

**TRAPA NATANS AS GREEN DRUG TO PATHOGENIC ESCHERICHIA COLI**Gagandeep Kaur, Joginder Singh, Anfal Arshi¹, Madhu Bala¹, Manoj Kumar*

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ABSTRACT

Trapa natans is a medicinal plant and is used for the screening of antimicrobial property. *T. natans* seed was extracted in different solvents like ethanol, methanol, butanol and water. The antimicrobial activities of all the extracts were determined by agar well diffusion method. The antimicrobial activity was checked against pathogenic strain of *E. coli*. The inhibitory effect of extracts was compared with the standard antibiotic tetracycline. The physiochemical tests were done to check secondary metabolites which were present in different solvent extracts. TLC was carried out to separate secondary metabolites and further analyzed by different solvents. DPPH assay was conducted to confirm the antioxidant property of solvent extracts at different concentrations.

Keywords: Antimicrobial property, *Trapa natans*, TLC, DPPH assays, antioxidant activity.

INTRODUCTION

The incidence of life threatening infections caused by pathogenic microorganisms has been increased worldwide, and is becoming an important reason of morbidity and mortality in immunocompromised patients in developing countries¹. The gradual rise of bacterial resistance against existing drugs is a serious problem; as a result sustainable source from nature is needed in different classes of antibacterial import². Medicinal plants continue to be an important therapeutic help for alleviating the ailments of human population. The ancestral approach towards the natural herb and its extracts has been a proven approach in India. In this modern age ayurvedic therapeutics has been re-defined and being practiced at international level inspired by our rich charka samhita. The reinforcement in order to signify the plant derived drugs is mainly due to the prevalent experiences that “green medicine” is safe and more sustainable than the costly synthetic drugs which have adverse side effects.

Nearly all the identified components from plants active against microorganisms are aromatic or saturated organic compounds, they are obtained through ethanol or methanol extraction³. Natural antimicrobials can be derived from bark, stem, leaves, roots, fruit rind etc⁴. Considering need of hour, we are in search for new antimicrobials. Some antibiotics are no longer in use because of microbial persistency to the drugs seems to be drug resistance⁵. *T. natans* seed extracts are found to be active against pathogen and have no side effects. Different solvents extracts of seeds of *T. natans* were used for analyzing the antimicrobial activities against *E. coli* by using well diffusion method. The inhibition zones were formed which tells about the antimicrobial activity of different solvent extracts of seeds of *T. natans*. The physiochemical tests were performed to check which secondary metabolites are present in different solvents extract. Solvents used were ethanol, methanol, butanol and water. Thin layer chromatography was performed to analyse which solvent was best for the extraction of secondary metabolites that will have antimicrobial activities. The secondary metabolites were alkaloid, flavonoid, saponin, tannin, photobalanin and anthraquinone.

Comparing the activities of enzymes present in the seeds of *T. natans* in the aerobic and anaerobic conditions, we

analyzed the result that the level of enzyme involved in metabolic processes of *T. natans*. The enzyme assay is performed and the levels of these enzymes were checked spectrophotometrically. DPPH (1,1-Diphenyl 1,2-picrylhydrazil) assay was performed to check the antioxidant and free radical scavenging activity of different solvent extracts of *T. natans*.

The present work is focused on antimicrobial activity of seed extracts of *T. natans* wherein *E. coli* was the model system for study.

MATERIALS AND METHODS**Sample collection**

Fresh seeds of *T. natans* were collected from fields of Haryana after proper identification. Seeds were washed with distilled water and dried sample was grounded into fine powder with the help of mortar and pestle.

Bacterial strain and culture preparation

Pathogenic strain, *Escherichia coli*, available at Lovely Professional University, Phagwara (Jalandhar) was sub-cultured and used throughout the study.

Preparation of plant extract

Antimicrobial metabolites from the dried seed powder was extracted in various solvents such as Ethanol (70%) , Methanol (80%), Butanol (80%) and Water. 10 g of powdered sample was extracted in 100 ml of the respective solvents (1:10) at 190-220 rpm for 24 h. Thereafter, it was filtered through 8 layers of muslin cloth and centrifuge at 5000 g for 15 min. The supernatant was collected and the solvent was evaporated to make the final volume⁶. It was stored at 4°C in airtight bottles for further studies. The dried metabolite extract was dissolved in double volume of DMSO (Dimethyl sulfoxide) thus giving the final concentration of extract to 500 mg/ml⁷.

Antimicrobial Susceptibility Assay

Antimicrobial Susceptibility Assay was carried out by well diffusion method, wherein sterile nutrient agar plates were prepared and spread with 50 ml of the available bacterial culture against which antibacterial activity was tested. Thereafter 2 wells of 8 mm diameter were dug with the help of sterile borer. Four plates were prepared for *E. coli* strain⁸. Different plates containing tetracycline versus different chemical derived extracts were tested as 2nd well was filled

with ethanolic extract, methanolic extract, and butanolic extract respectively. Since 1st well was having constant feeling with tetracycline.

Plates were incubated at 37°C for 24 hrs. The antibacterial activity of each extract was expressed in standard format of antibiotic screening approach which is accounted as zone of inhibition (mm) produced by each extract at the end of incubation period.

Qualitative determination of secondary metabolites present in different solvents extract

The phytochemical screening was done for ethanol, methanol, butanol and water extracts through standard procedure as given in Table 1.

Determination of antioxidant and radical scavenging property of solvent extracts of seeds of *T. natans*

1, 5, 10 and 50 µg/ml of different solvent extracts was taken in different test tubes. To each test tube 1000 µl of 0.1 mM DPPH-ethanol solution and 450 mM Tris HCl was added and tubes were kept at incubation for 30 minutes at room temperature. The reduction of DPPH free radical was

measured by taking absorbance at 517 nm. L-Ascorbic acid was taken as +ve control^{9,10}. Antioxidants donate hydrogen atom to the free radicals of DPPH to give rise to a reduced form with the loss of violet color of DPPH, which was detected by spectrophotometry at 570 nm¹¹.

TLC (Thin Layer Chromatography) analysis of extracts

TLC plates were made by dissolving 5 gm of Silica G powder in 15 gm of distilled water. Then the plates were allowed to air dry, and kept in oven for 10 minutes. Further with the capillary, the drop of solvent extract as a single dot was planted on the TLC plate. The spot of extract was placed at the bottom edge of the thin layer plate. Once the spot got dried, the plate was incubated in closed glass chamber having solution of ethyl acetate, formic acid, water in the ratio 100:11:27 respectively, as a solvent for running of solvent extracts along the TLC plate. Soon after solution had travelled a distance more than half of TLC plate, the plate was taken out and kept in the iodine chamber comprising iodine vapors. The iodine vapors helped to note the distance travelled by metabolites present in the solvent extract¹².

Table 1: Test performed for phytochemical components in *T. natans* seeds taken into consideration along with procedure and references

Tests	Procedure	References
Tests for alkaloids	Hager's	Evans and Trease, 2002
Tests for Flavonoids	Conc. H ₂ SO ₄ Test	Evans and Trease, 2002; Kokate et. al., 2009
Tests for Tannins	Braemer's Test	Evans and Trease, 2002; Kokate et. al., 2009
Tests for Saponins	Foam Test	Evans and Trease, 2002
Tests for Photobalanins	Reducing Test	Evans and Trease, 2002
Tests for Anthraquinone	Borntrager's Test	Kokate et. al., 2009; Sofowara, 1993; Kokate et. al., 2009
Tests for Carbohydrates	Fehling's Test	Trease and Evans, 1984; Kokate et. al., 2009; Sofowara, 1993

Table 2: Percentage inhibition of DPPH by different extracts at different concentrations in 30 minutes

Extract Concentration (µg/ml)	Ethanol		Methanol		Butanol		Water	
	O.D	%I	O.D	%I	O.D	%I	O.D	%I
5	0.279	19	0.276	20	0.302	11.17	0.289	15
10	0.165	51.4	0.167	50.8	0.292	14.11	0.210	38.2
15	0.120	64	0.124	63.5	0.140	58.8	0.186	45.2
50	0.030	82	0.110	67.6	0.123	63.8	0.169	50.2

O.D = Optical density; %I = percentage inhibition

Table 3: Analysis for the presence of different phytochemical compounds in various solution extracts

S. No.		Ethanol	Methanol	Butanol	Water
1	Alkaloid	+	+	+	-
2	Flavonoid	+	-	+	+
3	Saponins	-	+	-	+
4	Tannins	+	-	-	-
5	Photobalanins	+	+	-	-
6	Anthraquinone	+	+	+	-
7	Carbohydrates	-	-	-	+

+ = Present; - = absent

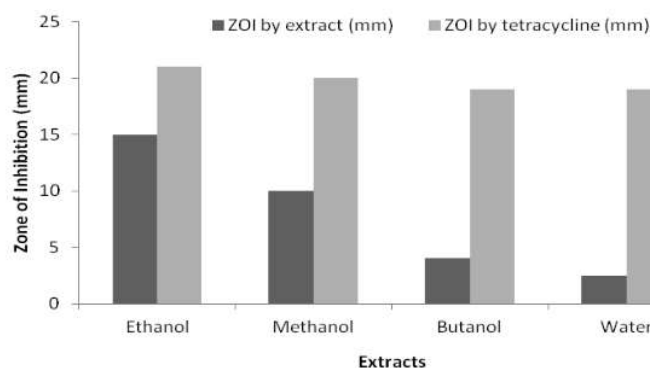


Figure 1: Comparative histogram of ZOI produced by different extracts with respect to positive control tetracycline

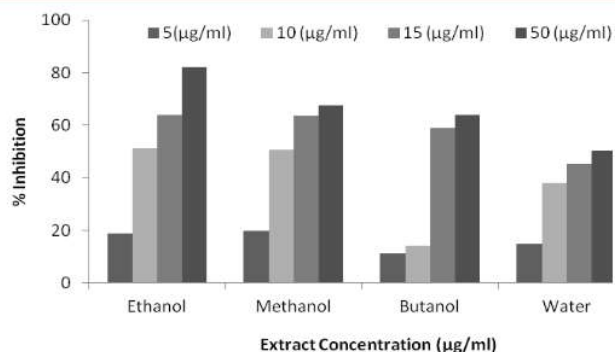


Figure 2: Assessment of antioxidant activity percentage inhibition of DPPH by 50 µL volume of different extracts of *Trapa natans* (30 minutes)

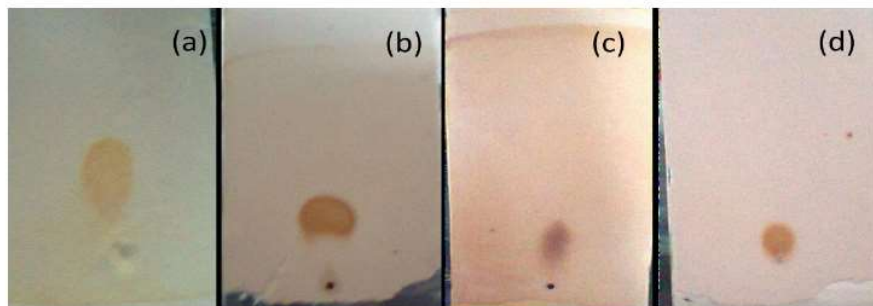


Figure 3: TLC images of (a) Ethanolic (b) methanolic (c) Butanolic (d) Aqueous extract of *Trapa natans*

RESULTS

Comparative Antimicrobial susceptibility

Antibiogram analysis revealed the facts that extract from *T. natans* is highly resistant as compare to tested antibiotic tetracycline. Pathogenic *Escherichia coli* was subjected for comparative analysis against tetracycline and natural extracts (Trapa) which shown the clear zone of inhibition that reflects the antibiotic significances (Figure 1). Ethanolic extracts was showing the maximum ZOI while cold aqueous extract was showing minimum ZOI.

Antioxidant scavenging assay

Table 2 shows the DPPH scavenging activity & antioxidant activity of different extracts at different concentration. Most promising result was in case of Ethanolic extracts (Figure 2)

Qualitative determination of secondary metabolites

As it is represented in Table 3 that the presence of different secondary metabolites in different extracts determined the best significances in terms of metabolic activity against any microbial activity.

Thin Layer Chromatography

TLC analysis was showing the best evidence in ethanolic extract as distance travelled by sample was comparatively more than other extracts eventually it is confirmed that ethanolic extract having high viability and feasibility in terms of physiological properties (Figure 3).

DISCUSSION

This study reveals the promising antioxidant and antimicrobial activities of different extracts of seeds of *T. natans*. As earlier reporting¹³ has already elucidated the similar antibacterial properties from fruit pulp extract though it was susceptible to the pathogenic strain. Present study is focused on seed extracts those have direct effect on pathogenic *E. coli*.

Tetracycline is used in this study as a reference antibiotic due to its broad spectrum and wide use in clinical trial and treatments. Researchers have found the pattern of antibiotic

activity of *T. natans* Fruit Peel Extract against Streptozotocin induced Diabetic Rats explaining the antioxidant behavior too which has narrow acceptance in pathogenic bacterial disease¹⁴. Therefore, newly introduced parameter based on metabolic and physiological activity has tried to show the universal acceptance against any pathogenic attack.

The parameter of different aqueous extract was taken and validated with published data and found that activity was quite low in cold extract¹⁵.

The quantitative and qualitative analysis of different phytochemical extracts from Trapa have already been done though reported alkaloids had least influence on *E. coli* as compare to Trapa seed extract¹⁶.

TLC analysis has revealed the fact that retention factor is quite low in the ethanolic seed extract which is superior in many folds over the available data based on DPPH free radical¹¹.

CONCLUSION

The seed extract of *T. natans* holds tremendous potential for antimicrobial activity. Significances at primary level lead to the future prospect will target the broad spectrum of microbes. Green herbal drug will be invigorated with high-throughput techniques based on the present threshold. At the end, the result claims the best part of natural ingredients extracted from Trapa is sustainable and eco-friendly approach towards the research world. The extracts could be further explored at molecular level to understand the in-depth phytochemical properties.

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