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Phytochemical analysis and bioactivity of selected medicinal plant of butterfly-pea (*Clitoria ternatea* L.) used by Kolam tribe Addjoing region of Telangana and Maharashtra states

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Abstract
Medicinal plants are of great importance to the health of individuals and communities. A large number of plants are claimed to possess the anti-diabetic, anti-fertility, anti hyperlipidaemic, anti-inflammatory, anti-cancer, hepato protective and immune modulatory activities in the traditional therapeutic systems. It is now believed that nature has given the cure of every disease in one way or another. Butter fly-Pea (*Clitoria ternatea*) a valuable medicinal plant possess many bioactive principles which includes diabetes mellitus, chronic bronchitis, goitre, mucous disorders and leprosy. The ethanolic extract of leaves of *C. ternatea* was investigated for its phytochemical properties and analysis for its active chemical ingredients. For qualitative and quantitative phytochemical analysis the ethanol extract of *C. ternatea* acts as a source of therapeutic agent the ethanolic extract of leaves of *C. ternatea* was investigated for its phytochemical properties and analysis for its active chemical ingredients. For qualitative and quantitative phytochemical analysis the ethanol extract of *C. ternatea* acts as a source of therapeutic agent.

Keywords: Butter fly-Pea *C. ternatea* chemical constituents, alkaloids, phytochemical screening, ethanol, extract, antidiabetic

Introduction
Traditional medicines derived from medicinal plants are used by about 60% of the world's population. Though there are various approaches to control diseases and their secondary complications, herbal formulations are preferred due to lesser side effects and low cost. The use of and search for drugs and dietary supplements derived from plants has been increased in recent years. Botanists, Ethno pharmacologists, microbiologists, and chemists are combing the earth for phytochemicals and drugs which could be developed for treatment of highly infectious diseases in a natural way. While 30 to 50% of current pharmaceuticals are derived from plants, only few of them are used as antimicrobials.

Traditional healers have long used plants to prevent or cure infectious conditions. Plants are rich in a wide variety of secondary metabolites, such as Terpenoids, Tannins, Alkaloids, Flavonoids, saponins and Anthraquinones which have been found *in vitro* to have antimicrobial properties. *Clitoria ternatea* (Family- Liguminoceae or Papillioneceae), a perennial herbaceous twiner, stems terete, more or less pubescent. Leaves imparipinnate, petioles 2.1-2.6 cm long; stipules 4 mm long, linear, acute. Leaflets 5-7, sub coriaceous, 2.5-5 by 2-3.2 cm, elliptic-oblong, obtuse, stipules filiform. Flowers axillary, solitary, standard bright or blue or sometimes white, with an orange centre, seed- 6-10, yellowish brown, smooth. Two types- white variety and blue flowered variety; widely distributed throughout India, used as an ornamental plant.

In Southeast Asia the flowers are used to colour food. In Malay cooking, an aqueous extract is used to colour glutinous rice. In Kelantan it is used to colour white rice for *Nasi kerabu*. In Thailand, a syrupy blue drink is made called *nam dok anchan, it* is sometimes consumed with a drop of sweet lime juice to increase acidity and turn the juice into pink-purple. The chemistry, biochemistry and molecular biology of the biosynthesis of flavonoids are identified, that is the largest and important group of flower color pigments is anthocyanin. It is a subclass of flavonoids which not only functional as coloring agents, but also contain an array of health-promoting benefits.

Nowadays, there are numbers of research done in order to identify flavonoids present in fruits and plants such as sweet potato, berries, hibiscus, and many more due to their potential-

promoting effects, besides the technological reasons and organoleptic properties. Numerous publications had identified various methods used for the extraction and storage condition to maintain the stability, as well as reducing the production cost. Freeze drying, drum drying, spray drying, tunnel drying are examples of drying method had been used in order to convert the juice into powder, to extending the shelflife, compared being left in suspension.

Anthocyanin

Derived from Greek words *anthos* (flower) and kyanos (blue), the word anthocyanin was inventively used to express the blue pigments of cornflower, *Centura cyanus*. Anthocyanin is an antioxidant eaten in large amounts by primitive human that capable of slowing or preventing the oxidation of other molecules. They are of strongest physiological effects of any plant compound. Apart from that, they are also things of beauty as their capability to produce pigments for pansies, petunias and plums. Anthocyanin that can be found in roots, caudexes, leaves, flowers and also fruits, apply a role as substitutes for synthetic pigments as they have the physiological functionality.

Structure and Characteristics

Anthocyanin are glycosides which consist of an aglycone called anthocyanidin (Figure 1) that linked to sugars (Figure 2) and many cases, acyl group (Figure 3). Anthocyanidin is unstable to light and insoluble in water, leading to no occurance in the Free State. The linkage between anthocyanidin and sugars provides better stability and water solubility. With structure of $C_6C_3C_6$ skeleton, anthocyanins are positively charged at acidic pH and this equilibrium form is called flavyliumcation (2-phenylbenzopyrylium). At some condition, anthocyanin can be differed to each other by glycoslyation of hydroxyl group, nature of glycosyl units, substitution pattern and potential aliphatic and aromatic acylation.

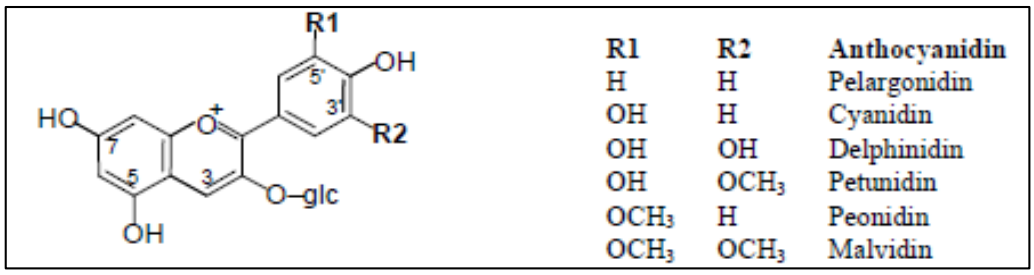

Fig 1: Structure of Anthocyanidins (aglycone)

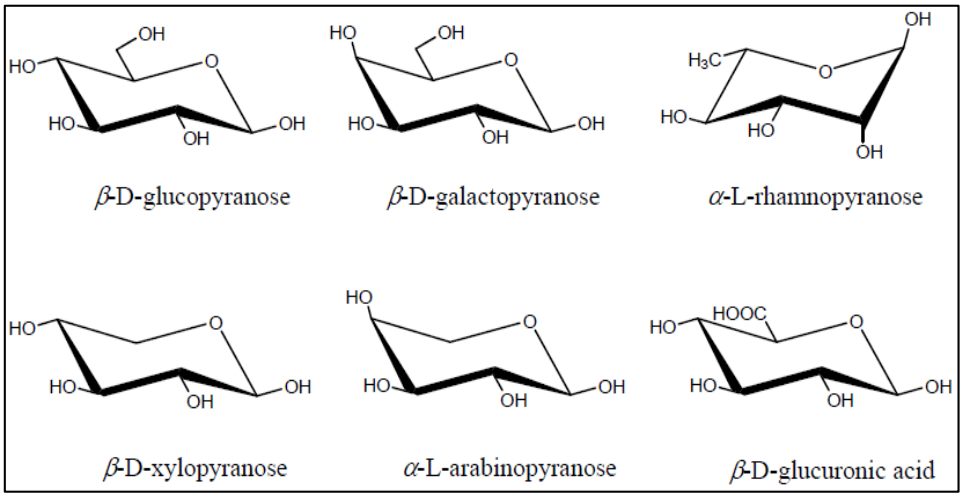

Fig 2: Monosaccharides found in Anthocyanin structures

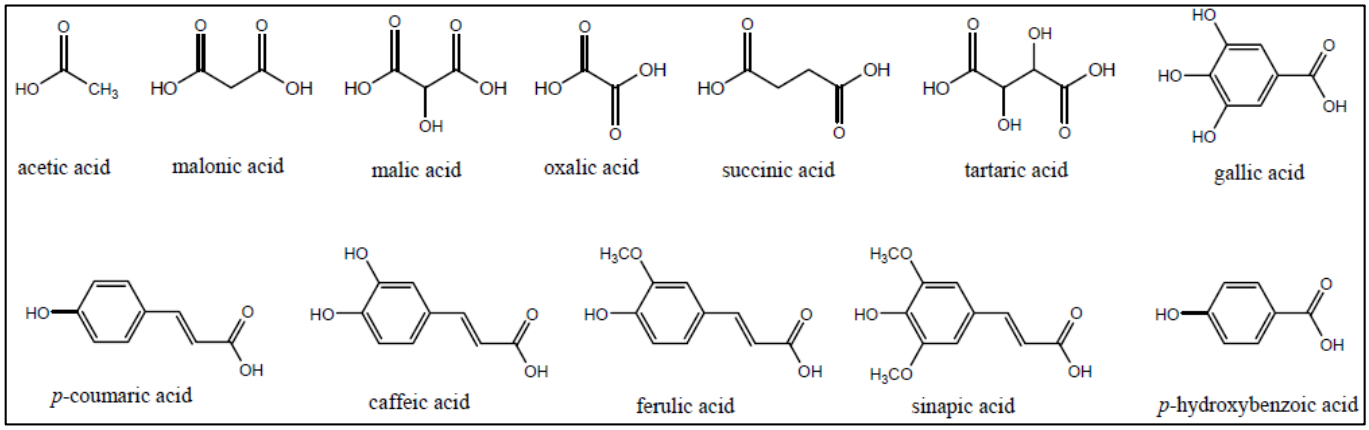

Fig 3: Structures of Acyl substituents found in Anthocyanin

Anthocyanin as a phenolic compound and non-toxic pigment presents a spectrum from orange to blue in color in the natural world, which satisfies the consumer needed in food colors. This phenol structure capability of capturing free-radicals, to play a role as antioxidant is reported to be higher than vitamin C and E. Their hue and structure are relying on pH and presence of co-pigments. The same anthocyanin may have different colors in different pH condition, depending on the pH of the organelle. For pH condition, at pH 1-3 the flavylium cation is red, and colourless at pH 5. When it comes to pH 7-8, the blue purple quinodial base is present. Cyanidin, delphidinin, and pelargonnidin are three non-methylated anthocyanidin, which have most abundant glycosidesin nature; in pigmented leaves (80%), 69% in fruits and 50% in flowers.

The extraction of anthocyanin from plant or fruits currently employed using methanol, ethanol, acetone, water or mixtures as solvents. However, the stability of these anthocynin is affected by the structural modifications with hydroxyl, methoxyl, glycosyl, and acyl groups. Not to forget, also by environmental factors such as temperature and light. Therefore, anthocyanin experience color decline during storage and process.

Materials and Methods

Butter fly-Pea (*C. ternatea* Linn) plants were collected from various places in and around the areas of Kolam tribe addjoing region of Telangana and Maharastra states. Whole Plants were collected from mature plants and identified by comparing with herbarium specimens. The plants were air-dried and powdered. The dry powder was extracted by reflexed in 100 ml methanol for 24 h, using a Soxhlet apparatus. The extract was filtered using Whatman filter paper, No. 1. The filtrate was then evaporated using rotatory evaporator and dried at 55 °C. Ethanol, methanol, hexane and distilled water extracts are obtained and all the extracts are preserved. Dried extract was stored at 20 °C in labeled, sterile capped bottles. Stock cultures of microbes are maintained at a temperature of 4^0 centigrade, active cultures are prepared by growing in tubes

Phytochemical screening: Phytochemical testing is done for the promising extract of all the four types of extracts as it has shown the interesting activity.

1) Braemer's test for Tannins.
2) Liebermann-burchardt test for Steroids.
3) Liebermann-burchardt test and Salkowski test for Terpinoids.
4) Dragendroff's reagent test for Alkaloids.
5) Shinoda test for Flavanoids
6) KOH test FOR Anthraquinones
7) Keller-Kilianii test for Cardiac glycosides
8) Frothing test for Saponins

Test for Tannin

Braemer's test: Added 2 ml of water to 1 ml of extract boiled it and then filtered. Added few drops of 5% ferric chloride to the filtrate. A dark green, blue or brown color indicated the presence of Tannin.

Test for Steroids Liebermann-Burchard test: Extract (1ml) was treated with chloroform, acetic anhydride and drops of H2SO4 was added and observed for the formation of dark green colour.

Test for Sterols: Liebermann-Burchard test: Extract (1ml) was treated with chloroform, acetic anhydride and drops of H_2SO_4 was added and observed for the formation of dark pink or red colour.

Dragendorff's reagent: Is a color reagent to detect alkaloids in a test sample. Alkaloids, if present in the solution of sample, will react with Dragendorff's reagent and produce an orange or orange red.

Test for Terpenoids: Salkowski test gave a positive result hence confirms the presence of Terpenoids.

Salkowski test: The extract was mixed with 2ml of chloroform and concentrated H_2SO_4 (3ml) is carefully added to form a layer. A reddish brown colouration of the interface is formed to show positive result of the presence of terpenoids

Test for alkaloids: Few quantity of the each portion was stirred with 5 ml of 1% aqueous HCl on water bath and then filtered. Of the filtrate, 1 ml was taken individually into 2 test tubes. To the first portion, few drops of Dragendorff's reagent were added; occurrence of orange-red precipitate was taken as positive. To the second 1 ml, Mayer's reagent was added and appearance of buff-coloured precipitate will be an indication for the presence of alkaloids (Sofowora, 1993).

Shinoda's test for flavonoids: About 0.5 of each portion was dissolved in ethanol, warmed and then filtered. Three pieces of magnesium chips was then added to the filtrate followed by few drops of conc. HCl. A pink, orange, or red to purple colouration indicates the presence of flavonoids (Trease and Evans, 2002).

Bornträger's test for anthraquinone glycosides

1. Powdered drug+diluteacid+boil for 2min (hydrolysis of glycosides), filter & cool. Filtrate +organic solvent (Benzene, ether or chloroform) +shake.
2. 3.Organiclayer(aglycones)+ammoniumhydroxide(10%)+ shakevigorouslyimmediaterosepinkorcherryredcolorinaqueouslaye

Keller-killiani Test for Cardiac glycosides: Have an 80% alcohol extract equivalent to ten grams of plant material. Evaporate until dryness over a water bath. Defat extraction by trituration with hexane to remove as much of the color pigments as possible. Discard the hexane solution. Warm the defatted residue over a water bath to remove the residual hexane solvent. Add three ml of Fe Cl Reagent. Stir to mix well and transfer to a test tube. Hold the test tube in a slant position, and carefully add an ml of Concentrated Sulfuric Acid that allows the acid to roll inside the walls of the test tube. Allow the mixture to stand for a while. Determine any change of color in the junction. (+) result is presence of reddish brown color which may gradually become bluish or purplish color indicate the presence of two de oxy sugars.

Test for Saponinsa: Foam test 1ml solution of extract was diluted with distilled water to 20 ml and shaken in a graduated cylinder for 15 minutes. Development of stable foam suggests the presence of saponins. b) 1ml extract was treated with 1% lead acetate solution. Formation of white precipitates indicates the presence of saponins

Results and Discussion

The results of the phytochemical screening to test the presence of tannin, antharaquinone, alkaloid, saponin, phlobatannin, flavonoid, cardiac glycosides, volatile oils, terpenoids and steroids in the metanolic extracts from various parts of *C. ternatea* are shown in (Table 1) The preliminary phytochemical screening study revealed that the leaf of *C. ternatea* contains intermediate amounts of tannin, cardiac glycosides and steroids and small amounts of alkaloids. There were no secondary metabolites noted in the stem. Both the flowers of *C. ternatea* contain phlobatannin, flavonoid, terpenoid in moderate amounts. The roots contain small amount of flavonoid, volatile oil and terpenoids. Antharaquinone and saponins were found to be absent in the entire plant.

Medicinal plants are the richest bio-resource for drugs of traditional medicines, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. The total ash value of leaf of *C. ternatea* is 4.18% respectively. The ash value is indicative of the impurities present in the drug. Since the ash value is constant for a given drug, this value is also one of the diagnostic parameters of the drug. The sample has more water soluble ash than acid insoluble ash. These ash values are generally considered as the index of the purity as well as identity of the drug. Extractive values are useful for the evaluation of phyto constituents especially when the constituents of a drug cannot be readily estimated by any other means. Further, these values indicate the nature of the active constituents present in a crude drug. Phytochemical study of the leaf branch root and flower extract of *C. ternatea* showed that leaf branch root and flower comprised a wide range of active chemical constituents such as alkaloids, flavonoids, free amino acids, glycosides, phenols, proteins, reducing sugars, Phlobatanins, Anthraquinones and saponins and oils were absent in leaf branch Root and flower.

The plant has many medicinal values and has been in use from the ancient times. The present study revealed the presence of Phyto chemicals like Resins Flavonoids Alkaloids, Tannins Glycosides, Reducing sugars and Steroids. All the phytochemicals help in preventing many diseases. It should be noted that steroids compounds are of importance and interest in Pharmacy due to their relationship with each compounds as sex hormones (Okwa 2001) [1]. The presence of proteins, glycosides and carbohydrates also indicate the palatability of the material. The roots has been evaluated for the medicinal values like antidiarrhea (Nitin kumar *et al.* 2010) [2] anti histanic (Dnyaneshwer *et al.* 2011) [3] cholinergic activity (Vyawahare *et al.* 2011) [4] etc.

Table 1: Phytochemical Screening of Secondary Metabolites from Butter fly-Pea (*C. ternatea* L.)

Chemical components	Name of the test	Leaf	Branch	Root	Flower
Alkaloids	Dragendorff test	+	-	2+	2+
Tannins	Braemer's test	2+	+	+	+
Flavonoids	Shinoda test	-	-	+	2+
Anthraquinones	KOH test	-	-	-	-
Phlobatanins	-	-	-	-	-
Saponins	Frothing test	-	-	-	-
Glycosides	Keller-Kilianii test	2+	-	-	-
Reducing sugars	+	-	+	+	+
Steroids	Lieberman Burchardt test	-	-	+	-
Terpenoids	LiebermannBurchar dt test	+	+	2+	2+
Phenol	Salkowski test	+	+	+	2+

'2+' Moderate, '+' Small amounts, '-' absent

As already stated the plant has many medicinal values, so it is an essential factor that the plant should not have any toxicity. The acute oral toxicity study showed no toxicity up to a range of 3000mg/Kg body weight therefore, oral administration of the root of the plant will not affect the animal in terms of its mortality hence the plant here can be seen as a potential source of useful drug.

Bioactivity

Recently, several biologically active peptides called cliotides have been isolated from the heat-stable fraction of *C. ternatea* extract. Cliotides belong to the cyclotides family and activities studies show that cliotides display potent antimicrobial activity against *E. coli, K. pneumonia, P. aeruginosa* and cytotoxicity against Hela cells. These peptides may have potential to be developed as antimicrobial and anti-cancer agents. Additional cyclotides from this plant were identified by RNA-sequence technology and shown to have cyclotide sequences possessing different biophysical and functional properties expressed in different organs. This layer of complexity to the properties of the cyclotides of *C. ternatea* illustrates the biological specialization that cyclotides have undergone in this plant species. Cyclotides from aerial organs possess tighter binding activity to insect-like membranes, whereas cyclotides from roots and seed, two organs that contact soil, had relatively higher effectiveness against juveniles of the model nematode *Caenorhabditis elegans*. Indeed, the isolated Cter M cyclotide that is highly expressed in aerial organs was shown to effectively slow the growth and kill moth larvae. Thus, these cyclotide genes and the peptides they encode are potentially valuable molecules for use in agriculture and plant protection.

The enzyme responsible for the biosynthesis and backbone cyclization of cliotides has recently been isolated. It was named butelase 1 in accordance to its local name in Singapore (*Bunga telang* ligase). Butelase is the fastest peptide ligase known capable of catalyzing peptide cyclization at an extraordinary efficiency.

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