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Preparation of Mouthwash Using Blue Tea and Its Antioxidant Activity

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: *Clitoria ternatea* also known as the "blue pea," is a perennial twinning herbaceous plant in the Fabaceae family. The medicinal properties of this plant have been scientifically validated, especially at an international level, and it has been recorded to have a variety of biological activities, including antioxidant, antidiabetic, and hepatoprotective properties.
Aim: The aim of this study was to evaluate the antioxidant property of blue tea extract based mouthwash.
Materials and Methods: The study was performed as an in vitro study under a laboratory setting. Synthesis of the mouthwash was performed using dried leaves of the butter pea plant subsequently the mouthwash was tested for its antioxidant activity and was assessed via the DPPH assay. The obtained values were compared with that of a known standard. The obtained data was sorted in Microsoft Excel and statistically analyzed using Statistical Package for Social Sciences (SPSS Software, Version 23.0). Unpaired t test was done for the comparison of blue tea extract with the standard and one way ANOVA followed by Tukey's post hoc test was done to compare various concentrations with standard.

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Results: The blue tea extract mouthwash was tested with 10µL initially and then was increased to 20µL, 30µL, 40µL and 50µL. Based on DPPH assay, as the concentration of the extract solution was increased by 10µL each time tested, their antioxidant activity also increased over a scale. It was seen that the blue tea extract mouthwash had significantly less antioxidant capacity when compared to the standard ($p<0.05$).
Conclusion: The blue tea extract mouthwash is found to be a potent antioxidant agent however the antioxidant property was less effective when compared to the standard.

Keywords: Blue tea; antioxidant; mouthwash; green synthesis; innovative.

1. INTRODUCTION

Natural products have always been the go-to option for consumers all around the world in all sections of need let it be pharmaceuticals or daily use products [1,2]. The hype around such products are of the highest order due [3,4] to its properties which have less or no side effects when compared to the current chemical products which have at least one or more agents ,which if taken at higher doses can turn lethal [5-7].

The 'Butterfly Pea' (*Clitoria ternatea*), also known as Asian Pigeon Wings, Blue Bell Vine, Blue Pea, Kordofan Pea, and Darwin Pea [8] is an amazing brain power enhancing herb native to tropical equatorial Asia. *Clitoria ternatea* has been used as a memory enhancer, brain booster, anti-stress, and calming agent in traditional Chinese [9] and Ayurvedic medicine for centuries. Butterfly Pea has commonly been used as a vegetable in cooking, to colour deserts, or to make a vividly bright coloured tea because of its luminous indigo colour. Butterfly Pea [10] is high in health-promoting antioxidants, flavonoids, and peptides, and has shown promise in animal studies as a natural remedy for a variety of ailments. Because of the effects of the flavonoid quercetin on skin and hair, Butterfly Pea has been used in a variety of beauty products. Many health benefits have been attributed to Butterfly Pea [11] in both Chinese and Ayurvedic medicine, many of which have been confirmed by recent clinical studies. The herb has shown efficacy in studies for its brain-boosting properties and a wide range [12] of neurological benefits, including depression, anxiety, and fever reduction.

Furthermore, the medicinal properties of this plant have been scientifically validated, especially at an international level, and it has been recorded to have a variety of biological [13,14] activities, including antioxidant, antidiabetic, and hepatoprotective properties. Medicinal plants are a good natural source for finding cures for existing non-communicable diseases around the world. Furthermore, numerous studies have shown that antioxidant-rich foods are essential in the prevention and management of a variety of oxidative stress-related chronic diseases. Scavenging of free radicals [15] inhibition of oxidative enzymes, chelation of metal ions, and acting as antioxidant enzyme cofactors are all mechanisms that antioxidants use to manage oxidative stress in biological systems. Since oxidative stress plays a major role in the development of diabetes mellitus and its complications, diets rich in antioxidants may be a better alternative source for managing diabetes mellitus. And since Blue pea has proven to have its antioxidant properties, using it for various other areas can also be proven to be helpful and efficient. Mouthwashes are known for keeping the breath fresh and preventing the build-up of bacteria [16-20] in between the teeth. Mouthwashes come in a variety of formulations, including everyday-care formulas, alcohol-free versions, and herbal blends, all of which are intended to encourage oral health, and good hygiene.

Since use of herbal mouthwashes have been in practice since a long time, introduction of the same but with blue tea extracts will only be addressed if it shows properties better than those currently in market. Constant check must be kept to ensure product value to make it more than the other mouthwashes being preferred. The extract can not only be incorporated in mouthwashes alone but also in topical gels which can be used at home or even as mouth sprays.

The aim of this study is to bring out a natural product i.e blue pea flower known to everyone but in form of a mouthrinse or even in form of topical gels. This would help in widening the range of areas where the blue tea extract can be used and also reach out to a wider population.

Our team has extensive knowledge and research experience that has translated into high quality publications [21-42]. In this context, the aim of this study was to prepare a mouthwash using blue tea and study its antioxidant properties.

2. MATERIALS AND METHODS

The study was performed in the Blue Lab, Saveetha Dental College and Hospitals, Chennai. Ethical clearance required for the study was obtained from the institutional committee. The study was performed from January 2021 to February 2021.

2.1 Preparation of the Mouthwash

5g of the dried leaves of *Clitoria ternatea* (blue tea) plant was taken and 100ml of water was added and boiled until the colour changed into dark blue. The solution was then taken and filtered and concentrated to 10%. In a separate beaker, 0.3 grams sucrose was taken as the sweetening agent, 0.001 grams of preservative and 0.01 grams of sodium lauryl sulfate were taken as the foaming agent. To this mix, 1ml of the 10% concentrated blue tea extract and 10 ml of distilled water was added and mixed well to obtain the blue tea extract mouthwash.

2.2 Antioxidant Activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was used to test the antioxidant activity of the blue tea extract mouthwash. Different concentrations (10µl, 20µl, 30µl, 40µl and 50µl) of mouthwash having the blue tea extract was mixed with 1 ml of 0.1 mM DPPH in methanol solution and 450 µl of 50 mMTrisHCl buffer (pH 7.4) and incubated for 30 minutes. After incubation, the reduction in the number of DPPH free radicals was measured based on the wavelength at 517 nm. BHT (butylated hydroxytoluene) was used as control. The percentage inhibition was calculated from the following equation:

$$\% \text{ inhibition} = \frac{[\text{Absorbance of control} - \text{Absorbance of sample}] \times 100}{\text{Absorbance of Control}}$$

2.3 Statistical Analysis

The obtained data was sorted in Microsoft Excel and statistically analyzed using Statistical Package for Social Sciences (SPSS Software, Version 23.0). Unpaired t test was done for the comparison of the standard and one way ANOVA followed by Tukey's post hoc test. Results depicted in graphs and tabulations.

3. RESULTS

The blue tea extract mouthwash was tested with 10 µL initially and then was increased to 20µL, 30 µL, 40 µL and 50 µL. Based on DPPH assay, as the concentration of the extract solution was increased by 10µL each time tested, their antioxidant activity also increased over a scale. It was seen that the blue tea extract mouthwash had significantly less antioxidant capacity when compared to the standard ($p<0.05$) (Fig. 1).

One way ANOVA followed by post hoc analysis, revealed that there was a concentration dependent increase in the antioxidant capacity of the extract mouthwash. Antioxidant capacity was least at 10 µL concentration and it was more at 20 µL which was statistically significant as observed in this study. The antioxidant activity was found to be increased significantly with increase in concentration of the extract mouthwash which was statistically significant, however it was not as effective as the standard (Table 1).

4. DISCUSSION

The purpose of this study was to evaluate whether the prepared blue tea extract mouthwash had antioxidant activity and to compare its efficacy to existing standards.

The antioxidant activity of five different concentrations of plant samples was analyzed and compared to the very same concentration of standard drugs. The activity of the formulated blue tea mouthwash was convincingly demonstrated in the concentration-dependent assay. The results revealed that the sample mouthwash had antioxidant properties. Despite the fact that the blue tea extract mouthwash demonstrated a concentration-dependent increase from lower to higher concentrations at all levels, it was significantly less effective than the standard. Although the antioxidant activity of the blue tea extract mouthwash was not superior to that of the standard, the antioxidant activity of the blue tea extract mouthwash increased significantly with increasing concentrations from 10µL to 20µL, and the effectiveness of the standard mouthwash was almost the same.

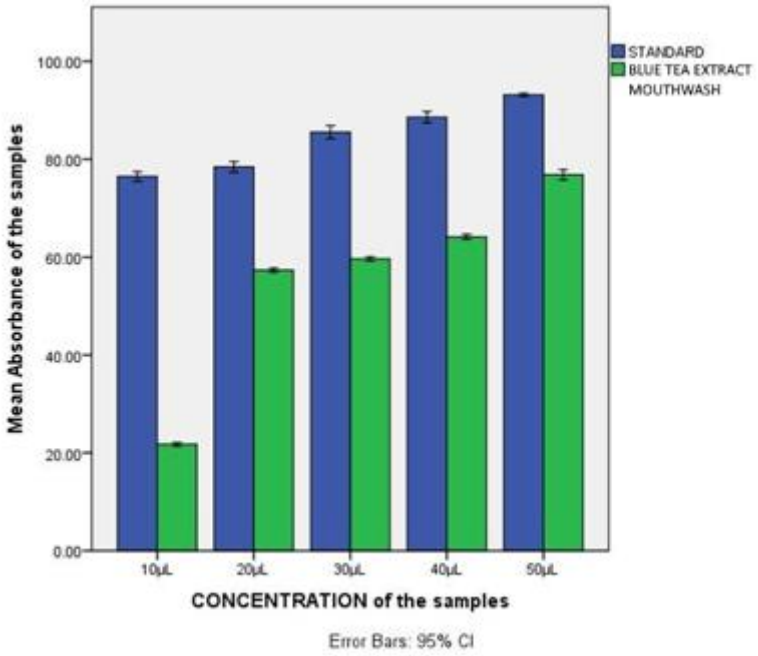

Fig. 1. Graph depicts the antioxidant activity of blue tea extract mouthwash compared to the standard

The Y axis depicts the mean absorbance value of the samples and the X axis denotes the various concentrations of the standard and samples. The blue bar depicts the standard while the greenbar depicts the blue tea extract based mouthwash. Blue tea extract based mouthwash had less antioxidant activity as compared to the standard at all concentrations, however there was a significant increase in absorbance values with increase in concentration, p<0.05, statistically significant (Unpaired t test, error percentage has been set upto 95%)

Table 1. Table depicts the statistical comparison of antioxidant activity at various concentrations using one way ANOVA analysis followed by post hoc. (p<0.05)

Concentration (I)	Concentration (J)	Significance (p value)
10µL	20µL	0.000*
	30µL	0.000*
	40µL	0.000*
	50µL	0.000*
20µL	30µL	0.000*
	40µL	0.000*
	50µL	0.000*
30µL	40µL	0.000*
	50µL	0.000*
40µL	50µL	0.000*

**(p<0.05)*

The *C. ternatea* extract mouthwash may have had more promising results if the components were used to its full potential rather than just depending on concentration levels. With increase in levels of certain components such as triterpenoids, flavanol glycosides and anthocyanins at varied scales may have brought a different result which could have been satisfactory. Studies show that certain components when extracted from the blue pea flower petals have eye opening results. The blue flower petals of *C. ternatea* contain quercetin glycosides and ternatin anthocyanins, which may be useful in the development of medications or nutraceuticals to protect against chronic inflammatory illnesses [11] by reducing the excessive synthesis of pro-inflammatory mediators by macrophage cells.

Certain authors have stated that the addition of the blue pea flower extract into daily consumed food items like yogurt has significantly increased

the antioxidant properties of the yoghurt [43]. The flower extract of C. ternatea serves as a direct antioxidant that may protect against free radicals produced by external or endogenous biological events [44]. It could be because of increased concentration of the extract and may also be due to certain chemical components add ons which can increase the efficacy of the blue pea extract. Since the *C. ternatea* plant is widely grown in various habitats, the extraction of its components which are important are not much of a tedious task. A study shows that it also helps in regeneration of the nitrogen level in the soil and also as a great source of novel phytochemicals. Butelase-1, a biotechnological tool [45] for peptide ligation and cyclization generated from C. ternatea pods, is also generating a lot of discussion.

The plant is not under observation for its antioxidant properties alone but also for its antimicrobial properties. Antimicrobial activity against Staphylococcus aureus, Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Streptococcus agalactiae, and Aeromonas hydrophila has been found in all sections of *C. ternatea* [12]. Authors of various studies have shown that the C. ternatea extracts have antibacterial, antipyretic, anti-inflammatory, analgesic, diuretic, local anaesthetic, antidiabetic, insecticidal, blood platelet aggregation-inhibiting, and vascular smooth muscle relaxing capabilities, among other things. This plant has a long history of usage in traditional Ayurvedic medicine for a variety of ailments, and scientific research has [46] proved so in the current times.

Previous studies [47] done to check for various characteristics of the *C. ternatea* flower extract show that when used as a commercial product and also as a folkloric medicine [48], it does show promising results of antioxidant activity but not as much as the other commercial products which were currently in use. The results of the present study are in accordance with the previous studies. However, more clinical trials need to be conducted and hence can be used for medicinal purposes.

5. CONCLUSION

The blue tea extract mouthwash is found to be a potent antioxidant agent however the antioxidant property was less effective when compared to the standard [49-58].

CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

The study was performed in the Blue Lab, Saveetha Dental College and Hospitals, Chennai. Ethical clearance required for the study was obtained from the institutional committee.

NOTE

The study highlights the efficacy of "ayurveda" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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