

## EVALUATION OF ANTIMICROBIAL ACTIVITY OF ETHANOLIC EXTRACT OF *CLITORIA TERNATEA*

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### ABSTRACT

*Clitoria ternatea* is known for its antimicrobial activity, but the antifungal effects of Ethanolic extract on growth of *Aspergillus niger* have been observed. The extract showed a favorable antifungal activity against *Aspergillus niger*. Ethanolic extract of *Clitoria ternatea* were examined for their phytochemical compounds and antimicrobial potential against three standard bacteria (*Escherichia coli*, *Klebsiella Pneumonia*, *Staphylococci*,) and one standard fungus (*Aspergillus niger*). The phytochemical analysis showed the presence of some active principle which correlates with the antifungal activity of ethanolic extract of *Clitoria ternatea*. The ethanolic extract of *Clitoria ternatea* shows the maximum antifungal activity compared to Griseoflavin.

**KEYWORDS:** *Clitoria ternatea*, *Aspergillus niger*, *Escherichia coli*, *Klebsiella Pneumonia*, *Staphylococci*, antifungal activity, Griseoflavin.

### INTRODUCTION

Infectious diseases are the leading cause of death world-wide. Antibiotic resistance has become a global concern.<sup>[1]</sup> The number of multi-drug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics are continuously increasing. This increase has been attributed to indiscriminate use of broad-spectrum antibiotics,

immunosuppressive agents, intravenous catheters, organ transplantation and ongoing epidermis of human immunodeficiency virus (HIV) infections.<sup>[2, 3]</sup> This situation provided the impetus to the search for new antimicrobial substances from various sources like medicinal plants.<sup>[4]</sup> Synthetic drugs are not only expensive and inadequate for the treatment of diseases but are also often with adulterations and side effects. Therefore, there is a need to search for new infection-fighting strategies to control microbial infections.<sup>[5]</sup>

The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens.<sup>[6]</sup> Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. There is a continuous and an urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases.<sup>[7]</sup> Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections.<sup>[8]</sup> The increasing failure of chemotherapeutic and antibiotic resistance exhibited by pathogenic microbial infectious agents has lead to the screening of several medicinal plants for their potential antimicrobial activity.<sup>[9,10]</sup>

India is a varietal emporium of medicinal plants and is one of the richest countries in the world in regard to genetic resources of medicinal plants. It exhibits a wide range in topography and climate, which has a bearing on its vegetation and floristic composition. Moreover, the agro-climatic conditions are conducive for introducing and domesticating new exotic plant varieties.<sup>[11]</sup> In recent years, secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents.<sup>[12]</sup> Thus, it is anticipated that photochemical with adequate antibacterial efficacy will be used for the treatment of bacterial infections.<sup>[13]</sup> Since time immemorial, man has used various parts of plants in the treatment and prevention of various ailments.<sup>[14]</sup>

Plants were the mainstay of medicine and credited with mystical and almost supernatural powers of healing. The practice of herbal medicine dates back to the very earliest periods of known history. There is evidence of the herb have been used in treatment of diseases and for revitalizing body systems in almost all ancient civilizations. Medicinal plants were existing

even before human beings made their appearance on the earth. It is therefore often said that where ever we are born, we have around us herbs, shrubs and plants which are useful for us.<sup>[15]</sup>

The use of plants, plant extracts or plant derived pure chemicals to treat disease is therapeutic modality, which has stood the test of time. Indeed, many pharmacological classes of drugs, including a natural product prototype. Aspirin, Atropine, Ephedrine, Digoxin, Morphine, Quinine, Reserpine and Tubocurarine are a few examples of drugs, which were originally discovered through the study of traditional cures and folk knowledge of indigenous people. There is a revived interest in herbal products (botanicals) at a global level and conventional medicine is now beginning to accept the use of botanicals once they are scientifically validated. Isphagula, Garlic, Ginseng, Ginger, Ginkgo, St. John's Wort and a Saw palmetto are a few examples of botanicals which are gaining popularity amongst modern physicians and this trend is likely to continue, partly due to the high cost involved in the development of patentable chemical drugs. There is growing evidence to show that medicinal plants contain synergistic and/or side-effects neutralizing combinations. Ethnopharmacology has already played an important role in the development of conventional medicine and is likely to play more significant role in the years to come<sup>[16]</sup>

During the later part of this century the practice of Herbalism has become main stream throughout the world. This is due in part to the recognition of the value of traditional medicinal systems, particularly of Asian origin, and the identification of medicinal plants from indigenous pharmacopoeias that have been shown to have significant healing power, either in their natural state or as the source of new pharmaceuticals. Generally, these formulations are considered moderately in efficacy and thus less toxic than most pharmaceutical agents. In the western world, in particular, the developing concept that 'natural' is better than 'chemical' or 'synthetic' has led to the evaluation of Neo-western Herbalism that is the basis of an ever expanding industry. In the U.S, often used as food or food supplements, known as nutraceuticals, these formulations are readily available for those that wish to self medicate.<sup>[17]</sup>

A team work amongst Ethno botanist, Ethno pharmacologist, physicians and phytochemist is essential for the fruitful outcome on medicinal plant research, while the Ethno pharmacologist has greater role to play in the rationalization of the combination of activities, the phytochemist's role will slightly shift towards standardization of botanicals.<sup>[18]</sup>

The main aim of the present study is “Evaluation of Antimicrobial activity of Ethanolic extract of *Clitoria ternatea*”. *Clitoria ternatea* vine has documented to possess antidepressant activity and anticonvulsant activity, but the effect of *Clitoria ternatea* vine for Antimicrobial and Antifungal activity is still not reported. Hence it was thought worthwhile to screen extract *Clitoria ternatea* as an Antimicrobial and Antifungal activity.

## MATERIALS

**Collection of plant materials:** *Clitoria Ternatea* vine was collected from the Botanical Garden attached to University College of Science, Osmania University, Hyderabad, Telangana, India and authenticated at the Department of botany of the same University.

## METHODS

**Preparation of plant extracts:** The leaves were dried under shade and later pulverized in a mechanical grinder. The 100 g of powder was then extracted with 95% ethanol for one week. The mixture was then filtered and the filtrate was concentrated under reduced pressure to yield semisolid 3.70% (weight ratio) extract. The extract was preserved in a refrigerator for further use.<sup>[19]</sup>

**Phytochemical Analysis:** Phytochemical analysis for major phytoconstituents of the plant extracts was undertaken using standard qualitative methods as described by Rizk and Bashir. The plant extracts were screened for the presence of biologically active compounds like glycosides, phenolics, alkaloids, tannins, flavonoids, saponins and steroids.<sup>[20]</sup> *Clitoria ternatea* was evaporated to residue and diluted HCl was added into it. After shaking, the extract was filtered and filtrate tests were performed for the detection of following constituents.

**Identification test:** The test were carried out to find the presence of the active chemical constituents such as glycosides, Phenolics, Alkaloids, Tannins, Flavonoids, Saponins and Steroids by the following procedure.

**Test for Alkaloids:** 2ml filtrate of the plant extract was mixed with 2ml of HCl and about 6 drops of Mayor's reagents. A creamish or pale yellow precipitate indicates the presence of alkaloids.<sup>[21]</sup>

**Test for steroids:** One ml of the extract was dissolved in 2 ml of acetic anhydride which is then added to 2 ml of H<sub>2</sub>SO<sub>4</sub>. The color changes from violet to blue or green in some samples, indicating the presence of steroids.<sup>[22]</sup>

**Test for tannins:** One ml of the extract was treated with a few drops of 1% ferric chloride solution and observed for brownish green or a blue-black coloration, confirming presence of tannins.<sup>[23]</sup>

**Test for flavonoids:** Four ml of filtrate was added to 5-6 drops of conc. HCl and 1.5 ml of methanol solution. Pink-to-mato red color indicates the presence of flavonoids.<sup>[24]</sup>

**Test for glycosides:** Two ml of extract is mixed with 2 ml chloroform. Then 2ml acetic anhydride and 2 drops of conc. H<sub>2</sub>SO<sub>4</sub> was added from the side of the test tube. First red, then blue and finally green colour appears indicating presence of glycosides.<sup>[25]</sup>

**Test for Phenolics:** Two ml of ethanol was added to the test solution and a few drops of ferric chloride solution. Blue coloration indicates the presence of Phenolics.<sup>[26]</sup>

**Test for Saponins:** 2ml of distilled water was added to 2ml of the test solution and shaken well and observed for frothing. Presence of various constituents in different solvent was determined.<sup>[27]</sup>

**Study of antimicrobial activity:** The selected compounds prepared in the course of the present investigation are screened for antibacterial activity against the following bacteria's. *Staphylococcus aureus* (Gram +ve) (MTCC 737), *Escherichia coli* (Gram -ve) (MTCC 1687), *Streptococci* (Gram +ve) (MTCC 3086), *Pseudomonas* (Gram -ve) (MTCC 1035) and antifungal activity against following fungi, *Aspergillus niger* (MTCC 2638).

**Antibacterial Activity:** The antibacterial activities are performed by cup, plate method (diffusion technique). The fresh culture of bacteria is obtained by inoculating bacteria into peptone water liquid media and incubated at  $37 \pm 2^{\circ}\text{C}$  for 18 – 24 hours. This culture mixed with nutrient agar media (20%) and poured into petri dishes by following aseptic techniques. After solidification of the media five bores are made at equal distance by using sterile steel cork borer (8 mm diameter). Into these cups different concentrations of standard drugs and synthesized compounds are introduced. Dimethyl formamide was used as a control. After the introduction of standard drugs and synthesized compounds, the plates were placed in a refrigerator at  $8^{\circ} - 10^{\circ}\text{C}$  for proper diffusion of drugs into the media. After two hours of cold incubation, the petri plates are transferred into incubator and maintained at  $37^{\circ} \pm 2^{\circ}\text{C}$  for 18-24 hours. After the incubation period, the petri plates were observed for the zone of inhibition by using vernier scale. The results evaluated by comparing the zone of inhibition shown by the

synthesized compounds with standard drugs. The results are the mean value of zone of inhibition measured in millimeter of two sets. The standard drugs and synthesized compounds were dissolved in a minimum quantity of N N' dimethyl formamide (DMF) and adjusted and the volume with distilled water to get 50mg/ml and 100mg/ml concentrations. The procaine penicillin, streptomycin used against *Staphylococcus aureus*, *Escherichia coli*, and Cetazolin Sodium, Sporfloxin used against *Pseudomonas, strepto cocci*.<sup>[28]</sup>

**Table 1 Preparation of subculture media**

Ingredients	Quantity
Peptone	10 gm
Beef extract	10 gm
Sodium chloride	5 gm
Distilled water	Q. S. 1000 ml

**Table 2 Preparation of assay medium**

Ingredients	Quantity
Nutrient agar media	28.0gms
Agar and agar	4.0gms
Distilled water	Q. S. 1000 ml

**Antifungal activity:** The synthesized compounds are screened against two selected fungal strain *Aspergillus niger* by using diffusion method. The 48 hours old fungal culture inoculated into a nutrient broth by following aseptic techniques and incubated for 48 hours at  $37^{\circ} \pm 2^{\circ}\text{C}$  in an incubator. This culture mixed with Potato-dextrose agar media (20%) and poured into petri plates. After solidification five bores are made at equal distance by using sterile steel cork borer (8 mm in diameter). Into these cups different concentrations of standard drug and synthesized compounds along with control DMF (N N' Dimethyl formamide) introduced. After the introduction of standard drug and compounds, these plates are placed in a refrigerator at  $8^{\circ}\text{--}10^{\circ}\text{C}$  for two hours for proper diffusion of the drugs. After 2 hours of cold incubation, the petri plates are transferred to an incubator and maintained at  $37^{\circ} \pm 2^{\circ}\text{C}$  for 24-36 hours. After the incubation period, the plates were observed in the zone of inhibition by using vernier scale. The results evaluated by comparing the zone of inhibition shown by the synthesized compounds with standard drug. The results are the mean value of zone of inhibition measured in millimeter of two sets. The results are tabulated in the Table No. 7. The standard drug and synthesized compounds were dissolved in a minimum quantity of DMF and adjusted, to make up the volume with distilled water to get 50 $\mu\text{g/ml}$  and 100 $\mu\text{g/ml}$  concentrations. The Griseoflavin used as a standard drug.<sup>[29]</sup>

**Table 3 Composition of nutrient broth**

Ingredients	Quantity
Peptone	10.0 gm
Yeast extract	6.0 gm
Potassium dihydrogen phosphate	3.0 gm
Sodium chloride	5.0 gm
Glucose (anhydrous)	10.0 gm
Distilled water	Q. S. 1000.0 ml

**Table 4 Potato – dextrose agar medium**

Ingredients	Quantity
Potato Dextrose media	39.0 gm
Agar agar	4.0 gm
Distilled water	Q. S. 1000.0 ml

## RESULTS AND DISCUSSION

A review of the chemistry, the pharmacology and therapeutic potency of different *dactolyniem* species has been reported. *Clitoria Ternatea* has particularly shown some interesting biological activities related to its worldwide uses in traditional medicine.

Preliminary phytochemical analysis revealed the presence of alkaloids and saponins. The other secondary metabolites like tannins, flavonoids, steroids etc. were present in trace amounts in some of the plants and results is shown in table 4.

**Table 4 Phytochemical Tests**

S.NO.	Chemical Test	Aqueous Extract
1.	Alkaloids	—
	Mayer's test	+++
	Dragondroff's test	+++
	Wagner's Test	+++
	Hager's Test	+++
2.	Steroids	+++
3.	Tannins	++
4.	Fixed oils	++
5.	Tannins	++
6.	Phenols	—
a).	Ferric chloride	++
b).	Lead acetate	++
c).	Gelatin solution	++
7.	Saponins	—
a).	Foam test	+
9.	Flavoniods	—
a).	Aqueous NaOH Test	++
b).	Conc. H <sub>2</sub> SO <sub>4</sub> Test	++
c).	Schinodo's test	++

+++ Indicates High, ++ Indicates Intermediate, + Indicates Low, \_ Indicates Absent.



The potential for developing antimicrobials from higher plants appears rewarding as it will lead to the development of a phytomedicine to act against microbes. Plant-based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials.

Continued further exploration of plant- derived antimicrobials is needed today. Further research is necessary to determine the identity of the antibacterial compounds from within these plants and also to determine their full spectrum of efficacy. However, the present study of in vitro antimicrobial evaluation of some plant forms a primary platform for further phytochemical and pharmacological studies. Ethanolic extract of *Clitoria Ternatea* showed zone of inhibition . There is a dose dependent increase in zone of inhibition. *Clitoria Ternatea* (200mg/kg) inhibits more colonies than that of *Clitoria Ternatea aegyptium* (100mg/kg). The ethanolic extract of *Clitoria Ternatea* was found to be effective in the dose of 100mg/kg and 200mg/kg respectively, and results are shown in table 5 and figure 1.

**Table 5 Antibacterial activity**

Sl. No	Name of the compounds	Mean zone of inhibition (in mm)*							
		<i>Staphylococcus aureus</i>			<i>Escherichia coli</i>		<i>Enterococci faecalis</i>		<i>Pseudomonas auruginosa</i>
		100µg	200µg	100µg	200µg	100µg	200µg	100µg	200µg
1	Procaine penicillin	18	20	-	-	-	-	-	-
2	Streptomycin	-	-	28	39	-	-	-	-
3	Sporofloxin	-	-	-	-	33	40	-	-
4	Ciprofloxacin	-	-	-	-	-	-	18	24
5	<i>Clitoria Ternatea</i>	-	-	-	-	-	-	-	-

The ethanolic extract of *Clitoria Ternatea* 200mg/kg inhibits the fungal growth and it is found that the Ethanolic extract of *Clitoria Ternatea* at a dose of 200mg/kg and 100mg/kg leads to significant inhibition of fungal growth and it also inhibits the microbial growth in *Aspergillus* at a dose of 200mg/kg. Although the dose 100mg/kg showed less efficacy as the results shown in table 6 and figure 1.

**Table 6 Antifungal Activity (*Aspergillus niger*)**

S No	Name of the compounds	Mean zone of inhibition (in mm)*	
1	<i>Aspergillus niger</i>		
2	Griseoflavin	100µg	200µg
3	<i>Clitoria Ternatea</i>	28	33





**Figure1: Zone of inhibition**

## CONCLUSION

In conclusion, the present study indicated a significant effect of the ethanolic extract of *Clitoria Ternatea* and supports its traditional usage as an antimicrobial agent. Further, studies is required for the detailed studies in isolation of the compounds and pharmacological investigations of constituents, which have many pharmacological activity reported in traditionally and its exact mechanism of action and the results are confirm that *Clitoria Ternatea* has a great potential as an Antimicrobial and may be useful in clinical conditions.

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