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Original Article

Phytochemical screening and in vitro antimicrobial activity of *Typha angustifolia* Linn leaves extract against pathogenic gram negative micro organisms

Ramesh L. Londonkar, Umesh Madire Kattegouga, Kirankumar Shivsharanappa, Jayashree V. Hanchinalmath*

Department of P.G. Studies and Research in BioTechnology, Gulbarga University, Jnana Ganga Campus, Sedam Road, Gulbarga 585106, Karnataka, India

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ABSTRACT

Background: *Typha* is a monocot genus of the monotypic family Typhaceae with about 12 species distributed in the tropical and temperate regions of the world in marshes and wetlands of varied depth. It a common plant of wetlands, is an unexploited taxon which can be used as a good source of food, medicines, and, fibres.

Methods: In the present study phytochemical evaluation and antimicrobial potency of *Typha angustifolia* has been investigated against 4 g negative standard reference bacterial strains i.e., *Enterobacter aerogenes* (MTCC111), *Salmonella typhimurium* (MTCC98), *Klebsiella pneumonia* (MTCC 109), *Pseudomonas aeruginosa* (MTCC 424) and *Escherichia* coli (clinical isolates). The antibacterial activity was determined by agar well diffusion method and broth dilution methods were used to determine the minimum inhibitory concentration (MIC) of the above strains.

Results: It is estimated that total ash value in leaves is 10.83%, acid insoluble ash and water soluble ash shows the value 4.66% and 3.16% respectively. Preliminary phytochemical screening of the aerial part of the *T. angustifolia* reveals the presence of different secondary metabolites in these extracts. The diameter of the zones of inhibition of the extracts ranged from 13 to 17 mm for *E. aerogenes*, 0–19 for *S. typhimurium*, 12–16 for *K. pneumoniae*, 9-19 for *P. aeruginosa* and 9–20 mm for *E coli*. The minimum inhibitory concentration (MIC) of the different extracts ranged from 0.78 mg/ml to 12.5 mg/ml.

Conclusion: The results obtained with methanol extract was particularly significant as it strongly inhibited the growth of *S. typhimurium*, *P. aeruginosa* and *E. coli*. The overall results of this study provided informative data for the use of *T. angustifolia* for the treatment of infections associated with the studied micro organisms.

* *Corresponding author*. Tel.: +91 (0) 9880416391.
E-mail addresses: londonkarramesh53@rediffmail.com, proframeshlondonkar@gmail.com (R.L. Londonkar).

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1. Introduction

Nature has been a source of medicinal agents since times immemorial. Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic value.[1] It is estimated that there are about 250,000–500,000 species of plants are existing on Earth.[2] The traditional medicine still plays an important role in the primary health care in India. Approximately 60–80% of the world's population were relies on traditional medicines for the treatment of common illnesses.[3] Medicinal plants contain large varieties of chemical substances which contain value added therapeutic properties that can be utilized in the treatment of human diseases. The studies of medicinal plants used in folklore remedies have attracted the attention of many scientists in finding solutions to the problems of multiple antibiotics resistances organisms. Most of the synthetic antibiotics now available in the market have major setback due to the multiple resistance developed by pathogenic micro organisms against these drugs. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions. In present situation the development of microbial resistance to antibiotics has lead the researchers to investigate the alternative source for treatment of resistant strains.[4] Thus, there is a need for search of new and more potent antimicrobial compounds of natural origin to combat the activities of these pathogens which is the basis for this study.

Typha angustifolia are herbaceous, colonial, rhizomatous, perennial plant with long, slender, green stalks topped with brown, fluffy, sausage-shaped flowering heads. It is a perennial growing up to 3 m (9ft) often forming extensive colonies along shores of shallow ponds, lakes and marshes. The results of Varpe SS reveals that the aqueous and 70% methanol extracts of *T. angustifolia* pollen grains exhibits anti-inflammatory activity.[5] In the present situation it has been proposed that *Typha* could be utilized as a biomass crop for renewable energy.[6] The present study aimed to screen for phytochemicals and to evaluate antibacterial activity of crude extracts of *T. angustifolia* leaf and to find out minimum inhibitory concentration (MIC) of different extracts against Garm negative bacteria.

2. Materials and methods

2.1. Collection of plant materials and extraction

Aerial part (leaves) of *T .angustifolia* was collected in and around the Gulbarga, Karnataka, India in the month of January 2012 and the plant was duly identified and authenticated in the Herbarium of the Department of Post Graduates Studies and Research in Botany, Gulbarga University, Gulbarga, Karnataka, India.

The collected leaves were washed with running tap water and allowed to air dry. The plant materials were dried in shade for two to four weeks. Precaution was taken to avoid direct sun light otherwise it will destroy the active compounds of plant leaves. After drying, the plant leaves were grinded finely and stored in airtight container. The air dried leaf powders (50 g) were successively extracted by soxhlet extraction with solvents of increasing polarity i.e., petroleum ether (60–80 °C), chloroform, methanol and distilled water. The extracts were dried and stored in a sterile container for further use.

2.2. Physicochemical analysis

The finely powdered leaves of *T. angustifolia* Linn was subjected to various physicochemical studies for determination of ash value like total ash, acid insoluble ash and water soluble ash.[7] Extractive values like water soluble, methanol soluble, chloroform soluble and petroleum ether soluble were determined.

2.3. Phytochemical screening of crude extracts

The phytochemical components of the *T. angustifolia* leaves were screened for using the standard method described by Harbone.[8] The components analyzed are alkaloids, proteins, glycosides tannin, steroids, phenol, saponins, flavonoids, carbohydrates, oils and fats.

2.4. Source of micro organisms

The micro organisms used for testing were *Enterobacter aerogenes* (MTCC111), *Salmonella typhimurium* (MTCC 98), *Klebsiella pneumonia* (MTCC 109), *Pseudomonas aeruginosa*(MTCC 424), *Escherichia coli* (Clinical strain). The above organisms were obtained from the department of Microbiology and Biotechnology, Gulbarga University, Gulbarga, Karnataka, India.

2.5. Standardization of micro organisms

200 μl of overnight cultures of each micro organisms was dispensed into 20 ml of sterilized nutrient broth and incubated at 37 °C for 4–6 h to standardize the culture to 10^6 CFU/ml. A loopful of the standard cultures was used for the antimicrobial assay.[9]

2.6. Screening for antibacterial activity (agar well diffusion assay)

In vitro antibacterial activities of all different extracts of *T. angustifolia* were determined by standard agar well diffusion assay.[10] Muller–Hinton Agar (MHA) plates were seeded with 18 h old culture of the isolates. Different extracts were dissolved in 1% Tween 80 in deionized water and made the final concentration of 50 mg/ml, from this 50 μl of different extracts were added into the sterile 6 mm diameter well. 1% Tween 80 and sterilized distilled water were used as negative controls while chloramphenicol antibiotic disc (30 mcg, Hi-Media) was used as positive control. A loopful each of the standardized culture of test organisms was streaked on the solidified medium and incubated for 24 h at 37 °C. Antibacterial activity was assayed by measuring the diameter of the zone of inhibition formed around the well using standard (Hi-Media) scale. The experiment done in triplicate and the average values were calculated for antibacterial activity.

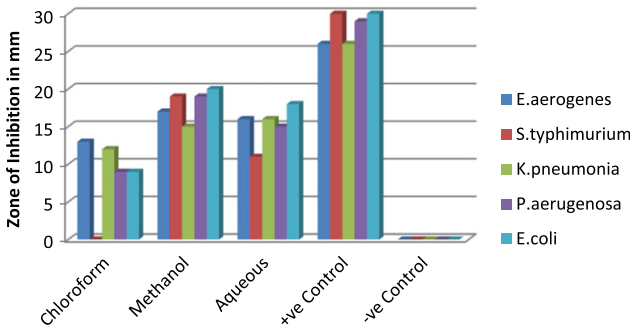

Fig. 1 – Effect of different extracts of *Typha angustifolia* leaves on five different gram negative bacterial strains. +ve Control represents the chloramphenicol antibiotic disc (30 mcg, Hi-Media). +ve Control represents 1% tween 80 in sterilized distilled water. The methanol and aqueous extract of *T angustifolia* shows the maximum zone of inhibition compared to chloroform extract.

2.7. *Determination of minimum inhibitory concentration*

Minimum inhibitory concentration (MIC) was defined as the lowest concentration where no visible turbidity was observed in the test tubes. The concentrations were determined by the method described by Vollekova[11] with minor modification was employed. The MIC was determined for the micro organisms that showed maximum sensitivity to the test extracts. In this method the broth dilution technique was used, where the leaf extract was prepared to the highest concentration of 25 mg/ml (stock concentration). By adding sterile distilled water serially diluted (two fold dilutions) using the nutrient broth and it is later inoculated with 0.2 ml standardized suspension of the test organisms. After 18 h of incubation at 37 °C, the test tubes were observed for turbidity .The lowest concentration of the tube that did not show any visible growth can be considered as the minimum inhibitory concentration.

3. Results

It is estimated that total ash value in leaves is 10.83%, acid insoluble ash and water soluble ash shows the value 4.66% and 3.16% respectively. The extractive value of methanol is more followed by aqueous, chloroform and petroleum ether with 20.12%, 6.98%, 4.36% and 2.14% respectively. Phytochemical screening of crude extracts of the aerial part of the *T. angustifolia* reveals the presence of alkaloids, tannin, steroids, phenol, saponins, flavonoids in aqueous and methanolic extracts where as carbohydrates, tannins, oils and fats were present in Petroleum ether and chloroform extract. In addition to this chloroform extract also contains flavonoids and phenols.

The antimicrobial activity of different extracts against the test organisms with varying zones of inhibition ranging from 09 to 20 mm (Fig. 1) has revealed the antimicrobial potency of this plant. Methanolic extract showed highest zone of inhibition against *E. coli (20 mm)* followed by *P aeruginosa, S typhimurium, E aerogenes and K pneumonia*. The aqueous extract showed greater potential against *E. coli* > *E. aerogenes* > *P. aeruginosa* > *K. pneumonia* > *S. typhimurium*. Chloroform extract shows moderate inhibitory effect on these organisms. The result of MIC assay is shown in Table 1 Methanol extract of *T. angustifolia* exhibited the highest antibacterial efficacy against *E. coli* at 0.78 mg/ml and least efficacy was shown by chloroform against *S. typhimurium, P. aeruginosa and E. coli* which was inhibited at 12.5 mg/ml concentration.

4. Discussion

Plants are important source of potentially bioactive constituents for the development of new chemotherapeutic agents. It

Table 1 – Minimum inhibitory concentration (Mic in mg/ml) of plant extracts on different bacterial strains.

Organisms		Concentration (mg/ml)								
	EXT	0.098	0.195	0.39	0.78	1.563	3.125	6.25	12.50	25.00
Enterobacter aerogenes	CE	+	+	+	+	+	+	***	–	–
	ME	+	+	+	+	+	***	–	–	–
	AE	+	+	+	+	+	***	–	–	–
Salmonella typhimurium	CE	+	+	+	+	+	–	–	***	–
	ME	+	+	+	+	***	–	–	–	–
	AE	+	+	+	+	+	+	***	–	–
Klebsiella pneumonia	CE	+	+	+	+	+	+	***	–	–
	ME	+	+	+	+	+	***	–	–	–
	AE	+	+	+	+	+	***	–	–	–
Pseudomonas aeruginosa	CE	+	+	+	+	+	+	+	***	–
	ME	+	+	+	+	***	–	–	–	–
	AE	+	+	+	+	+	***	–	–	–
E. coli	CE	+	+	+	+	+	+	+	***	–
	ME	+	+	+	***	–	–	–	–	–
	AE	+	+	+	+	***	–	–	–	–

CE is a chloroform extract, ME is a methanol extract, AE is an aqueous extract + indicates turbidity is observed, – indicates turbidity is not observed, *** represents the MIC value. Methanol extract significantly inhibit the growth of micro organisms at a low concentration followed by aqueous extract and chloroform extract is least.

has been well documented that the antimicrobial compound are abundantly present in medicinal plants.[12] The percentage of yield of methanol extract was more than that of the petroleum ether, chloroform and aqueous. The polar solvent was able to extract more of the extractives than non-polar solvents (petroleum ether, chloroform). Phytochemical constituents such as tannins, flavonoids, alkaloids, phenols and several other aromatic compounds are secondary metabolites of plants that serve as defence mechanisms against predation by many micro organisms, insects and herbivores.[13] Few researchers reported that several phytochemicals present in the plant extract exhibits antibacterial activity.[14,15]

The antimicrobial activities of all the three extracts tested, methanol extract significantly inhibited the growth of the organisms with 20 mm zones of inhibition. The result of this work however agrees with the findings of Alexeyena Varghese[16] who showed that the methanolic extract of *T. angustifolia* was active against *E. coli*, *S. aureus*. It is therefore conceivable that this extract can be used against *E. aerogenes, S. typhimurium, K. pneumonia and P. aeruginosa*. The antibacterial activity of the methanol and aqueous extracts of *T. angustifolia* may be due to the presence of secondary metabolites like alkaloids, tannin, steroids, phenol, saponins, flavonoids compounds, which are previously reported for their antimicrobial property.[16] The results of the minimum inhibitory concentration showed that the methanolic and aqueous extracts of *T. angustifolia* have potent bactericidal properties against the tested organisms. The inhibitory effects of the extracts are most likely due to the presence secondary metabolites. The results obtained indicated the existence of antimicrobial compounds in the crude methanolic extracts of *T. angustifolia* and showed a good correlation between the reported use of these plants in traditional medicine against infectious diseases.

5. Conclusion

The present study has revealed that methanol and aqueous extracts of *T. angustifolia* leaf exhibited significant antibacterial activity against gram negative organisms this is due to presence of different secondary metabolites in these extracts. Methanolic extract of the leaf exhibited maximum zone of inhibition for the tested organisms with minimum MIC values. Hence, this work justifies the use of *T. angustifolia* in ethnomedicine and further this plant can be exploited for new potent antimicrobial agent.

Conflicts of interest

All authors have none to declare.

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