

Research Article**PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITY OF CLITOREA TERNATEA LINN AGAINST EXTENDED SPECTRUM BETA LACTAMASE PRODUCING ENTERIC AND URINARY PATHOGENS**

*BABU UMA, Research scholar, Division of Microbiology, Rajah Muthiah Medical College and Hospital, Annamalai Nagar -608001 Tamilnadu, India. E-mail: amu_sri2003@yahoo.co.in
KESANI PRABHAKAR, Professor of Microbiology, Division of Microbiology, Rajah Muthiah Medical College and Hospital, Annamalai Nagar -608001, Tamilnadu, India.
SADAYAPPAN RAJENDRAN, Professor of Microbiology, Division of Microbiology, Rajah Muthiah Medical College and Hospital, Annamalai Nagar -608001, Tamilnadu, India.

ABSTRACT

The *in vitro* antimicrobial activity of various extracts of *Clitoria ternatea*. Linn flower was screened against some ESBL producing enteric and urinary pathogens isolated from patients. The antimicrobial activity was carried out by disc diffusion method and minimum inhibitory concentration by two-fold serial dilution method. Aqueous, methanol and chloroform extracts exhibited activity against uropathogenic *E.coli*, Enteropathogenic *E.coli*, Enterotoxigenic *E.coli*, *typhimurium*, *Klesiella pneumoniae* and *pseudomonas aureginosa*. Petroleum ether and hexane extracts did not exhibit any activity. Antibacterial activity was compared with standard antibiotics.

KEY WORDS *Clitoria ternatea*, Antimicrobial activity, Enteric pathogen.

INTRODUCTION

Clitoria ternatea. Linn (Fabaceae) is known as *Aparajitha* and *Sangupusbi* in India, is a flowering plant. It is a persistent, herbaceous perennial legume and it is native to south-east Asia and widely distributed throughout the world mainly in tropical countries.¹ *Clitoria ternatea* has diuretic and laxative effects. It also has anthelmintic and anti-ulcer properties. The leaves and flowers have the cooling effects.² Phytochemical screening of methanol extract of *Clitoria ternatea* roots shows the presence of tannins, resins, starch, taraxerol, and ternatins.³ The present study was therefore undertaken to evaluate antimicrobial activity and phytochemical screening of crude methanol extract of *Clitoria ternatea* blue flowers against ESBL producing enteric and urinary pathogens.

Above values are the means of three assays. -: no activity, ETEC- Enterotoxigenic *E.coli*, EPEC- Enteropathogenic *E.coli*, S.t - *Salmonella*

typhimurium, S.e - *Salmonella enteritidis*, K.p - *Klesiella pneumoniae*, and P.s - *Pseudomonas aureginosa*,

The blue flowers of *Clitoria ternatea* was collected from Chidambaram, Tamilnadu, India and was authenticated by the Department of Botany, Annamalai University, Annamalai Nagar, Tamilnadu, India. The flowers were shade dried, powdered and extracted with various solvents, aqueous, methanol, chloroform, petroleum ether and hexane using maceration techniques. Twenty-five grams of powdered flowers were extracted with 125 ml of solvent with occasional shaking for 3 days at room temperature. The extracts were filtered, concentrated and dried at 50°C and the weight of each residue was recorded and percentage yield was calculated. The antimicrobial screening was evaluated against ESBL producing uro-pathogenic *E.coli*, Enterotoxigenic *E.coli*, Enteropathogenic *E.coli*, *Salmonella typhimurium*, *Salmonella enteritidis*,

Klesiella pneumoniae and *pseudomonas aureginosa* isolated from patients with urinary tract infection and acute gastroenteritis attending Rajah Muthiah Medical College and Hospital. The activity of the above mentioned extracts were tested by disc diffusion method.⁴ The extracts were freshly reconstituted with 5% dimethyl sulphoxide (DMSO) at a concentration 200 mg/ml. Sterile 6mm discs were impregnated with 20µl of the extracts were placed aseptically over Muller-Hinton agar plates (Himedia), which were previously inoculated with the test strains. Amikacin 10 µg/disc was used as positive control and 5% dimethyl sulphoxide (DMSO) impregnated disc was used as negative control. The plates were incubated at 37° for 24 hours. Each experiment was carried out in triplicate. The diameters of zone of inhibition surrounding the discs were recorded. The extracts that showed antimicrobial activity were subjected to minimum inhibitory concentration (MIC) assay by using two-fold serial dilution method. MIC's were interpreted as the lowest concentration of the sample, which showed clear fluid without development of turbidity. All the MIC tubes, that did not show any turbidity, were streaked over the Muller-Hinton agar plates. The minimum bactericidal concentration was recorded as the lowest concentration (MBC) that did not permit any visible growth on the plates after the period of incubation. Observation and results are given in Table 1 and Table 2.

Above values are the means of three assays. -: no activity, ETEC-Enterotoxigenic *E.coli*, EPEC-Enteropathogenic *E.coli*, K.p - *Klebsiella pneumoniae*, and P.s - *Pseudomonas aureginosa*.

The antimicrobial assay showed that aqueous, methanol and chloroform extracts of *Clitoria ternatea* blue flowers exhibited activities against ESBL

producing uropathogenic *E.coli*, Enterotoxigenic *E.coli*, Enteropathogenic *E.coli*, *Klebsiella pneumoniae* and *pseudomonas aureginosa*. No activity was observed against *Salmonella typhimurium* and *Salmonella enteritidis*. Methanol extract of *Clitoria ternatea* exhibits comparatively high activity as compared with chloroform and aqueous extracts. The inhibitory zone produced by methanol extract at a concentration of 4 mg/disc was between 16 to 26mm, whereas chloroform extract produced between 14mm to 18 mm. Aqueous extract exhibited 12 mm zone of inhibition. Petroleum ether and hexane extracts did not exhibit any activity. Minimum inhibitory concentration of active extracts is shown in Table. 2. The MIC values observed for methanol extract was between 1.25 and 2.5mg/ml. The MBC values observed were 0.625 and 1.25 mg/ml. Chloroform extracts exhibited MIC and MBC values were 2.5 and 5 mg/ml and for aqueous extract MIC and MBC values were 5 and 10 mg/ml respectively. The study reveals that the extracts of *C.ternatea* were effective against gram negative urinary pathogens than enteric pathogens. All the extracts were inactive against *Salmonella* species. A previous study by Khadatkhar *et al.*, showed that the methanol extract of *C.ternatea* roots exhibits anti-anthelminitic property.⁵

The emergence of extended-spectrum beta-lactamases (ESBLs) in gram negative bacteria is an increasing problem world wide. Several disease causing urinary and enteric bacterial pathogens has now become resistant to one or more antibiotics. The rate of development of resistance in gram negative bacteria due to production of ESBLs in 3rd generation cephalosporins (viz). Cefazidime, cefotaxime and ceftriaxone have found to be increasing.⁶ Medicinal plants are playing an important role in the health care immemorial. Activities of medicinal plants against the pathogenic bacterial strains were due the

presence certain secondary metabolites. It is primary interest that the green medicine

Table- 1 Antimicrobial activity of *clitoria ternatea*. Linn flower extracts by disc diffusion assay

Solvent extracts	Conc./disc	Zone of inhibition in mm						
		E.coli	ETEC	EPEC	S.t	S.e	K.p	P.s
Aqueous	4 mg	12	12	12	-	-	12	12
Methanol	4 mg	20	16	16	-	-	26	26
Chloroform	4 mg	18	14	14	-	-	18	16
Petroleum ether	4 mg	-	-	-	-	-	-	-
Hexane	4 mg	-	-	-	-	-	-	-
Amikacin	10 µg	32	28	26	30	30	22	28
DMSO	-	-	-	-	-	-	-	-

Table- 2 Antimicrobial activity of *clitoria ternatea*. Linn flower extracts by two-fold serial dilution method

Extracts		MIC and MBC values are in mg/ml				
		E.coli	ETEC	EPEC	K.p	P.s
Aqueous	MIC	5	10	10	5	5
	MBC	2.5	5	5	2.5	2.5
Methanol	MIC	1.25	2.5	2.5	1.25	1.25
	MBC	0.625	1.25	1.25	0.625	0.625
Chloroform	MIC	5	5	5	2.5	2.5
	MBC	2.5	2.5	2.5	2.5	2.5

is safe and dependable, compared with costly synthetic drugs that have adverse effects.⁷. Preliminary phytochemical screening of the methanol extract showed presence of alkaloids, flavonoids, saponins, tannins, carbohydrates, flavonoids and proteins. The detailed chemical nature of the active principles responsible for antibacterial activity is not known. Hence, further studies should be carried out to elucidate the active principles of *Clitoria ternatea*.

REFERENCES

- Jain NN, Ohal CC, Shroff SK, Bhutada RH, Somani RS, Kasture VS. *Clitoria ternatea* and CNS. Pharmacol Biochem Behav 2003; 75: 529-36.
- Rai KS, Murthy KD, Karanth KS, Rao MS. *Clitoria ternatea* root extract treatment during growth spurt period enhances learning and memory in rats. Indian J Physiol Pharmacol 2001; 4: 305-13.
- Terahara N, Saito N, Matsui T, Osmajima Y, Saito N. five New anthocyanins A3, B4, B3, B2 and D2 from *Clitoria ternatea*. J Nat Prod 1996; 59: 139-144.
- Sahoo S, Kar DM, Mohapatra S, Rout SP, Dash SK, Antibacterial activity of *Hybanthus enneaspermus* against selected UTI pathogens, Indian J Pharm Sci 2006; 68: 653-655.
- Khadatkar SN, Manwar JV, Bhajipale NS. In-vitro anthelminitic activity of root of *Clitoria ternatea*. Phcog Mag 2008; 4: 148-150.
- Bush K. β -Lactamases of increasing clinical importance. Curr Phar Des 1999; 5: 839-845.
- Cowan MM. Plants products as antimicrobial agents. Clin Microbiol Rev 1999; 12: 564-582.