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Preliminary Phytochemical Analysis and Antibacterial activity of *Clitoria ternatea* Linn. Against Veterinary Infection

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Abstract

Clitoria ternatea (C. ternatea) commonly known as butterfly pea, is a tropical leguminous plant with many medicinal properties. This study aimed to conduct preliminary phytochemical analysis and evaluate the antibacterial activity of C.ternatea against veterinary pathogens. Phytochemical analysis of ethanolic leaf extract revealed notable levels of secondary metabolites like alkaloids, tannins, glycosides, steroids, saponins, flavonoids, and phenols. The antibacterial potential of ethanolic leaf extract was evaluated against veterinary pathogens such as E.coli, Klebsiella, Salmonella and Shigella Sps. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values were determined, indicating bactericidal properties of the extract. These findings support the potential use of C.ternatea as a natural antimicrobial agent in traditional veterinary medicine. This research investigates the capabilities of C.ternatea flowers as a plentiful source of anthocyanins, which can serve as a natural colorant. The flowers have a compound called ternatin, which gives them their unique and naturally occurring blue colour. The research also assessed the variations in colour of C.ternatea extract under various environmental pH levels, ranging from pH 1 to 14. Further studies are

recommended to isolate and identify specific bioactive compounds and to assess their efficacy and safety in clinical settings. Overall, this study highlights the potential of C.ternatea as a natural remedy for veterinary infections.

Keywords: Phytochemical, Clitoria ternatea, Anthocyanin, Antibacterial activity, Veterinary pathogens

Introduction

C. ternatea is an attractive perennial vine that features striking blue or white blossoms. It is widely recognized as Aparajita, butterfly pea or Shankhapuspi and is classified under the Fabaceae family. Traditionally, this plant is utilized to address various health issues (Darsini *et al.* 2013). Originating from Southeast Asia, it is found in tropical regions, including India, Philippines and Madagascar. This whole plant extract possesses potential medicinal properties, including anti-helminthic (Esmail *et al.* 2016), anti-inflammatory, antipyretic, antibacterial (Haripriya *et al.* 2010), analgesic (Indumathi *et al.* 2014), as well as anti depresent, anxiolytic, sedative, anticonvulsant, anticancer and hypoglycemic effects (Kamtekar *et al.* 2014), (Kavitha *et al.* 2013). In traditional Ayurvedic medicine, it has been utilized for centuries as a memory booster, nootropic, antistress agent, anxiolytic, antidepressant, anticonvulsant, and sedative. The key active components consist of resin, tannins, taraxerone, and starch, along with taraxerol (Lakshmia *et al.* 2015). The plant contains various secondary metabolites, including kaempferol and the glucoside clitorin, taraxerol, and the lactone Aparajita (Barik *et al.* 2007). The current research focuses on the quantitative and qualitative analysis of the leaves of *C. ternatea* to identify the presence of alkaloids, tannins, glycosides, steroids, saponins, flavonoids, and phenols. *C. ternatea* is a member of the Fabaceae family and studies indicate that it exhibits significant antibacterial activity against *E. coli, Klebsiella*, *Salmonella* and *Shigella*. These peptides may hold promise for development as antimicrobial and anti-cancer agents. In animal studies, the methanolic extracts of *C. ternatea* have shown anxiolytic, antidepressant, anticonvulsant, and antistress properties (Mukherjee *et al.* 2002). The key compounds present consist of tannins, resins, starch, taraxerol, and taraxerone. This study aims to investigate the antibacterial properties of *C. ternatea* against a specific group of microbes, along with the extraction and isolation of the compounds responsible for these properties in the plant. The phytochemical components of these plants indicated that different secondary metabolite similar to flavonoids, anthocyanin, glycosides, Pentacyclic triterpenes and

phytosterols has been separated from this plant (Mukherjee *et al.* 2002). *C. ternatea* seeds and documented to possess antifungal, antibacterial, and insecticidal properties (Kelemu *et al.* 2004). There is a chance that, this substance was primarily accountable for the noted antibacterial effects in this research (Kamilla *et al.* 2009). The aggregated data regarding the morphologica land chemical traits of this plant will assist innumerous new medical therapies like pharmacotherapy, radiation therapy and physical therapy. The flowers of *C. ternatea* have anthocyanins as a pigment which are accountable for the red violet-blue hue of the flower Esmail *et al.* 2016). There exist six primary anthocyanins ternatins (A1, A2, B1, B2,D1 and D2) (Gupta *et al.* 2010) identified as malonylated delphinidin 3,3',5'-triglucosides containing 3',5'- side chains using alternating D-glucose and p-coumaric acid (Haripriya *et al.* 2010). The diverse hues of flower colours arise from a limited variety of distinct pigments. These pigments include the identical carbon frame work, differing solely in the types of substituent groups (Indumathi *et al.* 2014). The stability of colour in Anthocyanins are influenced by their structure, pH, temperature, oxygen levels, light exposure, and water activity (Kamtekar *et al.* 2014). The hue Anthocyanins appear red in highly acidic solutions and blue in alkaline solutions (Esmail *et al.* 2016 & Kavitha *et al.* 2013). The usage of blue colour flowers that contain anthocyanins such as *C. ternatea* have not yet reached their full potential. This research sought to determine the anthocyanins found in the blossoms of *C. ternatea* and utilize its extract as an indicator in acid-base titration based on characteristics of anthocyanins.

Materials and Methods

Collection

C.ternatea leaves and flowers that were disease-free and in good condition were gathered from the V.H.N. Senthikumara Nadar College campus in Virudhunagar district, Tamil Nadu. Out of the two flowering plant varieties the blue and white the leaves and flowers of the blue type were collected for the research.

Extract Preparation

The mature, healthy, and fresh leaves and flowers of *C. ternatea* were rinsed with sterile distilled water and allowed to air dried. The flowers were extracted with ethanol for separation of anthocyanin pigment. The dry leaves were crushed into a fine powder. The powdered leaves are extracted with ethanol and ethyl acetate using Soxhlet apparatus. For 72 hours, the flasks were maintained at room temperature. The extracts were then kept in a refrigerator at 4°C after being separately filtered through Whatman No. 1 filter paper.

Fig.1. Leaf Extraction Using Soxhlet Apparatus

Fig.2. Extract filtration

Phytochemical analysis

Phytochemical analysis of plant extract by Qualitative method

The following tests have been done to detect the presence of the bioactive Constituents (Harborne1973, Sofowra 1993 & Trease and Evans 1989).

Test for Alkaloids

The crude extract was combined with 1% HCl and gently heated. Mayer's and Wagner's reagents were then added, resulting in a precipitate. The formation of this cloudy precipitate indicated the presence of alkaloids.

Test for phenol and tannins

The crude extract was treated with 2% $FeCl_3$ solution and the appearance of a blue-green or black color confirmed the presence of phenols and tannins.

Test for steroid

The crude extract was mixed with chloroform and concentrated H_2SO_4 was added, resulting in a red color in the chloroform layer, indicating steroid presence.

Test for Saponins

The crude extract was mixed with distilled water, shaken vigorously, and observed for stable foam formation, which indicated the presence of saponins.

Test for flavonoids

Shinoda test: The crude extract turned pink-scarlet after adding magnesium ribbon and concentrated HCl, indicating flavonoid presence.

Test for Terpenoid

The crude extract was tested for Terpenoid by dissolving it in chloroform,

evaporating to dryness, and then treating it with concentrated H_2SO_4. The appearance of a greyish color after heating confirmed the presence of Terpenoid.

Test for glycosides

Liebermann's test: The crude extracts turned violet, blue, and then green after adding chloroform, acetic acid, and H_2SO_4, indicating a steroidal nucleus (glycone portion).

Antibacterial activity

The bacteria were isolated from infected cow urine sample. The four different bacteria were isolated from the urine sample such as *E.Coli, Klebsiella, Salmonella and Shigella Sps* by Streak plate method. Then the plates were incubated at 37^0C for 24 hrs. After incubation the colonies grown on the plate were sub cultured on the selective agar plates and maintained as a pure culture. Antibacterial activity was determined using the standard disc diffusion method. The crude ethanolic and ethyl acetate extracts and flower ethanolic extracts were used for bioassay against test organisms. The sterile Mueller Hinton Agar medium was poured into sterile petri dishes and the plates were allowed to solidify. The organisms were uniformly swabbed on the plates and sterile disc were placed into the agar medium using sterile forceps. The sterile disc were later filled up with the plant extract at a quantity of 40µl 60µl. The plates were allowed to stand on for 1 hour to allow proper diffusion of the plant extract and to prevent spillage onto the surface of the agar medium and then incubated at 37°C for 24 hours. After that, the plates were observed for the zone of inhibition. The same procedure was followed for all the bacterial strains. The zone of inhibition was measured.

Anthocyanin as a pH indicator

The pH range of 1.0 to 14.0 was used to evaluate the effect of pH on anthocyanin colour change. Distilled water was used to dilute a 2.5 ml anthocyanin extract to 25 ml. Test tubes holding buffer solutions with different pH values were then filled with 3 ml of the solution. Colour changes were noted and recorded after fifteen minutes.

Preparation of pH solutions

A pH 1.0 solution was made by diluting an 18.75 ml solution of 37% HCl with distilled water. In order to get solutions with pH values between 2 and 14, several dilutions were carried out. A series of alkaline solutions were prepared, starting with a pH 14.0 solution made by dissolving1g of NaOH in water. The solution was then diluted to create subsequent solutions with progressively lower pH levels, approaching neutrality. The pH of each solution was confirmed using a pH meter.

Result and Discussion

Preliminary phytochemical analysis of *C. ternatea*

The present study carried out on the plant *C. ternatea* revealed the presence of bioactive constituents. The phytochemical constituent of the selected plant investigated is summarized in Table 3.1. Analysis of plant extract revealed the presence of Alkaloids, flavonoids, phenols, proteins, steroids and tannins.

Table 1: Preliminary phytochemical analysis in ethanolic and ethyl acetate extract of leaves of *C. ternatea*

Test name	Leaves Extract	
	Ethanol	Ethyl acetate
Alkaloids	+	+
Flavonoids	+	+
Amino acids	+	+
Phenol	+	+
Terpenoid	-	+
Saponins	-	-
Tannins	+	-
Glycosides	+	+

Note: (+) Present; (-) Absent

Biochemical Test

Biochemical tests were conducted to identify genus of bacterial isolates. Indole, triple sugar iron, citrate utilization, Methyl red, Voges Proskauer and urease tests were performed. The results were showed in Table 2.

Table 2: Biochemical test

Test name	***Salmonella***	***Shigella***	***E. coli***	***Klebsiella***
Indole	+	-	+	-
Methyl red	-	+	+	-
Voges Proskauer	-	-	-	-
Citrate	+	-	-	+
TSI	K/AwithH2S & gas production	A/A with gas production	A/A with gas production	A/A with gas production
Catalase	+	+	+	+
Oxidase	-	-	-	-

Note: (+) Present (-) Absent

Antibacterial activity

The result of antibacterial activity of *C. ternatea* was shown in table 3.3. The antibacterial activity demonstrated that leaf and flower extracts of *C.ternatea* are resistant to the tested microbial strains. Disk diffusion assay revealed that ethanol extract of *C. ternatea* leaves and flower exhibited greater antibacterial activity compared to ethyl acetate extract. Specifically, the ethanol and ethyl acetate leaf extract and ethanolic flower extract showed significant inhibition zones against *Salmonella, shigella, Klebsiella sps* and *Escherichia coli.*

Table 3: Antibacterial activity of leaf and Flower extract

Organisms	Leaves extract				Flower extract	
	Ethanol		Ethyl acetate		Ethanol	
	40 µl	60 µl	40 µl	60 µl	40 µl	60 µl
E.coli	10mm	21mm	7mm	12mm	12mm	16mm
Klebsiella sps	11mm	18mm	9mm	13mm	11mm	14mm
Salmonella	9mm	15mm	6mm	15mm	12mm	13mm
Shigella	12mm	12mm	12mm	18mm	10mm	15mm

Anthocyanin as a pH indicator

Table 4: CTAs extracts have different Colour intensities

pH	Colour observed
1	Pale pink
2	Pink
3	Red
4	Dark blue
5	Blue
6	Ink blue
7	Sky blue
8	Bluish green
9	Greenish yellow
10	Green
11	Green
12	Darkgreen
13	Yellow
14	Darkyellow

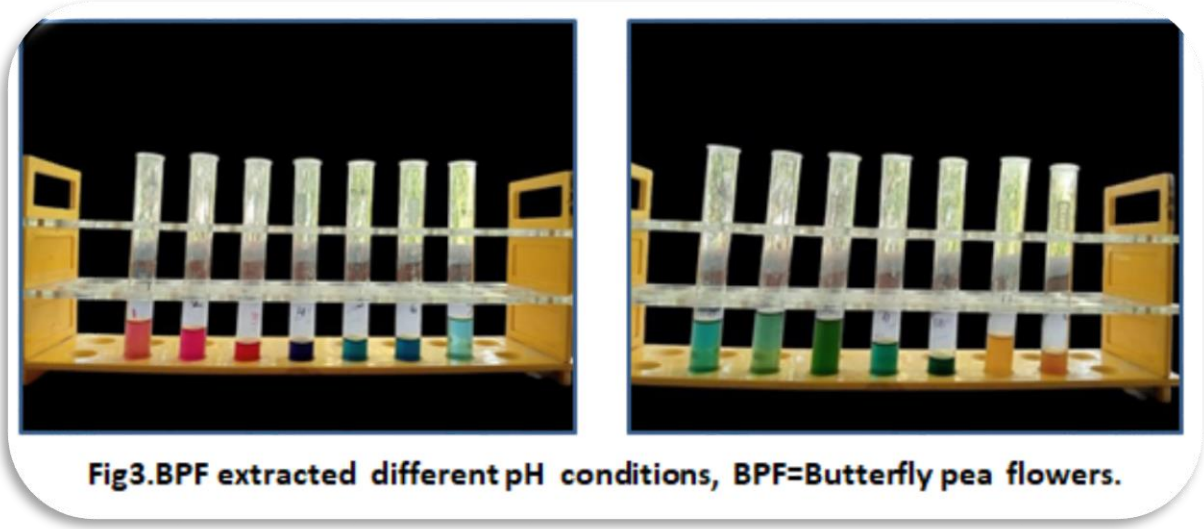

Fig3.BPF extracted different pH conditions, BPF=Butterfly pea flowers.

Conclusion

This study highlights *C. ternatea* as a valuable medicinal plant, attributing its significance to the diverse bioactive compounds present in its leaf extracts. The Antibacterial properties of *C. ternatea* against veterinary infection underscore its significance. The butterfly pea extract can serve as a natural pH indicator for acid and base titrations. Future research isolating and identifying the active compounds in *C. ternatea* extract may uncover valuable preservatives for food and natural medicines. This could pave the way for developing novel treatments for various veterinary diseases.

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