RESEARCH
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PREPARATION OF *TERMINALIA CHEBALA* ETHANOLIC EXTRACT BASED MOUTHWASH AND ITS ANTIMICROBIAL AND CYTOTOXICITY EFFECT

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Abstract
Antimicrobial activity is the process of killing or inhibiting the disease causing microorganisms. Many antimicrobial agents are used for this purpose. The effect of being harmful to cells caused by toxic agents is called cytotoxicity. Exposing cells to a cytotoxic agent may result in various outcomes in the functional and structural ability of cell. No challenges are faced in previous research. The research is needed to find whether the ethanolic extract of terminalia chebula extract mouthwash has antimicrobial activity and cytotoxicity. The research also fulfils the deficiency of work on comparing its antimicrobial activity on different families.The aim of the research is to find the antimicrobial and cytotoxicity effect of ethanolic extract of terminalia chebula extract mouthwash. For cytotoxicity effect, a total of 6 wells with salt water and 10 nauplii is taken and one well is controlled and the others are filled with 5, 10,20,40,80µl concentration of prepared mouthwash. left for 24hrs and the alive nauphlii in each. For antimicrobial activity, 25 , 50, 100 µl of prepared mouthwash and a mouthwash already available in the market are taken in the culture medium of 4 different microorganisms. and left for 24 hrs.the ethanolic extract mouthwash of t.chebula has higher antimicrobial activity than the mouthwash present in the market. And also has less cytotoxicity in its less concentration.
Keywords: *Terminalia chebala;* antimicrobial activity; Mouthwash

INTRODUCTION

Antimicrobial activity is the process of killing or inhibiting the disease causing microorganisms. Many antimicrobial agents are used for this purpose. Antimicrobial agents may be anti-bacterial, antifungal or antiviral(1). They all have different modes of action by which they try to kill or inhibit microbes.The main classes of antimicrobial agents are disinfectants (non-selective agents, like bleach), which kill a large range of microbes on non-living surfaces to forestall the spread of illness, antiseptics (which apply to living tissues and reduces the infection during surgery), and antibiotics(which kills the microorganisms within the body)(2,3). The term antibiotic originally describes only those formulations derived from the living microbes but now it is applied to synthetic agents too, like sulfonamides or fluoroquinolones(4)(5). Though the term wants to be restricted to antibacterials (and is usually used as a synonym for them by medical professionals and in medical literature), its context has broadened to incorporate all antimicrobials(2,6). Antibacterial agents will be further subdivided into bactericidal agents, which kill bacteria, and bacteriostatic agents, which hamper or stall bacterial growth. In response, further advancements in antimicrobial

technologies have resulted in solutions that may transcend simply inhibiting microbial growth(7)(8). The effect of being harmful to cells caused by toxic agents is called cytotoxicity. Exposing cells to a cytotoxic agent may result in various outcomes in the functional and structural ability of cells. At this point, the affected cells will actively move into the death phase(9)(10). Cytotoxic agents are known as the substances that are toxic to the cells, which include the factors that inhibit or stop their growth and cause death, They are also used to treat certain disorders like cancer(11)(12). Chemical substances or biological substances or physical agents can also be cytotoxic that affect the cells in varying degrees(13,14).Cells undergoing necrosis show rapid swelling, lose membrane integrity, stop metabolism, and release their contents into the environment. Cells that undergo rapid necrosis in vitro don't have sufficient time or energy to activate apoptotic machinery and can not express apoptotic markers. Apoptosis is characterised by well defined cytological and molecular events including a change within the ratio of the cell, cytoplasmic shrinkage, nuclear condensation and cleavage of DNA into regularly sized fragments(15,16)(17). Cells in culture that are undergoing apoptosis eventually undergo secondary necrosis. they're going to close up metabolism, lose membrane integrity and lyse. Cytotoxicity testing is employed within the development of the many products starting from drugs to cosmetics. Plant products (that are going to be used for extracts, etc.) are tested for toxicity(18)(19)(20). A term called "selectivity index" explains the ratio between potential biological activity of a plant specimen in relation to its potential cytotoxicity. Terminalia chebula is used to cure high cholesterol and digestive disorders like diarrhoea, constipation, dysentery and indigestion(21,22)(23). They have also been used for HIV infection and sore eyes. it's used as a douche for treating vaginal infections. Also used topically as a mouthwash and gargle.Terminalia chebula and Emblica officinalis is used to prevent death of heart tissue. Mouthwashes are also called oral rinse, it is a liquid product used to rinse your oral cavity. Some use this mouthwash to fight against bad breath, some use it to in a try to prevent tooth decay(24)(25)(26)(27). In this study the antimicrobial activity and cytotoxicity effects of the ethanolic extract of the terminalia chebula mouthwash is seen.No challenges are faced in previous research. The research is needed to find whether the ethanolic extract of terminalia chebula extract mouthwash has antimicrobial activity and cytotoxicity. The research also fulfils the deficiency of work on comparing its antimicrobial activity on different families.The aim of the research is to find the antimicrobial and cytotoxicity effect of ethanolic extract of terminalia chebula extract mouthwash.(28)(29)

Materials and methods

Ethanolic extract of t. chebula is prepared from the dried seeds of it. Then a mouthwash is prepared using this extract. sucrose , sodium benzoate, sodium lauryl sulfate are used to prepare the mouthwash. Sucrose is used as sweetening agent, sodium benzoate is used as preservative, and sodium lauryl sulfate is used as foaming agent. For antimicrobial activity, 25 , 50, 100 mue l of prepared mouthwash and a mouthwashalready available in the market are taken in culture medium of 4 different microorganisms. and left for 24 hrs. the zone of inhibition for e.fecalis, c. albicans, s.mutans, s.aureus. For cytotoxicity effect, a totalof 6 wells with salt water and 10 nauplii is taken and one well is controlled and the others are filled with 5, 10,20,40,80 mue l concentration of prepared mouthwash. left for 24hrs and the alive nauphlii in each.

RESULTS

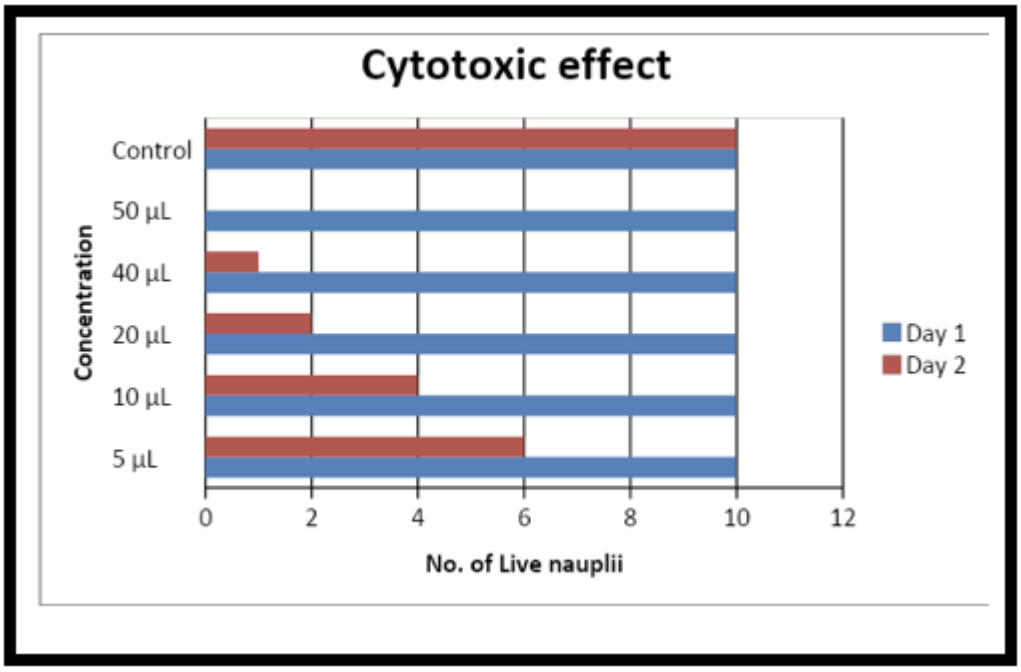

Figure 1: This bar graph shows the cytotoxicity of the mouthwash prepared from the ethanolic extract of terminalia chebula. X axis represents the number of live nauplii, Y axis represents the concentration. On day 1, in all the wells the mortality rate is 0%. In day 2, the 5 microliter concentration has 40% mortality, 10 microliter concentration has 60% mortality, 20 microliter concentration has 80% mortality, 40 microliter concentration has 90% mortality, 80 microliter concentration has 100% mortality, control has 0% mortality

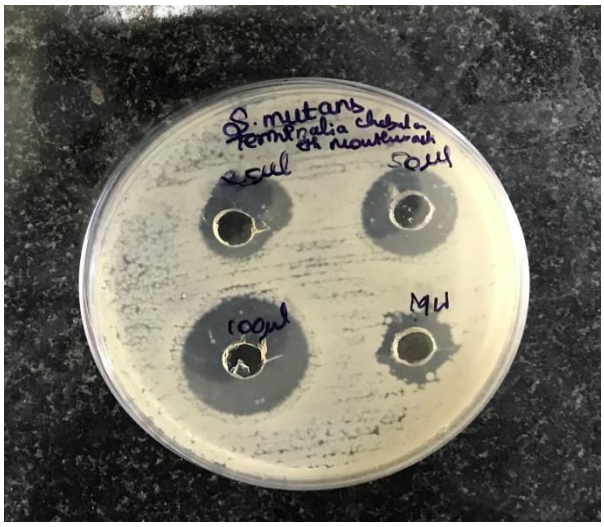

Figure:2

Figure: 4

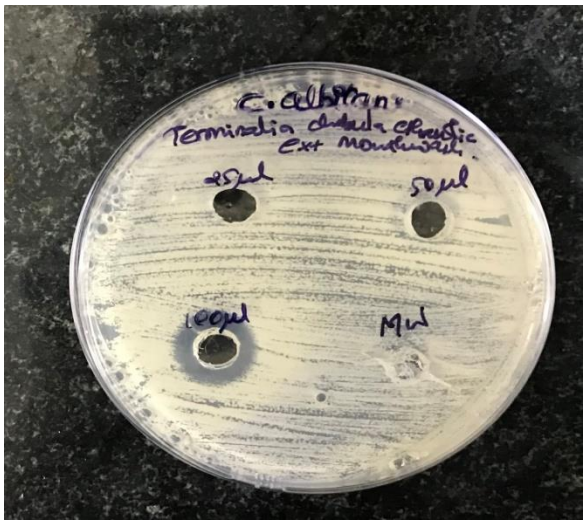

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Figure: 5

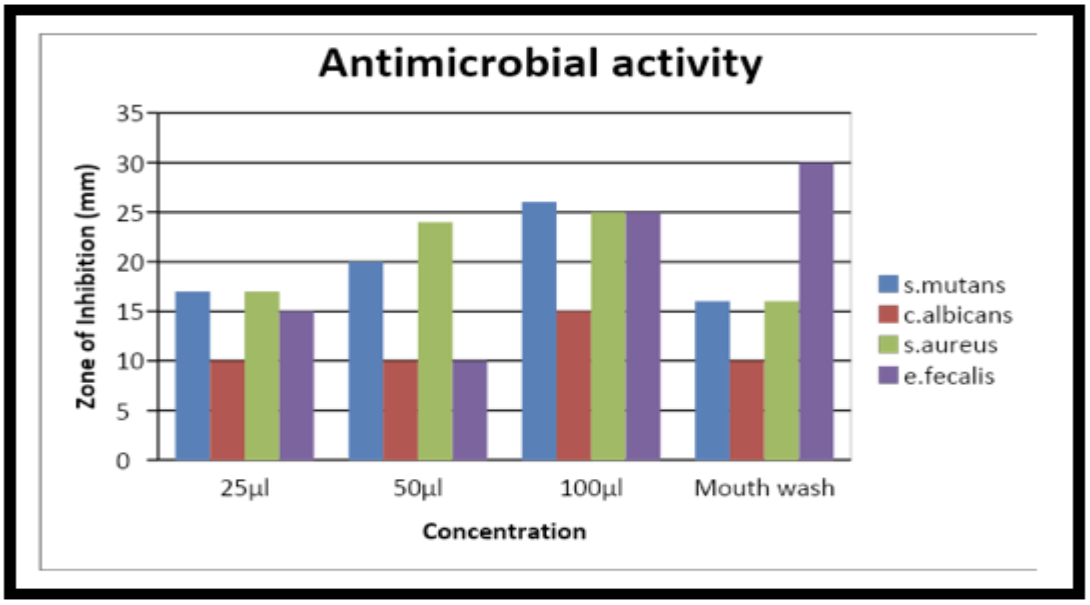

Figure 6 the given graph shows the antimicrobial activity of the mouthwash derived from ethanolic extract of terminalia chebula on 4 different microorganisms. The x axis represents theThe organisms that are taken for experiment here are s.aureus , s.mutans, c.albicans, e.faecalis. And is also compared with the antimicrobial activity of the market mouthwash. The antimicrobial activity is calculated by the diameter of the zone of inhibition on different concentrations and the market mouthwash.

Discussion:

This study shows that the antimicrobial activity of the mouthwash prepared from the ethanolic extract of the terminalia chebula is much higher than the market mouthwash. It shows a 17mm zone of inhibition for s.mutans(fig.1) and s.aureus(fig.5), 10mm zone of inhibition for c.albicans(fig.3), 15mm for e.faecalis at 25 microliter(fig.4). In a 50 microliter it shows 20mm zone of inhibition for s. Mutans(fig.2), 10 mm zone of inhibition for c.albicans(fig.3) and e.faecalis(fig.4), 24mm zone of inhibition for s.aureus(fig.5). In 100 microlitre it shows a 26mm zone of inhibition for s. Mutans(fig.2), 25mm zone of inhibition for s.aureus(fig.5) and e.faecalis(fig4), 15mm zone of inhibition for c.albicans(fig.3). The market mouthwash, shows a 16mm zone of inhibition for s.mutans(fig.2), 10 mm zone of inhibition for c.albicans(fig.3) and e.faecalis(fig.4), 30mm zone of inhibition for s.aureus(fig.5). Hence the antimicrobial activity of the mouthwash prepared ethanolic extract of terminalia chebula is higher than the market mouthwash on s.aureus, s.mutans, c.albicans even in its lower concentrations. This extract mouthwash has lesser antimicrobial activity on e.faecalis when compared to market mouthwash. Even though there is a difference, the difference in the antimicrobial activity of the market mouthwash and ethanolic extract of terminalia chebula mouthwash has no greater difference. They almost have the same antimicrobial activity on e.faecalis.

The cytotoxicity effect test has also given great results, that the mouthwash shows less cytotoxicity. It shows only a 40% cytotoxicity effect on 5 microlitre concentration. Cytotoxicity effects can be reduced to an even lesser concentration.on higher concentration it is completely cytotoxic that is 100% mortality just like other market mouthwashes on higher concentration. The cytotoxicity is compared between prepared mouthwash and the controlwell after adding extract mouthwash and after 24 hours. Then life and mortality is counted. This study is limited with the antibacterial activity of specific types of microorganisms. Further researches on the antimicrobial activity of this extract for more other types of microorganisms' can be done. further research can be done in cytotoxicity of this extract in lower concentrations. Different types of chemical mouthwashes can also be compared with this *Terminalia chebala* ethanolic extract based Mouthwash.

Conclusion

Thus, the ethanolic extract mouthwash of t.chebula has higher antimicrobial activity than the mouthwash present in the market. And also has less cytotoxicity in its less concentration. It will be great option for the market mouthwashes.further studies have to be done to make it have a better taste and smell

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