



Study on the Antibacterial Activity of *Clitoria ternatea* Leaves – Against some of Pathogenic Bacteria

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Abstract:

Infections caused by gram positive and negative bacteria are important causes of morbidity and mortality and have a wide effect on public health. Extracts of plants especially from the herbs legume like *Clitoria ternatea* (Butterfly pea) can be used as antibacterial material. This study was carried out to investigate the antibacterial properties of *Clitoria ternatea* leaves. The Methanol extract from the leaves of this plant was tested against the Gram-positive and Gram-negative bacteria. Gram-positive bacteria included *Staphylococcus aureus* and *Streptococcus agalactia*, while the Gram-negative bacteria included *Escherichia coli* and *Salmonella typhi*. The results showed promising antibacterial activity against all of the tested pathogenic bacteria. Among these, methanol extract of *Clitoria ternatea* leaves was more potential inhibitory activity effect against *Staphylococcus aureus* when compared to the other pathogenic bacteria under study.

Keywords: Inhibition Zone, Pathogens, *Escherichia coli*, Anti microbial.

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Introduction:

The *Clitoria ternatea* (Papilionaceae) perennial herbaceous plant has been widely used in Ayurveda medicine. The plant is native to tropical equatorial Asia, while has been introduced to Africa, Australia (Dighe et al, 2009). It's as promising medicinal plant with wide range of pharmacological activities which could be utilized in various medical applications because of its effectiveness and safety (Al-Snafi, 2016). The protein of 'finotin' has been isolated from *C. ternatea* seeds and reported it have antifungal, antibacterial and anti insect properties (Kelemu et al, 2004). Also it provides bioactive compounds for medicinal use and it is also an ornamental plant and cover crop. And this plant adaptable to a wide range of

temperature, rainfall and altitude (Gomez and Kalamani, 2003).

The resistance of pathogenic bacteria to human drug has been commonly reported from all over the world. In the present scenario of emergence of multiple drug resistance to human pathogenic organisms, this has necessitated a search for a new antimicrobial material from other sources including plants (Nitha and Indira, 2012). Chauhan et al., 2012 reported the pharmacognostical, phytochemical and pharmacological evaluation of *Clitoria ternatea* for anti asthmatic activity. The study showed that *Clitoria ternatea* extract possesses many pharmacological activity such as anxiolytic activity, anti microbial activity, anthelmintic activity, effects on general behavior, CNS depressant activity, anti stress activity, immune modulatory

effect, larvicidal effect, proteolytic activity, diuretic activity. Its extracts possess a wide range of pharmacological activities including antimicrobial, antipyretic, anti-inflammatory, analgesic, blood platelet aggregation-inhibiting and for use as a vascular smooth muscle relaxing properties (Mukherjee et al, 2008). Various secondary metabolites such as flavonoids, anthocyanin glycosides, pentacyclic triterpenoids and phytosterols have been isolated from this plant (Mukherjee et al, 2002).

In addition The *Clitoria ternatea* plant is traditionally used for food coloring, stress, infertility and gonorrhoea. It is an anxiolytic, anti-inflammatory, analgesic, anti-microbial and anti-carcinogenic (Lijon et al, 2017). In some of the Asian countries traditional eating the flowers of *Clitoria ternatea* L. or drinking their infusion as herbal tea is to defend against skin aging (Zakaria et al, 2018).

Anarthe et al, 2017 were reported the methanolic extract of *Clitoria ternatea* (MECT) showed significant immunostimulating activity with specific and non-specific mechanism which may be due to the presence of prominent amount of secondary metabolites such as of flavonoids and phenols. In addition the flower extract consists of antioxidant activity and protective effect and it has been commonly used as anti-stress, anti-depressant, anti-microbial, and in anti-inflammatory treatments (Karel et al, 2018). Its root extracts have been reported to exhibit analgesic, anti-pyretic, anxiolytic, anti-depressant, anti-convulsant, anti-diarrhoea, anti-stress, anti-asthmatic and wide importantly in the treatment of central nervous system (CNS) disorders (Chan et al, 2017). (Kapoor and Veena, 2013), they reported the Ethyl ether and alcoholic extracts of leaves of *Clitoria ternatea*, showed positive reactions against bacterial pathogens such as *Staphylococcus aureus*, *Escherichia coli* and a fungal pathogen *Candida albicans*. Indira and

Shamshad, 2015, they recorded The extracts from the whole plant of *Clitoria ternatea* (butterfly pea) showed prominent antibacterial activity against the tested microbial pathogens include *Salmonella typhimurium*, *Proteus vulgaris*, *Shigella dysenteriae* and a fungal pathogen *Candida albicans*. In addition the methanolic flower extract of this plant was tested against *Staphylococcus aureus* and showed significant anti-microbial activity (Bhushan et al, 2013). In addition methanol and chloroform extracts of *Clitoria ternatea* flower exhibited activity against uropathogenic *E.coli*, Enteropathogenic *E.coli*, Enterotoxigenic *E.coli*, *typhimurium*, *Klesiella pneumoniae* and *pseudomonas aureginosa* (Babu et al, 2009).

The Objective of the study was:

To evaluate the effect of *Clitoria ternatea* leaves Methanolic extract on gram positive (*Staphylococcus aureus* *Streptococcus agalactia*,) and gram negative bacteria (*Escherichia Coli* and *Salmonella typhi*).

Material and Methods:

Plant material:

Clitoria ternatea plants were collected from the farm of College of the Agricultural Studies, department of agronomy; Sudan University of Science and Technology at Shambat Khartoum North; Sudan.

Antimicrobial activity analysis of *Clitoria ternatea* leaves:

Preparation of leaves extract: The dried leaves of plant were powdered into fine particles using an electrical blender. The fine powder was stored in air tight containers. Fifty grams of leaf material was soaked in 250 ml methanol for 24 hours then put on rotary shaker (220 rpm) for 24 hours, then filtered using standard filter paper. The filtrate was transferred into vials and allowed to evaporate until completely dry and left in the refrigerator at (4° c).

Test microorganisms:

The following Gram-positive and Gram-negative bacteria were used for antibacterial activities studies: Gram-positive bacteria included *Staphylococcus aureus* and *Streptococcus agalactia*; Gram-negative bacteria included *Escherichia coli* and *Salmonella typhi*. The test organisms were supplied by Microbiology lab, College's campus of Veterinary medicine and Animal Production Science and Technology; Sudan University of Science and Technology.

Antibacterial activity:

Agar Well Diffusion Method:

The antimicrobial test was performed by following agar well diffusion method (Perez *et al.*, 1990); with some modifications by using Mueller Hinton Agar No. 2 medium. Muller Hinton agar was prepared as per the instructions by the manufacturer, once the media solidified. The media was punched with 3 mm diameter hole, and then the plates were inoculated with bacterial species with size of 10^6 colony-forming units (CFU)/ml of bacteria were spread with an L-shaped glass rod; then the well was filled with extract. Microbial growth was determined by measuring the diameter of the inhibition zone (cm) (Mean \pm SD). Inhibition zone around the wells were measured after 24 hours of incubation at 37°C by measuring the diameter of the inhibition zone (Mean \pm SD) in cm.

Preparation of concentrations for antibacterial assay:

Serial dilution

The leaf extract of each plant were subjected to a serial dilution using sterile distilled water as a diluents. 1gm from crude extract was added into a tube containing 9 ml of sterile distilled water, from this tube, a serial dilution was done and covered a dilution range of 10^{-1} to 10^{-4} .

This helped to determine the minimum inhibitory concentration (MIC) of extract on each strain (Uwimbabazi et al, 2015).

Bacteria inoculation and disc-diffusion method:

Filter papers discs were prepared (3mm diameter); then were sterilized and soaked in different concentration of extract, and then they were aseptically placed over the media with specific bacteria. The plates were incubated in an upright position at 37 °C for 24 hours. The diameters of inhibition zones were measured in cm (Uwimbabazi et al, 2015).

Statistical Analysis:

Data collected were presented as Mean \pm Standard deviation and were analyzed using SPSS (Version 17.0) (2008) computer software program as one way analysis of variance (ANOVA), treatments means were separated by the least significant difference (LSD) method at ($p\leq 0.05$).

Results:

Well Diffusion Method:

The results showed the methanol extract induced high range of inhibition zones in *Staphylococcus* (2.63 ± 0.78 cm) followed by *Streptococcus agalactia* (1.60 ± 0.36 cm), *Escherichia coli* (1.30 ± 0.20 cm) and *Salmonella typhi* (1.27 ± 0.25 cm) (Table1).

Disc-Diffusion Method:

The result showed the methanol extract induced high range of inhibition zones in *Staphylococcus aureus* (0.90 ± 0.1 cm), followed by *Streptococcus Agalactia* (0.40 ± 0.17 cm), *Escherichia coli* (0.40 ± 0.1) and *Salmonella typhi* (0.23 ± 0.06 cm) for first dilution (Table 2). Also in the second dilution *Staphylococcus aureus* recorded higher inhibition zone (0.05 ± 0.01 cm), compared with no inhibition for the others. While weren't inhibition zones in all bacteria tested for dilution Third, Fourth and Fifth (Table 2).

Table (1): Antibacterial activity of leaf extracts of *Clitoria ternatea* by well diffusion method:-

Microorganisms	Zone of Inhibition (cm) (Mean ± SD)
<i>Staphylococcus aureus</i>	2.63± 0.78 ^a
<i>Streptococcus</i>	1.60± 0.36 ^b
<i>E. coli</i>	1.30± 0.20 ^b
<i>Salmonella typhi</i>	1.27± 0.25 ^b
Sig	**

Table (2): Minimum inhibitory concentration (MIC) for *Staphylococcus aureus*, *Streptococcus Agalactia*; *Escherichia coli* and *Salmonella typhi*:-

Dilution concentration of leaf extracts	Zone of Inhibition (cm) (Mean ± SD)				Sig
	<i>Staphylococcus aureus</i>	<i>Streptococcus Agalactia</i>	<i>Escherichia coli</i>	<i>Salmonella typhi</i>	
10 ⁻¹	0.90± 0.10 ^a	0.40±0.17 ^b	0.40±0.10 ^b	0.23± 0.06 ^b	**
10 ⁻²	0.05±0.01	Growth	Growth	Growth	**
10 ⁻³	Growth	Growth	Growth	Growth	NS
10 ⁻⁴	Growth	Growth	Growth	Growth	NS

Discussion:

Well Diffusion Method:

Clitoria Ternatea methanol extract showed highly significant differences at (p≤0.01), between tested bacteria; the higher inhibition zone was recorded by *Staphylococcus* (2.63± 0.78cm) followed by *Streptococcus agalactia* (1.60± 0.36cm), *Escherichia coli* (1.30± 0.20cm) and *Salmonella typhi* (1.27± 0.25cm); this matched with (Kapoor and Purohit, 2013), (Babu Uma et al, 2009) and Indira and Shamshad (2013); they reported that the *Clitoria Ternatea* have antibacterial activity.

Disc-Diffusion Method:

The result showed the methanol extract of *Clitoria Ternatea* showed high range of inhibition zones in first dilution for *Staphylococcus aureus* (0.90± 0.1cm), followed by *Streptococcus Agalactia* (0.40±0.17cm), *Escherichia coli* (0.40±0.10) and *Salmonella typhi* (0.23± 0.06 cm). Also in the second dilution *Staphylococcus aureus* recorded higher inhibition zone (0.05±0.01cm), compared with no inhibition for the others. While weren't inhibition zones for all bacteria tested in Third and fourth dilutions; this may be due low concentration of extract content; this agreed with Uwimbabazi., et al (2015) they reported the bactericidal

activity increased with the increase of the extract concentration. Gram positive bacterial strains were more susceptible to the extract when compared to gram negative bacteria. This may be due to the fact that these two groups differ in their structure of the cell wall components; this agreed with Lakna, (2017); reported the Gram positive bacteria are more susceptible to antibiotics due to the lack of an outer membrane; whereas since gram negative bacteria contain an outer membrane, they are less susceptible to antibiotics.

Conclusion:

Methanol extract of *Clitoria ternatea* leaves was more potential inhibitory activity effect against *Staphylococcus aureus* when compared to the other pathogenic bacteria under study. While the extract was effective against Gram positive bacteria include *Staphylococcus aureus*, *Streptococcus*; compared Gram-negative bacteria included *Escherichia coli* and *Salmonella spp.*

References:

Al-Snafi, A. E. (2016). Pharmacological importance of *Clitoria ternatea*- A review. *IOSR Journal of Pharmacy*, Volume 6, Issue 3 (March 2016), PP. 68-83.

- Anarthe, S. J., Spoorthisudha P., Swetha M and Ganga Raju M. (2017), In vitro and in vivo screening of *Clitoria ternatea* (Linn.) for Immunomodulatory activity. *International Journal of ChemTech Research*, Vol.10 No.1 pp 116-125, 2017.
- Babu U, Kesani P, Sadayappan R. (2009). Phytochemical Analysis and Antimicrobial Activity of *Clitoria Ternatea* Linn against Extended Spectrum Beta Lactamase Producing Enteric and Urinary Pathogens. *Asian Journal of Pharmaceutical and Clinical Research*, Vol.2 Issue 4, October- December 2009.
- Bhushan P, Kamlesh N, Kishor D, Megha B and Vijayashree R,(2013). Antimicrobial Activity of *Clitoria ternatea* L. flower extract and use as a natural indicator in acid base titration. *J. Nat. Prod. Plant Resour.*, 2013, 3 (2):48-51
- Chan Yi Ling, Fui Joo Bong, Sreeramanan Subramaniam And Bee Lynn Chew. (2017). The Effects of Indole-3-Butyric Acid and 1-Naphthaleneacetic Acid on The Induction of Roots From *Clitoria Ternatea* L. *Journal of Sustainability Science And Management Volume 12 Number 2*, December 2017: 63-70
- Chauhan N , Rajvaidhya S and Dubey B.K. (2012). Pharmacognostical, Phytochemical And Pharmacological Review On *Clitoria Ternatea* For Antiasthmatic Activity International journals of pharmaceutical sciences and research, IJPSR, 2012; volume 3 (2):398-404.
- Dighe, N. S; Pattan, S. R; Nirma, S. A, Dake, S. G, Shelar, M.U, Dhasade, V.V and Musmade, D.S. (2009). A Review on Phytochemical and Pharmacological Profile of *Clitoria ternatea*. *Pharmacologyonline* 3: 204-210 (2009).
- Gomez, S., M., Kalamani, A.,(2003). Butter-fly Pea (*Clitoria ternatea*): A Nutritive Multipurpose Forage Legume for the Tropics- An Overview, *Pakistan Journal of Nutrition*, 2 (6): 374-379, (2003).
- Indira, P .D, Shamshad, S. (2015). Antimicrobial Activity and Phytochemical Evaluation of *Clitoria Ternatea*. *International Journal of Science and Research (IJSR)*, Volume 4 Issue 5, May 2015.
- Kapoor.B.B.S. and Veena P, (2013). Antimicrobial Screening of Some Fabaceous Plant Species of Rajasthan Desert. *International Journal of Pharmacy and Biological Sciences (e-ISSN: 2230-7605)*, IJPBS |Volume 3| Issue 3 |JUL-SEP|2013|201-203
- Karel A, Kumar H and Chowdhary B. (2018). *Clitoria ternatea* L. A Miraculous Plant. *Int.J.Curr.Microbiol.App.Sci.* 7(09): 672-674.
- Kelemu S, Cardona C, Segura G. (2004). Antimicrobial and insecticidal protein isolated from seeds of *Clitoria ternatea*, a tropical forage legume. *Plant Physiol Biochem.* 2004; 42: 867-873.
- Lakna, P. (2017). Difference between Gram Positive and Gram Negative Bacteria. <http://pediaa.com/differencebetween/gram-positive-and-gram-negative-bacteria/>
- Lijon MB, Meghla NS, Jahedi E, Rahman MA and Hossain I (2017). Phytochemistry and pharmacological activities of *Clitoria ternatea*. *International Journal of Natural and Social Sciences*, 4(1): 01-10.
- Mukherjee PK, Kumar V, Kumar NS, Heinrich M .(2008). The Ayurvedic medicine *Clitoria ternatea* from traditional use to scientific assessment. *J Ethnopharmacol* 2008;120: 291-301.
- Mukherjee PK, Saritha GS, Suresh B(2002). Antimicrobial potential of two different *Hypericum* species available in India. *Phytother Res.* 2002; 16: 692-695.

Nitha. B., A.B. Remashree and Indira Balachandran, (2012). Antibacterial Activity of some Selected Indian Medicinal Plants. *IJPSR*, 2012; Vol. 3(7): 2038-2042

Perez. C., M. Paul and P. Bazerque, (1990). An antibiotic assay by the agar-well diffusion method. *Acta Biol. Med. Exp.*, 15: 113–115.

SPSS (2008). *Statistical Package for the Social Science*. Version 17.0 SPSS .Inc Chicago.

Uwimbabazi F, Uwimana J, Rutanga, J. P(2015). Assessment of antibacterial

activity of Neem plant (*Azadirachta indica*) on *Staphylococcus aureus* and *Escherichia coli*. *Journal of Medicinal Plants Studies* 2015; 3(4): 85-91

Zakaria NNA, Okello EJ, Howes M-J, Birch-Machin MA, Bowman A. (2018). In vitro protective effects of an aqueous extract of *Clitoria ternatea* L. flower against hydrogen peroxide-induced cytotoxicity and UV-induced mtDNA damage in human keratinocytes. *Phytotherapy Research*. 2018; 32: 1064–1072.

دراسة النشاط المضاد للبكتيريا لأوراق الكلايتوريا تيرناتا – ضد بعض البكتريات الممرضة

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المستخلص

الإصابات التي تسببها البكتريا الموجبة والسالبة الجرام ذات أهمية كبيرة في حدوث الاصابات المرضية والوفيات وذات تأثير علي الصحة العامة. إن المستخلصات النباتات خاصة من الاعشاب البقولية مثل الكلايتوريا تيرناتا يمكن إستخدامها كمادة مضادة للبكتريا. أجريت هذه الدراسة لتقييم النشاط المضاد للبكتيريا لأوراق الكلايتوريا تيرناتا. تم استخدام المستخلص الميثانولي لأوراق الكلايتوريا تيرناتا ضد البكتريا الموجبة والسالبة الجرام. شملت البكتريا الموجبة الجرام البكتريا العنقودية الذهبية والبكتريا العقديية، أما البكتريا السالبة الجرام فشملت بكتريا الايكولاي و السالمونيلا. اظهرت النتائج وجود تأثير مضاد للبكتريا، حيث أن المستخلص الميثانولي لأوراق الكلايتوريا تيرناتا كان له التأثير الاكبر كمضاد علي بكتريا العنقودية الذهبية مقارنة بالبكتريات الاخرى في هذه الدراسة.

