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Phytochemical Analysis, Antimicrobial and Antioxidant Activity of *Clitoria ternatea* Blue and White Flowered Leaves

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Authors' contributions

This work was carried out in collaboration between all authors. Author AD performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author JA designed the study and managed the literature searches. Authors JA and *SAS managed the analyses of the study. All authors read and approved the final manuscript.*

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ABSTRACT

The present study was conducted to screen the phytochemical constituents, identifying the compounds present by Gas Chromatography-Mass Spectrometric analysis (GC-MS), functional groups present by Fourier Transform Infrared Spectrophotometer (FT- IR) in the ethyl acetate extract of white-flowered leaf *Clitoria ternatea*, explicit the antibacterial activity, antioxidant assay of using methanol and ethyl acetate extract of *Clitoria ternatea* blue and white flowered leaves. *Clitoria ternatea* blue flowered leaves possess 7.5% of total ash whereas white flowered leaves possess 8.5%. The phytochemical analysis revealed that blue flowered leaves contain alkaloids, flavonoids, phenols, terpenoids, glycosides, coumarins, catechol, quinines, gum and mucilage while, in white flowered leaves had alkaloids, glycosides, catechol, gum and mucilage were present. A total of 8 compounds such as 1- Decanol, 2- ethyl, 1- Eicosanol, Sulphurous acid octadecyl 2- propyl ester, Eicosanoic acid, L- (+)- ascorbic acid 2,6- dihexadecanoate, Sulphurous acid, pentadecyl 2-propyl ester, Oleic acid, 1-Hexyl-2 nitrocyclohexane were detected in the ethyl acetate extract of white-flowered leaves of *C. ternatea* using GC-MS. FT-IR analysis of the same had characteristic bands

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at 2983.88 cm^{-1}. indicating the presence of C-H stretching. The ethyl acetate extract of white-flowered leaves *C. ternatea* had antibacterial and antifungal activity against *E. coli, S. aureus, K. pneumoniae, P. aeruginosa, P. mirabilis, C. albicans, C. tropicalis, C. kruzi*. The total phenolic content of methanolic extract of *C. ternatea* blue flowered leaf was 25 mg Gallic Acid Equivalent/ g Dry Weight (mg GAE / g DW), and white flowered leaf was 18 mg GAE / g DW. The total flavonoid content of methanolic extract of *C. ternatea* blue flowered leaf was 10 mg Quercetin Equivalent/g Dry Weight (mg QE / g DW), and white flowered leaf was 5 mg QE/g DW. Maximum scavenging activity of 86.6% was observed in the blue flowered leaf ethyl acetate extract (DPPH assay). Maximum scavenging activity of 82% (H_2O_2 assay) and 95% (total antioxidant) was observed in the white flowered leaf ethyl acetate extract.

Keywords: Clitoria ternatea; phytochemicals; GC-MS; FT-IR; antimicrobial; antioxidant activity.

1. INTRODUCTION

Medicinal plants have been used from time immemorial in daily life to treat diseases all over the world. In herbal medicines, the one or more active ingredients are derived from the aerial and non-aerial parts, juices, resins and oils of the plant either in crude state or as pharmaceutical formulation [1]. *Clitoria ternatea* belongs to the family of Fabaceae. It is commonly known as Asian pigeonwings, bluebelluine, butterfly pea [2].

In the present era of pharmalogical activities, this leaf possesses various pharmacological effects like anti- asthmatic, anti-depressant, anti-convulsing, anti- stress, memory enhancer, nootropic, anxiolytic like activities. It is also used for treating diabetes mellitus, burning sensation, inflammations, skin diseases, pulmonary tuberculosis, eye infections, urogenital disorders and as an anti-dote for treating toxicity [3].

Soxhlet extraction has been used to remove lypodial materials from powdered *Clitorea ternatea* flowers using petroleum ether at 60°-80°C, resulted in 2.2% yield w/w [4]. Further extraction of the marc with ethanol ascertained the presence of alkaloids and saponins [4] in *Clitorea ternate* flowers [5].

Blue leaves contain a number of phytochemicals like alkaloids, flavonoids, tannins, phenols, steroids, terpenoids, glycosides, coumarins, catechol, reducing sugars and proteins [6].

Clitoria ternatea could be useful for the development of new tools as antimicrobial agents for the control of infectious diseases [7]. Methanol, ethyl acetate and petroleum ether extracts of leaves were tested with the *Bacillus cereus, Staphylococcus aureus, Klebsiella pneumonia, Proteus vulgaris* and *Salmonella typhi* have shown more potent inhibitory activity in methanol when compared to the other extracts such as ethyl acetate and petroleum ether [8]. In the present study, *Candida albicans* (IFM 40009), *Candida tropicalis* (IFM 46521) and *Candida kruzi* (IFM 55058) were used to determine the antifungal activity.

The quantitative estimation of ethanolic extract of *Clitoria ternatea* was found to contain total phenols (245.14±6.97 mg Tannic Acid Equivalent TAE/g), highly compared to tannins (78.75±2.09 mg TAE/g) and flavonoids (20.48±0.96 mg Rutin Equivalent /g) [6]. *C. ternatea* blue and white leaves were evaluated by determining the levels of enzymatic and non- enzymatic antioxidants. It was determined by using different assays such as ferric reducing power assay (FRAP), reducing activity assay, diphenypicrylhydrazyl (DPPH) assay and hydroxyl radical scavenging activity. It was determined that the white flowered leaves had a higher content of all the enzyme antioxidants analysed than the blue flowered leaves [2].

The present study was conducted to determine the total ash content, phytochemical constituents, identifying the compounds in GC-MS and functional groups in FT- IR using ethyl acetate extract of white flowered leaf *Clitoria ternatea*, antimicrobial activity, antioxidant activity of methanol and ethyl acetate extract of *Clitoria ternatea* blue and white flowered leaves.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation of Blue and White Leaf Extract

Blue flowered leaves of *Clitoria ternatea* were purchased commercially from Koyambedu market, Chennai. White flowered leaves of *Clitoria ternatea* were collected from Kerala and Kanchipuram district and authenticated by Dr. N.K. Uday Prakash, Department of

Biotechnology, Vels University, Chennai and deposited in the herbarium (MLHA 1163 for blue flowered leaf, MLHA 1164 for white flowered leaf) at Marina labs. Leaves of *Clitoria ternatea* were shade dried for and stored in air tight containers. 20 g of each leaf were extracted in Soxhlet equipment with 250 mL of methanol and ethyl acetate separately. The extracts were collected, evaporated by rotary evaporator and stored in air tight bottles at 4°C.

2.2 Total Ash Content

Silica crucible was heated to red-hot for 30 minutes and it was allowed to cool in desiccators. About 1.0g of powdered sample was weighed accurately and evenly distributed in the crucible. The content was shared over a heater and then burnt in a muffle furnace for 2 hours at 600±10°C. The crucible was allowed to cool in desiccators and weighed with the ash [9].

$$\% \text{ Total ash} = \frac{\text{Weight of the ash}}{\text{Weight of the sample}} \times 100$$

2.3 Phytochemical Screening

The blue and white flowered leaves of *C.ternatea* were subjected to different phytochemical analysis.

2.3.1 Test for alkaloids

a Mayer's test: To 250 µl of crude extract, 250 µl of 1% HCl in water and 6 drops of Mayer's reagent was added. An organic precipitate indicated the presence of alkaloids in the sample [10].

b Dragendroff's test: To 250 µl of crude extract, 250 µl of 1% HCl and 6 drops of Dragendroff's reagent. Appearance of red or orange precipitate indicated the presence of alkaloids in the sample [11].

2.3.2 Test for flavonoids

a To 250 µl of crude extract, 250 µl of 2% NaOH in water was added. Intense yellow turns colourless on addition of few drops of acetic acid indicated the presence of flavonoids in the sample [12].

b Shinoda test: Pieces of magnesium ribbon and concentrated HCl were mixed with crude plant extract. After few minutes pink colour scarlet appeared which indicated the presence of flavonoids [13].

2.3.3 Test for tannins

a To 250 µl of crude extract, 500 µl of water and 1 or 2 drops of ferric chloride solution was added. The appearance of blue colour indicated the presence of gallic tannins. The appearance of green black colour indicated the presence of catechol tannins in the sample [14].

b Lead acetate test: To 250 µl of crude extract, few drops of 1% lead acetate solution in water was added. Appearance of white precipitate indicated the presence of tannins in the sample [10].

2.3.4 Test for steroids

To 250 µl of crude extract, 250 µl of chloroform and 250 µl of concentrated sulphuric acid were added. Appearance of red colour precipitate indicated the presence of steroids in the sample [14].

2.3.5 Test for phenols

To 250 µl of crude extract, 250 µl of 2% ferric chloride solution was added. Appearance of black colouration indicated the presence of phenols in the sample [15].

2.3.6 Test for phenolic compounds

Magnesium hydrochloride reduction test: Pieces of magnesium ribbon and concentrated hydrochloric acid were mixed with the 250µl of crude extract. After few minutes the appearance of orange colour indicated the presence of phenolic compounds [10].

2.3.7 Test for saponins

To 250 µl of crude extract, 250 µl of water was added. The appearance of stable foam indicated the presence of saponins in the sample [14].

2.3.8 Test for terpenoids

Salkowski's test: To 250 µl of crude extract, 250 µl of chloroform and 250 µl of sulphuric acid was added. Appearance of reddish brown colour indicated the presence of terpenoids in the sample [12].

2.3.9 Test for glycoside

a Keller-killani test: To 250 µl of crude extract, 250 µl of glacial acetic acid and 1 to 2 drops of 2% ferric chloride reagent in water was added

and poured into a test tube containing concentrated sulphuric acid. The presence of brown ring at the interphase indicated the presence of glycoside in the sample [12].

b Glycoside-Libermann's test: To 250 µl of crude extract, 250 µl of acetic acid and 250 µl of chloroform was added. The mixture was then cooled and concentrated sulphuric acid was added. Green colour indicated the presence of a glycone steroidal part of glycoside [16].

2.3.10 Test for antraquinones

To 250 µl of crude extract, was shaken with 250 µl of benzene and filtered. To this, 1 mL of 10% ammonia solution (in water) was added and shaken well. Appearance of pink red or violet colour indicated the presence of antraquinones in the sample [13].

2.3.11 Test for phlobatinins

The extract was boiled with 1% HCl (in water). Appearance of red colour indicated the presence of phlobatannins in the sample [14].

2.3.12 Test for coumarins

To 250 µl of crude extract, 10% NaOH (in water) was added. Appearance of yellow colour indicated the presence of coumarins in the sample [17].

2.3.13 Test for anthocyanins

To 250 µl of crude extract, 100 µl of 2 N ammonium chloride and ammonia solution was added. Appearance of pink red colour which turns blue violet colour indicated the presence of anthocyanins [18].

2.3.14 Test for leuco-anthocyanins

To 250 µl of extract, 250 µl of isoamyl alcohol was added. The presence of red colour on the upper layer indicated the presence of leuco-anthocyanins in the sample [18].

2.3.15 Test for catechol

To 250 µl of extract, 6 to 8 drops of Erlich reagent was added and mixed well. To this few drops of concentrated hydrochloric acid was added. Appearance of green black colour indicated the presence of catechol in the sample [19].

2.3.16 Test for quinines

To 250 µl of extract, 250 µl of concentrated sulphuric acid was added. Appearance of red colour indicated the presence of quinines in the sample [12].

2.3.17 Test for reducing sugar

To 250 µl of crude extract, 500 µl of water and 5 to 8 drops of Fehling solution was added and heated in water bath. Appearance of black red precipitate indicated the presence of reducing sugar in the sample [20].

2.3.18 Test for carbohydrates

a 250 µl of Fehling A and 250 µl of Fehling B were mixed together and 500 µl of it was added to 500 µl of crude extract and gently boiled. Appearance of brick red precipitate indicated the presence of carbohydrates [21].

b Benedict's test: To 250 µl of crude extract, 250 µl of Benedict's reagent was added. The appearance of reddish brown precipitate indicated the presence of carbohydrates [21].

2.3.19 Test for proteins

Million's reagent: To the 250 µl crude extract, 6 to 7 drops of Million's reagent was added. The precipitate turned red upon heating which indicated the presence of proteins in the sample [10].

2.3.20 Test for aminoacids

To the 250 µl of extract, 250 µl of Ninhydrin solution was added. Appearance of purple colour indicated the presence of aminoacids in the sample [10].

2.3.21 Test for gum and mucilage

250 µl of extract was dissolved in 250µl of distilled water and 100µl of absolute alcohol was added.

Appearance of white or cloudy precipitate indicated the presence of gum and mucilage [10].

2.4 Gas Chromatography – Mass Spectrometry (GC-MS)

GC-MS analysis (Model - SHIMADZU) was carried out only for ethyl acetate extract of white

flowered leaf *C. ternatea* as it showed better antimicrobial and antioxidant activities. Identification of specific components was done by comparing their mass spectra and the retention time with the data provided in the NIST library. The peak area percentages in the chromatograph represent the abundance of the compounds in the extracts [22].

2.5 Fourier Transform Spectro-photometer (FTIR)

Only ethyl acetate extract of white flowered leaf *Clitoria ternatea* were subjected to FTIR analysis (Model - JASCO) as it showed better antimicrobial and antioxidant activities. The characteristic peak values and their functional groups were recorded [23].

2.6 Estimation of Total Phenolic Content

Total phenolic content of methanol and ethyl acetate extracts of blue and white flowered leaves were estimated using Folin Ciocalteau method. To 1 mL of each extract (100 µg/mL) in methanol, 5 mL of Folin Ciocalteau reagent (diluted tenfold) and 4 mL (75 g/l) of Na_2CO_3 were added. The mixture was allowed to stand at 20°C for 30 min and colour developed was recorded at 765 nm using UV-VIS spectrophotometer. Total phenolic content was expressed as mg/g gallic acid equivalent (GAE) per dry weight of the extracts using standard plot of gallic acid [12].

2.7 Estimation of Total Flavonoid Content

Total flavonoid content of methanol and ethyl acetate extracts of blue and white flowered leaves were estimated using aluminium chloride method. To 1 mL of each extract (100 µg/mL) in 4 mL of distilled water, 0.3 mL $NaNO_2$ (5%) was added. After 5 min, 0.3 ml of 10% $AlCl_3$ and 2 mL of 1 M NaOH were added and the volume rinsed 10 mL. The absorbance was measured at 510 nm. A standard calibration plot was generated using the concentrations (0.01-0.05 mg/mL) of quercetin. The concentrations of total flavonoid content were calculated as quercetin mg/g [12].

2.8 Antimicrobial Assay

The Gram positive organism *Staphylococcus aureus* (ATCC 25923) and four Gram negative organisms *Klebsiella pneumoniae* (ATCC 700603), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (clinical isolate) and *Proteus mirabilis* (ATCC 25933) was used to determine the antibacterial activity. *Candida albicans* (IFM 40009), *Candida tropicalis* (IFM 46521) and *Candida kruzi* (IFM 55058) were used to determine the antifungal activity. All the test organisms were obtained from Department of Biotechnology, University of Madras, Guindy campus, Chennai.

Antibacterial activity and antifungal activity was determined by agar well diffusion method on Muller Hinton agar and Sabouraud Dextrose agar plates respectively. The extracts (40 mg) were dissolved in 5% DMSO. Streptomycin sulphate (50 µl) and Nystatin (50 µl) were used as positive control for antibacterial and antifungal activity respectively. DMSO was used as negative control for both. Suspensions of test organisms were made in saline and adjusted with McFarland standard solution. The plates were uniformly spread with 100 µl of the culture organisms. Wells were cut with sterile cork borer. Different concentrations (10 mg, 12 mg, 14 mg, 15 mg) of methanol and ethyl acetate extracts of blue and white flowered leaves were added to the well. The plates were incubated at 37°C in incubator for 24 hours and the zone of inhibition was measured to determine the antibacterial and antifungal activity [24].

2.9 Antioxidant Activity

2.9.1 DPPH free radical scavenging activity

1 mL of extract (0.2-1 µg/mL in ethanol) was added to the 1 mL of 0.1 mm of DPPH solution. 1 mL of ethanol and 0.95 mL of Tris HCl was added to it and incubated at 37°C for 30 min in dark. Absorbance was measured at 517 nm. Ascorbic acid was used as reference standard. Percentage inhibition was calculated as [25]:

DPPH Scavenged (%) = (Absorbance $_{control}$ - Absorbance $_{test}$ / Absorbance $_{control}$) x 100

2.9.2 Hydrogen peroxide scavenging activity

A solution of H_2O_2 (43 mM) was prepared in phosphate buffer (0.1 M, pH 7.4). To the extract of different concentrations (0.2-1 mg/mL), 3.4 ml phosphate buffer and 0.6 mL of H_2O_2 solution (0.6 mL, 43 mM) was added to it and incubated for 10min. The absorbance was measured at 230 nm. Ascorbic acid was used as reference standard. Percentage inhibition was calculated as [26]:

H_2O_2 scavenging activity (%) = (Absorbance $_{control}$ - Absorbance $_{test}$ / Absorbance $_{control}$) x 100

2.9.3 Total antioxidant activity

The total antioxidant activity was estimated by Phospho-molybdenum method. To the plant extract (0.5 mL), a reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) of 4.5 mL was added and the solution was maintained in a boiling water bath at 95°C for 90 min. Upon cooling the solution at room temperature, the absorbance was measured at 695 nm using UV- Visible spectrophotometer. The total antioxidants in the plant were expressed as mg TAE/g dry weight of the leaf extract. The percentage of inhibition was calculated with tannic acid as standard [27],

% Inhibition = (Absorbance $_{control}$ – Absorbance $_{test}$ / Absorbance $_{control}$) x 100

3. RESULTS AND DISCUSSION

3.1 Total Ash Content

Maximum content of total ash was found in the blue and white flowered leaves. *C. ternatea* blue leaves possess 7.5 % of total ash whereas white leaves possess 8.5 % of total ash which is in concordance with [28] who reported that the blue leaves found to contain 8.73 ± 0.22%, whereas white leaves contain 9.5 ± 0.23%.

3.2 Phytochemical Screening

The phytochemical screening of methanol and ethyl acetate extract of blue and white flowered leaves of *C.ternatea* were studied by specific qualitative tests revealed the presence of alkaloids, flavonoids, phenols, terpenoids, glycosides, coumarins, catechol, quinines, gum and mucilage in blue flowered leaves. In white flowered leaves only alkaloids, glycosides, catechol, gum and mucilage are present which is in concordance with [11] who reported that the methanolic extract of blue leaves contain the alkaloids, flavonoids, tannins, steroids phenols, terpenoids, glycosides.

3.3 Gas Chromatogrphy – Mass Spectrometry

The GCMS studies of ethyl acetate extract of white flowered leaf *C.* have revealed the presence of many compounds (Table 1).

Different peaks were obtained and each peak represents a compound of *C.ternatea* white leaf ethyl acetate extracts showed the presence of 1-Decanol, 2- ethyl, 1- Eicosanol, Sulphurous acid octadecyl 2- propyl ester, Eicosanoic acid, L- (+)-ascorbic acid 2,6- dihexadecanoate, Sulphurous acid, pentadecyl 2-propyl ester, Oleic acid, 1-Hexyl-2 nitrocyclohexane, Tetracontane, 3, 5, 24-trimethyl, erucic acid which is in concordance with [28] who has reported that *Clitoria ternatea* were analysed through GC-MS studies have identified nineteen chemical constituents are Z, Z-8, 10- hexadecadien-1-ol (28.69%), n-hexadecanoic acid (26.60%), pentadecane 2, 6, 10, 13-tetramethyl (15.87%), 9- octadecenoic acid (Z)-2-hydroxyl-1(hydroxymethyl) ethyl ester (14.51%), hexacontane (12.88%).

3.4 Fourier Transform Infrared Spectrophotometer (FTIR)

The ethyl acetate extract of white flowered leaves *C. ternatea* exhibited a characteristic band at 2983.88 cm^{-1} corresponds to C-H stretching denotes the presence of carbonyl compounds (Fig. 1) which is in concordance with [29] who has reported that different compounds was identified with a variation in the peaks ratio.

3.5 Estimation of Total Phenolic Content

The total phenolic content for *C. ternatea* blue flowered leaves using methanol and ethyl acetate extracts was found to be 25 mg/g GAE and 11 mg/g GAE respectively. In white flowered leaves the methanol and ethyl acetate extracts had yielded a phenolic content of 18 mg/g GAE and 10 mg/g GAE respectively (Fig. 2).

3.6 Estimation of Total Flavonoid Content

The total flavonoid content for *C. ternatea* blue flowered leaves of methanol and ethyl acetate were10 mg/g quercetin and 6mg/g quercetin, respectively and white flowered leaves using methanol and ethyl acetate have yielded 5 quercetin mg/g and 6 quercetin mg/g, respectively (Fig. 2) which is in concordance with [30] who has reported that flavonoid content was maximum in methanolic extract.

3.7 Antimicrobial Assay

3.7.1 Antibacterial activity

The methanolic extract of blue flowered leaf *Clitoria ternatea* had a zone of inhibition of

Table 1. Phyto-compounds identified in ethyl acetate extract of white flowered leaves of *Clitoria ternatea* by GC - MS analysis

S. No.	Retention time	Name of the compound	Molecular formula	Molecular weight (Da)	Peak area %
1.	17.114	1- Decanol, 2- ethyl	$C_{12}H_{26}O$	186	3.644
2.	17.429	1- Eicosanol	$C_{20}H_{42}O$	298	1.843
3.	18.805	Sulfurous acid octadecyl 2- propyl ester	$C_{21}H_{44}O_3S$	376	2.585
4.	19.260	Eicosanoic acid	$C_{20}H_{40}O2$	312	32.417
5.	19.645	Eicosanoic acid	$C_{20}H_{40}O_2$	312	5.672
6.	19.840	L- (+)- ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$	652	2.602
7.	20.440	Sulfurous acid, pentadecyl 2-propyl ester	$C_{18}H_{38}O_3$	334	2.078
8.	20.766	Oleic acid	$C_{18}H_{34}O_2$	282	33.894
9.	21.166	Oleic acid	$C_{18}H_{34}O_2$	282	2.752
10.	21.271	1-Hexyl-2 nitrocyclohexane	$C_{12}H_{23}O_2N$	213	6.444

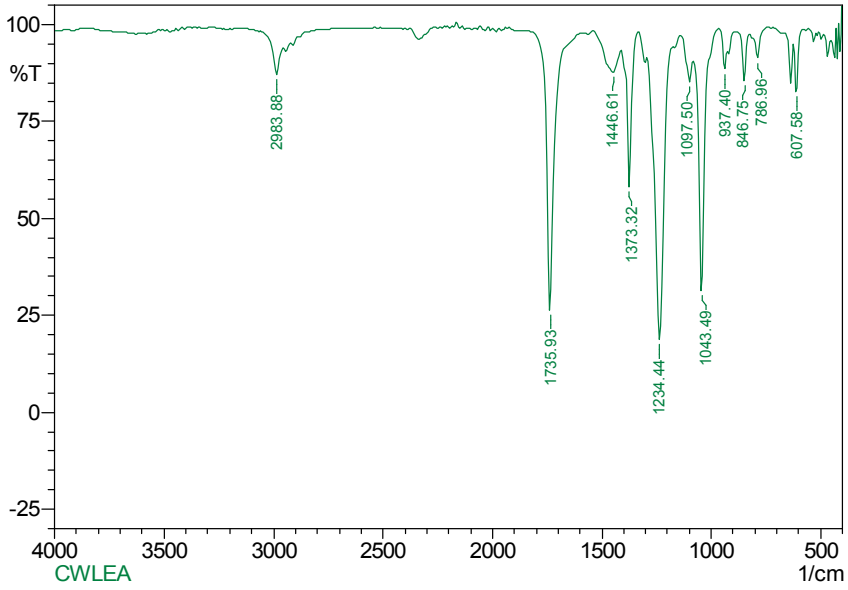

Fig. 1. FTIR spectrum of white flowered leaves ethyl acetate extract of *C. ternatea*

12 mm at 14 mg for *K. pneumoniae* and 13 mm at 10 mg for *P. aeruginosa* (Table 2). The ethyl acetate of *C. ternatea* exhibited an inhibition zone of 13 mm at 14 mg for *S.aureus* (Table 3), but for *K. pneumonia*e it was shown 14 mm for 15 mg, as well as for *P. aeruginosa* which showed an inhibition zone of 12 mm for both 14 and 15 mg. It also had an inhibition zone for *P. mirabilis* (12 mm) at 15 mg which is in concordance with [7] who have reported that the ethyl acetate affected the activity of *Salmonella typhi* with high range followed by *Bacillus cereus* (10 mm) and *Klebsiella pneumonia* (10 mm).

The methanolic extract of white flowered leaf of *Clitoria ternatea* showed a zone of inhibition at 15 mg for *K .pneumoniae* (11 mm) (Table 2), in the case of *P. aeruginosa* was 12 mm at 15 mg. Methanolic extract showed inhibition zone for *K. pneumoniae* and *P. aeruginosa* at all the concentrations (10, 12, 14, 15 mg). The ethyl acetate extract of white flowered leaves showed inhibition zone for *E. coli* (11 mm), *S. aureus* (11 mm), *K. pneumoniae* (14 mm) and *P. aeruginosa* (12 mm) at a concentration of 15 mg (Table 3). The ethyl acetate extract of white flowered leaf *Clitoria ternatea* had shown an inhibition zone against *E. coli S. aureus,*

K. pneumoniae and *P. aeruginosa* at all the concentrations (12, 14, 15 mg) as compared to ethyl acetate extract of blue flowered leaves.

3.7.2 Antifungal activity

The methanolic extract of blue flowered leaf *Clitoria ternatea* was effective for *C. tropicalis* (13 mm) at a concentration of 15 mg (Table 4). The ethyl acetate extract of *C. ternatea* was sensitive to *C. albicans* (13 mm), *C. tropicalis* (13 mm) at 15 mg concentration and for *C. kruzi* (11 mm) at 12 mg concentration. The results are in concordance with [13] who have reported that maximum zone of inhibition was obtained in methanolic extract of *Candida albicans* (11 mm).

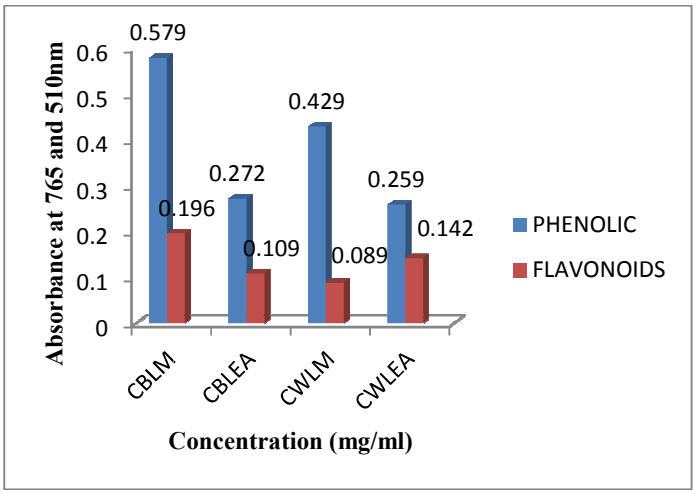

Fig. 2. Total phenol and flavonoid content of methanol and ethyl acetate extracts of blue (CBLM/CBLEA) and white flowered leaves (CWLM/CWLEA)

CBLM- C. ternatea blue flowered leaf methanol extract
CBLEA- C. ternatea blue flowered leaf ethyl acetate extract
CWLM- C. ternatea white flowered leaf methanol extract
CWLEA- C. ternatea white flowered leaf ethyl acetate extract

The methanolic extract of white flowered leaf *C. ternatea* was effective for *C. albicans* (15 mm) at 14 mg concentration (Table 4), *C. tropicalis* (15 mm), *C. kruzi* (13 mm) at 15 mg concentration. Ethyl acetate extract exhibited activity for *C. albicans* (20 mm) *C. kruzi* (12 mm) at 15 mg whereas, *C. tropicalis* had a zone of 14 mm at 10 mg. Overall, methanolic and ethyl acetate extracts of white flowered leaf *C. ternatea* was effective for *C. albicans*, *C. tropicalis* and *C. kruzi* as compared to blue flowered leaves. Positive control (Nystatin) showed a maximum an inhibition zone (30 mm) against *C. tropicalis*

3.8 Antioxidant Activity

3.8.1 DPPH free radical scavenging activity

The highest radical scavenging activity was observed in the ethyl acetate extract of blue flowered leaves of *C. ternatea* (86.6%) with a IC_{50} value of 8.53µg/mL (Table 5; Fig. 3) and the lowest DPPH radical scavenging activity was observed in the methanol extract of blue flowered leaves (58%) with a IC_{50} value of 93.43 µg/mL. Similarly, [8] reported that the petroleum ether, chloroform and methanol extracts of both blue and white flowered varieties of *C. ternatea* significantly inhibited the DPPH free radical concentrations ranging from 50-600 µg/ml.

3.8.2 Hydrogen peroxide scavenging activity

The highest radical scavenging activity was observed in the ethyl acetate extract of blue (79%) and white flowered leaves (82%) of *C. ternatea* with a IC_{50} value of 14.60 µg/ml and 10.34 µg/mL respectively. The lowest H_2O_2 radical scavenging activity was observed in the methanol extract of white flowered leaves (59%) with a IC_{50} value of 87.34 µg/mL (Table 6; Fig. 4). Similarly [31] reported that methanol extract of *C. ternatea* acts as a scavenger against free radicals under oxidative stress.

Table 2. Antibacterial activity of methanol extract of blue and white flowered leaves of *Clitoria ternatea*

Microorganisms	Blue flowered leaves				White flowered leaves				Positive control (Streptomycin sulphate)
	10 mg	12 mg	14 mg	15 mg	10 mg	12 mg	14 mg	15 mg	
	Zone of inhibition (mm)								
Gram positive									33
S. aureus	-	-	-	-	-	-	-	-	
Gram negative									
E.coli	-	-	-	-	-	-	-	-	29
K. pneumoniae	-	-	12	-	12	13	13	11	34
P. aeruginosa	13	-	-	-	14	12	11	12	27
P. mirabilis	-	-	-	-	-	-	-	-	25

Table 3. Antibacterial activity of ethyl acetate extract of blue and white flowered leaves of *Clitoria ternatea*

Microorganisms	Blue flowered leaves				White flowered leaves				Positive control (Streptomycin sulphate)
	10 mg	12 mg	14 mg	15 mg	10 mg	12 mg	14 mg	15 mg	
	Zone of inhibition (mm)								
Gram positive									
S. aureus	-	-	13	-	-	10	14	11	33
Gram negative									
E. coli	-	-	-	-	-	10	10	11	29
K. pneumoniae	-	-	-	14	15	13	16	14	34
P. aeruginosa	-	-	12	12	-	11	13	12	27
P. mirabilis	-	11	10	12	-	-	-	-	25

Table 4. Antifungal activity of methanol and ethyl acetate extracts of blue and white flowered leaves of *Clitoria ternatea*

Microorganisms	*Candida albicans*				*Candida tropicalis*				*Candida kruzi*			
	10 mg	12 mg	14 mg	15 mg	10 mg	12 mg	14 mg	15 mg	10 mg	12 mg	14 mg	15 mg
	Zone of inhibition (mm)											
Blue flowered leaves (methanol)	-	-	11	-	-	-	-	10	11	13	-	12
White flowered leaves (methanol)	12	-	15	-	-	-	12	15	14	-	-	13
Blue flowered leaves (ethyl acetate)	-	11	12	13	-	16	14	13	-	11	-	-
White flowered leaves (ethyl acetate)	-	11	13	20	-	14	-	-	-	11	12	12
Positive control (Nystatin)	26				30				27			

3.8.3 Determination of total antioxidant activity

The highest radical scavenging activity was observed in the methanolic extract of blue flowered leaves (94%) and ethyl acetate extract of white flowered leaves (95%) of *C. ternatea* with a IC_{50} value of 14.31 and 14.62 µg/mL respectively. The lowest radical scavenging activity was observed in the methanolic extract of white flowered leaves (59%) with a IC_{50} value of 38.06 µg/mL (Table 7; Fig. 5) which is in accordance with [31] have reported methanol extract of *C. ternatea* showed the highest radical scavenging activity.

Table 5. DPPH radical scavenging method (percentage inhibition) and IC_{50} values

C. ternatea extracts	Solvents	IC_{50} values (µg/mL)	Percentage inhibition
Blue flowered leaves	Methanol	93.43	58
	Ethyl acetate	8.53	86.6
White flowered leaves	Methanol	27.28	72.4
	Ethyl acetate	65.75	66.2

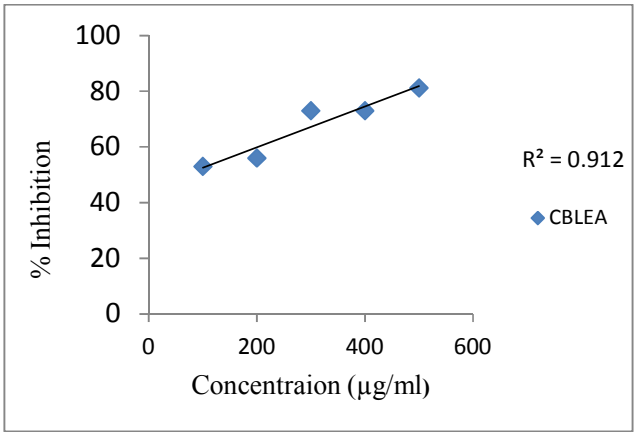

Fig. 3. DPPH free radical scavenging assay of *C. ternatea* blue flowered leaf ethyl acetate extract (CBLEA)

CBLEA- C. ternatea blue flowered leaf ethyl acetate extract

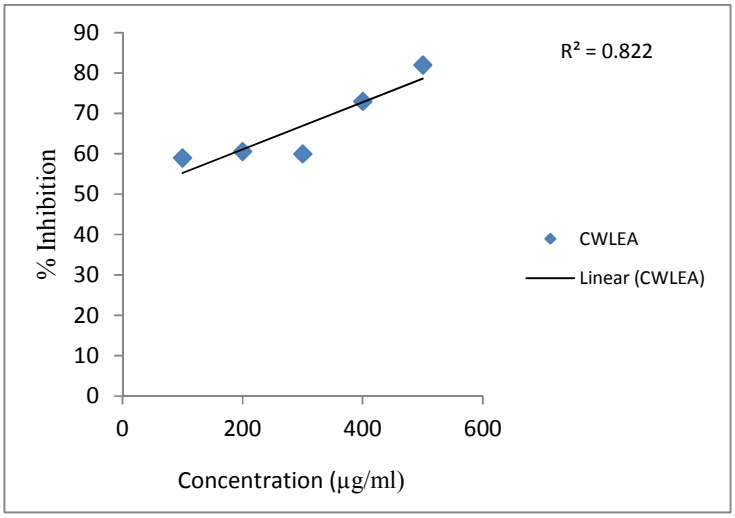

Fig. 4. H_2O_2 assay of *C. ternatea* white flowered leaf ethyl acetate extract (CWLEA)

CWLEA- C. ternatea white flowered leaf ethyl acetate extract

Table 6. H_2O_2 scavenging assay (percentage inhibition) and IC_{50} values

C. ternatea extracts	Solvents	IC_{50} values (µg/mL)	Percentage inhibition
Blue flowered leaves	Methanol	31.91	62
	Ethyl acetate	14.60	79
White flowered leaves	Methanol	87.34	54
	Ethyl acetate	10.34	82

Table 7. Total antioxidant activity (percentage inhibition) and IC_{50} values

C. ternatea extracts	Solvents	IC_{50} values (µg/mL)	Percentage Inhibition
Blue flowered leaves	Methanol	14.31	94
	Ethyl acetate	22.17	63
White flowered leaves	Methanol	38.06	59
	Ethyl acetate	14.62	95

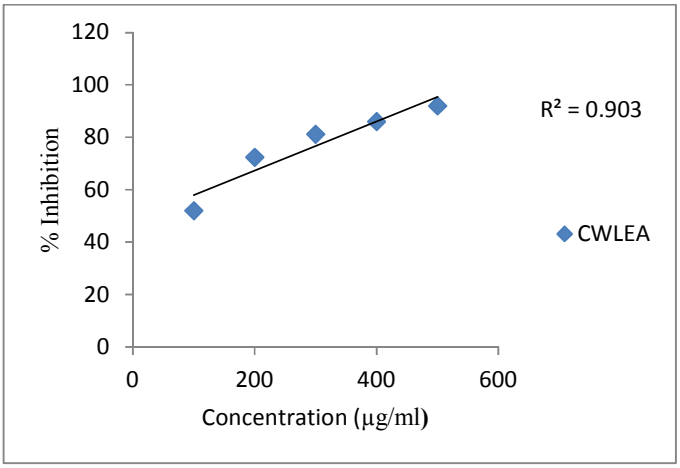

Fig. 5. Total antioxidant assay of *C. ternatea* white flowered leaf ethyl acetate extract (CWLEA)
CWLEA- C. ternatea white flowered leaf ethyl acetate extract

4. CONCLUSION

In this study, methanol and ethyl acetate extract of blue and white flowered leaves of *C. ternatea* were evaluated for the presence of phytochemicals, antimicrobial and antioxidant activity. The presence of various bioactive compounds indicates that these leaves extracts compounds can be used as therapeutic drugs for insect bites, skin diseases, asthma, burning sensation, ascites, inflammation, leukoderma, leprosy, hemicranias, amentia and pulmonary tuberculosis. Further studies needs to elucidate the molecular mechanism of interaction of plant-based drugs with human body in different diseases.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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