CYTOTOXIC EFFECT OF GINGER AND BLUE PEA HERBAL FORMULATION BASED MOUTHWASH - AN IN VITRO STUDY

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ABSTRACT:
INTRODUCTION: Herbal mouthwash made from natural sources is used in periodontal therapy controlling bleeding, inflammation. The efficacy of herbal mouthwash is effective in reducing plaque and gingivitis when compared to chlorhexidine mouthwash. Clitoria ternatea (blue pea) to be an effective natural remedy for a variety of ailments. Aim of the study is to evaluate the cytotoxic effect of ginger and blue pea based herbal mouth wash. **MATERIALS AND METHODS:** 100ml of distilled water was taken in a measuring cylinder and 1g of neem and stevia was measured and taken using a weighing machine. The temperature of the heating mantle was set to about 50 degrees celsius and the time taken to heat is about 5 - 10 minutes.A six-well ELISA plate was taken and 5 mL of artificial seawater was added to each of the 6 wells and 10 nauplii are added to each well. Then, 5 different concentrations of dried blue pea and ginger were introduced to each of the 5 wells.
RESULTS : At incubation time after 24 hours, 100 % of live nauplii were present in 5ul concentration. And 90% of live nauplii were present in 10ul concentration. At incubation time after 48 hours, 100% of live nauplii were present in 5ul concentration and 90 % of live nauplii were present in 10 ul concentration. And 80 % of live nauplii were present in 20 ul, 40 ul, 80ul concentration.
CONCLUSION: In this study ginger and blue pea herbal formulation showed good cytotoxic effect as the concentration of the formulation increased, number of nauplis decreased.
Keywords: antimicrobial; blue pea; ginger; cytotoxic activity.

INTRODUCTION:

Mouthwash / rinse is a chemotherapeutic agent used as an effective home care system by the patients to enhance oral hygiene. Some manufacturers of mouthwash claim that antiseptic and

anti-plaque mouth rinse kill the bacterial plaque which causes cavities, gingivitis and bad breath. Anti-cavity mouth rinse uses fluoride to protect against tooth decay(Surya et al. 2024)(1). Mouth rinse with alcohol as a base is prone for addiction for people consuming alcohol regularly. Excessive use of such mouth rinse regularly may cause harmful effects on the internal organs like lungs, kidneys, intestines and in certain cases the brain may also get affected.(Soylu et al. 2024)(2)

Studying the above mentioned drawbacks of the commercial mouthwash, people are now more aware and inclined towards the use of non-alcoholic or herbal mouthwash formulations.(Tidke et al. 2022)(3) Herbal mouthwash is formulated by using the active constituents obtained from plant sources directly by the process of extraction and evaporation.Herbal mouthwash made from natural sources is used in periodontal therapy controlling bleeding, inflammation.(Mehmethanoglu et al. 2025)(4) The efficacy of herbal mouthwash is effective in reducing plaque and gingivitis when compared to chlorhexidine mouthwash.

Cytotoxicity tests are useful to screen chemicals for their intrinsic and relative toxicities. This helps in determining the potential toxic or harmful effect of such compounds to human health that may occur inadvertently during use It is therefore important to establish their safety. Clitoria ternatea (blue pea) to be an effective natural remedy for a variety of ailments. It has powerful antimicrobial activity against various pathogens such as Escherichia coli, Staphylococcus aureus, etc. (Kshetrimayum 2017)(5)

Ginger can help prevent cavities and remove plaque & strengthen the gum tissue around your teeth. It has been used in the oral cavity through solutions and sprays because of its healing and anti-inflammatory actions presenting activity against S mutans and C albicans as well as antioxidant and anticancer activities(Deshpande et al. 2021)(6). It was verified that Ginger oils and extracts inhibit Gram-positive and Gram-negative bacteria, but there are still few studies showing the effect of ginger on the microor-ganisms prevalent in the oral cavity.(Chapple and Hamburger 2019)(7) The aim of the study is to evaluate the cytotoxic effects of blue pea and ginger based herbal mouthwash.

MATERIALS AND METHODS:

Blue pea flowers and ginger were collected from the local fields and were dried in shady place (Fig 1 a&b). After getting dried, they were crushed and made into a powder using a grinder or a mixer . Then, 100ml of distilled water was taken in a measuring cylinder and 1g of blue pea and ginger was measured and taken using a weighing machine (Fig 2). The solution is labeled and heated by using a machine called the heating mantle. The temperature of the heating mantle was set to about 50 degrees celsius and the time taken to heat is about 5 - 10 minutes (Fig 3). A six-

well ELISA plate was taken and 5 mL of artificial seawater was added to each of the 6 wells and 10 nauplii are added to each well (Fig 4). Then, 5 different concentrations of dried blue pea and ginger were introduced to each of the 5 wells, and control containing only the seawater was taken in one well.

Fig 1: (a) Blue pea extract

Fig 1: (b) ginger extract

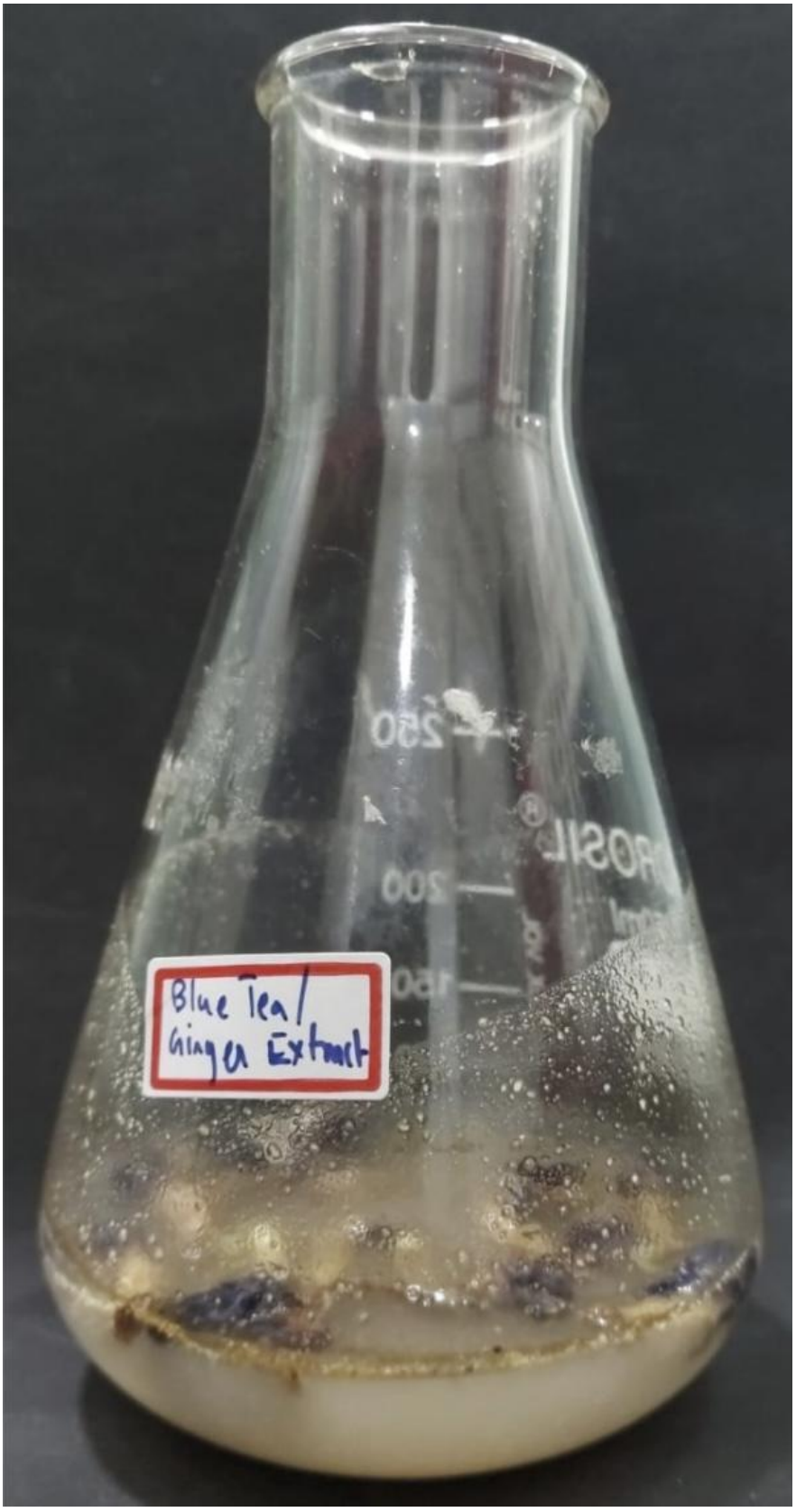
Blue Tea/
Ginger Extract

Fig 2: Blue pea and ginger extract


Blue Tea/
Ginger Extract
500 ml
LABQUEST
BY BOROSIL
HME500
0%
25%
50%
75%
100%

Fig 4: Heating mantle

Fig 5: centrifuged extract

RESULTS:

Incubation Time	Concentration (µL)	Percentage of Live Nauplii (%)
24 hours	5 µL	100%
24 hours	10 µL	90%
48 hours	5 µL	100%
48 hours	10 µL	90%
48 hours	20 µL	80%
48 hours	40 µL	80%
48 hours	80 µL	80%

Table 1: Explains the cytotoxic effects of blue pea and ginger with respect to the concentration and percentage of live nauplii.

At incubation time after 24 hours, 100 % of live nauplii were present in 5ul concentration. And 90% of live nauplii were present in 10ul concentration. At incubation time after 48 hours, 100% of live nauplii were present in 5ul concentration and 90 % of live nauplii were present in 10 ul concentration. And 80 % of live nauplii were present in 20 ul, 40 ul, 80ul concentration. In this study ginger and blue pea herbal formulation showed good cytotoxic effect as the concentration of the formulation increased, number of nauplius decreased.

DISCUSSION:

A study on Clitoria ternate extract suggested that the extract has substantial cytotoxic effect on cells and also has antioxidant effect.Our study results are in accordance with the previous studies (Haghgoo and Abbasi 2013)(8&9). The time of use of this mouthwash should be limited because of adverse factors such as palate changes, teeth staining, and burning. Ginger is one of the most popular and used spices in the world, and it is used as food seasoning because of its fresh aroma and spicy flavor, which may lead to an individual assessment of flavor (Kamilla et al. 2014)(10).

The characteristic odor and flavor of ginger are caused by a mixture of zingerone, shogaols, and gingerols, which are volatile oils that compose 1%-3% of the weight of fresh ginger.(Mansoor et al. 2024)(11) Pal et al. also conducted a BSLA on the leaf and stem extracts of *C. ternatea*, noting significant toxicity at various concentrations, with LC_{50} values calculated for leaves and stem extracts. The toxicity test showed that shrimp deaths increased with higher concentrations of green tea leaf extract after 24 hours, linking to the broader discussion on the cytotoxicity of natural extracts

Ginger powder has been reported to be effective in motion sickness.

It has been suggested that adsorbent, aromatic and carminative properties of ginger on G. I. tract cause adsorption of toxins and acid enhanced gastric motility. These may have probably blocking effects of G. 1. Reactions and nausea.(Devi et al. 2022) (12)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. The cells may stop actively growing and dividing or they may trigger a genetic programme that causes regulated cell death. Pharmaceutical companies also use cytotoxicity assays to assess cytotoxicity.(Müller et al. 2017) (13)

Herbal mouthwash having anti-inflammatory, anti-bacterial, anti-fungal, analgesic along with strengthening and mouth freshening properties and thus, this new formulated form of oral health care using various natural oils and extracts for getting the benefit of the intended to make the formulation effective use as well as easily acceptable by people. As such, seen in our country, oral hygiene is not given much focus, which in later stages of life leads to chronic diseases like cases of mouth cancer. Searching up for an herbal formulation in the market with all the said properties led to making of this product that is cost affordable and user friendly.(Sabarish et al. 2025) (14) The limitations of study is that anti inflammatory, antitoxic and antimicrobial activities were not done. It will be done in the further studies.

CONCLUSION:

In this study ginger and blue pea herbal formulation showed good cytotoxic effect as the concentration of the formulation increased, number of nauplis decreased. Oral pathogens from a wide variety cause severe infections in the oral cavity. So, there is a need for prevention of infections and diseases. As a preventive measure, mouthwash can be prepared. The herbal formulations with ginger and blue pea mouthwash preparation seems to be a better preventive measure.

CONFLICT OF INTEREST:

The authors have no conflict of interest.

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