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Evaluation of ethanolic and aqueous extract of *Clitoria ternatea* for antimicrobial activity

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In the recent years, there has been an emerge and spread of infectious diseases and also developing resistance to the antibiotics, so there is a great concern to find potentially effective, safer natural alternative for the infectious diseases. The present study was focused on the antimicrobial activity of ethanol and aqueous extract of *Clitoria ternatea* root. The *in-vitro* antimicrobial study was carried out against bateriea viz. *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and fungus viz. *Aspergilus niger*, *Candida albicans* by the cup-plate method. The extracts of *Clitoria ternatea* roots were subjected to preliminary phytochemical screening for detection of chemical constituents present in them. Preliminary phytochemical screening study revealed the presence of alkaloids, glycosides, tannins, phenol and flavonoids. The highest potential was observed in the ethanol extract of *Clitoria ternatea* roots. This study suggested that solvent polarity determined the phenol and flavonoid content significantly, hence affecting the antimicrobial activity. This plant extract which proved to be potentially effective can be used as a natural alternative for preventives to control infectious diseases causing due to the tested microorganisms.

Keywords: Antibacterial activity, Antifungal activity, Aqueous extract, *Clitoria ternatea*, Ethanol extract, Phytochemical screening

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Introduction

It has been noted that in the recent years there is increasing the incidence of infectious diseases and on the other hand, due to rapid development of resistance in a large number of bacteria along with undesired side effects of certain antibiotics created massive clinical problems thus management of infectious diseases becomes quite complicated[1]. According to the World Health Organization, 80% of the population in the world depend upon traditional remedies which includes plant extracts or the active constituent in plant[2]. Many plants which have been used in various infectious diseases because of the presence of phytochemicals synthesized in a wide variety of secondary metabolites which includes alkaloids, flavonoids, phenolic compounds, tannins which have *in vitro* antimicrobial action. So these phytochemicals can be used in treating various infectious diseases like urinary tract infection, gastric disorders, respiratory infections, and cutaneous infection which are most common in human population[3].

Thus due to fast increase in the infectious diseases along with multidrug resistance and undesirable side effects of synthetic antibiotics, there is an increase in demand of medicinal plants with adequate bacterial efficacy can be used against many infectious diseases[4].

Clitoria ternatea L. (Fabaceae) known as *Aparajita* or Butterfly pea, in south asia, is a well-known plant in the Indian traditional system of medicine and distributed throughout India[5]. The roots are used as a powerful laxative liver tonic. The roots are bitter, refrigerant, intellect promoting, diuretic, anthelmintic, tonic and are useful in dementia, hemicranias, burning sensation, leprosy, inflammation, leucoderma, bronchitis, asthma, pulmonary tuberculosis and fever. The plant is used for neuropharmacological actions such as enhancing acetylcholine content[6,7],

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nootropic, anticonvulsant, antistress, anxiolytic, antidepressant, tranquillizing and sedative activities[8]. It has antimicrobial[9], anti-inflammatory and analgesic[10].

The present study aims to evaluate the antimicrobial potential of ethanol and aqueous extracts of *C. ternatea* roots using cup-plate antimicrobial assay method against some microbial strains of human pathogenic bacteria and fungi along with the phytochemical screening of both the extracts.

Materials and Methods

Plant collection

The roots of *C. ternatea* were collected in 2019 from the garden of Ram Nagar of Wardha district, Maharashtra, India and authenticated by Dr Chaturvedi, Department of Botany, R. T. M. University, Nagpur, Maharashtra, India (Voucher No. 9346).

The roots of *C. ternatea* were washed, cleaned and dried at 50 ^{o}C in hot air oven till complete removal of moisture. The dried material was ground in a grinder and passed through sieve 10 and 40 mesh size. After sieving the coarsely powdered material was stored in an airtight container till its further use.

Extracts preparation

Aqueous extracts

The dried powder of roots of *C. ternatea* (50 g) was macerated with distilled water for 24 h. The extract was filtered through Whatman filter paper (0.45 µm) and the filtrate was evaporated to dryness at 45^{o} C in a hot air oven. The dried extract was dissolved in a small amount (5 mL) of DMSO and stored in the freezer.

Ethanolic extracts

The dried powder of roots of *C. ternatea* (50 g) was extracted with ethanol with the help of Soxhlet extractor. The extracts were filtered using Whatman filter paper (0.45 µm) and the filtrate was then evaporated and dried at 45° C in a hot air oven. The dried extract was dissolved in a small amount (5 mL) of DMSO and stored in the freezer.

Qualitative phytochemical screening

Qualitative phytochemical testing of the aqueous and ethanol extracts was done for the detection of major chemical groups. Extracts were screened for the presence of Tannins, Flavonoids using Shinoda test, Saponin using Frothing test, Salkowski test was used to test Steroids and Saponin[11].

Antimicrobial activity

Ethanol and aqueous extracts of *C. ternatea* roots were studied against *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* for antibacterial activity and Tetracycline was used as standard. Antifungal activity of both the extracts of *C. ternatea* roots was studied against *Candida albicans* and *Aspergillus niger* by using fluconazole as a standard. The cup-plate method was performed nutrient agar, cetrimide agar and Vogel Johnson agar (Hi-Media) medium were used for studying for antibacterial activity against *E. coli*, *P. aeruginosa*, *S.aureus* and Sabouraud's dextrose agar (Hi-Media) medium for antifungal activity.

To 10 mL of agar slant in previously sterilized test tubes, one loopful of the stock culture was inoculated and incubated at 37 ^{o}C for 24 and 72 h, respectively, for bacteria and fungi. A suspension of the culture was prepared by adding about 3 mL of distilled water to the test tube and the same was used for inoculation.

The medium, Petri plates and glassware used were sterilized by autoclaving at 121 ^{o}C (15 lb/in^{2}) for 30 min. To each sterilized Petri plate, 30 mL of medium inoculated with respective strains of bacteria and fungi was transferred aseptically. A single well of 6 mm diameter was made in each plate by using sterile cork-bore. Test sample and control sample (0.5 mL) were placed into the well. The plate is kept for 2 h for diffusion. For antibacterial assay plates were incubated at 37±1 ^{o}C for 24 h and antifungal assay at 28±1 ^{o}C for 72 h. Tetracycline (50 µg/mL) was used as positive antibacterial control, whereas Fluconazole (50 µg/mL) was used as positive antifungal control. The diameter of the zone of inhibition surrounding each well was recorded. The average of the lowest concentration showing no growth of the organism and the highest concentration showing visible growth by macroscopic evaluation was taken as MICs[12-14]. Each assay was performed in triplicate.

Results and Discussion

The extracts of *C. ternatea* roots were subjected to preliminary phytochemical screening for detection of chemical constituents present in them. In the preliminary phytochemical screening, the presence of alkaloids, glycosides, tannins, phenolics and flavonoids were reported in the extracts. Results are shown in Table 1.

Table 1 — Qualitative phytochemical Screening of ethanolic and aqueous extracts of *Clitoria ternatea*

Test for various phytochemicals	EE	AQE
Carbohydrates		
Molish's test	+ve	+ve
Fehling's test	+ve	+ve
Benedict's test	-ve	+ve
Proteins		
Million's test	+ve	+ve
Biuret test	-ve	+ve
Amino acids		
Precipitation test	+ve	+ve
Ninhydrin test	-ve	-ve
Alkaloids		
Dragendorff's test	+ve	+ve
Mayer's test	+ve	+ve
Hager's test	-ve	-ve
Steroids		
Salkowski reaction	-ve	-ve
Flavonoids		
Shinoda test	+ve	-ve
Lead acetate test	+ve	-ve
Tannins and phenolic		
$FeCl_3$ test	-ve	+ve
Iodine test	+ve	+ve
Dil. HNO_3 test	+ve	+ve
Potassium dichromate test	+ve	+ve
Acetic acid test	+ve	+ve

Where, + *ve- present,– ve- absent, EE-Ethanol extract, AQE- Aqueous extract*

The antibacterial, antifungal activity, and the percentage inhibition of the ethanol and aqueous root extracts of *C. ternatea* were evaluated in the present research work. The study of *in vitro* antimicrobial activity is an important step towards the development of new potential antimicrobial drugs. In the present study, the ethanol root extract of *C. ternatea* exhibited the highest antimicrobial activity and water extract showed minimum activity against three bacterial strains *E. coli*, *S. aureus* and *P. aeruginosa* (Fig. 1-3) and two fungal strains *A. niger* and *C. albicans* (Fig. 4,5)

Literature review on the phytochemical constituents of these plants revealed that various secondary metabolites like flavonoids, anthocyanin glycosides, pentacyclic triterpenoids and phytosterols have been isolated from this plant[15]. A protein designated as 'finotin' has been isolated from *C. ternatea* seeds and reported to have antifungal, antibacterial and insecticidal properties[16]. It is possible that this compound was mainly responsible for the observed antimicrobial effects in this study.

Antifungal activity of *C. ternatea* was studied and a little bit considerable activity was found. The present study indicated that the antibacterial and antifungal activity varies with the different solvents of plant root material used *C. ternatea* is effective even at low concentration against bacterial and fungal

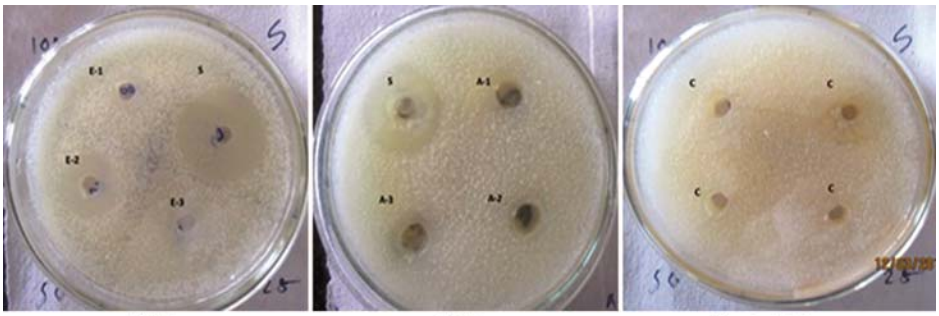

Fig. 1 — Antibacterial activity against *Escherichia coli*

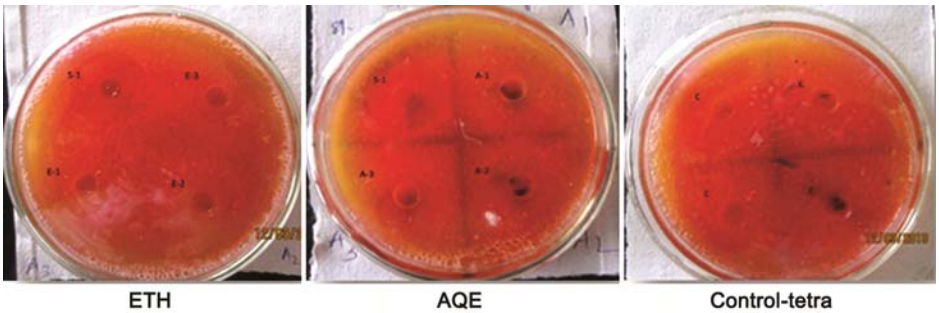

Fig. 2 — Antibacterial activity against *Staphylococcus aureus*

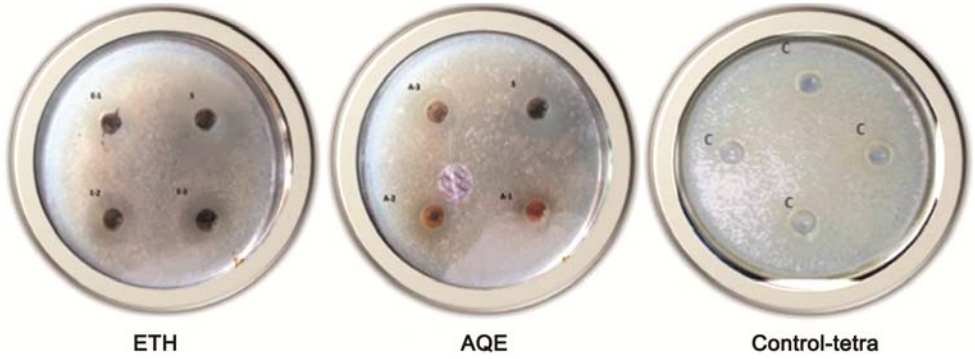

Fig. 3 — Antibacterial activity against *Pseudomonas aeruginosa*

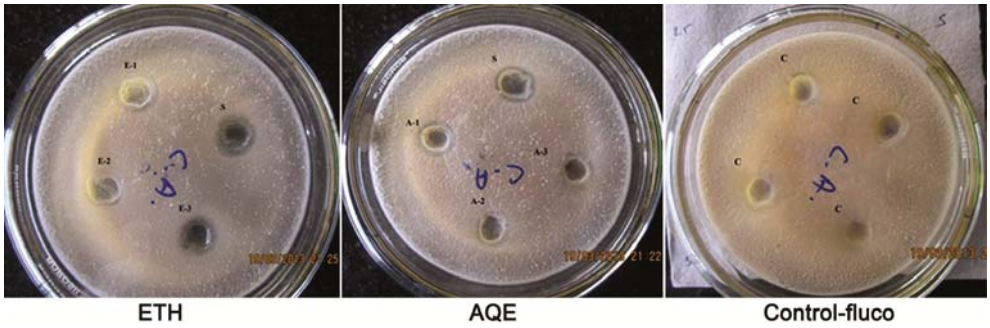

Fig. 4 — Antifungal activity against *Candida albicans*

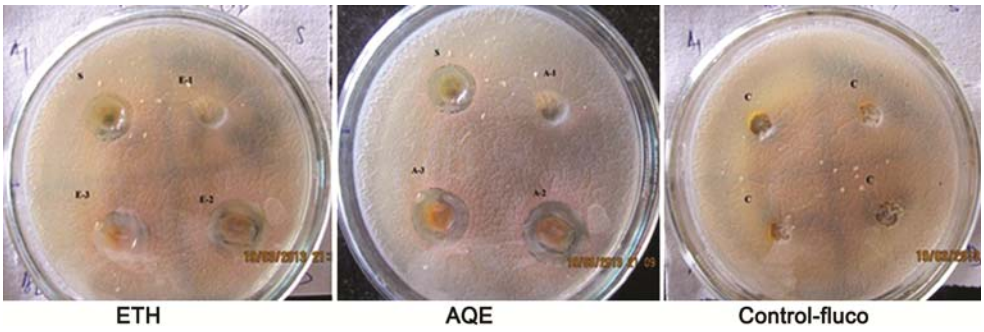

Fig. 5 — Antifungal activity against *Aspergillus niger*

pathogens. Generally, at present, with the spread of resistance against antibiotics almost at regular scale[17] and noticeable challenges confronted with medical physicians in the treatment of many infectious diseases[18]. Such plants should be considered to reap all the possible antimicrobial benefits intrinsic in them. In this way, the actual ingredients of having antimicrobial potential must be extracted and then identified. The tolerable level and toxic effects of such compounds on human as well as on animals should be properly investigated.

The results of the antimicrobial assay showed that the extracts of *C. ternatea* roots are effective against both gram+ve and gram-ve bacteria and the ethanol extract exhibited better activity when compared to aqueous extract against selected strain. However, the zone of inhibition exhibited by the plant extracts was less than that of the standard drug. Results for antimicrobial activity of extracts of *C. ternatea* against different microorganisms are shown in Table 2. *C. ternatea* extracts were potentially effective. Ethanolic extract of *C. ternatea* was found to be effective in concentration (60 µg/mL) against *E. coli*, *P. aeruginosa*, *S. aureus*, *C. albicans*, *A. niger* to prevent the microbial growth with the inhibition zones of 16, 18, 17, 12, 13 mm respectively. These results are in accordance with that of Manandhar S, Luitel S, *et al.*, (2019) and Rajni, Gautam S S, *et al.* (2014).

Conclusion

The outcome of this research work reaffirms the antimicrobial potential of the roots of *C. ternatea*. The antimicrobial assay of the ethanol and water extract of *C. ternatea* roots showed a significant antimicrobial activity. Present antimicrobial study of the *C. ternatea* roots showed that this plant possesses better antibacterial and antifungal activity which is more in ethanol extract than water extract. Further phytochemical studies are needed for determining the type of compound responsible for antimicrobial activities.

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Conflict of interest

The authors declared that there is no conflict of interest.

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