ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES Knowledge to Innovation

Vol 7, Suppl 1, 2014

ISSN - 0974-2441

Research Article

EVALUATION OF ANTIMICROBIAL POTENTIALITY OF 50% AQUEOUS ETHANOLIC LEAF EXTRACT OF *CLITORIA TERNATEA* L.

NEELA DAS, PADMA CHATTERJEE*

Pant Physiology, Biochemistry and Advanced Molecular Biology Laboratory Department of Botany, University of Kalyani, Kalyani, Nadia PIN-741235, West Bengal, India. Email: schatterjeecal2003@yahoo.co.in

Received: 26November 2013, Revised and Accepted:25 December 2013

ABSTRACT

Objectives: To study the antimicrobial property of 50% aqueous ethanolic leaf extract of *Clitoria ternatea* L. against few micro organisms.
Method: The leaves of *Clitoria* were sequentially soaked in petroleum ether (60-80^{0}C), chloroform, benzene and 50% aqueous ethanol, extracts were collected, filtered and concentrated. Antimicrobial potentiality of the extracts were tested against few micro-organisms.
Result: *Clitoria ternatea* L. exhibited antifungal effect against *Fusarium oxysporum ciceri* and antibacterial activity against *Serratia marcescens* and *Arthrobactor chlorophenolicus.*
Conclusion: Hence the plant leaf extract can be used as antimicrobial agent against the micro organisms.

Keywords: phytochemical products. antimicrobial property, bioassay.

INTRODUCTION

Medicinal plants are natural resources yielding valuable phytochemical products, which are often used in the treatment of various diseases. A substantial part of the population in developing countries, use folk medicines for their daily healthcare [1]. Some of the traditional medicine involves the use of crude plant extracts, which may contain an extensive diversity of molecules, often with indefinite biological effects [1]. However, most of the information available to the consumer with regard to the medicinal herbs is not backed by credible scientific data. For this reason, research is carried out, to determine the toxicity of medicinal plants.

Clitoria ternatea L. commonly known as Butterfly pea belonging to the family Fabaceae and sub-family Papilionaceae is a perennial leguminous twinner, which originated from tropical Asia and later was distributed widely in South and Central America, East and West Indies, China and India, where it has become naturalised. It is also commonly called as *Clitoria*, blue-pea, kordofan pea (Sudan), cunha (Brazil or pokindong (Philippines) and is a vigorous, summer growing, legume of old world origin. The mostly frequently reported species is *C. ternatea* L.

The major phyto-constituents found in the plant are the pentacyclic triterpenoids such as taraxerol and taraxerone.[2],[3].

The seeds contain nucleoprotein with its amino-acid sequence similar to insulin, delphinidin-3,3,5-triglucoside, essential amino-acids, pentosan, water soluble mucilage, adenosine, an anthoxanthin glucoside, greenish yellow fixed oil [4],[5], a phenol glycoside, 3,5,7,4-tetrahydroxy-flavone-3-rhamoglycoside, an alkaloid , ethyl D-galactopyranoside, p-hydroxy cinnamic acid polypeptide, a highly basic protein-finotin, a bitter acid resin, tannic acid, 6% ash and a toxic alkaloid. According to Yoganarasimhan seeds contain ß-sitosterol, and hexacosanol and anthocyanin glucoside [6] [7,8,9]. It also contains anti-fungal proteins and has been shown to be homologous to plant defensins [10]. Since the purified lectin was found to be potential tool for cancer studies so an attempt was made for the alternate high yielding purification method for *Clitoria ternatea* lectin designated CTL, present in the seeds of this member of leguminosae family [11,12].

From ancient times “Shankhpushpi” is known as reputed drug of Ayurveda and reported as a brain tonic, nervine tonic and laxative. It is considered as a Medhya-Rasayana in Ayurvedic texts. It comprises of entire herb with following botanicals viz *Convolvulus pluricaulis* (Convolvulaceae), *Evolvulus alsinoides* (Convolvulaceae), *Clitoria ternatea* (Papilionaceae) and *Conscora decusata* (Gentianaceae) those are used for treatment of neurological disorders [13].

The roots are being used as diuretic and seeds as cathartic [14]. In the traditional system of medicine particularly in Ayurveda, the roots, seeds and leaves of *C. ternatea* have long been widely used as a brain tonic and is believed to promote memory and intelligence. *C. ternatea* petals have been recognized to possess anti-oxidant activity.

Thus this plants contains a wide varieties of phytochemical constituents ranging from nucleoprotein to terpenoids, from flavonoids to alkaloids. However the claim that safe usage of *C. ternatea* L. *leaf* extract in folk medicine is unsubstantiated by scientific studies. Hence, the current study has been undertaken to investigate the antimicrobial property of 50 % aqueous ethanolic leaf extract of *C. ternatea* L. against few under mentioned microorganisms.

MATERIALS AND METHODS

Preparation of plant extract

100 gms of dried leaves of each plant were grounded into fine powder and was sequentially soaked into petroleum ether, chloroform, benzene and 50% aqueous ethanol for 7 days each in room temperature. The extracts were filtered, collected and condensed under reduced pressure. Dark residual solid were collected from each extract which were then subjected to antifungal and antibacterial bioassay.

Preparation of sample solution

The test solutions were prepared by dissolving the dark residual masses in few drops of propylene glycol and then diluting with sterile water in the concentration of 60 µg/ml. Few drops of propylene glycol diluted with sterile water was used as control. All the dilution was sterilized by filtration using membrane filter (0.02 µ pore size) in the laminar air flow.

Table 1: represents the names and characteristics of the micro organisms used

Name	Growth medium	Growth Condition	Temp. (^{0}C)	Incubation time (hrs)	Sub Culture (month)	Special features
Bacterial strains						
Serratia marcescens	No. 3	aerobic	30	48	2	-
Erwinia herbicola	No. 74	aerobic	37	24	1	-
Xanthomonas sp.	No. 3	aerobic	30	48	2	-
Arthrobactor chlorophenolicus	No. 3	Aerobic	28	48	1	Type strain, Degrades 4-chlorophenol
Fungal strains						
Botrytis cineria	PDA	aerobic	38	2	3	Produces hyphal mat
Fusarium oxysporum	PDA	aerobic	28	5-7	3	Produces macro and microconidia
Rhizoctonia solani	PDA	aerobic	32	2-3	3	Produces dark brown sclerotia
Aspergillus flavus	PDA	Aerobic	28	1-2	3	Type strain degrades 4-chloro- phenol

Preparation of media:

Growth media no.3:

Beef extract	1.0 gm
Yeast extract	2.0 gm
Peptone	5.0 gm
NaCl	5.0 gm
Agar	15.0 gm
Distilled water	1.0 Lt.

Growth media no.74:

Tryptone	10.0 gm
Yeast extract	5.0 gm
NaCl	10.0 gm
Distilled water	1.0 Lt.

Potato Dextrose Agar (PDA) medium:

Peeled potato	250 gms
Agar	20 gm
Dextrose	20 gms
Distilled water	1 lt., pH 6.8-7

Determination of antimicrobial activity of the crude leaf extracts of the plant by bioassay method:

Antibacterial assay by cup diffusion method: [15]

The bactericidal assay was done with the above prepared test solution following agar cup diffusion method of with certain modification.

Concentration of the bacterial culture used in the bioassay experiment was adjusted to 1×10^6 cfu /ml. Sugar tubes containing molten agar (10 ml) were sterilized and cooled to about 40-42^0 C.

The tubes were inoculated with 0.1 ml of the appropriate culture suspension of each bacterium, mixed gently and poured onto previously solidified nutrient agar plates. Wells were bored in each plate (9 cm diameter) with an aseptic cork borer of 7 mm when the seeded plates had solidified. With the aid of a micropipette each of the wells was filled with 0.2 ml of test solution and allowed to diffuse. A control plate was maintained by adding propylene glycol diluted with sterile water in the well only. The petridishes were sealed with a strip of parafilm and incubated at the required temperature and duration.

Antifungal assay by cup diffusion method: [16]

4-5 days old cultures of the fungal sps. were used for the bioassay experiments. Fungal suspension was prepared in such a way that the fungal concentration would be approximately 1×10^6 cfu/ml. An overnight broth culture was used as inoculums on sterile molten PDA medium. Small wells were bored in each plate (9 cm diameter) with an aseptic cork borer of 7 mm when the seeded plates had solidified. With the aid of a micropipette each of the wells was filled with 0.2 ml of test solution and allowed to diffuse. A control plate was maintained by adding propylene glycol diluted with sterile water in the well only. The petridishes were sealed with a strip of parafilm and incubated at the required temperature and duration.

Measurement:

After incubation the diameter of inhibition zone around the well was measured in cm. Antimicrobial studies were done in triplicates and diameters of zones of inhibition (cm) were expressed as means and standard errors of means.

RESULT AND DISCUSSION

Table 2: Screening of antimicrobial activity of different solvent fractions collected from *Clitoria ternatea* L. against the microbial strains selected:

Plant selected	Fractions	*Bacterial strains*			
		Serratia marcescens	*Erwinia herbicola*	*Xanthomonas sp.*	*Arthrobactor chlorophenolicus*
Clitoria ternatea L.	Pet. Ether	-	-	-	-
	Chloroform	-	-	-	-
	Benzene	-	-	-	-
	50% aq. ethanol	+	-	-	+
	Fractions	*Fungal strains*			
		Botrytis cinera	*Fusarium oxysporum ciceri*	*Rhizoctonia solani*	*Aspergillus flavus*
Clitoria ternatea L.	Pet. Ether	-	-	-	-
	Chloroform	-	-	-	-
	Benzene	-	-	--	-
	50% aq. ethanol	-	+ (2.21 cm)	-	-

Pet. Ether = petroleum ether(boiling point 60-80^0 C), 50% aq. Ethanol = 50% aqueous Ethanolic extract.

C. ternatea L. is a plant rich in wide range of phytochemical compounds. The data on table 2 exhibited that 50% aqueous ethanolic leaf extract of *Clitoria ternatea* L. possessed the antibacterial property against *Serratia marcescens, Arthrobactor chlorophenolicus* and antifungal property against *Fusarium oxysporum.*

CONCLUSION

It showed most promising antifungal and antibacterial effect against *Fusarium* and *Serratia marcescens, Arthrobactor chlorophenolicus* respectively. This study presents valuable data on antimicrobial property of *C. ternatea* L. leaf extract, which should be very useful for clinical study of this plant leaf extract.

ACKNOWLEDGMENTS

The authors express heartfelt gratitude towards the Department of Botany, University of Kalyani for providing laboratory facilities and University Grant Commission for financial assistance.

REFERENCES

1. Alkofahi A, Batshoun R, Owais W and Najib N. Biological activity of some Jordanian, medicinal plant extracts. Fitoterapia 1996; 68(5): 435.
2. Banerjee SK, Chakravarti RN, Taraxerol from Clitoria ternatea, Bull Calcutta School Trop Med, 1963; 11: 106-107.
3. Banerjee SK, Chakravarti RN, Taraxerone from Clitoria ternatea, Bull Calcutta School Trop Med, 1964; 12: 23.
4. Joshi SS, Shrivastava RK, Shrivastava DK, Chemical examination of *Clitoria ternatea* Seeds, Journal of American Oil Chemical Society, 1981; 58(6): 714-715.
5. Tiwari, RD., Gupta, RK., Chemical examination of the oil from the seeds of *Clitoria tematea* Linn. Journal of Oil Technology Association of India 1957;13: 9–13.
6. Yoganarasimhan SN, Medicinal Plant of India, Bangalore, India, Interline Publishing Co, 2000; 2: 146-147.
7. Sinha A, Studies on the unsaponifiable matter of the seeds of Clitoria *ternatea* Linn. And isolation of $^{\gamma}$sitosterol, Proceedings of the National Academy of Sciences, 1960; 29: 23-26.
8. Sinha, A. Studies on the unsaponifiable matter of the seeds of *Clitoria tematea* Linn. and isolation of -sitosterol. Proceedings of the National Academy of Sciences of India 1960; 29: 23–26.
9. Sinha, A. Mucilage from the leaves of *Clitoria ternatea*. Proceedings of the Institution of Chemists, India1960; 32:, 228–231.
10. Osborn RW, Samblanx GW. De, Thevissen K, Goderis I, Torrekens S, Van Leuven F, Isolation and characterisation of plant defensins from seeds of Asteraceae, Fabaceae, Hippocastanaceae and Saxifragaceae, FEBS Letters, 1995; 368: 257-262.
11. Naeem A, Ahmed E, Khan RH, An alternate high yielding purification method for *Clitoria ternatea* lectin, International Journal of Biological Macromolecules, 2007; 41: 481-486.
12. Naeem A, Haque S, Khan RH, Purification and Characterization of a Novel ß-D-Galactos I d es - Specific Lectin from *Clitoria ternatea*, The Protein Journal, 2007; 26(6): 403.
13. Girish Kumar Gupta , Jagbir Chahal , Manisha Bhatia , *Clitoria ternatea (L.)*: Old and new aspects, Journal of Pharmacy Research 2010; 3(11):2610-2614
14. Parimaladevi, B., Boominathan, R., Mandal, SC. Anti-inflammatory, analgesic and antipyretic properties of *Clitoria ternatea* root. Fitoterapia 2003;74: 345–349.
15. Anon. *Pharmacopiea* of India. 1996; 3rd Edition. Govt. of India, India, Ministry of Health and Family Welfare.
16. Kordali S., Kotan R., Mavi A. and Cakir A. Determination of the chemical composition and anti oxidant activity of the essential oil of *Artemisia dracunculus* L. and of the antifungal and antibacterial activities of Turkish *A. dracunculus, A. absinthium* and *santonicum* essential oil. J. Agric. Food Chem. 2005; 53:9452-9458