Evalution of Phytochemical and Antimicrobial Activity of Flowers of Clitoria Ternatea

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***Abstract*--**Cell damage caused by free radicals appears to be a major contributor to aging and to degenerative diseases such as cancer, cardiovascular disease, cataracts, and immune system decline and brain dysfunction. Plants have long been used in the treatment of cancer. The anti-cancer cells either kill cancer cells or modify their growth. The present investigation was undertaken to find out the unexplored cytotoxic effect of the flower Clitoria ternatea. All parts of Clitoria ternatea are useful for medical treatments and have been used in folk medicines and for curing different diseases. The primary objectives were to determine the Characterization of the Clitoria ternatea flower based on the phytochemical, antioxidant, antibacterial and antifungal activities. Twenty-five gram of air dried powdered samples was extracted with 300 ml of Solvent like Methanol using Soxhlet apparatus for ten hours of time. The extract was condensed using rotary evaporator. After condensation the samples were reconstituted in their respective solvents to obtain a stock of 100mg/ml and were stored in a refrigerator. Antioxidant activity assay is based on the reduction of 1,1-diphenyl-2-picrylhydrazyl (DPPH).Due to the presence of an odd electron it gives a strong absorption maximum at 517nm. Antibacterial activity was then followed using 5 microorganisms (E.coli, Staphylococcus aureus, Bacillus spp., Salmonella typhi and Pseudomonas aeruginosa).The Assay was performed by agar disc diffusion method. Then Antifungal activity assay was carried using (Candida albicans, Aspergillus niger, Rhizopus spp. and Trichoderma viride).Phytochemical analysis showed the presence for Tannins,saponins,flavonoids,proteins,steroids and anthroquinones. All values obtained are acquired from UV spectrophotometer/colorimeter for assays.

Keywords: *Clitoria ternatea, Phytochemical screening, DPPH Scavenging activity,Antimicrobial Activity,Flower extract.*

I. INTRODUCTION

Medicinal plants are the alternative remedies with incredible healing properties. Medicinal plants have pharmacological activities and thus may be a source for novel anti-tumor agents. The mechanism of action of anti-tumor agents include anti-proliferate and anti-oncogenic effects, induction of apoptosis, oxidative stress, oncogenes and tumor suppressor genes[1].Extracts of medicinal plants are believed to contain different chemo-preventive or chemotherapeutic compounds [2].Plants are the source of medication for preventive, curative ,protective or promote purposes [3].

Clitoria ternatea (Linn), commonly known as Shankupushpam, also known as 'Butterfly pea' or 'Aprajita'belongs to the plant family Fabaceae and is propagated through seeds. It is a perennial twinning herb with blue and white flowers [4].C.ternetea is widely used in traditional systems of medicines as a brain tonic [5]. Secondary metabolites act as a defence mechanism. Plants secondary metabolites yields products that promote in the growth and development of plants but these are not required by the plants. Flavonoids are one class of secondary metabolites. Antibiotics have been re-generated because the old ones are being resisted by the existing pathogens. Hence there is a need to develop new antibiotic which can be helpful for curing new emerging diseases. Plants are the natural and good resource of antibiotics with therapeutic potential.Some chemical substances are present in the plants which are beneficial for the human body,commonly known as Phytochemicals [6].The phytochemical investigations revealed the presence of phytosterols, phenolic compound, flavonoids and carbohydrates[7].Clitoria ternatea is the medicinal plant with all the secondary metabolites[8]. Therefore, an effort is made to contribute to establish scientific evidence in this regard.

II. MATERIALS AND METHODS

A. Collection of Sample

The plant samples (Flower) were collected which weighs 1kg.

B. Preparation of extracts

Twenty-five gram of air dried powdered samples was extracted with 300 ml of Solvent like Methanol using Soxhlet apparatus for ten hours of time. The extract was condensed using rotary evaporator. After condensation the samples were reconstituted in their respective solvents to obtain a stock of 100mg/ml and were stored in a refrigerator. The extract was used for the Qualitative phytochemical, antioxidant and antimicrobial studies.

III. I-PHYTOCHEMICAL ANALYSIS

A. TANNINS

1 ml of sample was taken, to that few drops of 0.1% ferric chloride was added and observed for blue colourization/brownish green.

B. SAPONINS

1 ml of sample was taken, to that 2 ml of H2O (shaken vigorously) was added and observed for foaming appearance.

C. FLAVONOIDS

1 ml of sample was taken, to that concentrated HCl and magnesium chloride was added and observed for pink tomoto red colour.

D. ALKALOIDS

1 ml of sample was taken, to that few drops of dragandoff reagent was added and observed for orange red colour.

E. PROTEINS

1 ml of sample was taken, to that few drops of Bradford reagent was added and observed for blue color development.

F. STEROIDS

1 ml of sample was taken; to that 10% concentrated H2SO4 was added and observed for green color.

G. ANTHRAQUINONES

1 ml of sample was taken, to that aqueous ammonia (shaking) was added and observed for change in colour of aqueous layer (pink, red or violet).(Table 1)

IV. ANTIOXIDANT ASSAY

A. II-DPPH free radical scavenging:

The ability of the extract to scavenge DPPH radical was determined by the method described by Naznin Araand HasanNur (2009).DPPH radical scavenging activity of various phenolics like, resveratrol, guercetin, myricetin, catechin, fistein, kaemferol, ellagic acid and naringenin at differentconcentrations were compared with respect to trolox and tocopherol. Quercetin, myricetin were found to have the strongest antiradical activity. Each molecule of quercetin and myricetin scavenged 10molecules of DPPH; catechin and fistein scavenged 5 molecules; resveratrol scavenged 3.6 molecules and ellagic could scavenge 3.3 molecules of DPPH. Quercetin, myricetin, catechin, fistein, resveratrol and ellagic acid were stronger than trolox and tocopherol which scavenged 2 molecules of DPPH. Kaemferol and naringenin were found to be the weakest antiradicals, which scavenged 1.7 and0.5 molecules of DPPH per molecule respectively.

B. REAGENTS

DPPH-1mg in methanol

BHT (standard)-1.6mg/ml in methanol

Samples-desired concentration from 1mg/ml –max of 5mg/ml (in methanol/DMSO)

C. PROCEDURE

Aliquot 3.7 ml of absolute methanol in all test tubes along with blank. Then add 100µl of absolute methanol to blank. Add 100µl of BHT to tube marked as standard and 100µl of respective samples to all other tubes marked as tests. Then, finally add 200µl of DPPH reagent to all the test tubes at room temperature add condition for minimum of 30minutes then, check absorbance of all samples 517nm.(Table 2)

S.NO	REAGENT	BLANK	STANDARD	TEST
1	Ethanol	3.8ml	3.7ml	3.7ml
2	BHT	_	100 µl	_
3	Sample	_	_	100 µl
4	DPPH	200µl	200µl	200µl
Incubation at dark for 30 mins				
OD at 517 nm				

D. DATA ACQUISITION &CALCULATION:

All values obtained are acquired from UV spectrophotometer/colorimeter for assays.

$$\%\text{Antioxidant activity.} = \frac{(\text{absorbance at blank}) - (\text{absorbance at test})}{(\text{Absorbance at blank})} \times 100$$

E. ANTIMICROBIAL ACTIVITY:

1) III-ANTIBACTERIAL ACTIVITY ASSAY:

Stock cultures were maintained at 4°C on Nutrient agar Slant. Active cultures for experiments were prepared by transferring a loop full of culture from the stock cultures into the test tubes containing nutrient broth , that were incubated at 24hrs at 37ºC.

The Assay was performed by agar disc diffusion method (Table 3).

F. AGAR DISC DIFFUSION METHOD:

Antibacterial extracts was determined by disc diffusion method on Muller Hinton agar (MHA) medium. Muller Hinton Agar (MHA) medium is poured into

The petriplate. After the medium was solidified, the inoculums were spread on the solid plates with sterile swab moistened with the bacterial suspension. The discs were placed in MHA plates and added 20 µl of sample were placed in the disc .The plates were incubated at 37ºC for 24 hrs. Then the antimicrobial activity was determined by measuring the diameter of zone of inhibition. Fig 1(1.1, 1.2, 1.3, 1.4, 1.5)

G. IV-ANTIFUNGAL ACTIVITY ASSAY:

Stock cultures were maintained at 4°C on Sabouraud Dextrose agar Slant. Active cultures for experiments were prepared by transferring a loop full of culture from the stock cultures into the test tubes containing Sabouraud Dextrose broth , that were incubated at 48hrs at 37ºC.

Antifungal extracts was determined by disc diffusion method on Sabouraud Dextrose agar (SDA) medium. Sabouraud Dextrose agar (SDA) medium is poured in to the petriplate. After the medium was solidified, the inoculums were spread on the solid plates with sterile swab moistened with the fungal suspension. The disc were placed in SDA plates and added 20 µl of sample (Concentration: 1000µg, 750µg and 500 µg) were placed in the disc .The plates were incubated at 37ºC for 24 hrs. Then the antimicrobial activity

was determined by measuring the diameter of zone of inhibition.Fig. 2(2.1,2.2,2.3,2.4).

NO.	PHYTOCHEMICALS	RESULT
1.	Tannins	Presence
2.	Flavanoids	Presence
3.	Saponins	Presence
4.	Proteins	Presence
5.	Steroids	Presence
6.	Alkaloids	Absence
7.	Anthroquinones	Presence

TABLE 2:ANTIOXIDAND ASSAY (DPPH)

S.NO	SAMPLE	O.D				DPPH activity (%)
		I	II	III	Average	
1	Sample	0.29	0.29	0.28	0.286	70.2
2	Standard-BHT	0.002	0.002	0.002	0.002	99.7

V. RESULTS

The phytochemical screening showed that it was rich in phenols, flavonoids followed by tannins, reducing sugars, and anthraquinones.(Table-1)

TABLE 1: QUALITATIVE PHYTOCHEMICAL PROPERTIES OF THE SAMPLE

Blank O.D: 0.96

TABLE 3: ANTIBACTERIAL EFFECT

Organisms	Zone of Inhibition (mm) Concentration(µg/ml)			Antibiotic (1mg/ml)
	1000	*750*	*500*	
Staphylococcus aureus	20	15	-	25
Bacillus spp.	16	15	12	39
E. coli	16	15	14	25
Salmonella spp.	30	28	19	34
Pseudomonas aeroginosa	20	19	15	40

TABLE 4: ANTIFUNGAL EFFECT

Organisms	Zone of Inhibition (mm) Concentration(µg/ml)			Antibiotic (1mg/ml)
	1000	750	500	
Candida albicans	11	8	8	13
Aspergillus niger	8	7	-	9
Trichoderma viride	9	8	-	10
Rhizopus spp.	9	7	6	11

A. Antibacterial Result:

Fig 1 (1.1)

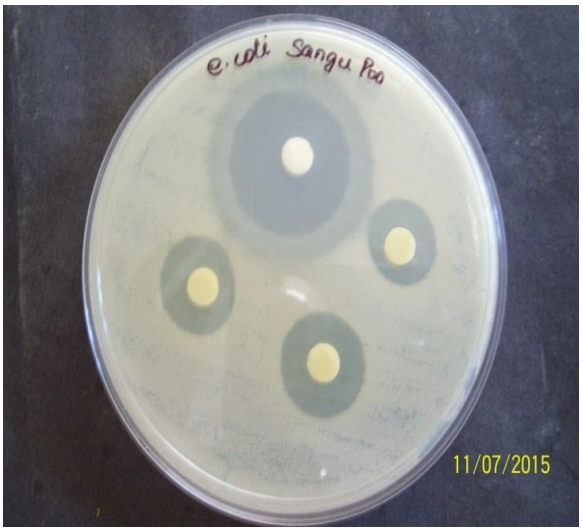

Fig 1 (1.2)

Fig 1 (1.3)

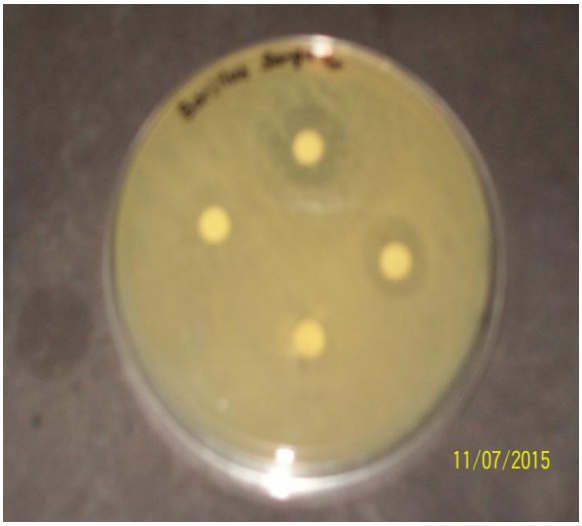

Fig 1 (1.4)

Fig 1 (1.5)

B. Antifungal Result:

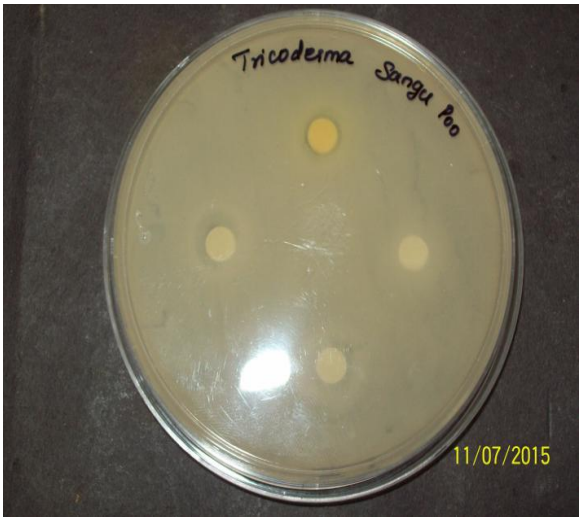

Fig 2(2.1)

Fig 2(2.2)

Fig 2(2.3)

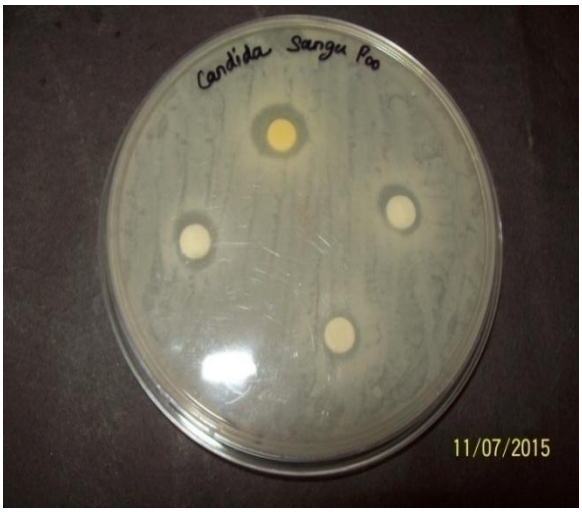

Fig 2(2.4)

VI. DISCUSSION

Phytochemical screening of medicinal plants is very important in identifying new sources of therapeutical and industrial importance [9].Plant extracts and their isolated constituents have always been an important part of various therapeutic systems [10]. Recent technological advances and efforts should be made towards isolation and characterization of the active principles. Herbs are found all over the world with natural chemical compounds and can be used for future studies. The use of various parts of the medicinal plants helps to decrease the cost of medication and also be made locally available with lesser side effects as compared to synthetic drugs. In this present study, preliminary screening for antimicrobial activity showed more zone of inhibition than any other. Results obtained from this study indicate that the flower of CT showed the strongest antimicrobial activity than the commercially available antibiotics.Many medicinal plants have been analyzed and reported for their DPPH scavenging activity.The DPPH scavenging ability of medicinal plants has been attributed to several components ,including phenolics[11].flavonoids[12]. The present study thus scientifically validates and strengthens the candidature of Clitoria ternatea in the preparation of medicinal aids to combat the wide spectrum of diseases.

VII. CONCLUSION

The flower of Clitoria ternatea was identified for their Phytochemical activity and was tested for free radical scavenging activity against DPPH. The flower extracts showed strongest antimicrobial activity than normal antimicrobes. The study conducted with the flower of Clitoria ternatea revealed different parameters that will be useful in scientific evaluation, identification and authentication of the drugs.

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