

Original Research



## PHYTOCHEMICAL INVESTIGATIONS AND IN VITRO EVALUATION OF *CLITORIA TERNATEA* LEAF EXTRACTS FOR ANTIMICROBIAL AND ANTIOXIDANT PROPERTIES.

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### ABSTRACT:

**Purpose:** The present investigation evaluates the antimicrobial and antioxidant potential of *Clitoria ternatea* (CT), which is a rich ethnomedicinal plant with wide range of medicinal properties confirmed through literature reviews.

**Methods:** The preliminary extraction of the dried leaves with various solvents (Petroleum ether, Dichloromethane (DCM), Ethylacetate (EA) and n-Butanol (NB)), followed by phytochemical screening, antimicrobial and antioxidant activities. The antimicrobial susceptibility studies were conducted against gram (-) and gram (+) bacteria. The antioxidant activity was performed through reducing power, ferric thiocyanate and thiobarbituric acid methods.

**Results:** The antimicrobial activity shows good result for butanolic fraction (zone of inhibition 14-20 mm and MIC 1-6 mg/ml). The antioxidant activity of butanolic extract of *C. ternatea* (BECT) shows good profile for total phenolic content (24.78 mg GAE/g MM), absorbance in reducing power method (1.89 nm), percentage inhibition for ferric thiocyanate method (79.97%) and thiobarbituric acid method (74.46%) respectively.

**Conclusion:** The current result supports the medicinal use of the leaf which acts as an antimicrobial and antioxidant agent. The present work has the nobility as no such work has been done with the above plant.

**Keywords:** Fabaceae, Blue Pea, lincomycin, BECT.

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**INTRODUCTION:**

The World Health Organisation explains that largest fractions of the people living in developing countries almost exclusively use traditional medicine. Medicinal plants form the principle component of traditional medicine. Medicinal plants used in traditional medicine should therefore be studied for safety and efficacy. Plants have the ability to produce a wide variety of chemical compounds that are used to perform important biological functions. Many of these phytochemicals have useful effects on long-term health when consumed by humans, and can be used to effectively treat human diseases. At least 14,000 such compounds have been isolated so far; a number estimated to be less than 15% of the total. Chemical compounds in plants mediate their effects on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs but with fewer side effects. This enables herbal medicines to be as effective as conventional medicines<sup>1</sup>.

The synergistic medicinal effect of the plant material typically results from the combination of secondary products present in the plants. In plants these compounds are mostly secondary metabolites such as alkaloids, steroids, tannins and phenol compounds. Many physiological and biochemical processes in the human body may produce oxygen-centered free radicals and other reactive oxygen species as by products. Over production of such free radicals can cause oxidative damage to biomolecules (e.g. lipids, proteins, DNA), eventually moving to many chronic diseases, such as atherosclerosis, cancer, diabetes, aging, and other degenerative diseases in humans. Plants (fruits, vegetables, medicinal herbs, etc.) and products made from plants may contain a wide variety of free radical scavenging molecules, such as phenolic compounds (e.g. phenolic acids, flavonoids, quinones, coumarins, lignans, stilbenes, tannins), nitrogen compounds (alkaloids, amines, betalains), vitamins, terpenoids (including carotenoids), and some other endogenous metabolites, which are rich in antioxidant activity. Epidemiological studies have shown that many of these antioxidant compounds possess anti-inflammatory, antiatherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial, or antiviral activities to a greater or lesser extent.

Flavonoids, steroids, resins, fatty acid and gums which are capable of producing definite physiological action on body. Many flavonoids are found to be strong free radical scavenger and antioxidants. The progress of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including plants. Screening of medicinal plants for phytochemicals, antimicrobial and antioxidant activity is important for finding potential new compounds for therapeutic use. Phenolic compounds have received considerable attention due to their beneficial effects on human health, such as a protective action against chronic degenerative diseases, cancer and cardiovascular diseases<sup>2</sup>.

In the present study of *Clitoria ternatea* species which belongs to the family fabaceae and are used in traditional folklore medicine to cure various ailments in Africa, Asia and Latin America. *Clitoria ternatea* is commonly called Aparajita, Blue pea etc. Previous studies have reported that the plant exhibits bioactive activities for fever, mouth infections, jaundice, guinea worm sores, cancer and joint rheumatism. This study was designed to examine antimicrobial activity and phytochemical screening of various extracts and antioxidant activity of n-butanolic extract of leaves of *Clitoria ternatea*. Successive extraction of the plant material was carried out using various organic solvents on the polarity basis (Petroleum ether, dichloromethane, ethylacetate and n butanol). The resulting extracts were subjected against some Gram-positive and Gram-negative bacterial strains by following disc diffusion methods. Lincomycin was taken as standard antibiotic to compare the results at a concentration between 0.5mg/ml and 10 mg/ml. Since leaves contain flavonoids an attempt has been made to evaluate antioxidant potential of the extracts from the leaves of *Clitoria ternatea* by reducing power (RP), thiobarbituric acid (TBA) and ferric thiocyanate (FTC) methods<sup>3</sup>.

**MATERIALS AND METHODS:****Collection and Identification of Plant Material**

The leaves of *Clitoria ternatea* (Fabaceae) were collected (during March 2012) from SIRD (State Institute of Rural Development, Kahikuchi, Guwahati, India.) and identified by Dr. G.C Sarma (Department of Botany, Gauhati University, India).

The voucher specimen (Acc.No: 17777) were deposited in the Departmental herbarium for future reference.

### Extraction of Plant material

#### Successive Extraction

Fifty grams (50g) of the dried leaf powder was exhaustively defatted with petroleum ether (60-80°) and successively extracted with dichloromethane, ethylacetate and then finally n-Butanol using the soxhlet extractor. All the extracted portions were concentrated in rotary vacuum evaporator and the concentrated brown mass which weighed 5.032 g equivalent to 10.06% (w/w) for DCM, 1.92 g equivalent to 3.84%(w/w) for ethyl acetate and 4.32 g equivalent to 8.64% (w/w) for n- Butanol. Each extracts were coded as DCM (Dichloromethane extract),EAE (ethyl acetate extract) and NBE (n-Butanol extract) respectively and was kept aseptically until use.

#### Phytochemical screening

The phytochemical screening of the sample was carried out as described by Herbune and Sofowora. The sample was screened for alkaloids, flavonoids, saponins, carotinoids, carbohydrates, glycosides and tannins.

#### Microorganisms

The species of bacterial & fungal organisms were *S.aureus*, *E.coli*, *K. pneumoniae*, and *Candida albicans*. They were clinical isolates obtained from Microbiology Department of Jaduvpur University, Kolkata. The cultures of bacteria were maintained on nutrient agar slants at 4°C in GIPS microbiology laboratory, re-identified by biochemical tests (Cheesbrough,1982; Cowan and Steel, 2004) and sub-cultured on to nutrient broth for 24 h prior to testing<sup>4</sup>.

#### Preparation of impregnated discs of extract and standard antibiotics

The discs of 7.25 mm diameter were prepared by punching of Whatman No.1 filter paper and were sterilized by dry heat at 160°C for an hour in batches of 100 in screw capped Bijou bottles. The DCM, EA and NB extracts of *Clitoria ternatea* leaf was weighed and dissolved in sterile distilled water to make the required stock solutions of concentration 10, 20 and 50 mg/ml. Similarly the stock solution of the control antibiotic (lincomycin) was prepared by

dissolving the required amount of lincomycin in sterile distilled water to prepare stock solutions 2.5mg/ml concentrations. All the stock solutions were then kept at 4°C and used for three months. For preparation of antibiotic impregnated discs 1.0 ml of the stock solutions of the antibiotic were added separately to each bottle of 100 discs. The procedure was repeated for preparation of impregnated discs of the plant extracts and their isolated compounds. The discs were used in wet condition and for further use they were stored at 4°C, as the discs can retain their moisture and potency for at least 3 months in the screw capped bottles<sup>4</sup>.

#### Antimicrobial activity

Microbial sensitivity tests were performed by disc diffusion method. The nutrient agar plates, containing an inoculum size of  $10^5$ - $10^6$  cfu/ml of bacteria were used. Previously prepared crude extract (Concentration 10-50 mg/ml) and antibiotic (concentration 2.5 mg/ml) discs were placed aseptically on sensitivity plates. All the plates were then incubated at  $37^\circ\text{C}\pm 2^\circ\text{C}$  for 18 h. The sensitivity was recorded by measuring the clear zone of inhibition on agar plate around the discs. The tests were carried out by using a stock concentration of 100mg/ml prepared by dissolving 1g of the extracts into 10 ml of distilled water. Nutrient agar was prepared and 25ml each was poured into sterile petridish. This was allowed to solidify and dry. Thereafter, the wells disc dipped with the extract solutions at varying concentrations of 50mg/ml, 20mg/ml and 10mg/ml respectively were placed on the plates. This was done in triplicate and the plates were incubated at 37°C for 18hours. The antibacterial activities were observed and measured using a transparent meter rule and recorded if the zone of inhibition was  $\geq 10$ mm. Antibiotic lincomycin at the concentration of 2.5mg/ml was taken as standard drug for this activity<sup>5</sup>.

#### Minimum Inhibitory Concentration (MIC)

MIC is defined as the lowest concentration where no visible turbidity is observed in the test tube (bacteriostatic concentration). The Vollekova *et al.*, (2001) method modified by Usman *et al.*, (2007) was employed. In this method, the broth dilution technique was utilized where the plant extract was prepared to the highest concentration of 500mg/ml (stock concentration) in sterile distilled water and

serially diluted (two-fold) to a working concentration ranging from 0.780mg/ml to 20mg/ml using nutrient broth and later inoculated with 0.2ml suspension of the test organisms. After 18 hours of incubation at 37°C, the test tubes were observed for turbidity. The least concentration where no turbidity was observed was determined and noted as the minimum inhibitory concentration (MIC) value<sup>6,7</sup>.

### Antioxidant Activity

#### Ferric Thiocyanate Method (FTC)

The FTC method was used to determine the amount of peroxide at the initial stage of lipid peroxidation. The FTC assay was carried out as described by Kikuzaki and Nakatani (1993) [9]. A mixture of 4 mg of extracts (final concentration 0.02% w/v) in 4 ml of 99.5% ethanol, 4.1 ml of 2.51% linoleic acid in 99.5% ethanol, 8.0 ml of 0.02 M phosphate buffer (pH 7.0) and 3.9 ml of distilled water, contained in a screw-cap vial (Φ 38 x 75mm) was placed in an oven at 40 °C and incubated in the dark. To measure the extent of antioxidant activity, 0.1 ml of the reaction mixture was transferred into a test tube (Φ13 x 150 mm). Then, 9.7 ml of 75% (v/v) aqueous ethanol was added to it, followed by 0.1 ml of 30% aqueous ammonium thiocyanate and 0.1 ml of 0.02 M ferrous chloride in 3.5% hydrochloric acid. Three minutes after the addition of ferrous chloride to the reaction mixture, the absorbance was measured at 500 nm. The measurement was taken every 24 hours until the absorbance of the control reached its maximum value. Ascorbic acid was used as a positive control.

#### Thiobarbituric Acid Method (TBA)

The test was conducted according to the method of Kikuzaki and Nakatani (1993). The same samples prepared for FTC method were used. To 2.0 ml of the sample solution, 1.0 ml of 20% aqueous trichloroacetic acid (TCA) and 2.0 ml of aqueous thiobarbituric acid (TBA) solution were added. The final sample concentration was 0.02% w/v. The mixture was placed in a boiling water bath for 10 minutes. After cooling, it was then centrifuged at 3000 rpm for 20 minutes. Absorbance of the supernatant was measured at 532 nm. Antioxidant activity was recorded based on the absorbance of the final day of the FTC assay. Both methods (FTC and

TBA) described antioxidant activity by percent inhibition:

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

#### Reducing Power Methods (RP)

For the determination of the reducing power, 1.0 ml of the extract (different conc.10-100µg/ml) suspended in distilled water, 2.5 ml of 0.2 M-phosphate buffer (pH6.6), and 2.5 ml of K<sub>3</sub>Fe(CN)<sub>6</sub> (1% w/v) were added. The mixture was incubated at 50 °C for 20 min. followed by addition of 2.5ml of TCA (10% w/v). The mixture was centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and 0.5 ml FeCl<sub>3</sub> (0.1% w/v), and the absorbance was measured at 700 nm against blank sample. The ascorbic acid is used as standard antioxidant compound<sup>8</sup>.

#### Determination of total phenolic (TP).

The total content of phenolic compounds in BEMM was determined by using the Folin–Ciocalteu reagent according to the colorimetric method described by Singleton and Rossi adapted to a 96-well microplate. Briefly, 5 µl of butanolic filtered extract were mixed with 60 µl of sodium carbonate solution at 7.5% (w/v) and 15 µl of Folin–Ciocalteu reagent. Subsequently, 200 µl of distilled water were added and the solutions were mixed. After that, the samples were heated at 60 °C for 5 min and were allowed to cool at room temperature. The absorbance was measured at 700 nm. A calibration curve was made from a gallic acid standard solution (10, 25, 50, 100, 200, 500 and 1000 mg/l) and the blank was prepared with distilled water. The total content of phenolic compounds was expressed as milligram gallic acid equivalent per dry weight material (mg GAE/g MM)<sup>9</sup>.

#### Statistical Analysis

Data were subjected to one-way analysis of variance (ANOVA) and treatments mean were compared to control by using Dunnett's Multiple Comparison Test. GraphPad Prism 5 software (GraphPad Software Inc., San Diego, CA) was used for all the statistical analyses<sup>10,11</sup>.

**RESULTS AND DISCUSSION:****Table 1 Results for phytochemical screening of *Clitoria ternatea***

Phytochemical Screening of various fractions of <i>Clitoria ternatea</i>					
Sl.No	Test	Pet.ether	Eth.Acetate	DCM	n-Butanol
1	Alkaloid	-	-	-	-
2	Flavonoids	-	-	-	+++
3	Saponin	-	+	++	+
4	Steroid	-	+	-	-
5	Tanin	+	-	-	-
6.	Mucilage	-	+	-	-
7.	Carbohydrates	-	-	-	-

**Table 2****Antimicrobial activity: zone of inhibition in mm  $\pm$  SD of three replicates.**

Sl.No	Pathogens	DCM			Ethyl acetate			n-Butanol			Lincomycin 2.5 mg/ml
		10mg/ml	20mg/ml	50mg/ml	10mg/ml	20mg/ml	50mg/ml	10mg/ml	20mg/ml	50mg/ml	
1.	S.aureus	8.8 $\pm$ 0.66	12.5 $\pm$ 1.2	10.5 $\pm$ 1.6	nd	2.9 $\pm$ 0.23	4.3 $\pm$ 0.43	17.3 $\pm$ 0.122	22 $\pm$ 1.15	20 $\pm$ 1.23	27 $\pm$ 0.89
2	<i>K. pneumoniae</i>	nd	Nd	12 $\pm$ 1.55	nd	nd	nd	15.4 $\pm$ 1.63	14.8 $\pm$ 1.43	15 $\pm$ 0.98	22 $\pm$ 0.56
3	E.coli	12.6 $\pm$ 1.34	14 $\pm$ 0.76	16.5 $\pm$ 0.32	nd	nd	5.4 $\pm$ 1.56	16 $\pm$	17 $\pm$ 1.26	16.5 $\pm$ 1.43	16 $\pm$ 0.76
4.	C.albicans	nd	Nd	nd	nd	nd	nd	23 $\pm$ 1.34	25 $\pm$ 1.43	25 $\pm$ 1.56	28 $\pm$ 0.75

1. nd: Not detected

**Table 3: Minimum inhibitory concentration (MIC) of DCM, Ethyl acetate and n-butanolic extract of *Clitoria ternatea* against bacterial pathogen at 600nm OD**

Test tubes	Pathogens	DCM (mg/ml)	Ethylacetate (mg/ml)	n-Butanolic (mg/ml)	Lincomycin ( $\mu$ g/ml)
1	S.aureus	6 $\pm$ 0.124	12 $\pm$ 0.154	6 $\pm$ 0.88	25 $\pm$ 0.001
2	<i>K. pneumoniae</i>	4.5 $\pm$ 0.326	6 $\pm$ 0.132	1.5 $\pm$ 1.43	12.5 $\pm$ 0.053
3	E.coli	3 $\pm$ 0.241	5 $\pm$ 0.432	2 $\pm$ 1.43	100 $\pm$ 0.012
4	C.albicans	1.5 $\pm$ 0.198	1.5 $\pm$ 0.012	1 $\pm$ 0.43	25 $\pm$ 0.001

MIC  $\pm$  SD of three replicates

**Table 4: In vitro free radical scavenging effect of *Clitoria ternatea* by reducing power determination.**

Sl.No	Extracts	4 $\mu$ g/ml	8 $\mu$ g/ml	15 $\mu$ g/ml	30 $\mu$ g/ml	60 $\mu$ g/ml	125 $\mu$ g/ml	250 $\mu$ g/ml	500 $\mu$ g/ml
1	DCM	0.088 $\pm$ 0.002	0.163 $\pm$ 0.002	0.272 $\pm$ 0.001	0.388 $\pm$ 0.001	0.434 $\pm$ 0.005	0.576 $\pm$ 0.002	0.705 $\pm$ 0.001	0.897 $\pm$ 0.005
2.	Ethyl acetate	0.054 $\pm$ 0.001	0.124 $\pm$ 0.001	0.235 $\pm$ 0.002	0.378 $\pm$ 0.005	0.568 $\pm$ 0.001	0.612 $\pm$ 0.005	0.689 $\pm$ 0.002	0.765 $\pm$ 0.001
3	n-Butanol	0.0423 $\pm$ 0.01	0.165 $\pm$ 0.001	0.287 $\pm$ 0.002	0.543 $\pm$ 0.001	0.785 $\pm$ 0.002	0.986 $\pm$ 0.001	1.254 $\pm$ 0.005	1.897 $\pm$ 0.001
		0.1 $\mu$ g/ml	0.2 $\mu$ g/ml	0.4 $\mu$ g/ml	0.8 $\mu$ g/ml	1.0 $\mu$ g/ml			
4.	Ascorbic acid	0.09231 $\pm$ 0.02	0.1854 $\pm$ 0.01	0.5243 $\pm$ 0.01	0.9567 $\pm$ 0.001	1.9894 $\pm$ 0.05			

Absorbance (Mean  $\pm$ SEM) of triplicates

**Table 5. Lipid peroxidation of *Clitoria ternatea* by Ferric Thiocyanate Method**

Sl.No	Extracts	4 $\mu$ g/ml	8 $\mu$ g/ml	15 $\mu$ g/ml	30 $\mu$ g/ml	60 $\mu$ g/ml	125 $\mu$ g/ml	250 $\mu$ g/ml	500 $\mu$ g/ml
1	DCM	19.21 $\pm$ 0.001	22.31 $\pm$ 0.05	27.2 $\pm$ 0.02	35.81 $\pm$ 0.05	47.24 $\pm$ 0.001	49.63 $\pm$ 0.002	52.55 $\pm$ 0.002	55.97 $\pm$ 0.001
2.	Ethyl acetate	12.54 $\pm$ 0.001	15.12 $\pm$ 0.05	19.35 $\pm$ 0.001	20.38 $\pm$ 0.002	25.36 $\pm$ 0.001	31.12 $\pm$ 0.05	37.29 $\pm$ 0.001	42.64 $\pm$ 0.05
3	n-Butanol	37.43 $\pm$ 0.001	40.65 $\pm$ 0.001	47.27 $\pm$ 0.05	56.53 $\pm$ 0.001	60.85 $\pm$ 0.002	67.86 $\pm$ 0.05	71.25 $\pm$ 0.05	79.97 $\pm$ 0.001
		0.1 $\mu$ g/ml	0.2 $\mu$ g/ml	0.4 $\mu$ g/ml	0.8 $\mu$ g/ml	1.0 $\mu$ g/ml			
4	Ascorbic acid	39.32 $\pm$ 0.05	55.65 $\pm$ 0.001	64.98 $\pm$ 0.002	69.66 $\pm$ 0.01	79.23 $\pm$ 0.05			

Percentage inhibition (Mean  $\pm$ SEM) of triplicates

**Table 6: In vitro free radical scavenging effect of *Clitoria ternatea* by Thiobarbituric Acid Method.**

Sl.No	Extracts	4 µg/ml	8 µg/ml	15 µg/ml	30 µg/ml	60 µg/ml	125 µg/ml	250 µg/ml	500 µg/ml
1	DCM	15.21±0.001	20.31±0.001	23.2±0.002	25.81±0.005	27.24±0.002	29.63±0.001	32.55±0.005	35.97±0.001
2.	Ethyl acetate	13.44±0.002	19.62±0.001	29.38±0.005	30.37±0.001	35.06±0.001	41.92±0.005	47.69±0.002	52.94±0.001
3	n-Butanol	32.93±0.01	44.79±0.001	49.87±0.002	53.93±0.001	64.95±0.001	69.32±0.005	71.85±0.001	74.46±0.001
		0.1 µg/ml	0.2 µg/ml	0.4 µg/ml	0.8 µg/ml	1.0 µg/ml			
4	Ascorbic acid	33.45±0.01	48.64±0.001	55.32±0.001	64.66±0.005	75.43±0.01			

**DISCUSSION:**

The search for new antimicrobials from natural sources has received much attention and efforts have been put in to identify compounds that can act as suitable antimicrobials agent to replace synthetic one from the market. Phytochemicals derived from plant products serve as an origin to develop less toxic and more effective medicines in controlling the growth of microorganism. These compounds have significant therapeutic application against human pathogens including bacteria, fungi, virus or protozoa. Many studies have been performed with the extracts of various plants, screening antimicrobial activity as well as for the discovery of new antimicrobial compounds.

In the present investigation, different extracts of *Clitoria ternatea* was evaluated for exploration of their antimicrobial activity against certain Gram negative and Gram positive bacteria, which was regarded as human pathogenic microorganism. Susceptibility of each plant extract was tested by serial micro dilution method (MIC) and agar well diffusion method was determined. The work will be further carried with the antioxidant potentiality of the various extracts of the plant *Clitoria ternatea*. In today's worlds the effects of environmental and habitual changes in human world initiating various oxidation process in the body, which finally triggers the concentration of free radicals. Thus introduction of a new potent antioxidant and antimicrobial agent in the era of herbal medicines will be a boon for the human society.

The phytochemical screening result exhibits the presence of flavonoids and saponin for the n-butanolic extracts. The presence of flavonoids in the butanolic fraction should be the reason behind its excellent antioxidant result (Table 1). The thorough review of literature supports the strong antioxidant activity of flavonoids and its derivative compounds. All three extracts of the plant tested showed varying degree of antimicrobial activities against the test bacterial species (Table 2). The antimicrobial activities of the extracts compared favorably with that of standard antibiotic (lincomycin) and have appeared to be broad spectrum as its activities were independent on gram reaction. The n-Butanolic extract (inhibition zone 14 - 20 mm) was found to be more effective than the DCM extract (inhibition zone 8 - 16 mm) and Ethylacetate (inhibition zone 0 - 5 mm) against all the organisms (Table 2). The DCM extract shows moderate zone of inhibition ( $10.05 \pm 1.6$  and  $11.23 \pm 0.32$  respectively) against *S. aureus* and *E. coli* growth, but shows no effect against *K. pneumonia* and *C. albicans*. The ethylacetate extract shows the lowest antimicrobial activity with inhibition zones ranging between 0 and 5 mm for different bacteria tested. The zone of inhibition of the n Butanolic extract provides a fascinating result and can eventually compared with the standard drug lincomycin (Table 2).

The minimum inhibitory concentration (MIC) of the n-Butanol extract for different organisms ranged between 1 and 6 mg/ml, while that of the DCM and

Ethylacetate extracts ranged between 1.5 and 10 mg/ml and 1.5 and 12 mg/ml. Also the MIC of lincomycin control ranged between 12.5 and 100 µg/ml (Table 3). The review of literature supports the MIC of n butanolic extract at the concentration of 1-6 mg/ml can be considered as a potent antimicrobial agent<sup>12</sup>.

The antioxidant activity for reducing power determination (RP) states that as the concentration of the extracts increased, the absorbance was also increased correspondingly, indicating that DCM, Ethylacetate and n-Butanolic extracts have potent anti-oxidant activity by reducing power ability. Butanolic extracts showed more reducing power ability than others, when compared with that of the standard anti-oxidant ascorbic acid (Table-4). The results for lipid peroxidation of *Clitoria ternatea* by Ferric Thiocyanate method exhibits in a concentration dependant manner up to a concentration of 500µg/ml. Butanolic extract showed more peroxidation effect than other extracts. The reference standard ascorbic acid also showed a significant radical scavenging potential in the concentration of 1 µg/ml. The lipid peroxidation of DCM, Ethyl acetate, n-Butanolic extracts and ascorbic acid was found to be 55.97%, 42.64%, 79.97 and 78.23% respectively (Table 5). In vitro free radical scavenging effect of *Clitoria ternatea* by Thiobarbituric Acid Method exhibits the good percentage inhibition for butanolic extracts. The free radical scavenging of DCM, Ethyl acetate, n-Butanolic extracts and ascorbic acid was found to be 35.97%, 52.94%, 74.46 and 75.43% respectively (Table 6). On the basis of the phytochemical result

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the butanolic fraction was subjected to the test for total phenolic content. The result gives a good outcome 34.78 mg GAE/g MM. All results of the present work fetch support for *Clitoria ternatea* to be a plant with strong antioxidant property<sup>13</sup>.

#### CONCLUSION:

The present study exhibits quite a good profile of phytochemical content and antimicrobial activity with the various leaf extracts of the plant *Clitoria ternatea*. The zone of inhibition and MIC shows maximum result for n-Butanolic extracts. The antioxidant activity (TP, RP, FTC and TBA) performed with the various plant extracts provides support to mark this plant with strong antioxidant activity. The strong antioxidant profile for the BECT comparatively provides evidences for its flavonoidal compounds to possess a good antioxidant property. Maximum value of phenolic compounds extracted from BECT were correspondent to 24.7 mg GAE/g MM. Thus the butanolic fraction was useful to produce extracts containing simultaneously high content of phenolic compounds and high antioxidant activity. Since antioxidant compounds provide health benefits, BECT could be of great interest for application in pharmaceutical products.

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