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Qualitative phytochemical analysis and inhibitory property of Blue Butterfly Pea flower extract against *Escherichia coli*

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Abstract

From ancient times, medicinal plants have been utilized to heal a variety of human illnesses. The development of antibiotics as a means of treating diverse bacterial illnesses sparked a revolution in medicine. Several therapeutic plant extracts are known to have antibacterial properties that are applied in the preservation of food and for therapeutic purposes. Blue butterfly pea (*Clitoria ternatea*) is a plant that is popularly used as medicine and recognized to treat a number of illnesses. Practically, every component of this plant is said to have therapeutic properties. Furthermore, *C. ternatea* plant has strong antibacterial effect as it contains biological compounds that act as best antimicrobial property against bacteria. Also, phytochemical analysis of *C. ternatea* flower extract showed the presence of flavonoids, steroids and tannins in which these phytochemical compounds contributed to the antibacterial ability of the flower extract. On the other hand, *C. ternatea* flower extract has a heavy amount of flavonoids and these results to a very active capacity to inhibit the growth of the *Escherichia coli*. Hence, flavonoids are reported that it has the capability against infectious degenerative disease, viral and bacterial. Certainly, this medicinal plant may be used for an alternative medicine considering the fact that this is harmless, low-cost production and its flower can be eaten raw.

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Introduction

Human illnesses are treated using medicinal plants and have been for a very long time. The development of antibiotics as a means of treating diverse bacterial illnesses sparked a revolution in medicine. However, their indiscriminate usage has caused an alarming growth in the number of microorganisms that are resistant to antibiotics, giving rise to multiresistant strains, which has raised concerns around the world (Shariff, 2001).

Clitoria ternatea L. is a species of *Clitoria* under Fabaceae family is often utilized as a traditional plant called as blue butterfly pea. It is Ayurvedic medicinal plant as a memory booster, stress reliever, and calming, antidepressant, anticonvulsant, and anxiolytic also a sedative (Ramkissoon *et al.* (2013). More so, *C. ternatea* has been utilized for a number of medical conditions. Uses for its roots include cure heartburn, bloating, constipation, fever, arthritic pain, sore throat, while its seeds are utilized as a treatment for eye and skin conditions, to treat colic and swollen joints with a laxative. The conventional Cuban culture employs either a single root decoction or paired with flowers to induce menstruation and encourage as well as to treat liver and intestinal problems, uterine contractions problems (Mukherjee *et al.*, 2008; Fantz 1991). Furthermore, its flower petal's nutritional value using anthocyanins as a naturally occurring blue coloring in a various cuisine. It is also act as anti-oxidant, antibacterial, anti-inflammatory, and other pharmacological properties that are present in the extract of *C. ternatea* (Gupta *et al.*, 2010).

Moreover, a large and increasing number of patients in the world used medicinal plants and herbs for health purposes. There are hundreds of biologically active compounds developed from traditional medicinal plants (Inoue, 2018). It is believed that the plant's medicinal crude extract is more biologically active that isolated compounds due to its synergistic effects (Jana and Shekhawat, 2010).

Based on the presented prior arts that *Clitoria ternatea* possess medicinal values and clearly benefit the users. This study explores on the screening of the antibacterial activity of phytochemical properties present in blue butterfly pea that cited that contains antibacterial composition against *Escherichia coli.* Hence, it can be an alternative medicine to cure various diseases that acts as anti-viral, anti-inflammatory, anti- allergic, has rich in antioxidants and good treatment for mental illness that contributes to the range of rediscovering essential uses of this plant.

Materials and methods

Research Design

The study was conducted with the use of Completely Randomized Design (CRD). According to Larry (2008), CRD is the simplest procedure in which the treatments are assigned to the experimental units completely at random.

Experimental Set-up

The experiment used only one (1) treatment with three (3) replications.

a. Treatment - Agar growth media + *C. ternatea* flower extract

b. Replicates 2- Replicates in each treatment

Collection site of Blue butterfly pea (C. ternatea) flower

The researchers collected blue butterfly pea (*C. ternatea)* flowers in the locality with in the province of Agusan del Sur, Philippines. The fresh flowers were washed under a moderate running tap water and cut into small pieces weighed 1 and ½ kilograms when dried.

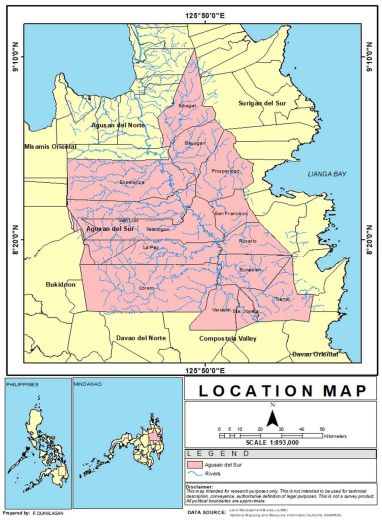

Fig.1. Map of the Province of Agusan del Sur, Philippines.

Drying Process of Blue butterfly pea (C. ternatea) flower

The collected blue butterfly pea (*C. ternatea*) flower was completely air dried in shade room temperature for 8 days. Then it was powdered using an electric blender that weighed 1 and ½ kilograms and put it in the plastic bag which was sealed carefully and brought to Regional Standards and Testing Laboratory, Department of Science and Technology, Caraga region, Butuan City, Philippines for Phytochemical test and Antibacterial test.

Extraction of Dried Blue butterfly pea (C. ternatea) flower

1. Materials

Blue butterfly pea (*C. ternatea*) flower, 375.0 g, dried 95% ethyl alcohol, 2.0 L

2. Methods

The dried 375.0 g of blue butterfly pea (*C. ternatea*) flower was pulverized using Wiley mill and soaked in 2.0 L of 40% ethyl alcohol for 48 hours. The mixture was filtered, and the filtrate obtained was concentrated using a rotary evaporator at 60°C under vacuum for 2 hrs. The concentrated extract was further evaporated using a water bath at 60°C to obtain a semi-solid extract.

3. Qualitative Phytochemical Screening of Crude Extract

Using the standard procedures all the obtained samples from extracted flower of blue butterfly pea (*C. ternatea*) underwent a chemical test for screening and identifying the phytochemical constituents qualitatively. Crude extraction of dried 375.0 g of blue butterfly pea (*C. ternatea*), plant produced 1.5 L ethanolic extract. The concentration of the filtrate yielded 31.0 g of a semi-solid extract with a percentage yield of 8.3%.

Preparation of the Test Organisms

The recommended set of assay organisms for the antimicrobial screening program will represent the major group of microbial flora: *Escherichia coli*- gram -positive cocci. The 0.5 McFarland standard was used to adjust the turbidity of the inoculums prior to the cotton swabbing of the agar plates for antimicrobial assays.

Preparation of 0.5 Mcfarland standard b

a. Materials

5 ml of screw-capped
Mechanical vortex mixer
0.5 Mcfarland standard

b. Procedure

First, the 0.5 ml of 0.048 M BaC_{12} (1.175%w/v $BaC_{12}.2H_2O$) was mixed to 99.5 ml of $0.36NH_2SO_4$ (1%V/V). Then, the 5 ml was distributed into screw-cap tubes of the same dimension as those to be used in preparing the culture suspension. The tubes were sealed and stored in the dark at room temperature. Before use, shake turbidity, standard vigorously on a mechanical vortex mixer.

This standard can be kept up to 6 months if stored in a tightly sealed screw capped tube in the dark.

The Broth Culture

1. Preparation of the Nutrient Broth

The preparation of a 1000 ml solution was referred to as label. The loop full of bacteria (gram positive) was taken from the culture slant and inoculated for 24 hours at 35 degree Celsius and was observed for turbidity which is indicative of microbial growth.

2. Adjusting the Turbidity of the Inoculum

The adjusted turbidity serves as the inoculum for the microbial assay. This served as the inoculums, which was swabbed onto agar plates. Use within 15 minutes after adjusting the turbidity.

Then, aseptically transfered 5 ml of the culture broth in sterile screw-capped tubes. Agitated on a vortex mixer the bacterial suspension and immediately compared against the 0.5 Mcfarland standard prepared. If the bacterial suspension does not appear to be of the same density as the Mcfarland standard, adjust the turbidity by adding sterile saline solution or culture broth and subsequently compare the resulting turbidity to the standard.

Preparation Treatment Agar

The approximately 15 ml of melted nutrient agar was poured into dry and sterile Petri dishes and until the

medium solidified. Moisten a sterile cotton swab into the *E. coli* (inoculum) suspension. Used cotton swab with wooden applicator handles. A sterile cotton swab was dipped into a suspension of the *E. coli* (inoculums. The moistened swab was pressed and rotated firmly against the inside wall of the tube just above the fluid level to remove the excess liquid.

Cotton swabbing

The *E. coli* is aseptically swabbed into solidified nutrient agar but streaking the swab over the entire surface of the agar plate 3x and rotated the plate 60 degrees after each application to ensure an even distribution of the inoculum on the surface of the medium and the swabbed plates stand for 5 minutes.

Data Gathering Procedure

Zone of Inhibition

A. The Plates

The "HALO" or "Clearing" around the disc was the zone of inhibition. It used ruler to measures the diameter of each inhibition zone in millimeters (mm). The result was expressed as mm diameter zone of inhibition.

B. Analyzing the Results

The analysis of the result was based on from Department of Science and Technology- Caraga region, Regional Standards and Testing Laboratory, Butuan City, Philippines.

<10 mm, inactive;
10 mm – 13 mm, partially active;
14 mm – 19 mm, active; and
>19 mm, very active

Results and discussion

Phytochemicals of Blue butterfly pea (C. ternatea) Ethanolic Extract

The phytochemicals are non-essential nutritive plant cloth that consists of shielding and disease preventive properties. The secondary metabolites of *C. ternatea* act as a defense mechanism against insects, microorganisms and use as an antibacterial treatment for patients in various infectious diseases (Jamil and Pa'ee, 2018). Furthermore, the phytochemical properties present in flower extract of *C. ternatea* are flavoids (+++), steroids (+) and tannins (+) which has light amount as shown in table 1.

Table 1. The Phytochemical properties of blue butterfly pea (*C. ternatae)* flower extract.

Phytochemical compounds	Results
Flavonoids	+++
Steroids	+
Tannins	+
Saponins	-
Alkaloids	-
Anthraquinones	-
Cyanogenic glycosides	-

Legend: (-) negative; (+) trace/light amount; (++) moderate amount; (+++) heavy amount).

Consequently, results of the analysis demonstrated that *C. ternatea* flower extract has heavy amount of flavonoids. Hence, flavonoids are reported that it has the capability against infectious degenerative disease, viral and bacterial diseases and it also activates human protective enzymes (Jamil and Pa'ee, 2018). According to Harborne and Williams (2000), flavonoids are wide phytochemical with various pharmacological effects including anti-inflammatory, anti- allergic, oestrogenic, anti- oxidant, enymes inhibition and anti- tumor activity. Conversely, plants are always containing flavonoids, which are a wide category of polyphenol compounds with a benzoyl-pyrone structure. The phenylpropanoid pathway is responsible for their synthesis. According to the findings that are now available, flavonoids and other secondary phenolic metabolites are primarily responsible for the wide range of pharmacological activity (Mahomoodally *et al.*, 2005; Pandey 2007). Moreover, steroids compounds also have noticed in the *C. ternatea* flower extract in light amount that was reported that it has a wide phytochemical and antibacterial properties fight against various bacteria. More so, steroids contain vast array of biological activities such as anticancer, antivirus and could fight against bacteria (Ke, 2018).

Other than that, the tannins compounds have also present in the flower extract of *C. ternatea* in light amount. Research results shown that tannins prevent the growth of bacteria either it is gram-negative or gram-positive bacteria (Vu *et al.*, 2017).

More so, tannins have the ability to heal the wounds faster and defense against microorganisms (Okwu and Josiah, 2006). Additionally, tannins are thought to provide a variety of pharmacotherapeutic actions (Ferreira *et al.*, 2008). According to Uma (2009), biological compounds can be found also from blue butterfly pea are ternatins, flavonoids, alkaloids, saponins, tannins, proteins, and carbohydrates.

The Degree of Inhibition of Blue butterfly pea (C. ternatea) Ethanolic Extract on the Growth of Escherichia coli.

Table 2 depicts the degree of the zone of inhibition of blue butterfly pea (*C. ternatea*) flower extract against *E. coli*. The replicate 1 indicates the results of 24 mm, the replicate 2 indicates the results of 23 mm, and replicate 3 indicates the results of 23 mm. The three trials resulted in average mean of 23 mm. Based on the parameter provided by the DOST Caraga Regional Standards and testing Laboratory, the result of mean of the degree of inhibition of Blue butterfly pea (*C. ternatea*) flower extract on the growth of *E. coli* is very active This implies that the Blue butterfly pea (*C. ternatea*) ethanolic extract can inhibit the growth of *E. coli* in a very active degree. Thus, *C. ternatea* flower extract has the potential pharmaceutical combat against *E. coli*. It was supported with the study of Alok *et al.*, 2015 that *C. ternatea* has a natural capacity to treat different diseases and shows bactericidal action and positive effect to inhibit *E. coli*. Moreover, based on its phytochemical components, this plant has anti-inflammatory, anticancer, anti-diabetic, hepatoprotective, anti-asthmatic, antioxidant, and antibacterial properties (Kamilla *et al.* 2009; Pratap Gowd *et al.* 2012; Lijon *et al.* 2017; Chusak *et al.* 2018; Widyarman *et al.* 2018; Kumar and More 2019; Lakshan *et al.* 2019; Haditio *et al.* 2021).

Table 2. Zone of inhibition (mm) of the growth of *E. coli* under blue butterfly pea (*C. ternatae)* flower extract.

Sample Description	Parameter	Result Zone of inhibition	Analysis of the Result
Blue butterfly pea (*C. ternatae)* flower extract	*Escherichia coli*		
	Trial 1	24 mm	Very active
	Trial 2	23 mm	Very active
	Trial 3	22 mm	Very active
	Average	23 mm	Very active

Fig. 2. Blue Butterfly pea plant.

Profile

Scientific Classification

Kingdom:	Plantae
Phylum:	Streptophyta
Order:	Fabales
Family:	Fabaceae
Genus:	*Clitoria*
Species:	*ternatea*

Conclusion

Blue butterfly pea (*C. ternatea*) flower extract possesses biological compounds such as flavonoids, steroids and tannins that are utilized to treat of neurological disorder, for intellectual enhancement and offers a promising. Certainly, this medicinal plant may be used for an alternative medicine considering the fact that this is harmless, low-cost production and its flower can be eaten raw.

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