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Ethnomedicinal and antimicrobial potential of *Tylophora asthamatica* Wight & Arn. with special reference to respiratory disorders

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ABSTRACT

The present study attempt to evaluate the antifungal, antibacterial activity and also to screen phytochemicals present in the *Tylophora asthamatica*. Disk diffusion method was used to study the antibacterial activity against *Streptococcus pneumoniae, Streptococcus pharyngitis, Corynebacterium diphtheriae, Pseudomonas aeruginosa, Mycobacterium tuberculosis, Bordetella pertussis* antifungal activity against *Candida albicans,* and *Aspergillus niger*. In this study, we observed that the samples in different solvent extract had antibacterial and antifungal activities by formation of inhibitory zone. Qualitative phytochemical analysis of samples in different solvent extract confirms the presence of various phytochemicals like Carbohydrates, Saponins, Tannins, Flavonoid, Alkaloids, Glycosides, Phytosterol & Steroids, Phenols, and Terpenoids. The solvent Petroleum ether, Chloroform and Ethyl acetate extract gave good results. In this study we concluded that different solvent extract of *Tylophora asthamatica* leaves showed good antibacterial activity and it may be attributed due to the presence of phytochemicals and may be used as antimicrobial agents.

Keywords: Antibacterial activity, antifungal activity, *Tylophora asthamatica*, zone of inhibition, Phytochemicals.

INTRODUCTION

Lifestyle of human population is changing fast in the present world. Man is continuously running for fulfilling his materialistic needs and forgets that he will only be able to enjoy when he is fit and healthy. Health will be affected if proper care is not taken. The common health disorder for anyone is respiratory disorder. Till we breathe we are alive, breathing in

disease free atmosphere is becoming hard due to change in lifestyle. To treat a disease properly you can opt two options a temporary one in which reoccurrence of disease can occur. The modern allopath tic drugs give initial good results but reoccurrence of disease is seen in many cases. The researchers are looking on plant drugs as permanent treatment due to their long lasting effect to treat ailments. The different parts of herbal medicinal plants, used to prepare the medicines for the treatment of urinary tract infections, piles, and jaundice (Ladda *et. al.* (2013). The traditional medicinal plants are identified, which parts are used to prepare the medicines or used by traditional practitioners for the treatment of urinary tract infections, piles, and jaundice (Ambhore *et. al*. 2013).

The plant drugs can be non-antibiotic drugs with potential antimicrobial properties that can create opportunities for innovative therapeutic approaches. The leaves of *Tylophora asthmatica* are used as the sourse of bioactive material (Bhavan, 1992). The present work is a step in this direction to study the ethnomedicinal and antimicrobial properties of *Tylophora asthamatica*. In Ayurveda, the plant has been used in the treatment of asthama. The alkaloids of *Tylophora asthmatica* in powder form, abot 400-500 miligram given once daily to asthmatic patients for six days to cure asthma (Shivpuri et al. 1968). *Tylophora asthamatic*a is a twinning perennial climber, roots many, fleshy, stem elongate, glabrous. Leaves elliptic-0blong, acute, often apiculate, glabrous, less pubescent beneath, base usually cordate, petiolate. Flowers large for the genus, in umbellate cymes, peduncle form between the petioles, pedicels filiform with hairy bract at base. Calyx hairy outside, divided to the base, segments lanceolate, acute. Corolla greenish yellow, oblong, acute, corona gibbous below, narrow apex, tapering to the base. Style apex not exserted beyond the anther tips. Follicle tapering to fine point at apex. Seeds broadly ovate.

MATERIALS AND METHODS

Plant material

The whole plant of *Tylophora asthamatica* was collected in fresh condition from the Melghat, Buldana District, Maharashtra, India. The plant was authenticated by well known taxonomist Dr. S. M. Bhuskute Principal, Bhavbhuti Mahavidyalaya, Amgaon, district Gondia, Maharashrra. Voucher specimen has been deposited at Bhartiya Mahavidyalaya, Amravati, Maharashtra. The tubers were washed under running water and dried under shade then ground into a fine powder using blender and stored in plastic bottle at room temperature.

Preparation of extracts

The extraction of soluble compounds from *Tylophora asthamatica* (Leaves) by the soxhlet method was performed using Petroleum ether, Benzene, Acetone, Chloroform and Ethyl acetate and Distilled water as solvents. 25 gm of dried powdered plant sample were taken in a Whatman No.1 filter paper cone and placed into soxhlet apparatus. 100 ml of above solvents were taken successively, in the round bottom flask attached to the soxhlet apparatus. A condenser was attached to this setup. Then the whole setup was placed on a heating mantle. The temperature was set in the range of 25-30^{0}C. solvents gets vaporized and rises up to the condenser where it condenses back into liquid. This liquid falls into the plant sample in the cone and extracts certain compounds and falls back into the round bottom flask. This process was continued till all the compounds get extracted from the plant. The extracts obtained from the above process was evaporated and stored in cap glass vials.

Phytochemical analysis

Preliminary qualitative phytochemical screening of samples was carried out with the following methods describe by Harborne (1973).

Test for Detection of carbohydrates

Molisch's Test: Small Quantity of Petroleum ether, Benzene, Acetone, Chloroform, Ethyl acetate, Distil water extract were taken separately in 10 ml of distil water and two drops of Ethanolic naphthol (20%) and 2ml of concentrated Sulphuric acid were added, formation of reddish violet ring at the junction indicates presence of carbohydrates.

Test for Detection of Saponins

Foam Test: 2 ml of Petroleum ether, Benzene, Acetone, Chloroform, Ethyl acetate, Distil water extract were taken and added two equal amount of distil water and shaken in a graduated cylinder for 15 minutes lengthwise. Formation of 1 cm layer of foam indicates the presence of saponins (Kumar *et al*., 2009).

Test for Detection of Tannins

Ferric Chloride Test: Small Quantity of Petroleum ether, Benzene, Acetone Chloroform, Ethyl acetate, Distil water extract were taken separately in water, 2-3 drops of 5% ferric chloride was added. Formation of black or green colour indicates the presence of tannins.

Test for Detection of Flavonoids

Sulphuric Acid Test: A fraction of extract was treated with concentrated sulphuric acid and observed for formation of orange colour.

Test for Detection of Alkaloids

Mayer's Test: To 2ml of plant extract, 2ml of concentrated hydrochloric acid was added then few drops of Mayer's reagent were added. Presence of green colour or white precipitate indicates the presence of alkaloids.

Test for Detection of Glycosides

Sulphuric Acid Test: To 2ml of plant extract, 1ml of glacial acetic acid and 5% ferric chloride was added then few drops of concentrated sulphuric acid were added. Presence of greenish blue colour indicates the presence of glycosides.

Test for Detection of Proteins and Amino acids

Ninhydrin Test: To 2ml of plant extract, few drops of 0.2% Ninhydrin was added and heated for five minutes. Formation of blue colour indicates presence of proteins.

Test for Detection of Steroids and phytosterols

Sulphuric Acid Test: To 1 ml of plant extract, equal volume of chloroform and few drops of concentrated sulphuric acid were added. Formation of brown ring indicates the presence of steroids and formation of bluish green colour indicates the presence of phytosterols.

Test for Detection of Phenols

Ferric Chloride Test: To1 ml of plant extract, 2ml of distilled water followed by few drops of 10% ferric chloride was added. Formation of blue or green colour indicates presence of phenols.

Test for Detection of Terpenoids

Salkowski test : 2ml of plant extract was mixed with 2ml of chloroform and 3ml of concentrated sulphuric acid was carefully added to form a layer. Reddish brown coloration of the interface is formed indicating the presence of terpenoids.

Antimicrobial Activity:

The role of medicinal plants is very important in treatment of various diseases in human being. Medicinal plants are the first home remedy that a person relies on for respiratory troubles. Ginger, clove and garlic are home remedies against respiratory disorders etc. Though antifungal and antibacterial drugs control infections but the pathogen become resistant to these allopathic drugs, second chance is the infection gets cured causing other complications and side effects. To prevent patient from drug resistant species and undesirable side effects plant medicine prove to be the best solution. Higher plants have shown to be potential source of new antimicrobial agent (Mitscer, 1987). Especially respiratory disorders are challenge before modern medicine, hence scientist are showing great interest for the discovery of new effective plant based drugs for treatment of these disorders.

Collection of Bacterial and Fungal Isolates

Bacterial isolates *Streptococcus pneumoniae, Streptococcus pharyngitis, Corynebacterium diphtheriae, Pseudomonas aeruginosa, Mycobacterium tuberculosis, Bordetella pertussis* the fungal isolates *Candida albicans,* and *Aspergillus niger* were obtained from Samruddhi Microbiology Diagnostic Laboratory, Amravati which is run by Dr. S. R. Gulhane (Microbiologist). The isolates were authenticated by biochemical tests as described by Cheesebrough (1985) preserved on potato dextrose agar and nutrient agar respectively and stored at 4^0C until ready to use.

Disc Diffusion Method

The bacterial isolates of *Streptococcus pneumoniae, Streptococcus pharyngitis, Corynebacterium diphtheriae, Pseudomonas aeruginosa, Mycobacterium tuberculosis, Bordetella pertussis* and fungal isolates of *Candida albicans* and *Aspergillus niger,* were subculture overnight at 37^0C on potato dextrose agar and nutrient agar plates respect-tively. Six plate per organism. The suspension of each bacterial and parasitic isolates were prepare as described by John *et al.* (1999) in isotonic sodium chloride solution. Solidified petridishes, for each microorganism for six solvents on Muller- Hinton agar were flooded with the appropriate suspension of bacterial isolates respectively.

Sterile 10 mm diameter absorbent filter papers disc (punched out from No.1 Whatman paper) were impregnated with respective solvents of plant extracts namely Petroleum ether, Benzene, Acetone, Chloroform, Ethyl acetate, Distil water extracts and placed on inoculated lawn. Six extracts from each plant parts in ten plants were tested for antimicrobial sensitivity. All the plates were kept for incubation period i.e. 24 hrs. for bacteria and fungi respectively at room temperature. Results were noted down in terms of sensitivity zone around the disc which is measured in millimeter (mm) and results were sequentially recorded in the tabular form.

RESULTS AND DISCUSSION

Table 1 represents various photochemical present in different extracts. The *Tylophora asthmatica* extract of leaves in petroleum ether shows presence of glycosides and terpenoids. While the extract in benzene shows the presence of Carbohydrates, alkaloids, terpenoids and phytosterols. Acetone extract depicts the presence of flavonoids, alkaloids, phytosterols and terpenoids. The presence of carbohydrates, saponins, flavonoids, alkaloids and phytosterols was determined in chloroform extract.

Table - 1 (Preliminary phytochemical screening of *Tylophora asthmatica* Wight & Arn.)

S. No.	Secondary metabolite	Petroleum ether	Benzene	Acetone	Chloroform	Ethyl acetate	Distilled water
1	Carbohydrates	---	+	---	+	+	++
2	Saponins	---	--	---	+	---	+
3	Tannins	---	---	---	---	---	+
4	Flavonoids	---	---	++	++	+	++
5	Alkaloids (Mayer's test)	---	+++	++	++	---	++
6	Glycosides	+	---	---	---	---	+
7	Proteins	---	---	---	---	---	---
8	Phytosterol & Steroids	---	++	+	+	---	+++
9	Phenols	---	---	---	---	---	---
10	Terpenoids	+++	+	+	++	+++	++

Table -2 (Antimicrobial activity of *Tylophora asthmatica* Wight & Arn.)

Microoganism	Petroleum ether	Benzene	Acetone	Chloroform	Ethyl acetate	Distilled water
Streptococcus pneumoniae	00	00	00	00	00	00
Streptococcus pharyngitis	00	00	00	00	00	00
Corynebacterium diphtheriae	00	00	00	00	16	00
Pseudomonas aeruginosa	00	00	00	00	00	00
Mycobacterium tuberculosis	00	00	00	00	00	00
Bordetella pertussis	00	00	00	00	00	00
Candida albicans	12	00	00	14	00	00

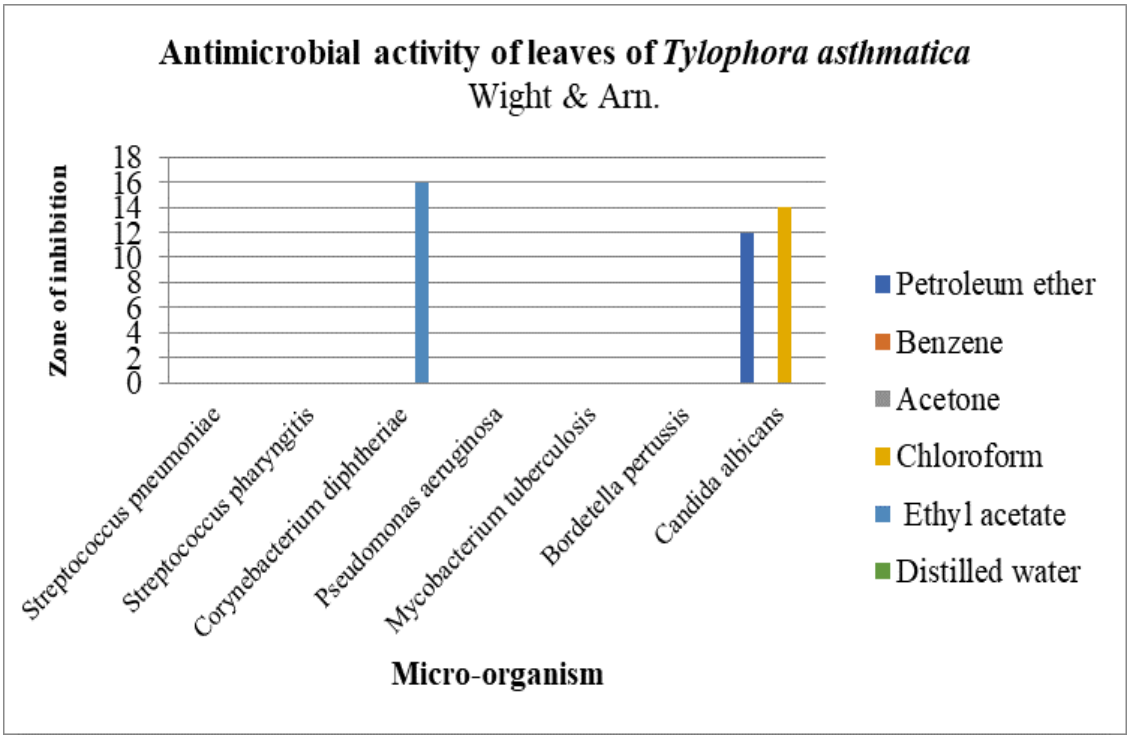

Fig. 1 Analysis of antimicrobial sensitivity of *Tylophora asthmatica* Wight & Arn.

The ethyl acetate shows the presence of carbohydrates, flavonoids and terpenoids. Significant amount of carbohydrates, saponins, tannins, flavonoids, alkaloids, glycosides, phytosterols and terpenoids were present in aqueous extract of *Tylophora asthmatica* leaves.

The petroleum ether extract showed inhibitory activity against *Candida albicans* with a zone of inhibition of 12mm. The extract in chloroform showed positive result against *Candida albicans* with a maximum zone of inhibition of 14mm. The ethyl acetate extract showed maximum inhibitory activity against *Corynebacterium diphtheriae* with a zone of inhibition of 16mm. The flavonoids, alkaloids and phytosterols extracted in moderate amount in maximum solvent. Terpenoids extracted in all solvent in abundant amount. Terpens have found to inhibit growth of micro-organism concentration (Gupta *et al*.2011). The extract in benzene, acetone and distilled water did not show any antimicrobial activity.

CONCLUSION

Based on the result obtained in this study, it is clear that the preliminary phytochemical analysis of *Tylophora asthmatica* shows presence of rich amount of metabolites like Carbohydrates, Saponins, Tannins, Flavonoid, Alkaloids, Glycosides, Phytosterol & Steroids, Phenols, and Terpenoids. Extract of *Tylophora asthmatica* in petroleum ether, chloroform and ethyl acetate showed good antibacterial and antifungal activity and it may be attributed due to the presence of phytochemicals and may be used as antimicrobial agents.

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Conflicts of interest: The authors stated that no conflicts of interest.

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