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ANTIBACTERIAL STUDIES ON LEAVES OF *CLITORIA TERNATEA* LINN. - A HIGH POTENTIAL MEDICINAL PLANT

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ABSTRACT: The aim of the present study was to investigate the antibacterial properties of *Clitoria ternatea.* The organic solvent (Petroleum ether, Ethyl acetate and Methanol) extracts from the leaves of *Clitoria ternatea* (Papilionoideae) were tested against *Bacillus cereus*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Proteus vulgaris* and *Salmonella typhi* by agar disc and well diffusion methods. The results showed promising antibacterial activity against the tested microbial pathogens. Among these, methanol extract was found to possess a more potent inhibitory activity effect when compared to the other extracts (Petroleum ether and Ethyl acetate). The results of this study validate the use methanol extract of this species in ethnomedicine, favouring the isolation of antibacterial agents from the leaf extracts of *Clitoria ternatea*.

Key words: Microbial pathogens, ethnomedicine, solvents, *Clitoria ternatea*.

INTRODUCTION

Infectious disease is the number one cause of death accounting for approximately one-half of all death in tropical countries. Death from infectious diseases, ranked 5th in 1981, has become 3rd leading cause of death in 1992, with an increase of 58% (Venkataswamy *et al*., 2010). The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug resistant pathogens (Doss *et al*., 2009). There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanism of action because there has been an alarming increases in the incidence of new and re-emerging infectious diseases (Parivuguna *et al*., 2008). Natural products of higher plants may give a new source of antimicrobial agents with possibly novel mechanism of action. Contrary to the synthetic drugs, antimicrobial of plant origin not associated with many side effects and have an enormous therapeutic potential to heal many infectious diseases (Geeta singh and Padma kumar, 2011). *Clitoria ternatea* (Family- Liguminoceae, previously known as Papillioneceae), a perennial twining herb, stems terete, more or less pubscent. Leaves imperipinnate, petioles 2-2.5 cm long; stipules 4 mm long, linear, acute. Leaflets 5-7, subcoriaceous, 2.5-5 by 2-3.2 cm, elliptic-oblong, obtuse or caute; stipules filiform. Flowers -axillary, solitary, standard bright or blue or sometimes white, with an orange centre, seed- 6-10, yellowish brown, smooth. Two types- white variety and blue flowered variety; widely distributed throughout Bangladesh, used as ornamental plant. Various parts of *C. ternatea* have been reported to have tranquilizing property, anti-inflammatory, analgesic activity, antipyretic, and immunomodulatory activities (Mukherjee *et al*., 2008). The flavonol glycoside present in roots is reported to have antibacterial activity (Yadava *et al*., 2003). Considering the high economical and pharmacological importance of secondary metabolites, industries are deeply interested in utilizing plant tissue culture technology. *C. ternatea* has been reported to have anti-inflammatory, hepatoprotective (Solanki and Jain, 2011), antihyperlipidemic (Solanki and Jain, 2010) and immunoinhibitory activities. The purpose of this study was to screen for the organic solvent extracts of *C. ternatea* that could be useful for the development of new tools as antimicrobial agents for the control of infectious diseases.

MATERIALS AND METHODS

Plant material

Clitoria ternatea plants were brought from the bank of river of Kollidam in Tiruchirappalli, Tamilnadu and its identity was confirmed by Botanical Survey of India (Southern Circle), Coimbatore, Tamilnadu, India.

Preparation of extracts

The dried leaves of *Clitoria ternatea* were powdered and sieved through a 40-mesh screen. The fine powder was stored in air tight containers and left in the refrigerator. Fifty grams of leaf material was soaked in 250 ml (methanol, ethyl acetate and petroleum ether) for 24 hours and filtered using standard filter paper. The filtrate was transferred into vials and allowed to evaporate until completely dry.

Antibacterial activity

The antimicrobial test was performed by following agar disc diffusion method (Maruzella and Henry, 1958) and well diffusion method (Perez *et al*., 1990) using Mueller Hinton Agar No. 2 medium. Microbial growth was determined by measuring the diameter of the inhibition zone (SD ± Mean).

RESULTS AND DISCUSSION

Many medicinal plants have been found effective in the cure of bacterial diseases. Due to increasing antibiotic resistance in microorganisms and side effects of synthetic antibiotics medicinal plants are now gaining popularity in the treatment of bacterial infections. Medicinal plants are considered as clinically effective and safer alternatives to the synthetic antibiotics. India has rich heritage of using medicinal plants in traditional medicines such as *Ayurveda, Siddha, Unani* besides folklore practices. The earliest mention of the medicinal uses of plants found in the Rigveda which is one of the oldest repositories of human knowledge.

The results showed that methanolic extract affected the activity of *Bacillus cereus* to a greater extent followed by *Klebsiella pneumoniae, Proteus vulgaris* and *Salmonella typhi* (Table -1). Petroleum ether extract affected the activity of *Salmonella typhi* to a great extent followed by *Proteus vulgaris, Bacillus cereus* and *Klebsiella pneumoniae.*

Table 1. Antibacterial activity of leaf extracts of *Clitoria ternatea* using different solvents by Disc diffusion method

Microorganisms	Extracts	Zone of Inhibition (cm) (Mean ± SD)
Bacillus cereus	Methanol	1.2 ± 0.8
	Petroleum ether	0.3 ± 0.1
	Ethyl acetate	0.1 ± 0.0
	Streptomycin	3.0 ± 0.8
Klebsiella pneumonia	Methanol	0.8 ± 0.2
	Petroleum ether	-
	Ethyl acetate	0.1 ± 0.0
	Streptomycin	2.0 ± 0.7
Proteus vulgaris	Methanol	0.1 ± 0.0
	Petroleum ether	1.0 0.3
	Ethyl acetate	-
	Streptomycin	0.1 ± 0.0
Salmonella typhi	Methanol	0.1 ± 0.0
	Petroleum ether	0.8 ± 0.3
	Ethyl acetate	0.6 ± 0.2
	Streptomycin	3.5 ± 0.9
Stapylococcus aureus	Methanol	0.2 ± 0.1
	Petroleum ether	-
	Ethyl acetate	-
	Streptomycin	2.0 ± 0.1

There was no inhibition zone in *Klebsiella pneumoniae*. Similar kind of result was made by Jeyachandran *et al.* (2003) in *Tinospora cordifolia* plant extract. Ethyl acetate extract affected the activity of *Salmonella typhi* with high range followed by *Bacillus cereus, Proteus vulgaris* and *Klebsiella pneumoniae*. There was no inhibition zone for *Proteus vulgaris* (Table - 1). Streptomycin (synthetic antibiotics) was maintained as a control.

For the Agar well plate method Streptomycin (antibiotic disc) was maintained as a control. The result showed the methanol extract induced high range of inhibition zones in *Proteus vulgaris* followed by *Klebsiella pneumonia, Bacillus cereus*, *Salmonella typhi* and *Staphylococcus aureus* (Table - 2). The result showed the petroleum ether extract induced the high range of inhibition zone in *Salmonella typhi* and *Proteus vulgaris*followed by *Bacillus aureus, Klebsiella pneumoniae* and *Staphylococcus aureus* have no inhibition zones. The result showed the ethyl acetate extract induced the high range of inhibition zones in *Staphylococcus aureus* and *Salmonella typhi*. Followed by *Proteus vulgaris, Bacillus cereus* and *Klebsiella pneumoniae* Jeyachandran and Anand (2005) reported same kind of observation in *Tinospora cordifolia* (Table - 2).

Table 2. Antibacterial activity of leaf extracts of *Clitoria ternatea* using different solvents by Well diffusion method

Microorganisms	Extracts	Zone of Inhibition (cm) (Mean ± SD)
Stapylococcus aureus	Methanol	0.2 ± 0.1
	Petroleum ether	-
	Ethyl acetate	0.8 ± 0.2
	Streptomycin	2.5 ± 0.6
Klebsiella pneumonia	Methanol	0.6 ± 0.3
	Petroleum ether	-
	Ethyl acetate	0.2 ± 0.4
	Streptomycin	2.5 ± 0.9
Proteus vulgaris	Methanol	1.0 ± 0.4
	Petroleum ether	0.3 ± 0.1
	Ethyl acetate	0.1 ± 0.0
	Streptomycin	2.0 ± 0.5
Salmonella typhi	Methanol	0.1 ± 0.0
	Petroleum ether	1.0 ± 0.2
	Ethyl acetate	0.2 ± 0.3
	Streptomycin	3.0 ± 0.7
Bacillus cereus	Methanol	0.2 ± 0.1
	Petroleum ether	-
	Ethyl acetate	0.1 0.0
	Streptomycin	2.5 ± 0.2

Amongst the gram positive and gram negative bacteria, gram positive bacterial strains were more susceptible to the extracts when compared to gram negative bacteria. This may be attributed to the fact that these two groups differ in their structure of the cell wall components. All the extracts showed varying degrees of antimicrobial activity on the microorganisms tested. Some of these crude extracts were more effective than traditional antibiotics to combat the pathogenic microorganisms studied. The chance to find antimicrobial activity was more apparent in methanol than other extracts. Further work is needed to isolate the secondary metabolites from the extracts studied in order to test specific antimicrobial activity.

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