Phytochemical analysis, Antioxidant and Antibacterial activity of Clitoriaternatea

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Abstract

Medicinal plants are of great interest today because of their vast source of phytochemicals having potent health promoting activities. In the present study we investigated the phytochemical screening, antioxidant and antibacterial activity of root extract of Clitoriaternatea (butter fly pea). The results suggest that the root extract possess potent antioxidant activities such as reducing power assay, metal chelating assay, total antioxidant activity andantibacterial activity against pathogenic bacteria such asP.aerogenosa and B.subtilis. It is also showed that both aqueous and ethanol extracts possess flavonoid and phenolics. These results conclude that the root extract may be used as a potential antioxidant source.

Keywords – C.ternatea (root), Phytochemical, flavonoid, antioxidant, Reducing power, ferrous chelating, antibacterial.

I. INTRODUCTION

Plant-derived substances have recently become of great interest due to their versatile applications in the medicine. Medicinal plants are the richest resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Ncube, Afolayan and Okoh, 2008)(Rice-Evans, 2004) and reduce the risk of certain diseases such as cancer, heart diseases and stroke (Prior and Cao, 2000). Oxidative stress is an imbalance between the production of reactive oxygen species and antioxidant defense mechanisms, causing oxidative damage to the cellular biomolecules including DNA, protein, nucleic acid, and membrane lipids. The increased oxidative stress of cellular physiology has been implicated in the pathogenesis of many diseases such as cancer, chronic kidney disease, and neurodegenerative diseases (Phaniendra .A et al 2015). Cells are equipped with different kinds of antioxidant defense systems to fight against ROS and to maintain the redox homeostasis of cell (Bergendiet al., 1999). When the mechanism of antioxidant protection becomes unbalanced in human body, antioxidant supplement may be used to help reduce oxidative damage. There are many synthetic antioxidants in use. However, they have several side effects (Ito et al., 1983), such as risk of liver damage and carcinogenesis in laboratory animals (Gao et al., 1999). There is therefore a need for more effective, less toxic, and cost effective antioxidants. Medicinal plants appear to have these desired comparative advantages, hence the growing interest in natural antioxidants from plants

Natural antioxidant increases the antioxidant capacity of plasma and reduces the risk of diseases. The most extensively used natural antioxidants are ascorbic acid and tocopherol. Synthetic antioxidants includebutylatedhydroxytoluene (BHT), butylatedhydroxyanisole (BHA), propylgallate (PG) and tertiary butyl-hydroquinone (TBHQ). They are produced by humans by way of synthesis or biosynthesis in the industries. Recent studies reported that synthetic antioxidants possess some carcinogenic properties and also have been suspected to promote some negative health effects. Therefore nowadays use of synthetic antioxidants are restricted and there is growing interest in the use of natural antioxidants(Esfahani et al, 2011 and Landete, 2013). So for the safety of human health, investigations of antioxidants are now focused more in natural origin.

Clitoriaternatea known as Butterfly pea is a perennial, tropical leguminous twiner. It belongs to the order, Fabales, family Fabaceae and sub-family, papilionaceae. *C. ternatea* is a high-quality, protein-rich legume, a "tropical alfalfa" often referred to as a protein bank that can be grown at low cost (Cook et al., 2005). Itis commonly also called Clitoria, blue-pea, kordofan pea (Sudan), cunha (Brazil or pokindong (Philippines). This plant is known as Aparajit (Hindi), Aparajita (Bengali) and Kokkattan (Tamil) in Indian traditional medicine. It has several synonyms in Ayurvedic scriptures like, Sanskrit names: Aparajita, Girikarnu, Asphota and Vishnukranta. The plant 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 naturalized. Its sparsely pubescent stems are sub-erect and woody at the base and may be up to 5 m long. They root only at the tips (Cook et al., 2005; Staples, 1992). The leaves are pinnate, bearing 5-7 elliptical, 3-5 cm long leaflets. The flowers are solitary or paired, deep blue or pure white, about 4 cm broad. The fruits are flat, linear, sparsely pubescent pods that dehisce violently at maturity and throw 8-10 dark and shiny seeds (Cook et al., 2005; Staples, 1992). Livestock tend to prefer it over other legumes and grasses and it is therefore much valued as a pasture legume. It is also used for cut-and-carry feeding systems and cut for hay and silage (Gomez et al., 2003). It is used as a ley legume or as green manure. It is a valuable cover crop in rubber and coconut plantations. The young pods are edible and used as vegetables in the Philippines (Staples, 1992). The flowers of the plant *C. ternatea* resemble a conch shell; therefore it is commonly called "Shankpushpi" in the Sanskrit language Butterfly pea is used in fences and in trellises as an ornamental for its showy flowers, valuable for dyeing and in ethno-medicine (Cook et al., 2005).

All parts of *Clitoriaternatea* contain peptides called cliotides that have potent anti-microbial properties against Escherichia coli (Nguyen GiangKienTruc et al., 2011).*Clitoriaternatea* is one of four herbs traditionally used as ShankaPushpi, an Ayurvedic medicine used to promote neurological health. 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 is an ayurvedic drug used for its action on theCNS (Central Nervous System), especially for boosting memory and improving intellect. It is reported to be a good "Medhya" (brain tonic) drug and, therefore, used in the treatment of "ManasikaRoga" (mental illness)i.e., for treatment of neurological disorders. Roots, seeds and leaves are the reported plant part used from ancient times in ayurveda, but for slightly different purposes. The major phytoconstituents found in *Clitoriaternatea* are the pentacyclictriterpenoids such as taraxerol and taraxerone.

The *C.ternatea* extract possesses a wide range of pharmacological activities including anti-inflammatory, anti-diabetic, anti-microbial, and antioxidant antipyretic, anti-helminthic, and analgesic activities (Mukherjee PK et al., 2008 and Gupta GK et al., 2010) (Kamkaen, Wilkinson, 2009 and Mukherjee et al, 2008; Ramkissoon JS, et al., 2013). *C.ternatea* flower exerts anti-hyperglycemic effects in alloxan-induced diabetic rats (Soundrapandian et al., 2007). *C.ternatea* possess anxiolytic, antidepressant, anticonvulsant and antistress activity on CNS (Neeti N. et al, 2003).CT significantly increases the proliferation and growth of neurospheres and has growth promoting neurogenic effect and enhances learning and memory (Kiranmai S. Rai 2010 and 2001) by increasing the functional growth of neurons of the amygdala. (Kiranmai S. Rai, 2005) A highly basic small protein 'finotin' was purified from seeds of *C. ternatea* which has broad and potent inhibitory effect on the growth of various important fungal pathogens of plants (S. Kelemu et al, 2004). *C.ternatea* is diuretic and laxative. It is useful in ophthalmopathy, bronchitis and asthma (Dnyaneshwar, et al) tubercular glands hemicrania, burning sensation, starngury, helminthiasis, inflammation, vitiated condition of pitta vicerormegaly and fever (NeelmaniChauhan et al, 2012). *C. ternatea* showed the most promising mosquito larvicidal activity (Nisha Mathew et al, 2008).*C. ternatae* has significant hepatoprotective effects on drug-induced liver damage (Nithianantham et al., 2011). Blue and white flowers of *Clitoriaternatea* exhibited significant antioxidant activity and the (Sivaprabha .J et al, 2008).*C.ternatae* exhibits significant antitumour effects in DLA cell lines (Lijy Jacob and M.S. Latha, 2012 and VidhyaRamaswamy. et al, 2011).

The roots are most widely used and are bitter, refrigerant, laxative, intellect promoting, diuretic, anthelmintic and tonic and are useful in dementia, hemicrania, burning sensation, leprosy, inflammation, leucoderma, bronchitis, asthma, pulmonary tuberculosis, ascites and fever.*Clitoriaternatea L.* root has anti-pyretic effect comparable to that of paracetamol (Parimaladevi B, Boominathan R and Mandal SC). *C. ternatea* roots exhibited a significant inhibition in yeast-induced pyrexia in rats (Devi BP, Boominathan R and Mandal SC).*Clitoriaternatea* root extract enhances acetylcholine content and acetyl cholinesterase activity in a similar fashion to the standard cerebro protective drug Pyritinol (Taranalli AD and Cheeramkuzhy TC). The roots of *Clitoriaternatea* have anti-inflammatory properties and are useful in severe bronchitis, asthma (Dnyaneshwar J Taur and Ravindra Y Patil, 2011).

Antioxidants or "free radical scavengers" are nutrients as well as enzymes that are believed to play a vital role in preventing the development of chronic diseases such as cancer, heart disease, Alzheimer's, diabetics etc., by blocking or slow down the oxidation process by neutralizing free radicals.Bacteria are very common in the body and in the surroundings. Less than 1% of these bacteria is harmful and gives rise to bacterial infection. Bacterial infections are usually treated with antibiotics. Recently, antibiotic resistance has become a global concern as the clinical efficacy of many existing antibiotics is being threatened by the

emergence of multi drug resistant pathogens. Over the last centuries, intensive efforts have been made to discover clinically useful antibacterial drugs. Green plants possess the broadest spectrum of pharmacological activity and have been the source of many useful compounds like flavonoids, alkaloids, tannins and other phenolic compounds which have been established as the most important bioactive compounds called secondary metabolites. This study focused mainly on the determination of antioxidant and anti-bacterial property and GC-MS analysis of the components present in the roots of *C.ternatae*.

II. MATERIALS AND METHODS

A. Extraction of plant material

The plant roots were collected from Pondicherry University and the plant was authenticated by Prof. Pardhasaradhy, Department of Ecology, Pondicherry university. The plant material was washed in warm water and cut into small pieces and then dried. The dried roots were homogenized into fine powder and stored in an airtight container.

1. Aqueous extract

10 grams of root powder was taken and mixed with 100 ml sterile water and placed in a shaking water bath for 1 hour. The mixture was filtered through whatmen filter paper No.1. The filtrate was dried in oven. The dried extract was collected by scratching and stored. 0.420 grams of extract was obtained.

2. Ethanol extract

5.6 grams of the root powder was mixed with 56ml of 75 % ethanol in a conical flask and was kept for 48 hours and mixed in between. The mixture was filtered using whatmen filter paper No.1. The filtrate was stored in refrigerator.

B. Qualitative phytochemical analysis

Preliminary chemical tests were carried out using crude powder extract to identify different phyto-constituents.

1. Alkaloids

A known quantity of extract was dissolved in dilute HCl and filtered. The filtrate was used for the following tests

*a) Mayer's test - T*o a few ml of the filtrate added 1 or 2 drops of mayer's reagent. Colored precipitate indicates presence of alkaloids.

b) Wagner's test - To a few ml of the filtrate added few drops of wagner's reagent. A reddish brown precipitate indicates presence of alkaloids.

c) Hager's test - To a few ml of filtrate added 1 or 2 ml of hager's reagent Yellow colored precipitate indicates presence of alkaloids.

2. Carbohydrate

Few grams of extract was dissolved in 5 ml of distilled water and filtered. The filtrate was used for the following tests.

a) Molisch's test - To a few ml of the filtrate added 2 drops of alcoholic solution of alpha-naphthol. The mixture was shaken well and added 1 ml of concentrated sulphuric acid slowly along the side of the test tube. The tube was allowed to stand for some times. Formation of violet indicates presence of carbohydrate.

b) Fehling's test **-** To 1 ml of the filtrate added 1ml of fehlings reagent A and 1ml of fehlings reagent B. the tubes were then boiled in water bath. Red colored precipitate indicates presence of carbohydrate.

c) Benedicts test **-** To 1 ml of filtrate added 1 ml of Benedict's reagent. The mixture was heated for 2 minutes in a water bath. Orange red precipitate indicates the presence of reducing sugar.

3. Flavanoids

a) Alkaline reagent test **-** To the extract added few drops of sodium hydroxide to form a yellow colored solution and then added dilute hydrochloric acid. Formation of a yellow color solution which turns colorless on addition of dilute hydrochloric acid indicates the presence of flavonoids.

b) Aluminium chloride test **-** To 4ml of the extract added 1 ml of 1% aluminium chloride and shake well. Formation of yellow colored precipitate indicates the presence of flavonoids.

4. Saponins

a) Froth test **-** To the extract added distilled water and made up to 20 ml and shake the solution for 15 minutes. Appearance of a layer of 1 -2 cm of froth indicates the presence of saponins.

b) Foam test **-** To 0.5 grams of the extract added 2 ml of water and shaken. The formation of foam which persists for 10 minute indicates the presence of saponins.

5. Proteins and Amino acids

100mg of the extract was dissolved in 10 ml of distilled water and filtered using whatman filter paper. The filtrate was used for the following tests.

a) Biuret test **-** To 2 ml of the filtrate added 1 drop of 2% copper sulfate solution. Then, 1 ml of 95% ethanol and potassium hydroxide pellet was added. Formation if pink colored ring was indicates the presence of protein.

b) Ninhydrin test - To 2 ml of the filtrate added few drops of ninhydrin solution. Formation of purple color indicates the absence of amino acids.

c) Xanthoproteic test - A few drops of concentrated nitric acid was added to the filtrate. The formation of yellow color indicates the presence of aromatic amino acids.

6. *Tannins*

a) Ferric chloride test - A quantity of extract was boiled with 45% ethanol for 5 minutes. The mixture was cooled and filtered. A few ml of distilled water is added to the filtrate and then 2 drops of 5% ferric chloride is added. Dark greenish to black color change indicates the presence of tannins.

7. Phenols

a) Ferric chloride test **-** A quantity of extract was boiled with 45% ethanol for 5 minutes. The mixture was cooled and filtered. A few ml of distilled water is added to the filtrate and then 2 drops of 5% ferric chloride is added. Dark greenish to black color change indicates the presence of tannins.

8. Resins

a) Precipitation test **-** To 0.2 g of the extract 15 ml of 96% ethanol was added and the mixture was added to 20 ml distilled water. Formation of precipitate indicates presence of resins.

9. Glycosides

50 mg of extract was to concentrated sulphuric acid and hydrolyzed the mixture in water bath for 2 hours and then filtered. The filtrate was used for the following tests.

a) Legal's test **-** 50 mg of the extract was dissolved in pyridine and added sodium nitroprusside solution. Then 10% sodium hydroxide was added to the mixture. Formation of pink color indicates the presence of glycosides.

b) Test for cardiac glycosides- To 5 ml of the filtrate added 2 ml of glacial acetic acid containing one drop of ferric chloride. Then, 1 ml of concentrated sulfuric acid was added. Appearance of green ring in the acetic acid layer indicates the presence of cardiac glycosides.

10. Anthroquinones

To 0.5 grams of the extract added 5 ml of chloroform and filtered. To the filtrate added equal volume of 10% ammonia solution. Formation of bright pink color in the aqueous layer indicates the presence of free anthroquinone.

11. *Diterpenes*

a) Copper acetate test **-** The extract was dissolved in water and added 3-4 drops of copper acetate solution into it. Formation of emerald green color indicates presence of terpenoids.

12. Gums and Mucilages

The extract was dissolved in 10 ml distilled water and added 25 ml of absolute alcohol with constant stirring. White or cloudy precipitate indicates of presence of gums and mucilages.

C. Quantitative phytochemical analysis

1. Determination of total phenolic content

The amount of total phenol content was determined by Folin-Ciocalteu reagent method. Gallic acid was used as standard with a working standard concentration of 2.5 mg per 100 microliter. The working concentrations of plant extract (both ethanol and aqueous) 50µg, 100µg, 150µg, 200µg, 250µg, 300µg, 350µg, 400µg, 450µg, 500µg were diluted with distilled water and made upto 10µl. To this 25µl of Folin-Ciocalteu reagent was added and incubated for 5 minutes in room temperature. Then added 25µl of 7.5 % sodium carbonate and made up the volume to 200µl and incubated in dark for 30 minutes. The absorbance was measured at 760nm using UV-vis spectrophotometer.

2. Determination of Total Flavanoid Content

The plant extract solution of varying concentration were (both ethanol and Aqueous) (20-200µg) taken and was made up to 100µl with the solvent(DMSO).Then 0.3ml of distilled water was added followed by 0.03ml $NaNO_2$ and was incubated for 5 min at RT. This was followed by the addition of 0.03ml of $AlCl_3$ (10%) and was incubated for 5 min at RT and 0.2 ml of 1 mMNaOH was added and the total volume was made up to 1ml with distilled water and the absorbance was measured at 510 nm. Various concentrations of standard quarcetin solution were used to make a standard calibration curve. The flavonoid content was expressed in equivalents ofquercetin.

D. In vitro antioxidant assay

1. Reducing power assay

The reducing power ability of the extracts were assessed by the method of Yildirim,Mavi,and Kara(2001).The extract (5mg,10mg,15mg,20mg) in 1ml of the corresponding solvent was mixed with 2.5ml of phosphate buffer (0.2M,pH 6.6) and 2.5ml $K_3Fe(CN)_6$ (10g/l).The mixture was then incubated at 50°C for 30 min. After incubation ,2.5ml of TCA (100g/l) was added and the mixture was centrifuged at 1650g for 10 min.Finally ,2.5ml of the supernatant was them mixed with 2.5ml of distilled water and 0.5ml of $FeCl_3$ (1g/l) .The absorbance was measured at 700nm. High reducing power was indicated by high absorbance.

2. Total antioxidant activity

The assay is based on the reduction of Mo (VI) to Mo(V) by the plant extract. At acidic pH there is formation of a green phosphate/Mo(V) complex. The total anti-oxidant capacity of the extracts was assessed by the method ofPrieto, Pineda & Aguilar, 1999.

Aliquots of 0.1 ml of sample solution (containing 50, 250, 500 µg of plant extract in corresponding solvent) was added in a test tube with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were capped and incubated in a thermal block at 95 C for 90 min. The samples were then allowed to cool at room temperature; the absorbance was then measured at 765nm against a blank.

3. Ferrous (Fe2+) chelation activity

Most reactive oxygen species (ROS) are generated as by-products during mitochondrial electron transport and other metabolic reactions. In addition, ROS are formed as necessary intermediates of metal catalyzed oxidation reactions. The transition metal ion Fe^{2+} possesses the ability to perpetuate the formation of free radicals by gain or loss of electrons. Therefore, the reduction of the formation of reactive oxygen species can be achieved by the chelation of metal ions with chelating agents.The working concentrations of plant extract ranging from 50µg-500µg was taken in different test tubes and made up the volume to 1 ml with corresponding solvent. 50 µl of ferrous chloride and 200ml of ferrozine were added to all the tubes. The tubes were incubated for 20 minutes at RT and the absorbance was measured at 562nm.

E. Evaluation of antibacterial activity

The compound added diffuses from the well into the agar. The concentration of the compound will be highest next to the disk, and will decrease as distance from the disk increases. If the compound is effective against bacteria at a certain concentration, no colonies will grow where the concentration in the agar is greater than or equal to the effective concentration. This is the zone of inhibition. This along with the rate of antibiotic diffusion is used to estimate the bacteria's sensitivity to that particular antibiotic.

The extracts of *C. ternatae*root were used for bioassay against various bacteria. The agar well diffusion method was used for antimicrobial assay. The inoculum was prepared from 24 hours old culture of bacterial isolation in LB broth. LB agar plates were prepared and inoculum was seed by spread plate method. The wells were made on agar plates seeded with the organism using gel puncture. The wells were filled with different concentrations (50µg, 100µg, 150µg, 200µg) of the extract (aqueous and ethanol). The plates were incubated in at 37oC for 1-24 hours. Antimicrobial activity was evaluated by measuring the inhibition zone in millimeter in diameter and tabulated. The positive control (antibiotic disc) was kept in separate plate and DMSO was used as the negative control.

III. *RESULTS*

A. Qualitative phytochemical screening

The results of qualitative phytochemical analysis of the aqueous and ethanolic root extract of *C.ternatae* are shown in Table 1. The aqueous extract of *C.ternatae* shows presence of flavonoids, alkaloids, reducing sugar, diterpines, saponins and glycosides. Ethanol extract of *C.ternatea* shows presence of terpenoid, flavonoid, tannin which may act as principle antioxidants and also contains alkaloids, reducing sugar, amino acids and glycosides.

Table: 1. Qualitative analysis of aqueous and ethanol extract of *Clitoriaternatea*

S.No	Phytochemicals	Tests	Aqueous extract	Ethanol extract
1	Alkaloid	Mayer's test Wagner's test Hager's test	+ + +	+ + +
2	Carbohydrates	Molish test	-	-
3	Reducing sugar	Fehling's test Benedicts test	- +	- +
4	Saponins	Froth test Foam test	+ +	- +
5	Flavanoids	Alkaline reagent test Aluminium chloride test	+ +	+ -
6	Phenols	Ferric chloride test	+	+
7	Amino acids	Ninhydrin test Xanthoproteic test	- +	- +
8	Tannins	Ferric chloride test	+	+
9	Resins	Precipitation test	-	+
10	Glycosides	Legal's test: Cardiac glycosides	- +	+ -
11	Proteins	Biuret test	-	-
12	Free anthraquinones		-	-
13	Diterpenes		+	+
14	Gums and Mucilage		-	-

However,both the extracts were found to be negative for biuret test representing the absence of protein. In addition both the extracts were found to do not contain free anthroquinones and gums and mucilages.

B. Qualitative phytochemical analysis

1. Total phenolic content

Phenolic compounds are among the most widely occurring secondary metabolites in the plants which are known to have antioxidant and anti-tumor properties. Phenols make intestinal mucosa more

resistant and reduce secretion, stimulate normalization of deranged water transport across the mucosal cells and reduction of the intestinal transit, blocks the binding of B subunit of heat-labile enterotoxin, resulting in the suppression of heat-labile enterotoxin-induced diarrhea, astringent action. Thus it is also known to pocess antimicrobial, anthelmintic and antidiarrhoeal activity. The total phenolic content of the aqueous and ethanolic extracts of *C. ternatae* was spectrophotometrically determined by Folin-Ciocalteu reagent method described by Singleton and Rossi (1965) using galic acid as the standard. The total phenolic content in the examined extracts is expressed in terms of µg of Galic acid/gram of extract.Gallic acid standard curve is shown in figure 1. Total phenol content of aqueous extract and ethanolic extract of *C. ternatae*were shown in Fig. 2. The amount of total phenol of aqueous extract was 42.14 ± 1.38 µg/g, while that of ethanolic extract was found to be 62.27±1.06 µg/g.

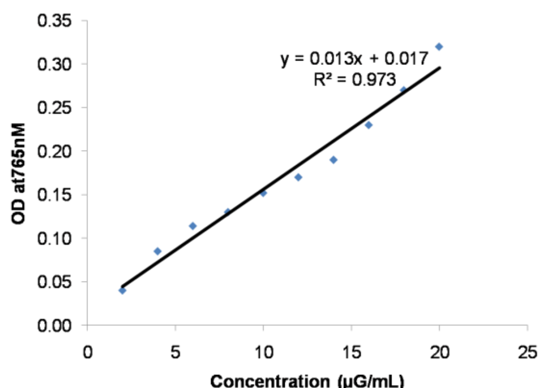

Fig 1. Standard graph of Gallic acid

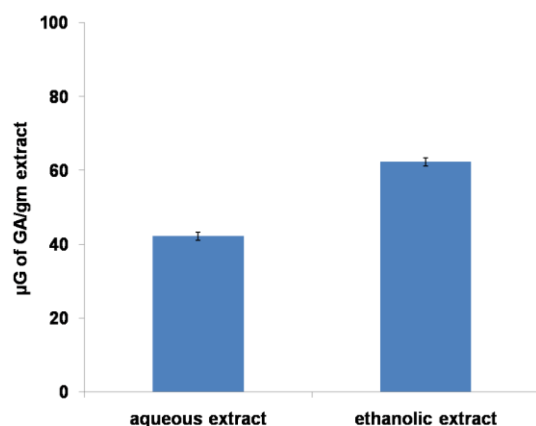

Fig: 2.The phenolic content of aqueous and ethanolic extracts of *C. ternatae*

2. *Total flavonoid content*

The total flavonoid content of the aqueous and ethanolic plant extract was determined spectrophotometrically using quercetin as standard and is expressed in terms of µg of quercetin/g of extract. The amount of total flavonoids in aqueous and ethanolic extract was found to be 74.35 and 93.7 µg/gm respectively (figure 4). The standard curve of quercetin was shown in figure 3.

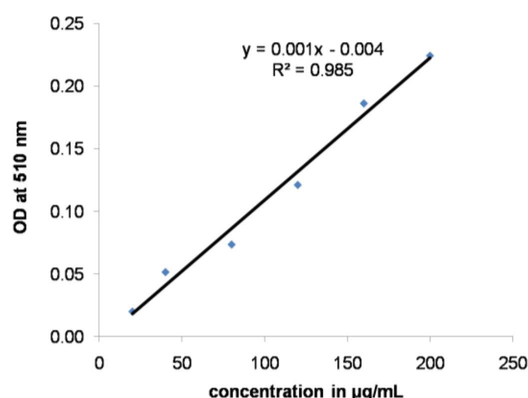

Fig: 3. Standard graph of quercetin.

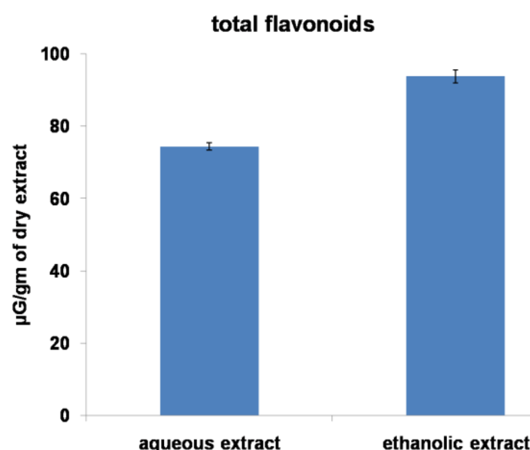

Fig: 4.The total flavaniod content of aqueous and ethanolic extracts of *C.ternatae*

C. *Invitro antioxidant assay*

1. *Reducing power assay*

The reducing power assay for aqueous and ethanolic extract of *Clitoriaternatae* root were assessed by the method of Yildirim,Mavi,and Kara (2001) spectrometrically. The reducing power assessment of compounds may serve as significant indicators of its potential antioxidant activity. In the reducing power assay, the presence of antioxidants in the extract result in the reduction of Fe^{3+} to Fe^{2+} by donating an

electron. The amount of Fe^{2+} can then be monitored by measuring the formation of blue color at 700 nm. Ascorbic acid was used as the standard antioxidant. Higher absorbance of a reaction mixture indicated greater reducing power. From the figure 5, it is observed that the reducing power of aqueous and ethanolic root extract of *C. ternatae*showed an increase in reducing capacity with the increase in concentration. The ethanolic extract is found to be having higher reducing power compared to aqueous extract.

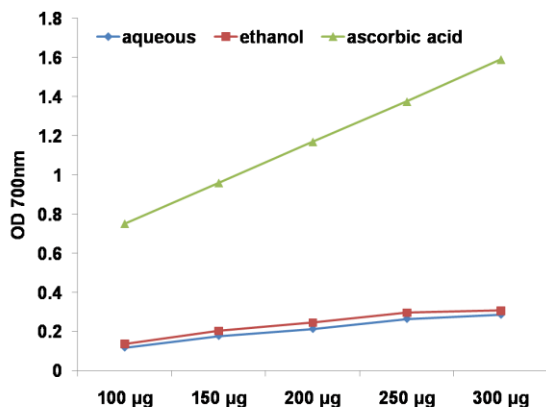

Fig: 5. Reducing power assay of aqueous and ethanol extracts of *C.ternatae*

1. *Total antioxidant activity*

The total anti-oxidant capacity of the aqueous and ethanolic root extracts of *Clitoriaternatae* was assessed by phoshomolebdate method of Prieto, Pineda & Aguilar, 1999. The total antioxidant capacity was expressed as µg equivalents of ascorbic acid by using the standard ascorbic acid graph (figure 7). Total antioxidant capacity is expressed as µg of AA equivalent per gm of extract. Total antioxidant activity of ethanolic extract (38.20)of *Clitoriaternatae* showed higher compared to aqueous extract (8.0) (fig: 8).

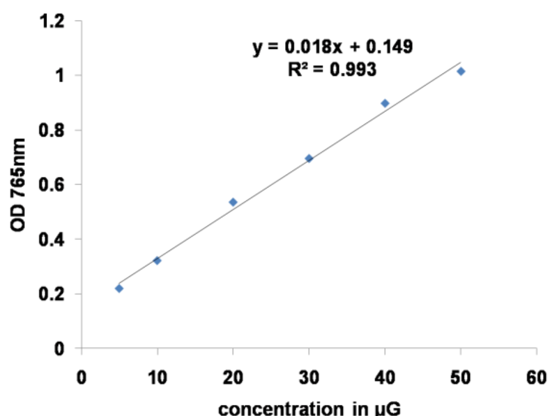

Fig: 6. Standard graph for total antioxidant activity using ascorbic acid.

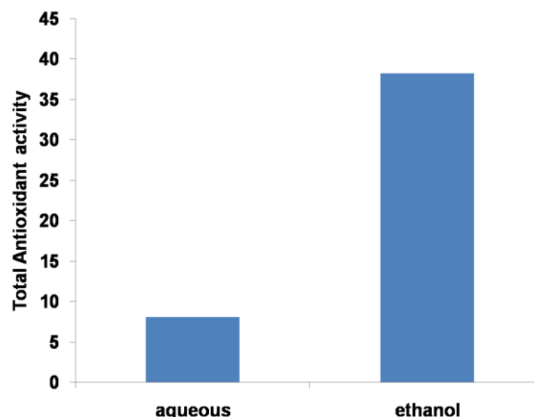

Fig: 7. Total antioxidant activity of aqueous and ethanolic extract of *C.ternatae*.

2. *Ferrous (Fe^{2+}) chelation activity*

Most reactive oxygen species (ROS) are generated as by-products during mitochondrial electron transport and other metabolic reactions. In addition, ROS are formed as necessary intermediates of metal catalyzed oxidation reactions. The transition metal ion Fe^{2+} possesses the ability to perpetuate the formation of free radicals by gain or loss of electrons. Therefore, the reduction of the formation of reactive oxygen species can be achieved by the chelation of metal ions with chelating agents. The ferrous chelation activity of *C.ternatae* root extract was assessed and EDTA, a known chelating agent was used as standard. Addition of the aqueous and ethanolic extracts of *Clitoriaternatae* interferes with the ferrous-ferrozine complex and the purple colour of the complex decreased with the increasing concentrations of the fractions. Both the extracts captured ferrous ions before ferrozine and thus have ferrous chelating ability. The aqueous extract showed higher chelating property than the ethanolic extract (fig: 9).

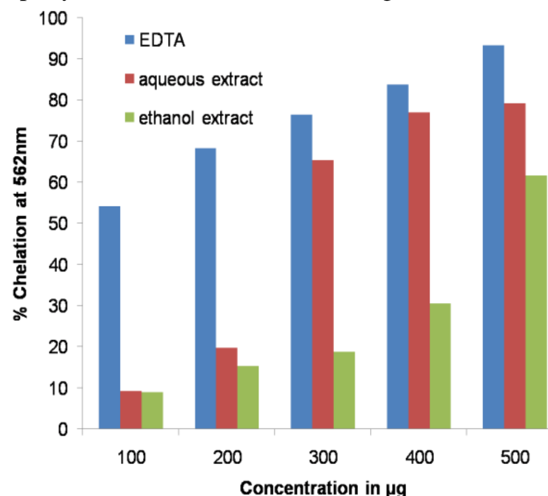

Fig: 8. Ferrous chelating activity of aqueous and ethanolic extract of *Clitoriaternatae*

D. Evaluation of antimicrobial activity

The antimicrobial activity of the *C. ternatae* root extracts (aqueous and ethanol) were evaluated for antimicrobial activity against *Bacillus subtilis,* and *Pseudomonas aeruginosa*. The aqueous extract showed antimicrobial activity against *P. aeruginosa,*and *B. subtilis*.

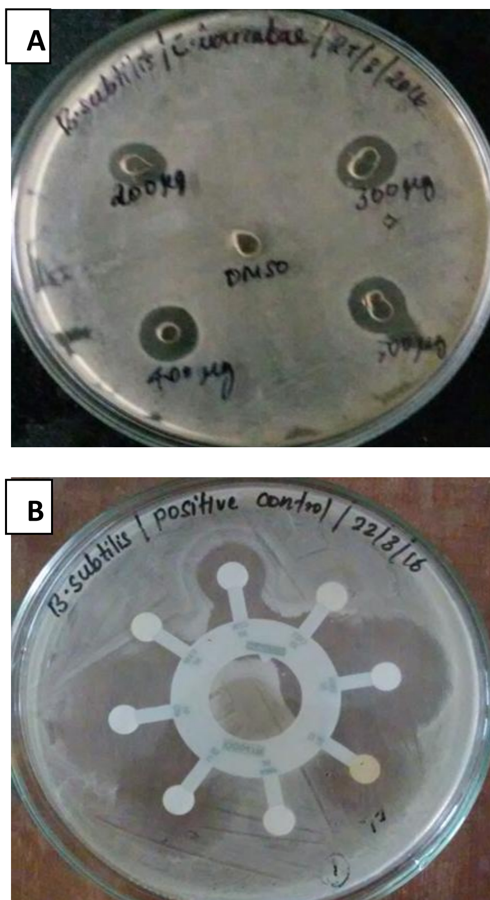

Fig: 9.
A) Antibacterial activity of *C. ternatae* root extract (aqueous) showing 13mm zone of inhibition at 200µg concentration against *Bacillus subtilis*.
B) Positive control for *B.subtilis* showing zone of inhibition.

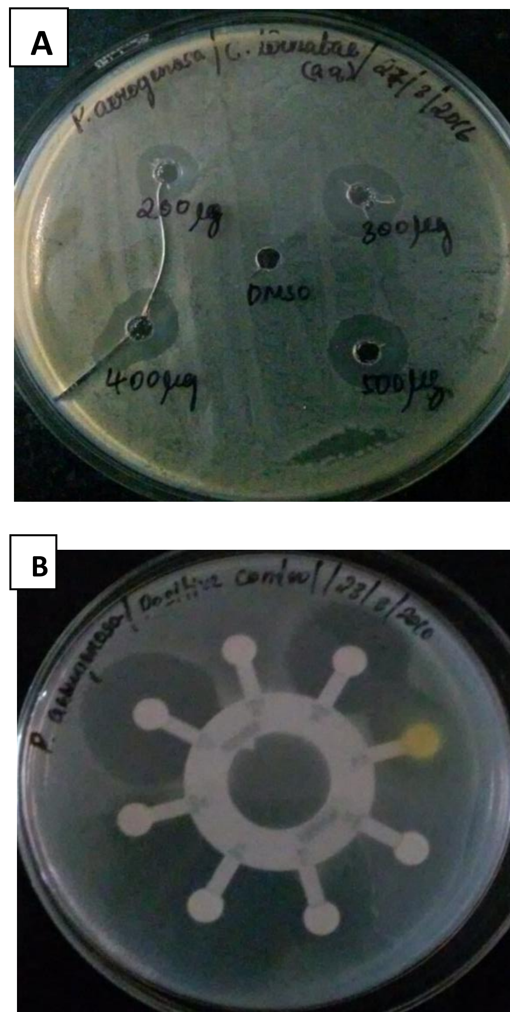

Fig: 10.
A) Antibacterial activity of CT root extract (aqueous) showing 15mm zone of inhibition at 200µg concentration against *Pseudomonas aerogenosa*
B) Positive control for *P.aerogenosa* showing zone of inhibition.

The aqueous extract showed antibacterial activity against *P.aerogenosa* and *B.subtilis*. The zone of inhibition increased in a dose dependent mannar. The aqueous extract of *C. ternatae*at 50µg showed a zone of inhibition of 11mm, at 100µg and 150µg showed 13mm and at 200 µg showed 15mm against *P.aerogenosa*. Aqueous root extract showed zone of inhibition of 10mm at 50µg concentration, 11mm at 100µg and 13 mm at 150µg and 200µg concentrations against *B. subtilis.*However, the ethanolic extract did not show any inhibition. The result suggest that the aqueous extract is found to possess potent antibacterial activity than ethanolic extract.

IV. DISCUSSION

In a human body system living cells produces free radicals and other reactive oxygen species (ROS) as byproducts of various physiological and biochemical process. Mostly free radicals are produced as a byproduct of mitochondrial electron transport of aerobic respiration. It can also be generated as a result of metal catalyzed oxidation reactions or by oxidoreductase enzymes. Antioxidant increases the antioxidant capacity of plasma and reduces the risk of diseases.

A. Phytochemical analysis

C.ternatae roots were rich in alkaloids, flavonoids, phenols, tannins, diterpenes, saponins and cardiac glycosides while proteins and quinones were absent. It is possible that these secondary metabolites might be responsible for the bioactivity of the plant extract (Nino et al., 2006). The presence of alkaloids and saponins in the plant indicates that the plant extract could be used for antifungal activity (Rani and Murty, 2006). Secondary metabolites may be used for the preparation of drug in a systematic way which may lead to the cure of many ailments in the future (Shanthi and Amudha, 2010).

B. Total phenolic content

Plant polyphenols possess an ideal structural chemistry for free radical scavenging activity. Antioxidative properties of polyphenols arise from their high reactivity as hydrogen or electron donors from the ability of the polyphenol derived radical to stabilize and delocalize the unpaired electron (chain-breaking function), and from their potential to chelate metal ions (termination of the Fenton reaction) (Rice-Evans et al., 1997). The amount of total phenol content can be determined by Folin-Ciocateu reagent method (McDonald et al., 2001). Gallic acid, tannic acid, quercetin, chlorogenic acid, pyrocatechol and guaiacol can be used as positive controls (Chanda and Dave, 2009). The total phenolic content is expressed in terms of standard equivalent (mg g-1 of extracted compound).

The extract had high total phenol content. Phenolics, phenols or polyphenolics are chemical components that occur ubiquitously as natural colour pigments responsible for the colour of fruits of plants. Plant phenols are one of the major groups of compounds acting as primary antioxidant free radical terminators. The antioxidant property of phenols is due to the hydroxyl functional group. The antimicrobial property of phenols is by binding to adhesins, enzyme inhibition, substrate deprivation, complex with cell wall, membrane disruption and metal ion complexation. Phenols in plants are mostly synthesized from phenylalanine via the action of phenylalanine ammonia lyase (PAL).These compounds possess a wide spectrum of chemical and biological activities including radical scavenging properties.

C. Total flavonoid content

The antioxidative properties of flavonoids are due to several different mechanisms, such as scavenging of free radicals, chelation of metal ions, such as iron and copper, and inhibition of enzymes responsible for free-radical generation (Benavente-Garcia et al., 1997). Depending on their structure, flavonoids are able to scavenge practically all known ROS. The amount of total flavonoid content can be determined by Aluminum chloride method (Chang et al., 2002). Quercetin and catechin can be used as positive controls (Chanda and Dave, 2009). The flavonoid content is expressed in terms of standard equivalent (mg g-1 of extracted compound).

The antioxidant capacity of flavonoids depends upon their molecular structure (the position of hydroxyl groups and other features in the chemical structure). The flavonoids forms complex with cell wall, binds to adhesins, inhibits the release of autocoids and prostaglandins, inhibits contractions caused by spasmogens and stimulates normalization of the deranged water transport across the mucosal (Cowan MM, 1999).They inhibit GI release of acetylcholine showing antimicrobial and antidiarrheal properties. (Kumar R. et al, 2010).

The quantitative phytochemical assay results indicate that *C. ternatae* plant extract contains significant amounts of flavonoids and phenolic compounds. Both these classes of compounds have good antioxidant potential and their effects on human nutrition and health are considerable. The mechanism of action of flavonoids is through scavenging or chelation (Cook NC and Samman S, 1996). Phenolic compounds are also very important plant constituents because their hydroxyl groups confer scavenging ability (Yildirim A.et al, 2000).

D. In vitro antioxidant activity

The antioxidant reactions involve multiple steps including the initiation, propagation, branching and termination of free radicals. The antioxidants which inhibit the formation of free radicals from their unstable precursors are called preventive antioxidants, and those which interrupt the radical chain reaction (propagation and branching) are the 'chain-breaking' antioxidants (Ou et al., 2001). Generally, there are various methods for determination of antioxidant activities. We have used reducing power, total antioxidant and ferrous chelating activity to assess the antioxidant activity of CT root extract.

E. Reducing power assay

Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity (Oktay et al., 2003). Compounds with reducing power indicate that they are electron donors, and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants (Yen and Chen, 1995). The reducing power can be determined by the method of Athukorala et al., (2006). Ascorbic acid, butylatedhydroxyanisole (BHA), α-tocopherol, trolox and butylatedhydroxytoluene (BHT) can be used as positive controls (Chanda and Dave, 2009).

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Many reports have revealed that there is a direct correlation between antioxidant activities and reducing power of certain plant extracts (Yildirim A.et al, 2001b). Compounds with reducing power indicate that they are electron donors, and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can actas primary and secondary antioxidants (Yen and Chen, 1995). However, the activities of antioxidants have been attributed to various mechanisms such as prevention of chain initiation, decomposition of peroxides, reducing capacity and radical scavenging. (Yildirim A. et al, 2000). The results suggest that both aqueous and ethanolic root extracts of CT pocess almost equel amount of reducing capacity with ethanolic extract showing slightly higher activity. Although they pocess the reducing capacity, the capacity was lesser when compared to the standaerd ascorbic acid.

F. Total antioxidant activity

The total anti-oxidant capacity of the aqueous and ethanolic root extracts of *Clitoriaternatae* was assessed by phoshomolebdate method (Prieto, Pineda & Aguilar, 1999). The total antioxidant capacity was expressed as µg equivalents of ascorbic acid by using the standard ascorbic acid graph. The total antioxidant activity of ethanolic extract of *Clitoriaternatae* showed higher antioxidant activity than aqueous extract.

G. Ferrous chelating activity

Iron can stimulate lipid peroxidation by the Fenton reaction (H_2O_2+ Fe^{2+}= Fe^{3+}+ OH^-+ OH) and can also accelerate lipid peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals that can perpetuate the chain reaction. Metal chelating capacity is significant since it reduces the concentration of the transition metal that catalyzes lipid peroxidation. According to the results, the plant extract is not as good as the standard EDTA but the decrease in concentration dependent color formation in the presence of the extract indicates that it has iron chelating activity.

H. Evaluation of antibacterial activity

The aqueous extract investigated possessed antibacterial activity against *B. subtilis* and *P. aeruginosa* strains of bacteria. The antibacterial activity against *B.subtilis* shows that the plant can be used for treatment of GI infections and diarrhea (Roggers YS, general microbiology, 5th edition). The inhibitory activity against *P.aerugenosa* shows it can be used for boils, sores and wounds since *P.aerugenosa* is known to cause these diseases (Braude AI, Microbiology, 1982).

V. SUMMARY AND CONCLUSION

Recent studies reported that synthetic antioxidants possess some carcinogenic properties and also have been suspected to promote some negative health effects. Thus the use of synthetic antioxidants is restricted and use natural antioxidants are promoted. Hence the replacement of synthetic antioxidants like BHT, BHA and PG with natural compounds is an advantage.Preliminary phytochemical screening revealed that *C.ternatae* roots are rich in phenols, flavonoids, alkaloids, cardiac glycosides, tannins, terpenes and saponins.

From the quantitative phytochemical analysis it is confirmed that *C.ternatae* roots contain high amount of total phenolsthan flavonoids.

In vitro antioxidant studies showed that ethanolic extract has a slightly higher reducing capacity. From the total antioxidant activity assay and ferrous metal chelating assay it is observedthat the ethanolic extract has more activity than aqueous extract. The phenolic content of ethanolic extract of *C. ternatae* showed correlation with all the antioxidant assays done. The study shows the ethanolic extract of *C. ternatae* exhibits high antioxidant activity than aqueous extract but aqueous extract showed more chelation activity.

The evaluation of antimicrobial property of *C. ternatae* revealed that it has significant inhibitory effect in the growth of many pathogenic organisms.

However, the component responsible for the antioxidant, reducing power, metal chelation and antimicrobial properties of *C.ternatae* is still undefined. Therefore, characterization of specific antioxidant components of *C. ternatae* and evaluation of their therapeutic significance is an open area of research.

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