

Evaluation Of Anti-Inflammatory Property of Mouthwash Using Ethanolic Extract of Clitoria Ternatea- An In Vitro Study

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ABSTRACT:

BACKGROUND: Clitoria ternatea, commonly known as butterfly pea, is a plant species belonging to the family Fabaceae.C. ternatea ethanolic extract (CTEE) is effective against oral pathogen, Streptococcus mutans. Clitoria ternatea is a well-known bioactive plant used to treat several inflammatory ailments in Ayurvedic system of medicine in India. Inflammation is caused by release of chemicals from tissues and migrating cells. Anti-inflammatory agents block certain substances in the body that cause inflammation.

AIM: To evaluate the anti-inflammatory activity of mouthwash using ethanolic extract of Clitoria ternatea.

MATERIALS AND METHODS: The research involved preparing mouthwash using ethanolic extract of Clitoria ternatea. These were tested for anti-inflammatory activity by BSA assay and EA assay.

RESULTS: The study shows that the anti-inflammatory activity of mouthwash increased as the concentration increased in a dose dependent manner when compared with the control, 50 μ l showed the highest % of inhibition 80% in both BSA and EA Assay. Hence can act as a good anti-inflammatory control.

CONCLUSION: Mouthwash using ethanolic extract of Clitoria ternatea were proved to possess strong level of anti-inflammatory activity. Also, the results of the study reinforce the opinion that medicinal properties of the plant are promising sources of potential anti-inflammatory agents which may be useful for various dental therapeutic procedures.

INTRODUCTION:

Clitoria ternatea Linn. is a perennial, twining plant of the Fabaceae family. Alkaloids, tannins, glycosides, resins, steroids, saponins, flavonoids, and phenols are among the bioactive chemicals found in C. ternatea. The pentacyclic triterpenoids taraxerol and taraxerone are the most abundant phytoconstituents discovered. C. ternatea contains flavonoid glycosides such as rutin, delphidin, kaempferol, quercetin, and malvidin, according to phytochemical studies. In India, the root, stem, and flower are used to heal snakebite and scorpion sting. Rheumatism and ear disorders are treated using a decoction or powder of C. ternatea root. Traditional uses of the herbs include the treatment of tonsillitis, cough, asthma, vermin, worn infestation, skin diseases. and infertility.¹ Numerous researchers investigate the pharmacological properties numerous of substances, including those with anti-hyperglycemic, anti-inflammatory, anti-diarrheal,

antioxidant. hepatoprotective, immunomodulatory, anti-histamic, and many more. Clitoria ternatea fresh flower was used in an early investigation that revealed hypolipidemic benefits. The inclusion of terpenoids, flavonoids, and triterpenoids such taraxerol, taraxerone, rutin, quercetin, delphidin, kaemferol, and malvidin may be the cause of the antiinflammatory and anti-arthritic properties of the ethanolic extract of C. ternate.

Studies on C. ternatea began in the 1950s aimed clarify and to its pharmacological properties, phytochemical makeup, and active ingredients.²⁻⁵ In 1985, scientists discovered the unique "ternatins" anthocyanins that give C. ternatea blooms their vibrant blue colour. The ternatins biosynthetic route was suggested ten years later after the isolation and structural characterization of multiple other ternatins. Although some recent researches have attempted to clarify the pharmacological properties of C. ternatea, the impact of specific extract components on any bioactivity evaluated is still unclear. Studies to evaluate the ability of C. ternatea to fix nitrogen began in the 1970s.⁶ Nodulation was demonstrated to be more successfully induced at soil moisture levels between 25 and 45 percent, with light exposure times of 11 to 14 hours, and a light intensity of 11 to 17 W/m2. Sulfur addition to the soil has also been shown to help in nodule development. The advantages of C. ternatea to soil health have been reported in investigations. several Due to the emergence of COVID 19 a requirement for their development of ayruvedic based medicinal drugs for dental care has been practitioners.^{7,8} among perennial According to studies extracts of C. ternatea roots and leaves exhibit anti-inflammatory,

analgesic, and antipyretic effects.⁹ In vitro, ternatea has strong anti-rheumatoid arthritis effects. oral dosing of C. elegans' ethanolic and methanolic root and flower extract. According to studies, ternatea greatly reduces the oedema and vascular permeability caused by acetic acid and carrageenin in rats' paws.

Clitoria ternatea methanolic extract and root extract and their anti-rheumatic, antibacterial, antioxidant, and anti-diabetic effect. As the literature is regarding the use of Clitoria ternatea for dental use is novel and no recent research on the use of mouthwash containing an ethanolic extract of Clitoria ternatea was done. However, the aim of the study is to evaluate the antiinflammatory efficacy of mouthwash utilizing Clitoria ternatea ethanolic extract.

MATERIALS AND METHODS: Extract preparation:

1gm of dried Clitoria ternatea was added in 50 ml of ethanol and kept in stirrer for 24 hrs. Boiled it for 10-15mins at 40 degrees Celsius. After boiling, the plant extract was filtered by Whatman No 1 filter paper. Kept for condensation up to 5 ml and the solution was saved in refrigerator for further use. (Figure 1)



Figure 1: Shows Clitoria ternatea kept for condensation

Mouthwash preparation:

0.3gsucrose, 0.001g sodium benzoate and 0.01g of sodium lauryl sulphate was dissolved in 8ml distilled water. To that extract sample 2ml was added. And flavouring agent peppermint oil was added-50microlitres. (figure 2a, 2b,2c)



Fig: 2a



Fig: 2b



Fig: 2c

Figure 2a, 2b & 2c: preparation of mouthwash using ethanolic extract of clitoria ternatea.

ANTI-INFLAMMATORY ACTIVITY

ALBUMIN DENATURATION ASSAY:

The anti-inflammatory activity for ethanolic extract of Clitoria ternatea was tested by the following convention proposed by Mizushima and Kobayashi with specific alterations (Pratik Das et al.,2019). 0.05 mL of ethanolic extract of Clitoria ternatea of various fixation (10µL,20µL,30µL,40µL,50µL) was added to 0.45 mL bovine serum albumin (1% aqueous solution) and the pH of the mixture was acclimated to 6.3 utilizing a modest quantity of 1N hydrochloric acid. These incubated samples were at room temperature for 20 min and then heated at 55 °C in a water bath for 30 min. The samples were cooled and the absorbance was estimated spectrophotometrically at 660 nm. Diclofenac Sodium was used as the standard. DMSO is utilized as a control. Percentage of protein denaturation was determined utilizing following equation, % inhibition= Absorbance of control-Absorbance of sample×100

Absorbance of

control

ALBUMIN DENATURATION EGG ASSAY:

A 5ml solution was made which was comprised of 2.8ml of freshly prepared phosphate buffered saline of pH - 6.3, 0.2 ml of egg albumin extracted from hen's egg. Specific concentrations were prepared separately for mouthwash using ethanolic extract of Clitoria ternatea as (10µL,20µL,30µL,40µL,50µL).

Diclofenac sodium was used as the positive control. Then, the mixtures were heated in water bath at 37°C for 15 minutes. After which the samples were allowed to cool down to room temperature and absorption was measured at 660 nm.

RESULTS:



Concentration



Graph: 1



Graph 1 and graph 2 shows the comparison of anti-inflammatory activity of mouthwash using ethanolic extract of Clitoria ternatea at different concentrations. X axis represents concentration of mouthwash and Y axis represents percentage of inhibition. Blue colour denotes Clitoria ternatea ethanolic extract mouthwash and green colour denotes standard. From the above graph it has been observed that the antiinflammatory activity of mouthwash using ethanolic extract of Clitoria ternatea was seen to be increased as the concentration increased in а dosedependent manner, hence can act as good anti-inflammatory control.

DISCUSSION:

Anti-inflammatory activity of mouthwash using ethanolic extract of Clitoria ternatea

was done by BSA assay and EA assay. At 10µl concentration 54% is observed by prepared mouthwash and 47% by standard, anti-inflammatory 63% activity was observed by prepared mouthwash and 60% by the standard at 20 µl concentration, at 30µl concentration prepared mouthwash showed 73% anti-inflammatory property and 72% by standard, at 40µl concentration 78% was observed by prepared mouthwash and standard, at 50 µl concentration 83% 84% is observed by prepared and mouthwash and standard respectively in BSA assay. At 10µl concentration 54% is observed by prepared mouthwash and 55% standard. anti-inflammatory by 61% activity was observed by prepared mouthwash and 64% by the standard at 20 ul concentration, at 30µl concentration prepared mouthwash showed 65% antiinflammatory property and 69% by standard, at 40µl concentration 68% was observed by prepared mouthwash and 72% by standard, at 50 µl concentration 76% and 81% is observed by prepared mouthwash and standard respectively in EA assay.⁹ The present study shows that the antiinflammatory activity of mouthwash increased as the concentration increased in a dose dependent manner when compared with the control, 50 µl showed the highest % of inhibition 80% in both BSA and EA Assay. Hence can act as a good antiinflammatory control. (Graph 1 & Graph 2) The response of living tissues to stimuli produced by inflammatory agents, such as physical trauma, heat, microbial infections, and noxious chemical irritations, is known as inflammation. Cells' reactions to inflammation can result in pathological symptoms as redness, heat, swelling, and discomfort as well as compromised physiological processes.^{10,11,12,13} Numerous diseases, including arthritis, stroke, and

cancer, have inflammation as part of their pathological processes. Protein denaturation has been linked to inflammatory responses that are more common and lead to a variety of inflammatory disorders, including arthritis. According to Opie, denaturation of the protein components of cells or of ground material may very well be the cause of tissue harm over the course of a person's lifetime. ^{14,15,16} Similar studies by Terahara et al., Pasukamonset et al., Adhikary R et al, Kelemu S et al 2021 confirms Ternatea possesses significant anti-inflammatory and anti-arthritic activity due to presence of terpenoids, flavonoids and triterpenoids and release of inflammatory mediators (histamine and prostaglandins). ^{17,18,19}

Future research can be focused on testing the efficacy of mouthwash in caries, gingival and periodontal diseases against various agents in different animal models under different circumstances.

CONCLUSION:

Mouthwash using ethanolic extract of Clitoria ternatea were proved to possess strong level of anti-inflammatory activity. The results of the study reinforce the opinion that medicinal plants are promising sources of potential anti-inflammatory agents that may be useful for therapy. In future, it can be also used for orodental care as an intracanal medicament, mouthwash, and toothpaste.

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