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Blue Tea Extract Based Preparation Of Mouthwash And Its Cytotoxic Activity

Research Article

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Abstract

Background: Blue tea is a caffeine-free herbal infusion produced by dried or fresh *Clitoria ternatea* plant leaves. The blue tea is high in antioxidants, which shield the body from free radical damage.
Aim: The aim of this study was to prepare mouthwash using blue tea and to assess its cytotoxic activity.
Materials and Methods: 5 gms of the dried leaves of *Clitoria ternatea* plant was taken in 100 ml of water and then boiled. It is later cooled down, filtered and thus forming the extract. For the mouthwash, 0.3 grams of sucrose, 0.001 grams of preservative and 0.01 gms of sodium lauryl sulphate (SLS) was added to 1 ml of concentrated extract and then 10 ml of distilled water was added. Brine shrimp lethality assay technique was used to assess the cytotoxic effect.
Results: Unpaired t test showed that the number of brine shrimps alive after 24 hours in *Clitoria ternatea* mouthwash was significantly lesser when compared to the control at various concentrations even though the magnitude of difference was lesser at higher concentrations ($p>0.05$). Also, the comparison of the mean brine shrimp count alive after 24 hours between different concentrations and the control was calculated using a one way ANOVA test. There was a significant decrease in the number of brine shrimps from lower concentration to higher concentration ($p>0.05$).
Conclusion: The present study reveals that the prepared blue tea extract based mouthwash exhibited cytotoxic activity at various concentrations even though the magnitude of difference was lesser at higher concentrations.

Keywords: Blue Tea; Herbal Mouthwash; Green Synthesis; Cytotoxicity; Innovative Technique.

Introduction

Herbal teas are becoming more popular as more people become aware of the possible negative effects of caffeinated beverages such as daily tea and coffee[1-9]. Herbal teas made from flowers such as chamomile and blue pea have become popular among tea drinkers all over the world. Though chamomile tea is well-known for its mild, calming flavour and ability to relieve tension, blue pea flower tea is less well-known[10-11].

Blue tea, also known as butterfly pea flower tea, is a caffeine-free herbal infusion produced by infusing dried or fresh *Clitoria ternatea* plant leaves [12-14]. The best part about blue tea is that it contains no caffeine and is loaded with antioxidants. The butterfly-pea, also known as blue pea or pigeon wings, is a plant that can be found in Southeast Asia [15]. Catechins, found in blue tea, are said to help burn belly fat and aid weight loss. Drinking butterfly-pea flowers soaked in warm water is said to boost metabolism, causing the body to consume more calories [16].

The blue tea is high in antioxidants, making it an excellent addition to your detox diet. Antioxidants shield the body from free radical damage. Butterfly-pea flower tea is said to have a calming effect due to its earthy flavour [16, 17]. The tea is said to have stress-relieving properties and may also assist in the reduction of anxiety symptoms [18]. As a natural diuretic, blue tea is said to assist in the loss of water weight. While there isn't much clinical evidence to indicate that blue tea can help with diabetes manage-

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ment, it is said to help regulate blood sugar levels [19].

There is a lot of debate and discussion about the advantages of blue tea over green tea. On the same topic, research is ongoing, and it has been scientifically proven that blue tea contains more antioxidants than green tea [18-21]. Our team has extensive knowledge and research experience that has translated into high quality publications [22-41]. Therefore the aim of this study was to prepare mouthwash using blue tea and assess its cytotoxic activity.

Materials and Methods

Preparation of Blue tea extract

5 gms of the dried leaves of *Clitoria ternatea* plant was taken in 100 ml of water and then boiled. It is later cooled down, filtered and thus forming the extract. (Figure 1).

Preparation of mouthwash

For making the mouthwash, 0.3 grams of sucrose was added as the sweetening agent, 0.001 grams of preservative was added and 0.01 gms of sodium lauryl sulphate (SLS) as the foaming agent, to this 1 ml of concentrated extract and 10ml of distilled water was added (Figure 2).

Brine shrimp lethality assay

Brine shrimp eggs were purchased commercially. Brine shrimp eggs were incubated for 48 hours in a small water tank containing brine/seawater for hatching. The larvae were utilised for the experiment after 24 hours (nauplii). 10-12 mL of salt water was used to fill 6 well ELISA plates. To that end, 10 nauplii were slowly added to each well containing varying concentrations of mouthwash (control, 5µl, 10µl, 20µl, 40µl, and 80µl). After 24 hours, the plates were incubated. To achieve triplicate values, this technique was performed three times. After 24 hours, the ELISA plates were examined for the presence of live nauplii and the number of dead nauplii was determined using the formula (number of dead nauplii / number of live nauplii x 100) [42, 43]. (Figure 3).

Results

In the present study, brine shrimp lethality assay was done to assess the cytotoxic effect of various concentrations of blue tea extract based mouthwash. Unpaired t test was done to compare the number of brine shrimps alive after 24 hours between *Clitoria ternatea* mouthwash and the control. The number of brine shrimps alive after 24 hours in *Clitoria ternatea* mouthwash was significantly lesser when compared to the control at various concentrations even though the magnitude of difference was lesser at higher concentrations ($p>0.05$). (Figure 4).

Also, the comparison of the mean brine shrimp count alive after 24 hours between different concentrations and the control was calculated using a one way ANOVA test. Maximum mortality was noted in the concentration 80µl. There was a significant decrease in the number of brine shrimps from lower concentration to higher concentration ($p>0.05$). (Figure 5).

Discussion

The present study was done to evaluate the cytotoxic activity of the blue tea extract using *Clitoria ternatea*.

The results of the current study showed that maximum mortality was noted in the concentration 80µl. There was a significant decrease in the number of brine shrimps from lower concentration to higher concentration.

A study on *Clitoria ternatea* extract suggested that the extract has substantial cytotoxic effect on cells and also has antioxidant effect [44]. Our study results are in accordance with the previous studies. The presence of the cytotoxic compound in cells can result in a variety of cell fates. Necrosis occurs when cells lose their membrane integrity and results in cell death [45]. The cells may stop actively growing and dividing or they may trigger a genetic programme that causes regulated cell death [46]. Pharmaceutical companies also use cytotoxicity assays to assess cytotoxicity [46, 47]. Researchers can either look for cytotoxic compounds that aim to quickly separate cancer cells or they can test preliminary drugs for cytotoxic effects before investing in their pharmaceutical production [1]. In this research, one of the most important approaches to assessing cell viability and cytotoxic effects was assessing the lethality of brine shrimp nauplii.

However, the current study had certain drawbacks such as the use of brine shrimp lethality assay to assess the cytotoxic effect. Since there is a lack of toxicity studies and clinical trials addressing the cytotoxicity of *Clitoria ternatea*, further research is needed to fully understand the cytotoxic impact of *Clitoria ternatea*. However, this research has aided in proving the bioactive potential of inexpensive natural plant extracts. More in vitro and in vivo research is needed to better understand the uses of this extract in the treatment of various illnesses as a traditional medicine that can substitute antibiotic overuse, improve patient compliance and minimise systemic toxicity or other adverse effects.

Figure 1. Preparation of Blue tea extract.

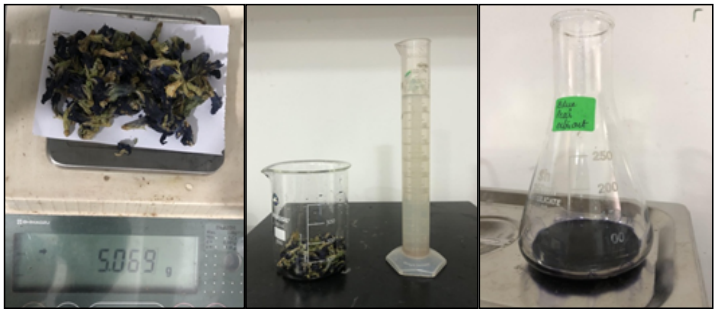


Figure 2. Preparation of Blue tea mouthwash.

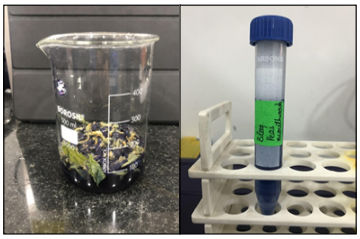


Figure 3. Isolation of brine shrimp eggs and incorporation of Blue tea mouthwash.

Figure 4. Bar graph shows the cytotoxic activity of *Clitoria ternatea* mouthwash and control against brine shrimp at various concentrations. The X axis represents the various concentrations of control and *Clitoria ternatea* extract in units of µl and the Y axis represents the number of brine shrimps. Blue represents the control and green represents the *Clitoria ternatea* extract. The graph shows that the number of brine shrimps alive in *Clitoria ternatea* mouthwash was significantly lesser when compared to the control at various concentrations even though the magnitude of difference was lesser at higher concentrations (p>0.05) (unpaired t test).

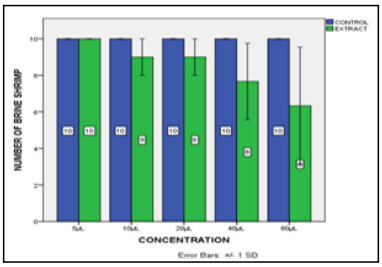
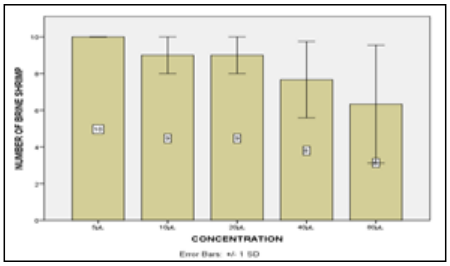


Figure 5. Bar graph shows the cytotoxic activity of *Clitoria ternatea* mouthwash against brine shrimp at various concentrations. The X axis represents the various concentrations of *Clitoria ternatea* extract in units of µl and the Y axis represents the number of brine shrimps. There was a significant decrease in the number of brine shrimps from lower concentration to higher concentrations. (p>0.05) (One Way ANOVA).



Conclusion

The present study reveals that the prepared blue tea extract based mouthwash exhibited cytotoxic activity at various concentrations even though the magnitude of difference was lesser at higher concentrations.

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