Muhammad Jahangir, 2002. and it is contrasting to Thube Smita et al., 2009.	Muhammad Saiq Ali and Muhammad Jahangir, 2002 Thube Smita et al., 2009
	TLC of alcoholic extracts of *Tylophora indica* leaves showed the presence of maximum number of spots, confirming the presence of different classes of phytoconstituents as revealed by previous studies.	Mohammad et al., 2007
	Due to break through barrier of outer membrane permeability,it is confirmed that the antibacterial compounds ascertain the property of destroying the protein profile of the bacteria including the respiratory dehydrogenases. Holt and Bard (2005) found that silver nanoparticles inhibited respiration in *E.coli* by determining the respiratory quotient in the culture system.	Holt and Bard (2005)
	Furthermore the terpenoids of T.procumbens and alkaloids of T.indica interfere with the DNA system of pathogens studied as reported by Yuk-Ching, (2009). .	Yuk-Ching, (2009)

7.0 CONCLUSION AND SCOPE OF THE FUTURE WORK	*Tridax procumbens* (Rf 0.66) and *Tylophora indica* (Rf 0.72) showed antibacterial activity on both gram negative and positive bacteria such as, *Escherichia coli*, *Pseudomonas aeruginosa*, *Vibrio cholera*, *Proteus mirabilis*; and *Staphylococcus aureus.* The result is validated as it interferes with the molecular domain of the pathogen including phosphodiester bond of DNA. Thus the experimental research confirmed that the phytochemicals isolated have potential bactericidal activity. The futuristic perspective demands animal toxicity studies so that the chemo preventive nature can be ascertained on both gram positive and negative bacteria.	

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