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# EVALUATION OF ANTIMICROBIAL POTENTIALS OF CLITORIA TERNATEA AND HIBISCUS ROSA SINESIS FLOWER EXTRACTS AGAINST ORAL MICRO ORGANISMS

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## ABSTRACT

The plants in ancient times, in the past decades played an important role in human life and health. Research conducted on plants as source of treatment for in curable diseases has resulted in a huge demand for medicinal plants. And have found and reported that plants have been considered as one of the most sought after sources for the discovery of antimicrobial agent. With modern advancement of Ayurvedic tradition and its scientific exploration have studied plants in order to evaluate their therapeutic potentials and to isolate the lead compounds. The mouth and oral cavity is house of over 700 species of bacteria. Phytochemical screening and chemical tests identify new sources of therapeutic and industrial importance. *Clitoria ternatea* and *Hibiscus rosa sinesis* has been reported to have anti-inflammatory, hepatoprotective, immunoinhibitory activities. The purpose of this study was to screen for solvent extracts of *Clitoria ternatea and Hibiscus rosa sinesis,* flowers extract for antimicrobial activity. Testing was done of oral microorganism using agar well diffusion and disc diffusion then zone of inhibition was measured.

**KEYWORDS:** Microorganism, antimicrobial agent, *Clitoria ternatea, Hibiscus rosa sinesis,* zone of inhibition.

## INTRODUCTION

Infectious diseases need to have a host to spread their transmission endogenous to the body system in body fluids or tissues. The cause of many new infectious diseases, for instance, Asian Lineage Avian Influenza A (H7N9), Ebola and Influenza A virus subtype H5N1, also known as H5N1, lead to serious harm to human health.[1] Microbes, a diverse group of generally minute simple life-forms that include bacteria, archaea, algae, fungi, protozoa, and viruses.[2] With the increase in world population, there is a corresponding increase in health issues due to high environmental pollution. Antimicrobial and antibacterial agents decrease infectious diseases in people and animals. The use of medicinal plants could be potential as a natural antimicrobial and antibacterial agent.[3] The development of new tools for antibacterial agents could be useful as Clitoria ternatea and Hibiscus rosa sinesis is harmless and beneficial to human health.[4] A research was done to investigate antimicrobial activity under minimum inhibitory concentration on C.ternatea flower and Hibiscus rosa sinesis using disc diffusion and well diffusion methods.[5]

*Clitoria ternatea* L.(CT), Fabaceae is very well known in the Ayurvedic medicine used for different and various ailments, which has been investigated scientifically in considerable detail. A number of bioactive secondary metabolites and pharmacological activities of the plant have been reported.[6] The blue flower of *C ternatea* and leaves of *Wedelia chinensis* were evaluated for antimicrobial activity using aqueous extract concentrations (5%, 10%, 25%, and 50%).[7]

Traditionally Hibiscus flowers have been reported to possess anti-tumour properties, as well as have been used as analgesic, anti-asthmatic and anti-inflammatory agents. Several studies have proved the presence of anti-oxidant, anti-fungal, and anti-microbial properties in flowers of *Hibiscus rosa sinesis*. Research on extracts of roots, stems, roots, leaves, and flowers from *Hibiscus*

*rosa sinesis* revealed that its phytochemical components contributed to beneficial findings to human health such as antioxidant activity, which is the removal of free radicals that can lead to DNA damage.[8]

The finely-tuned equilibrium of the oral ecosystem is disrupted, allowing diseases-promoting bacteria to manifest and cause conditions such as caries, gingivitis and periodontitis. For practitioners and patients alike, promoting a balanced microbiome is therefore important to effectively maintain or restore oral health.[9]

The cheek mucosa, the tongue, the gingival crevice and the tooth surface provide place and sites with different physicochemical and nutritional microenvironment that allows for the adherence and growth of selective microorganisms.

Thus oral microbiota can be used as targets to treat oral and systemic diseases. In the future and upcoming times, oral microbiota may become a new target for the treatment of certain diseases.[10]

The antimicrobial activity or susceptibility testing can be used for drug discovery, research, epidemiology and prediction of therapeutic outcome. In this present study, we focused on the use of antimicrobial testing methods for the in vitro investigation of extracts and pure drugs as potential antimicrobial agents. Plants and other natural present available sources can provide a huge range of complex and structurally diverse compounds. In recent times many researchers have focused of the investigation of plants and microbial extracts, essential oils, pure secondary metabolites and new synthetized molecules as a potential and effective antimicrobial agent. A variety of laboratory methods can be used to study, evaluate or screen the in vitro antimicrobial activity of an extract or a pure compound. The most common known basic methods are the disk-diffusion and broth or agar dilution methods. Other methods are used especially for antifungal testing, such as poisoned food technique.[11]

The different methods to find out antimicrobial activity are as follows

Different Methods
A. Diffusion methods
Agar disk – diffusion method
Antimicrobial gradient method (test)
Other diffusion methods

- Agar well diffusion method
- Agar plug diffusion method
- Cross streak method
- Poisoned Food Method

B. Thin layer chromatography (tlc)- bioautography
Agar diffusion
Direct bio autography
Agar overlay bioassay
C. Dilution methods
Broth dilution method
Agar dilution method

D. Time- kill test
E. ATP bioluminescence assay
F. Flow cytoflourometric method

**Antibiotics**
Antibiotics are the powerful medicines that fight certain infections and can save lives when used properly. They either stop bacteria from reproducing or destroy them.[12] example: Tetracyclines, Cefotaxime, Ceftriaxone

## MATERIALS AND METHODS

**Collection of Plants**
Healthy and diseases free *Clitoria ternatea (Family-* Fabaceae[13] *and Hibiscus rosa sinesis (Family-* Malvaceae) plant were collected around the area of Vijaynagar.

**Plant Part Used**
Flowers (blue variety). The blue variety flowering plants flower were taken for the further studies. The flower was collected and dried fully under shade drying and then used for the further preparation of extract of *Clitoria ternatea.*
The flower parts were initially separated from the main plant body and shade dried. The Flower part was subjected for preparation of extract of *Hibiscus rosa sinesis.*

**Authentication of the Plant**
The plants were identified and authenticated

**Preparation Of Flower Extract: (Refer Table No: 1)**
**A.1. Clitoria ternatea extract method 1**
Firstly, fine dried 20 flower petals were collected in mortar and pestle and formed into powder then the contents were boiled directly with 100ml distilled water then the extracts were boiled for at about 1-2 hours till the volume reduced to 20ml, the extracts was transferred to chain dish to evaporate to dryness. Then extract was scraped and collected. Then 1ml of alcohol added as preservative.

**2. Clitoria Ternatea Extrct Method 2**
Fine dried 20 flowers petals were cleaned thoroughly with purified water and then transferred to mortar and pestle. Triturated with pestle using small quantity of phosphate buffer of pH 7.0 for about 15-20 minutes. Then extract was filtered and collected.

**B. Preparation of Flower Extract of Hibiscus Rosa Sinesis**
***1.*** **Hibiscus Rosa Sinesis Flower Extract Method 1**: *Hibiscus rosa sinesis* flower was collected and dried completely. Complete flower was powdered using mortar and pestle. The extract was placed in beaker (250ml) with 100ml of distilled water for decoction. Allow the beaker to boil on wire gauze using burner and reduce the

volume up to 50ml. Transfer the contents into China dish and evaluate to dryness. Collect the aqueous extract and scrape down the extract. Add 1-2 ml of alcohol as preservative and store in a suitable container.

**2. Hibiscus Rosa Sinesis Flower Extract_Method 2:** *Hibiscus rosa sinesis* flower was collected. The flowers were dried completely, after drying flowers was taken in mortar pestle (flower petals) and make into powder. The powder was triturated by adding phosphate buffer pH 7.

Phosphate buffer pH 7.0 Mixed preparation; Dissolve 0.50g of anhydrous disodium hydrogen phosphate and 0.301g of potassium dihydrogen in sufficient water to produce 1000ml. Another 10ml of buffer solution was added to Mortar pestle and contents was filtered and collected pH 6.8 to 7.0 was checked and store extract in suitable container.

**Analysis of Physicochemical Parameters**

**Physical Properties:** The formulated extract has been subjected for different physical properties such as color, odor, texture, pH, appearance refer **table no: 2.**

**Chemical Evaluation:** Formulated extract has been subjected for Phytochemical Screening for evaluating different phytochemical constituent refer **table no: 3.**

**Isolation of Microoganisms**

The culture and growth of oral microorganism was done by the following procedure

Oral bacterial sample was collected using a sterile cotton bud and dissolved into 500µl phosphate buffer saline (PBS) buffer (0.12M Na2HPO4, 5mM KH2PO4[pH 7.5]. An aliquot (100 µl) of bacterial suspension was spread on Agar plate. Agar media shows blue colony in case of *Streptococcus mitis, S. salivarius, S. mutans, S. sanguis and Entercoccus (pink colour)* and subsequently cultured on fresh Agar medium. Refer **fig no: 1**

**Preparation of the Culture Media**

The preparation of the culture media for microorganism involves the following the way. Ingredients are dissolved in appropriate volume of distilled water. The pH of dissolved medium is adjusted; the medium is disposed into suitable container whose mouth are then closed with cotton plug or metal cap. Then the medium is then sterilized by moist heat by autoclave. Weigh accurately all the ingredients and mix them well in a conical flask and add water and heat to $100^{o}$ C to dissolve the ingredients. Transfer 30ml of preparation in a conical flask and autoclave for sterilization, then after sterilization transfer the whole 30ml medium in a sterile test tube aseptically or 10ml each separately into 2 test tube for stab and slant position and another in upright position. Allow for solidification.

**Antimicrobial Activity**

After the preparation of the culture media, two methods of were carried out for the testing of antimicrobial activity and finding out the zone of inhibition. Refer **table no: 4 & 5, fig no: 2 to 14.**

A. Agar disk-diffusion method.

B. Agar well diffusion method

So by both the methods the zone of inhibition for the antimicrobial activity was studied for the *Clitoria ternate and Hibiscus rosa sinesis* flower extracts. Procedure was carried in the following methods

**Agar disk-diffusion methods**

It's one of the official method used in many clinical microbiology laboratories for the routine antimicrobial activity testing. In this well-known procedure, agar plates are inoculated with a standardized inoculum of the test microorganism. Then, filter paper discs (about 6mm in diameter), containing the test compound at a desired concentration, are placed on the agar surface. Agar plates are incubated under suitable conditions. Generally, antimicrobial agent diffuses into the agar and inhibits germination and growth of the test microorganism and the diameters of inhibition growth zones are measured.

**Agar well diffusion method**

The antimicrobial activity of plants or microbial extracts is tested by this method. Similarly, here the agar plate surface is inoculated by spreading a volume of the microbial inoculum over the entire agar surface. Then a hole with a diameter of 6 to 8mm is punched aseptically with a sterile borer and a volume (20-100 µL) of the antimicrobial agent or extract solution at desired concentration is introduced into the well. Agar mediums are incubated under suitable conditions depending upon the test microorganism. The antimicrobial agent diffuses in to the agar medium and inhibits the growth of the microorganisms to be tested.

## RESULTS AND DISCUSSION

**Authentication Detailts**

The plant was identified and authenticated as *Clitoria ternatea* belonging to the family Fabaceae Ref No-Authentication/SMPU/CARI/BNG/2020-21/1253B

The Plant sample was identified and authenticated as *Hibiscus rosa sinesis* belonging to the family Malvaceae, Ref No: Authentication/SMPU/CARI/BNG/2020-21/1253C, RefRRCBI-1131.Both were authenticated at central ayurveda research institute at Uttarahalli, Kanakpura Main Road, Bengaluru- 560109.

**Preparation Of Extract Table No: 1.**

| Ingredients | Quantity of *Clitoria ternatea* | Quantity of *Hibiscus rosa sinesis* |
|---|---|---|
| *Clitoria ternatea* flowers | 20 flowers | - |
| *Hibiscus rosa sinesis* flowers | - | 20 flowers |
| Phosphate buffer of 7.0 (Method 1) | 10ml | 10ml |
| Distilled water (Method 2) | 100ml | 100ml |

**Physical Parameters: Table No: 2.**

| Parameters | Results of Clitoria ternatea | results of Hibiscus rosa sinesis |
|---|---|---|
| Colour | Deep blue colour due to anthocyanins | Dark Brown |
| Odour | Mildly phenolic | Odourless |
| Taste | Slighty bitter and mucilagious | Mucilaginous |
| Appearance | Dark purple semisolid matter | Dark brown wet mass |
| Texture | Fine semisolid in nature | Semisolid |
| pH | 7.0 | 7.0 |

**Phytochemical Screening Of Clitoria Ternatea & Hibiscus Rosa Sinesis Aqueous Extract: Table No 3.**

| Test | Result of clitoria ternatea | Result of hibiscus rosa sinesis |
|---|---|---|
| 1.Fehling's test for reducing sugars: | + | + |
| 2. Benedict's test: | + | ++ |
| 3. Iodine test for Non-reducing polysaccharides: | _ | - |
| 4. Foam test for Saponin glycosides: | ++ | + |
| 5. Dragendorff's test for Alkaloids: | _ | - |
| 6. Hager's test: | _ | - |
| 7. Wagner's test: | _ | - |
| 8. Murexide test for purine alkaloids: | _ | - |
| 9. 5% $FeCl_3$ solution: | _ | - |
| 10. Lead acetate solution test: | + | + |
| 11. Acetic acid solution: | + | - |
| 12. Bromine water test: | + | + |
| 13. Potassium dichromate test: | _ | - |
| 14. Dilute iodine test: | _ | - |
| 15. Dilute $HNO_3$ test: | +++ | + |
| 16. Dilute potassium permanganate solution: | + | ++ |
| 17. Shinoda test for flavonoids: | +++ | + |
| Absent (-) Slight (+) Moderate (++) Very high (+++) | | |

**Isolation of Oral Microorganism**

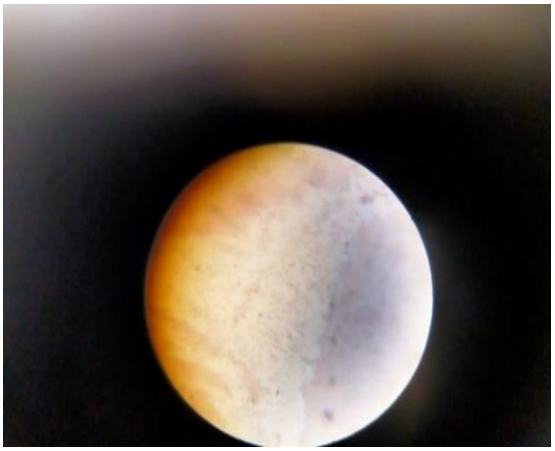

**Fig. 1: Microscopic view of stained isolated oral microorganism it was found to be gram positive bacteria.**

**Antimicrobial Study**

**Disc diffusion method and well (bore) diffusion methods: anti microbial activity zone of inhibition of flower extracts of Clitoria ternatea and standard antibiotic drugs. Table no: 4**

| Standard antibiotics | Zone of inhibition by disc diffusion method (Diameter) | Zone of inhibition well (bore) diffusion methods (Diameter) |
|---|---|---|
| Taxim- Cephalosporin (Cp) | - | - |
| Tetracycline (Te) | 9 mm | 8 mm |
| Monocef- Ceftriaxone (Ct) | - | - |
| *Clitoria ternatea* Flower extract (E) | 8 mm | 6 mm |

**Disc Diffusion Method (Clitoria Ternate)**

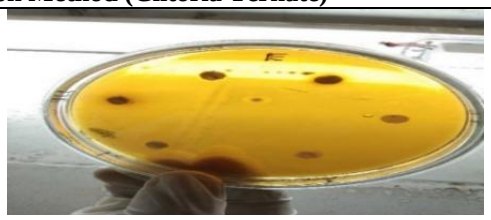

**Fig: 2: Antimicrobial study in disc diffusion method**

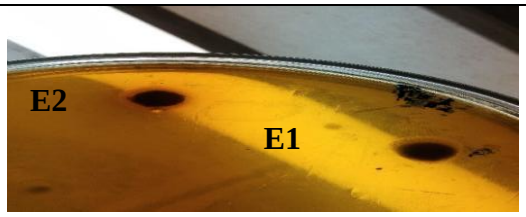


**Fig: 3: Zone of inhibition of extract(E1 & E2 ).**

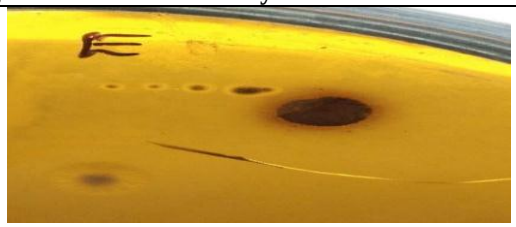

**Fig: 4: Zone of inhibition of *Clitoria ternate* flower extract(E2)**

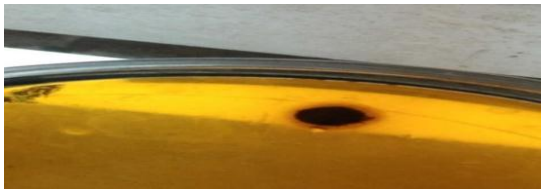

**Fig: 5: Zone of inhibition of tetracycline antibiotic.**

**Well (Bore) Diffusion Method (Clitoria Ternate)**

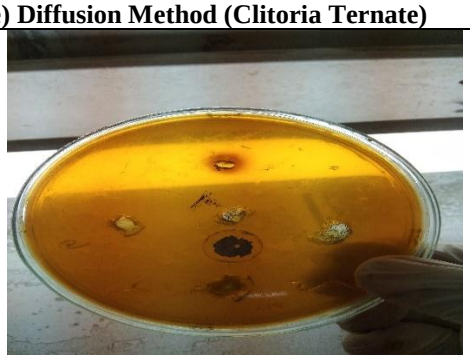

**Fig: 6: Antimicrobial study by well diffusion method.**

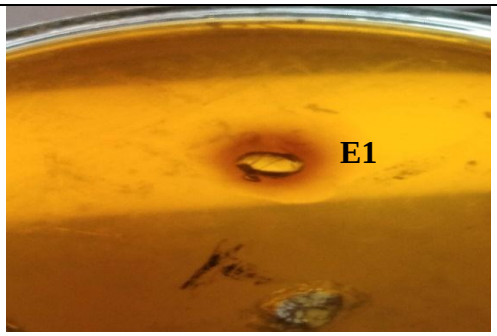


**Fig. 7: Zone of inhibition of *C. ternatea* extract (E1) (magnified).**

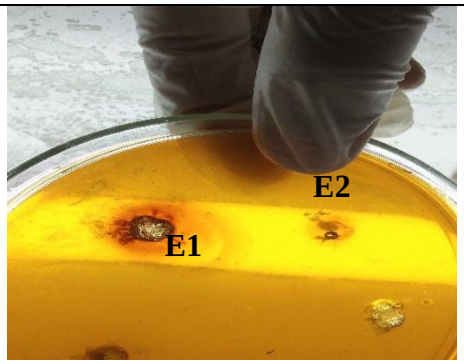


**Fig. 8: Zone of inhibition of extract (E1 & E2).**

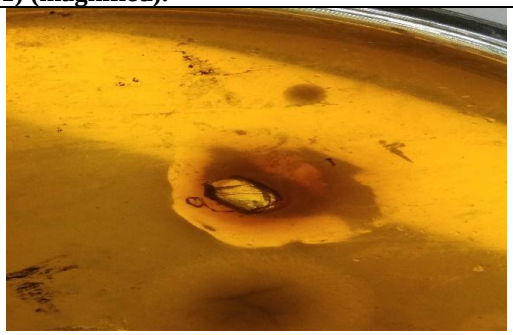

**Fig. 9: Zone of inhibition of Tetracycline antibiotic.**

**Antimicrobial activity of Hibiscus rosa sinesis flower extract and standard antibiotics by agar disc diffusion method and well (bore) diffusion methods. Table no: 5.**

| *Hibiscus rosa sinesis* | Zone of Inhibition by disc diffusion method Diameter (mm) | Zone of inhibition by well (bore) diffusion methods Diameter (mm) |
|---|---|---|
| Aqueous flower extract | 9 | 8 |
| Cefataxime | No inhibition | No inhibition |
| Tetracycline | 9 | 9 |
| Ceftriazone | No inhibition | No inhibition |

**Agar Well Diffusion Method (Hibiscus Rosa Sinesis)**

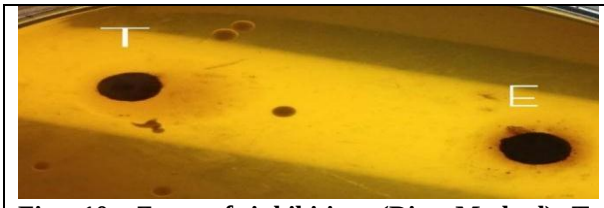


**Fig: 10: Zone of inhibition (Disc Method) T: tetracycline E: *Hibiscus rosa sinesis* flower extract**

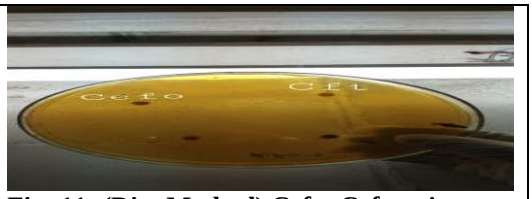


**Fig: 11: (Disc Method) Cefo: Cefotaxime, Cft: Ceftriaxone**

**Agar Well Diffusion Method (Hibiscus Rosa Sinesis)**

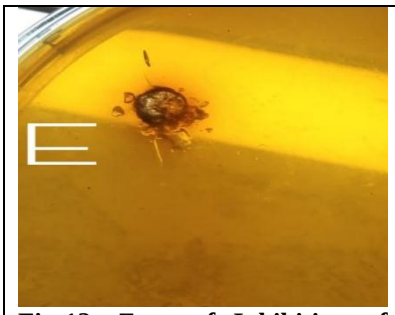


**Fig:12: Zone of Inhibition of *Hibiscus rosa sinesis* (Bore Method) *E: Hibiscus rosa sinesis***

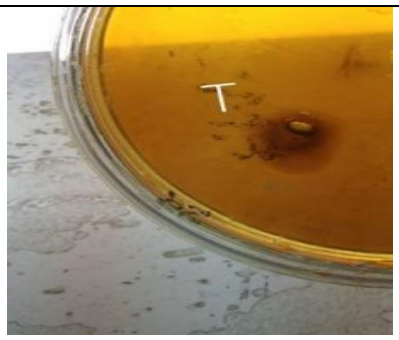


**Fig: 13: Zone of inhibition (Bore Method) T: Tetracycline**

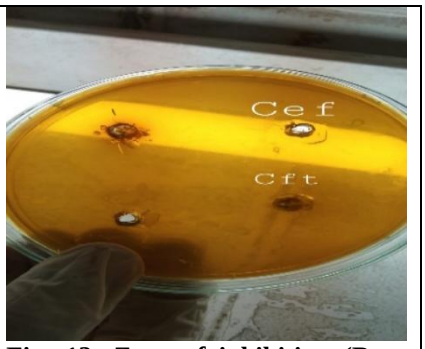


**Fig: 13: Zone of inhibition (Bore Method)T: Tetracycline**

**Duration of the study**

The study for the physiochemical parameters and antimicrobial activity of *Clitoria ternatea* flower extract was carried for about 3 months.

## DISCUSSION

In the present study, the culturing method employed was Agar Disc Diffusion Method and Agar well diffusion method which offers several advantages such as selective quantification of microorganisms. It has become more common over the past few years, due to increased rate of development of antibiotic resistance organism. The inhibition of bacterial growth invitro by the extracts of flower could be due to the presence of some active compounds in the extracts. Results from the assessment of antimicrobial activity of *Clitoria ternatea* and Hibiscus rosa sinesis flower extract against Oral microorganisms showed that low concentrations extract was found to be effective against oral microorganisms, probably due to the greater number of presence of active Phytochemical constituents is responsible for antimicrobial activity. Standard Antibiotics like Tetracycline, Cefotaxime, Ceftriaxone was used. Among these Tetracycline has shown zone of inhibition and Extracts (*Clitoria ternatea* and *Hibiscus rosa sinesis)* has shown zone of inhibition by Agar disc diffusion method. In other method, Agar well diffusion method Tetracycline and Extracts has shown zone of inhibition, which may be due to nature of chemical composition of plant flowers. Cefotaxime and Ceftriaxone has not shown zone of inhibition. Thus, it indicates the flower extracts of *Clitoria ternatea* and Hibiscus rosa sinesis showed zone of inhibition. These active compounds may act alone or in combination to inhibit the growth of bacteria. The crude extracts containing multiple organic components including flavonoids, tannins, alkaloids.

## CONCLUSION

The attempt has been made to determine the antimicrobial activity of *Clitoria ternatea* and Hibiscus rosa sinesis results suggests that the extracts showed better antimicrobial activity when compared to the standard Tetracycline drug. Furthermore, study is required with respect to the isolated compound present in the extract to know the active constituents responsible for the antimicrobial activity against the particular species of oral microorganisms.

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**REFRENCES**

1. Sun L, Chen J, Li Q. et al, Dramatic uneven urbanization of large cities throughout the world in recent decades. *Nat Commun*, 2020; 11(1): 5366.
2. Pelczar, Rita M. and Michael J. "Microbiology". Encyclopedia Britannica; Published, 2020 Dec 4.
3. Anand SP, Doss A, Nandagopalan V. Antimicrobial activity from leaf, flower, steam, root of Clitoria ternatea- A review. Int. J. Life Science, Rev., 2015: 1(1): 1-9.
4. Asma M. An update review of *Hibiscus rosa sinesis* Phytochemistry & Medicinal uses. J of Ayurvedic & Herbal Medicine, 2018; 4(3): 135-146.
5. Mukherjee PK, Kumar V, Kumar NS, Heinrich M. The Ayurvedic medicine *Clitoria ternatea*- From traditional use to scientific assessment. National library of medicine, 2008; 120(3): 291-301.
6. Kilian M, Chapple ILC, Hannig M, Marsh PD, Meuric V, Pedersen AML, Tonetti MS, Wade, WG, Zaura E. The oral microbiome- an update for oral healthcare professionals. British Dental J, 2016; (10)25: 657-666.
7. Wan N I et al. Oral microbes and its Environment. Esteem Academic J, 2013; 9(2): 67-75.
8. Maja Ptasiewicz et al, Armed to the teeth- The oral mucosa immunity system and microbiota. Int J Mol Sci., 2022; 23(882): 1-19.
9. Balouiri M, Sadiki M, Ibnsouda SK. Methods for *in vitro* evaluating antimicrobial activity: A review. National library of medicine, 2016; 6(2): 71-79.
10. Allen N, Sapadin M D & Raul Flieshmajer MD- Tetracycline; Nonantibiotic properties and their clinical implications, J of American Academy of Dermatology, 2006; 54(2): 258-65.
11. Mollie C, Hossein A, Tetracyline. Stat Pearls, 2021 July 17: 1.
12. Richards DM, Heel RC, Brgden R N, Ceftriaxone; A review of its antibacterial activity, pharmacological properties & therapeutic uses, National Library of Medicine, 1984; 27(6): 469-527.
13. Thakur RS, Puri HS, Husain A. Major Medicinal Plants of India; CIMAP, 1989.