Bindura University of Science Education

Faculty of Science

Biological Sciences Department

Phytochemical characterization and formulation of a herbal oral care product from Governor's plum (*Flacourtia indica*) and Powderbark gardenia (*Gardenia jovis-tonantis)*

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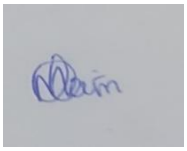
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May 2026

APPROVAL FORM

The undersigned certify that they have read the dissertation titled **"Phytochemical characterization and formulation of a herbal oral care product from Governor's plum (*Flacourtia indica*) and Powderbark gardenia (*Gardenia jovis-tonantis*)"**, and confirm that it is suitable for submission to the Biological Science Department, Faculty of Science, for assessment.

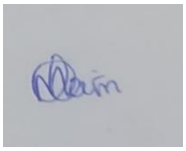
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DECLARATION

I, Keith Takudzwa Kwangwari (B224621), declare that this research herein is my own work and has not been plagiarized from another source(s) without acknowledgement of the concerned authors either electronically or otherwise

Signature:  Date: 08 June 2026

Supervisor

I, J. Ndava , declare that l have supervised this thesis and I am satisfied that it can be submitted to the Biological Science Department, Faculty of Science, at Bindura University of Science Education.

Signature:  Date: 15 June 2026

DEDICATION

Dedicated to my mother, sister, and aunt who have been the cornerstone of all I am, including my academic and life, for their unconditional support that has been flawlessly, maintained over the years.

ACKNOWLEDGEMENT

I would like to express my deepest gratitude to God Almighty, for giving me the strength, wisdom, and tenacity to finish this dissertation; without His direction and favour, this accomplishment would not have been feasible. I am sincerely grateful to my lecturer, Mr J. Ndava, for his invaluable guidance, support, and encouragement throughout this research. His knowledge, patience, and constructive feedback played a significant role in shaping this work. I would also like to extend my deepest appreciation to my family for their endless love, sacrifices, and unwavering support. Their encouragement and belief in me have been my greatest motivation. Finally, I am grateful to everyone who, in one way or another, contributed to the completion of this thesis.

LIST OF ABBREVIATIONS

ADA	American Dental Association
ANOVA	Analysis of Variance
AWD	Agar Well Diffusion
CAMHB	Cation-adjusted Mueller-Hinton Broth
Conc	Concentration
hGF	Human Gingival Fibroblast
MIC	Minimum Inhibition Concentration
MHA	Mueller-Hinton Agar
mm	millimetre
SD	Standard Deviation
SLS	Sodium Lauryl Sulfate
WHO	World Health Organization
ZOI	Zone of Inhibition

LIST OF FIGURES

Figure 1: Formulated toothpaste 29

Figure 2: Before and after brushing with formulated toothpaste 29

TABLE OF CONTENTS

Table 1: Qualitative phytochemical constituents of plant extracts 24

Table 2: Mean zone of inhibition (mm) against S. mutans 24

Table 3: Mean zone of inhibition (mm) against S. mutans 25

Table 4: Activity of extracts against oral pathogens at 75% (Mean ± SD, mm) 26

Table 5: MIC values (mg/mL) 26

Table 6: Physicochemical properties 27

Table 7: Activity of herbal toothpaste 28

Table 8: Stability over 4 weeks 28

Table 9: ANOVA: Two-Factor with Replication plants extract against S.mutans 30

Table 10: ANOVA: Two-Factor with Replication plant extract against C.albicans 30

List of Appendices

Appendix A *F.* indica, Governor's plum, Munhunguru .....**Error! Bookmark not defined.**9

Appendix B *G. jovis*-tonantis, Powder-bark gardenia, Mutarara ..... **Error! Bookmark not defined.**9

Appendix C Phytochemical tests 40

Appendix D Zone of inhibition for *F. indica* 1:5 extract.....40**Error! Bookmark not defined.**

Appendix E Zone of inhibition of *G. jovis-tonantis* leaves...**Error! Bookmark not defined.**1

Appendix F Zone of inhibition of *F. indica* 1:10.....**Error! Bookmark not defined.**1

Appendix G Zone of inhibition of *G .jovis-tonantis* Bark.....**Error! Bookmark not defined.**2

Appendix H Minimum inhibition concentration against *C. albicans*.... **Error! Bookmark not defined.**2

Appendix I Minimum inhibition concentration against *S. mutans*..... **Error! Bookmark not defined.**2

Appendix J Normality test .....**Error! Bookmark not defined.**3

Appendix K One way ANOVA for minimum inhibition concentration **Error! Bookmark not defined.**3

Abstract

The increasing prevalence of oral diseases, such as dental caries, gingivitis, periodontal infections, and oral candidiasis, has become a major public health concern. The growing problem of antimicrobial resistance and the side effects associated with synthetic oral care products, have increased interest in the development of safe, affordable and plant-based alternatives. This study investigated the phytochemical composition and formulation of a herbal oral care product using Governor's plum, *Flacourtia indica* bark extracts and Powderbark gardenia, *Gardenia jovis-tonantis* leaf and bark extracts. The study also evaluated the antimicrobial activity of plant extracts against microorganisms associated with oral diseases. Plant samples were collected and authenticated before extraction. Ethanolic extracts were prepared using maceration method. Standard microbiological techniques were used to isolate and identify microorganisms associated with oral infection. Qualitative phytochemical screening was carried out to determine the presence of bioactive compounds. The antibacterial and antifungal activities of the extracts against *Candida albicans* infection and *Streptococcus mutans* infections were evaluated using the agar well diffusion (AWD) and minimum inhibition concentration (MIC) methods. All experiments were performed in triplicate. Phytochemicals analysis revealed the presence of flavonoids, phenolics, saponins, tannins and terpenoids. *F. indica* extract at a 1:5concentration showed the highest antimicrobial activity against *C. albicans* with an average zone of inhibition of 20.33 ± 0.58mm. *G. jovis-tonantis* barks extract showed the highest activity against *S. mutans* with an average zone of inhibition of 23.67 ± 1.53mm. Combined extracts at 75% concentration produced average inhibition zones of 21.67 ± 0.58mm against *S. mutans* and 20.33 ± 0.58mm against *C. albicans*. The combined extracts also recorded the lowest MIC values of 1.56 ± 0.00mg/ml against *C.* albicans and 1.30 ± 0.45mg/ml against *S. mutans* indicating synergistic antimicrobial activity. Statistical analysis using two-way ANOVA showed significant differences among the antimicrobial effects of the extracts ($p < 0.05$). The findings of this study suggest that *F. indica* and *G. jovis-tonantis* extracts have potential use in herbal toothpaste formulation as alternatives to

synthetic oral care products. The study also supports the use of locally available medicinal plants in the development of affordable oral healthcare products and provides scientific evidence for their traditional medicinal use.

Contents

APPROVAL FORM i
DECLARATION ii
DEDICATION iii
ACKNOWLEDGEMENT iv
LIST OF ABBREVIATIONS v
LIST OF FIGURES vi
Figure 1: Formulated toothpaste 29 vi
Figure 2: Before and after brushing with formulated toothpaste 29 vi
TABLE OF CONTENTS vii
Table 1: Qualitative phytochemical constituents of plant extracts 24 vii
Table 2: Mean zone of inhibition (mm) against S. mutans 25 vii
Table 3: Mean zone of inhibition (mm) against S. mutans 25 vii
Table 4: Activity of extracts against oral pathogens at 75% (Mean ± SD, mm) 26 vii
Table 5: MIC values (mg/mL) 27 vii
Table 6: Physicochemical properties 27 vii
Table 7: Activity of herbal toothpaste 28 vii
Table 8: Stability over 4 weeks 28 vii

Table 9: ANOVA: Two-Factor with Replication plants extract against S.mutans 30 vii
Table 10: ANOVA: Two-Factor with Replication plant extract against C.albicans 30 vii
List of Appendices viii
Abstract ix
CHAPTER 1 1
1.1 BACKGROUND TO THE STUDY 1
1.2 PROBLEM STATEMENT 2
1.3 JUSTIFICATION 2
1.4 AIM OF THE STUDY 2
1.5 OBJECTIVES OF THE STUDY 3
1.6 RESEARCH QUESTIONS 3
1.7 HYPOTHESES 3
1.8 SIGNIFICANCE OF THE STUDY 3
1.9 LIMITATION 4
1.9.2 DELIMITATION 4
1.10 DEFINITION OF TERMS 4
CHAPTER 2 5
2.1 Introduction 5
2.2 *Flacourtia indica* Classification 5
2.2.2 Botanical Description 6
2.2.3 Ethnomedicinal uses of *Flacourtia indica* 6
2.3 *Gardenia jovis-tonantis* Classification 7
2.3.2 Botanical Description 7
2.3.3 Ethnomedicinal uses of *Gardenia jovis-tonantis* 8
2.4 Phytochemical and Antimicrobial Profile of *Flacourtia indica* 8
2.4.1 Phytochemical constituents 8
2.4.2 Antimicrobial Activity 9
2.4.3 Antioxidant and Anti-inflammatory Properties 9
2.5 Phytochemical and Antimicrobial Properties of *Gardenia jovis-tonantis* 9
2.5.1 Phytochemical constituents 10
2.5.2 Antimicrobial Activity 10

2.5.3 Antioxidant and Anti-inflammatory Properties 10
2.6 Formulation of Herbal Oral Care Products 11
2.7 History of toothpaste 11
2.8 Chemical based toothpaste 13
2.9 Side effects of chemical based toothpaste 14
2.10 Herbal Toothpaste 15
2.11 Gaps, Challenges and Opportunities 15
2.11.1 Gaps 15
2.11.2 Challenges 15
2.11.3 Opportunities 16
CHAPTER 3 17
MATERIALS AND METHODS 17
3.1 Sample collection and preparation 17
3.2 Extraction of sample 17
3.3 Phytochemical determination 17
3.3.1 Flavonoid / Shinoda's test 17
3.3.2 Alkaloids / Wagner's reagent test 18
3.3.3 Phenolic / Ferric chloride test 18
3.3.4 Saponins / Foam test 18
3.3.5 Terpenoids / Salkowski's test 18
3.4 Antimicrobial Evaluation 19
3.4.1 Agar well diffusion 19
3.4.2 Minimum inhibition concentration 19
3.5 Formulation of toothpaste 20
3.6 Evaluation of formulated product 21
3.6.1 Physical examination (colour, odour, taste, and smoothness) 21
3.6.2 pH 21
3.6.3 Formability 21
3.6.4 Spreadability 21
3.7 Microbiological Evaluation 22
3.8 Statistical analysis 22

CHAPTER 4 23
RESULTS 23
4.1 Phytochemical results 23
4.2 Antimicrobial activity of plant extracts Against *Streptococcus mutans* 24
4.3 Antimicrobial activity of plant extracts against *Candida albicans* 25
4.4 Comparative Antimicrobial Activity at 75% Concentration 25
4.5 Minimum inhibition concentration (MIC) 26
4.6 Formulation of Herbal Toothpaste 27
4.6.1 Composition and Development 27
4.7 Antimicrobial Activity of Formulated Toothpaste 28
4.9 Statistical Analysis 30
CHAPTER 5: 31
DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS 31
5.1 Discussion 31
5.1.1 Phytochemical screening interpretation 31
5.1.2 Antimicrobial Susceptibility of *F.indica* and *G.jovis-tonantis* 32
5.1.3 Minimum inhibition concentration 32
5.1.4 Properties of formulated herbal toothpaste 33
5.1.5 Statistically Analysis 33
5.2 Conclusions 33
5.3 Recommendations 34
REFERENCES 35
APPENDICES 39

CHAPTER 1

1.1 BACKGROUND TO THE STUDY

Oral hygiene refers to the maintenance of a clean and healthy oral cavity through regular brushing, flossing and frequent dental check-ups. Good oral hygiene is essential in preventing oral diseases and maintaining overall health. According to Oluwasina *et al*., (2023) oral disease and conditions are major global health challenges affecting nearly 47.3% of the world's population as reported by the World Health Organization. According to the World Health Organisation oral diseases affect a significant proportion of the global population with conditions such as dental caries, periodontal diseases, endodontic infections, mouth odour, oral cancer, tooth loss, oral manifestation of HIV infection, noma, oral mucosal lesion, and congenital abnormalities such as cleft lip and palate being common worldwide.

Danavale *et al*., (2024), reported that the primary causes of dental caries are cariogenic bacteria such as *Streptococcus mutans* and *Streptococcus sobrinus* together with fermentable carbohydrates. The carbohydrates are metabolised to produce acids that demineralise tooth structures resulting in enamel erosion, gum damage and increased susceptibility to opportunistic pathogens. Conventional toothpastes containing synthetic agents such as triclosan, parabens, diethanolamine, fluoride, peroxide and chlorhexidine are common used to control oral pathogens and improve oral health. However prolonged use of these chemical-based products has been associated with adverse effects including tooth discolouration, taste alteration, mucosal irritation, antimicrobial resistance, nausea, and disruption of the normal oral.

The increasing concern over side effects of synthetic oral care products has stimulated interest in the development of herbal alternatives. Historically natural plant materials were widely used for oral cleaning before the introduction of commercial toothpaste formulation. Medicine plants are known to contain bioactive components which possess antimicrobial, anti-inflammatory and antioxidant properties may be useful in oral health management. This study therefore focuses on the phytochemical characterization of *Flacourtia indica* and *Gardenia jovis-tonantis* with the aim

of developing a safe, effective and affordable herbal toothpaste formulation for oral healthcare applications.

1.2 PROBLEM STATEMENT

Oral diseases including dental caries, gingivitis, periodontitis, tooth discolouration, and tooth loss continue to pose major public health challenges globally. These conditions are often associated with poor oral hygiene practices and the presence of pathogenic oral microorganisms. Despite the increasing interest in herbal oral products, many consumers still rely on conventional toothpaste formulations containing synthetic chemicals whose prolonged use may result in undesirable side effects. In addition several medicinal plant species with potential oral health benefits remain underutilized and insufficiently studied. There is therefore, a need to investigate locally available medicinal plants as potential sources of safer and cost-effective bioactive compounds for herbal oral care product development.

1.3 JUSTIFICATION

This study is justified on scientific, health, socioeconomic, and innovative grounds. The study will contribute to knowledge on the phytochemical and antimicrobial properties of *F. indica* and *G. jovis-tonantis*. The research may support the development of safer plant-based oral care products with fewer side effects compared to synthetic products. Utilization of locally available medicinal plants may reduce dependence on imported chemical products and promote sustainable use of indigenous resources. The study may contribute to the formulation of novel herbal toothpaste with potential commercial applications.

1.4 AIM OF THE STUDY

To investigate the phytochemical and antimicrobial properties of *F. indica* and *G. jovis-tonantis* and formulate a herbal toothpaste for oral healthcare applications.

1.5 OBJECTIVES OF THE STUDY

- To extract and characterize bioactive compounds present in *F. indica* and *G. jovis-tonantis*.
- To evaluate the antimicrobial activity of the plant extracts against selected oral pathogen using agar well diffusion (AWD) and minimal inhibition concentration (MIC).
- To formulate a prototype herbal toothpaste and evaluate the physiochemical properties of the formulated herbal toothpaste.

1.6 RESEARCH QUESTIONS

- Do the extracts of *F. indica* and *G. jovis-tonantis* possess bioactive compounds and antimicrobial properties that can benefit oral hygiene?
- Can herbal oral care product be formulated using these plant extracts and what are their physiochemical and microbiological properties?

1.7 HYPOTHESES

H_o hypothesis

- *F. indica* and *G. jovis-tonantis* have no antimicrobial effects against oral microorganisms.

H_1 hypothesis

- *F. indica* and *G. jovis-tonantis* have antimicrobial effects against oral microorganisms.

1.8 SIGNIFICANCE OF THE STUDY

The study provides scientific validation for traditional use of *F. indica* and *G. jovis-tonantis* in oral health and the development of affordable safe and natural oral care alternatives. In addition it encourages the sustainable use of indigenous medicinal plants and the potential for commercialization thus employment creation and community health impact.

1.9 LIMITATION

The limitations of the study are variability in phytochemical composition due to environment factors such as soil composition, climate and harvesting season and resources and financial constraints, limited funding and access to laboratory reagents, equipment, and skilled personnel may restrict the scale and comprehensiveness of the study. In addition limited analytical resources for example HPLC and GC MS limited the depth of phytochemical characterization and identification of specific active compounds. The study was confined to laboratory testing due to lack of clinical validation hence the safety, efficacy and acceptability of the herbal oral care product in human population cannot be fully confirmed.

1.9.2 DELIMITATION

The delimitations of the study included provenance, as plant samples will be collected from a specific geographical area within Zimbabwe and variations in phytochemical composition due to different climates and regions outside the study area are not considered. In addition laboratory based evaluations and clinical trials involving human participants are beyond the scope of this study. The study was conducted within a limited time frame which does not allow for long-term stability studies or extended shelf-life evaluation of the formulated product.

1.10 DEFINITION OF TERMS

- Antimicrobial Susceptibility Test: is a procedure performed to measure the effectiveness of compounds against pathogens. (Bayot and Bragg, 2022)
- Cariogenic bacteria: these are bacteria which play a crucial role in the formulation of dental cavities by metabolizing sugars and producing acids the demineralize tooth enamel. (Lamont and Egland, 2015).
- Oral hygiene: is the practice of maintaining oral hygiene by brushing and flossing to prevent tooth decay and periodontal diseases. (Iba and Adamu, 2021).

CHAPTER 2

2.1 Introduction

Oral diseases including dental caries, gingivitis, and periodontitis remain major global public concerns. According to Kowalska *et al*., (2024) oral diseases are primarily driven by bacterial biofilm formation and oxidative stress in oral cavity. According to Oluwasina *et al*., (2023) oral disease and conditions are major global health challenges affecting nearly 47.3% of the world's population as reported by the World Health Organization. According to Kanouté *et al*., (2022), convention oral care products containing synthetic antimicrobial agents such as chlorhexidine, triclosan, parabens, and fluoride have demonstrated efficacy; however prolonged use has been associated with adverse effects including tooth staining, mucosal irritation and microbial resistance. This has driven interest in plant-derived substances with antibacterial, astringent properties, anti-inflammatory and antioxidant for formulation of oral care products (Danavale *et al*., 2024). Plant-based oral care products offer biocompatibility, affordability, accessibility and lower toxicity profiles as compared to synthetic products. Medicinal plants contain diverse phytochemicals including alkaloids, tannins, flavonoids, saponins, phenolic and terpenoids that exhibit antimicrobial, antioxidant, anti-inflammatory and wound-healing activities. According to Ahmad *at el*., (2020) and Ngbolua *et al*., (2015) *Gardenia jovis-tonantis* and *Flacourtia indica* are ethnomedicinal plants with reported phytochemical and antimicrobial profiles that make them candidates for herbal oral care formulations.

2.2 *Flacourtia indica* Classification

According to Lim, T. K. (2012), the taxonomical classification of *Flacourtia indica* is as follows:

Kingdom: Plantae

Phylum: Streptophyta

Class: Equisetopsida

Subclass: Magnoliidae

Order: Malpighiales

Family: Salicaceae

Genus: Flacourtia

Species: *Flacourtia indica*

Common names: Batoka plum, Governor's plum, Indian plum, Mududwe, Munhunguru, Mutombototo, Mutudza, Mutunguru.

2.2.2 Botanical Description

Flacourtia indica, referred to as governor's plum or Indian plum, is a spiny shrub or small tree that typically attains heights of 3–6 meters, although it can reach up to 15 meters in tree form. It is esteemed for its edible fruits, medicinal attributes, and ecological applications (Koperuncholan and Kulandaivel, 2022). The plant features a spiny trunk and branches, with ovate, glossy leaves measuring 3–12 cm in length, often exhibiting serrated edges. It produces diminutive white flowers in clusters, which may be unisexual or occasionally bisexual, followed by globose drupes that mature from green to vibrant red, orange, or reddish-black, measuring up to 2.5 cm in diameter. The seeds are predominantly dispersed by avian species.

2.2.3 Ethnomedicinal uses of *Flacourtia indica*

Culinary: The fruits are edible, tart, and consumed fresh or processed into jams and preserves. Medicinal: In Ayurveda and African traditional medicine, various parts of the plant are used for digestive support, wound healing, fever reduction, and treatment of infections. Active compounds include tannins, gallic acid, ellagic acid, flavonoids, and potassium salts. Historical records indicate its use in South Asia for diarrhoea, gonorrhoea, and toothache relief. The plant provides fuel, animal fodder, and invertebrate food, and has environmental and social applications.

2.3 *Gardenia jovis-tonantis* Classification

According to Albuquerque and Salgueiro (2024), the taxonomical classification of *Gardenia ternifolia subsp. jovis-tonantis* is as follows.

Kingdom: Plantae

Clade: Angiosperms, Eudicots, Asterids

Order: Gentianales

Family: Rubiaceae

Genus: Gardenia

Species: *Gardenia ternifolia*

Subspecies: *Gardenia jovis-tonantis*

Common names: Mutara, Mutarara, Mutarura, Powder-bark gardenia, Umvalasangwana, Wild gardenia.

2.3.2 Botanical Description

The evergreen shrub *Gardenia ternifolia subsp. jovis-tonantis* can reach heights of 4–7 meters. It has short, stiff side branchlets with whorls of three leaves that are roughly hairy on both surfaces and oblanceolate to obovate in shape (Hyacinthe *et al*., 2023). The white, solitary blooms at the tips of the branchlets have a pleasant perfume and turn yellow when they fade. The fruit is oval, 5 x 3.5 cm, velvety, yellowish brown, and has calyx remnants on top. This plant is indigenous to Tropical Africa and does well in tropical biomes that are seasonally dry. It may grow in full or partial light and needs a reasonable amount of moisture. It is frequently seen on termite mounds. The ripe fruit can be consumed raw and is edible.

2.3.3 Ethnomedicinal uses of *Gardenia jovis-tonantis*

It's used in treatment of infection diseases which include bacterial infections, sexually transmitted infections and skin diseases. It's used in treating oral sores, gum infections toothache, and used to manage swelling, pain and inflammatory conditions. Used as tradition medicine to treat dysentery, fever, and malaria. Its gastrointestinal uses include stomach disorders, constipation and abdominal pain and its haematological and systemic uses include the treatment of anaemia and blood cleansing remedies. Powder-bark gardenia is used in various ceremonies and rituals. The leaves and flowers are used to make a relaxing and sedative tea. The flowers are used in perfumes and potpourri and its fruit is edible.

2.4 Phytochemical and Antimicrobial Profile of *Flacourtia indica*

Flacourtia indica, also referred to as Governor's plum has been extensively studied for its phytochemical composition and bioactivity.

2.4.1 Phytochemical constituents

Gas-chromatography and mass spectra (GC-MS) analysis of acetone, chloroform, ethanolic, hexane and methanolic extracts of *F. indica* barks, leaves, fruits and roots revealed a diverse array of bioactive compounds. According to Eramma and Gayathri, (2013) the major constituents include flavonoids, saponins, tannins, terpenoids, phenols, alkaloids, steroids, reducing sugars and cardiac glycosides. In another similar study by Koperuncholan and Kulandaivel, (2022) phytochemical tests showed presence of steroids, alkaloids, saponins, terpenoids, phenols, flavonoids, and tannins which concur to the study carried out by TR and Siddiqha, (2017). According to Mal *et al*., (2025) the total phenolic content in methanolic extracts 34.72 ± 2.03µg/mg, and the total flavonoids content 9.56 ± 1.2103µg/mg. In another study by Al Bashera *et al*., (2025) the total phenolic content 2.3882 ± 1.47mg GAE/mg and the total flavonoids content 126.246 ± 4.252mg QE/gm.

2.4.2 Antimicrobial Activity

Methanolic, chloroform and ethanolic extracts of *F. indica* exhibit significant antimicrobial activity against human pathogens. In vitro studies have demonstrated that *Flacourtia indica* has antimicrobial activity against oral microbes. In a study by Koperuncholan and Kulandaivel, (2022), *Flacourtia indica* ethanolic leaves were found to possess antimicrobial activity against *C.albicans* with an average zone of inhibition of 16 mm at 10mg/ml which concurred with that of TR and Siddiqha, (2017). In another study carried out by Eramma and Gayathri, (2013) *Flacourtia indica* ethanolic roots also showed antimicrobial activity against common human pathogen bacteria and compared it to Tetracycline. In another similar study by Mal *et al*., (2025) methanolic fruits showed minimal antifungal activity against *C. albicans*. Another study carried out by Chingwaru *et al*., (2020) showed that ethanolic barks from *F. indica* possess antimicrobial properties. According to Eramma and Gayathri, the antimicrobial effects are primarily attributed to phenolic compounds and flavonoids that disrupt microbial membranes, inhibit enzymatic activity and inhibit nucleic acid synthesis.

2.4.3 Antioxidant and Anti-inflammatory Properties

In a study by Mal *et al*., (2025) the antioxidant activity showed an IC_{50} value was 124.65 ± 1.38µg/mg in ABTS assay and 189.14 ± 1.08µg/mg in DPPH assay. In a similar study by Eramma and Gayathri, (2021) the antioxidant activity showed an IC_{50} value 172.41 ± 0 in DPPH assay. Studies indicate that the strong antioxidant activities are due to its high phenolic and flavonoids. In a study by Makuttan *et al*., (2025) in vitro anti-inflammatory assays using protein denaturation and HRBC membrane stabilization showed up to 70% and 64.23% inhibition by ethanolic fruit extracts respectively. Studies show that tannins and flavonoids in *F. indica* possess anti-inflammatory properties capable of reducing gingival swelling and irritation.

2.5 Phytochemical and Antimicrobial Properties of *Gardenia jovis-tonantis*

Gardenia ternifolia subsp. jovis-tonantis commonly known as Gardenia powder-bark has limited studies for phytochemical and bioactivity.

2.5.1 Phytochemical constituents

In a study by Ngbolua *et al*., (2015) *G. jovis-tonantis* methanolic extracts showed presence of polyphenols, tannins, alkaloids, saponins, steroids, flavonoids, and terpenoids. In similar study by Hyacinthe *et al*., (2023), ethanolic extracts showed presence of polyphenols, flavonoids, terpenoids, phenolic and sterols. According to Ngbolua *et al*., (2015) anthocyanin and organic acid extracts from *G. jovis-tonantis* demonstrated strong antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*.

2.5.2 Antimicrobial Activity

In Vitro studies have demonstrated the *Gardenia jovis-tonantis* has antimicrobial activity against oral microbe and general human pathogen microbes. In a study carried out Ngbolua *et al*., (2015), *G. jovis-tonantis* leaves were found to have anti-sickling and antibacterial activity. In similar study by Mbela *et al*., (1992), *G. jovis-tonantis* barks were found to have antimicrobial activity against oral pathogen microbes for example *C. albicans*, *S. mutans*, *Actinomyces* and *S.sobrinus* the study also concurred with the study done by Trindade *et al*., (2023). In a similar study by Hyacinthe *et al*., (2023), the average zone of inhibition 10mm against *Escherichia coli* and *Staphylococcus aureus*.

2.5.3 Antioxidant and Anti-inflammatory Properties

In a study by Hyacinthe *et al*., (2023), the inhibition percentage for stem bark was 98.689 ± 0.109% for ethanolic extracts and the inhibition and 97.542 ± 0.262% for hydroethanolic extract. The IC_{50} values for aqueous and organic extracts ranged from 0.07833mg/ml to 0.45013mg/ml. In another study by Ngbolua *et al*., (2015) presence of phenolic and flavonoids may promote antioxidant properties that protect oral tissues from oxidative stress and neutralize free radicals. Plant-derived tannins and flavonoids reduce inflammatory mediators and may assist in the management of gingivitis and oral mucosal inflammation.

2.6 Formulation of Herbal Oral Care Products

According to Kanouté *et al*., (2022) plant-based products offer multi-target activity and better sensory profile compared to synthetic products which concur to the study by Cury and Tenuta, (2014). According to Senthilkumar *et al*., (2022) and Oluwasina *et al*., (2023) formulation should adhere to the following parameters pH stability, antimicrobial efficacy, viscosity, stability during storage, organoleptic properties and safety and biocompatibility. In a study by Senthilkumar *et al*., (2022) Vera gel, Neem powder, clove oil, and pomegranate peel powder were used to formulate herbal based toothpaste which resulted in an excellent performing product with high antimicrobial activity and good physicochemical properties which concur with the findings by Danavale *et al*., (2024). According to Danavale *et al*., (2024) fluorinated toothpaste lead to fluorosis which weakens the bone hence herbal toothpaste is the best choice for adults and children.

In a study by Oluwasina *et al*., (2023) the research provided information on the synthesis of human health-friendly dental antimicrobial from plant material *Allium cepa* L skin chaff, *Azadirachta indica* A seed, and *Tetrapleura tetraptera* pod extracts. The study showed the lowest MIC value of 1.25mg/ml against *Pseudomonas aeruginosa, Escherichia coli, Candida albicans* and *Candida parapsilosis*. According Sultana, (2025) herbal toothpastes play an essential role in maintaining oral hygiene and avoid dental caries than chemically based synthetic toothpastes. The combination of *F. indica* and *G. jovis-tonantis* offers complementary phytochemistry. *F. indica* provides high flavonoid and phenolic content with demonstrated antimicrobial, antioxidant and anti-inflammatory activity. *G. jovis-tonantis* contains flavonoids, terpenoids and phenylpropanoids with anti-biofilm and flavouring properties. The synergism aligns with findings that plant metabolites favour sensory acceptability and provide antimicrobial, antioxidant, flavouring activity in toothpastes.

2.7 History of toothpaste

Toothpaste is a gel or paste that is applied using a toothbrush to maintain oral hygiene and improve dental appearance. According to Lippert (2013) toothpaste formulations have changed considerably overtime, from early materials such as crushed egg shells and ashes to modern

products containing more than 20 different ingredients. According to Sultana, (2025), the contemporary practice of oral hygiene utilizing toothpaste and toothbrushes commenced in the 1950s, which is relatively recent in historical terms. However the pursuit of oral cleanliness dates back at least 5,000 years. Approximately 3,000 BC, the ancient Egyptians formulated dental paste made of myrrh, eggshells, water and pumice. It is reported that the early Chinese employed ground fishbone for dental care, while the ancient Greeks and Romans allegedly utilized crushed bones and oyster shells. During the Middle Ages, Arabs reportedly experimented with fines, and Europeans tested table salt. Such methods appear to have been quite abrasive to tooth enamel. It was not until the late 1700s that the first modern toothpaste was introduced, usually homemade. Some incorporated burnt bread into their formulations or a naturally occurring dark red plant resin and dragon's blood.

In 1824, a dentist by the name of Peabody became the first to introduce soap to dental paste, followed by John Harris in the 1850s, who included chalk as an additional component. Approximately 20 years later, Colgate initiated mass production of the first toothpaste in a jar (Lippert, 2013). A significant transformation occurred in 1892 when Dr.Washington Sheffield, an American dentist, deemed it unsanitary for multiple individuals to dip their toothbrushes into a single jar of toothpaste. Consequently, he invented the first toothpaste packaged in a collapsible tube that same year. In 1914, one of the most pivotal advancements in toothpaste history emerged with the introduction of fluoride, as documented in British Patent GB 3,034. Crest toothpaste, launched by Procter and Gamble, is believed to be the first fluoride toothpaste marketed on a large scale globally. The American Dental Association sanctioned the inclusion of fluoride salts in toothpaste in 1960, facilitating the worldwide distribution of fluoride toothpaste. The evolution of toothpastes is far from complete. The primary challenge that remains is generally inadequate intraoral substantivity of active agents, particularly fluoride.

According to Cury and Tenuta, (2014) ideal toothpaste designed for dental plaque control in oral and dental health must possess several key characteristics. The ingredients should be non-toxic, non-allergenic, and non-irritating to ensure safety during use. It must demonstrate efficacy in controlling dental plaque and reducing the formation of plaque-related periodontal diseases as (Kowalska *et al*., 2024). The antibacterial agents included in the formulation should specifically target pathogenic oral flora while minimizing disruption to beneficial microorganisms.

Additionally, the taste and odour of the toothpaste should be acceptable to the user, ensuring a pleasant experience during application.

2.8 Chemical based toothpaste

Toothpastes are composed of two primary categories of ingredients: active and inactive. Active ingredients include anticaries agents, desensitizing agents, and antimicrobial agents, while inactive ingredients consist of abrasives, detergents, humectants, thickening agents, and flavouring agents (Davies *et al*., 2010). Among the active ingredients, fluoride-based remineralization agents and anticaries agents play a crucial role. In addition to fluoride, other active ingredients with remineralization properties, such as sodium fluoride, amine fluoride, stannous fluoride, triclosan, arginine, and Novamin, are used in toothpaste formulations for caries prevention. In addition to their anticaries benefits, fluoride compounds have been shown to exert dose-dependent cytotoxic effects on oral cells.

Other compounds may be added to fulfill additional purposes, including whitening, erosion prevention, anti-tartar action, anti-malodor, anti-plaque, and anti-gingivitis effects. According to Lippert, (2013), triclosan and chlorhexidine are commonly included for their antiplaque and antimicrobial properties. Triclosan exhibits anti-inflammatory effects by suppressing cyclo-oxygenase and lipoxygenase pathways, and it is compatible with other toothpaste ingredients, such as fluoride and surfactants (Kanouté *et al*., 2022).Chlorhexidine, a powerful antiseptic, is effective in treating periodontal diseases and controlling plaque build-up on teeth. Inactive ingredients in toothpaste include abrasives, detergents, humectants, and thickeners. Abrasive insoluble particles such as silica, aluminium hydroxide, and calcium carbonate are commonly incorporated into toothpaste formulations. These abrasives are uniformly dispersed throughout the toothpaste's soluble matrix and primarily serve to aid in the mechanical removal of plaque and other dental deposits.

Detergents, including sodium N-lauryl sarcosinate and SLS, are added to generate a foaming action, which facilitates the dispersion and solubilisation of plaque and other dental accretions during the brushing process. Moore *et* al., (2008) reported that the foaming effect not only enhances the cleaning efficacy but also contributes to the even distribution of active ingredients

across the tooth surface. Humectants, such as glycerol, propylene glycol, and sorbitol, are incorporated into toothpaste formulations to prevent moisture loss and maintain the consistency and stability of the product. These compounds function by retaining water, thereby mitigating the evaporation of moisture during storage and use. Additionally, thickening agents or binders, including mineral or seaweed colloids, natural gums, and synthetic cellulose derivatives, are utilized to provide the necessary viscosity and stabilize the toothpaste formulation.

2.9 Side effects of chemical based toothpaste

Toothpastes contain a variety of chemical compounds, some of which may induce allergic reactions or irritation in susceptible individuals (Zirwas and Otto, 2010).Conditions such as perioral contact dermatitis, stomatitis, or cheilitis have been reported as adverse effects associated with toothpaste use (de Groot, 2017). These reactions are often attributed to certain ingredients, particularly essential oils like peppermint, cinnamon, and spearmint, which are commonly used for flavouring but may trigger hypersensitivity in some individuals. Toothpaste not only interacts with the hard dental tissues during mechanical plaque removal but also comes into contact with the gingival and other intraoral mucosal tissues.

Literature reviews have highlighted that the primary compounds incorporated into toothpaste formulations can exert cytotoxic effects on cellular structures (Neppelberg *et al*., 2007). Notably, detergents, particularly SLS and cocamidopropyl betaine, have been identified as the most significant contributors to cytotoxicity, with several studies documenting their harmful impact on cells (Birant *et al*., 2021). Despite their widespread use, there is a notable lack of studies investigating the cytotoxic, apoptotic, and genotoxic effects of toothpaste formulations with varying compositions on human gingival fibroblast (hGF) cells, as well as their comparative antibacterial efficacy.

To sum up SLS, irritates oral mucosa and worsens mouth ulcers, over time it may contribute to gum recession and sensitivity, Parabens and preservatives have been linked to potential hormonal imbalances and toxicity and Triclosan is linked to hormone disruption and antibiotic resistance (Cvikl *et* al., 2015). Harsh abrasives wear down enamel with excessive brushing making teeth more sensitive and prone to decay, fluoride overdose, can lead to fluorosis, a condition causing

white spots, enamel damage and even bone weakening in severe cases and artificial colour and flavours cause allergic reactions or irritation in sensitive individuals.

2.10 Herbal Toothpaste

The natural products obtained from medicinal plants have demonstrated to be rich source of biologically active compounds, many of which have served as the foundation for the development of new lead chemicals for pharmaceuticals. Kanouté *et al*., (2022) outlined that herbal toothpaste is a natural alternative to convention toothpaste formulated with plant-based ingredients to promote oral health, fresh breath and gum protection. Desirable characteristics of herbal toothpaste encompass effective abrasive action for comprehensive cleaning, non-toxic formulation for gentle oral care, and prevention of tooth staining to maintain a bright and natural appearance and the provision of a fresh and clean mouth feel after each application. Its enduring effects for on-going oral hygiene benefits are cost-effective and easily accessible, rendering it a practical option for diverse range of users.

2.11 Gaps, Challenges and Opportunities

2.11.1 Gaps

There's limited information on the phytochemical and pharmacological data for *G. jovis-tonantis* as well as limited human clinical data, bioavailability and toxicity data for both species especially in the formulation of oral products. No studies have been done for the interaction between the two extracts and *F. indica* has been fairly studied but it has limited data on formulated product, human trials and standardised extract.

2.11.2 Challenges

Different methods yield varying phytochemical composition making reproducibility difficult, and some active phytochemicals may degrade during processing, storage or formulation. Combining

two plant extracts may lead to unpredictable interactions complicating formulation and herbal product formulation and herbal product formulation requires safety, efficacy, and quality control before approval.

2.11.3 Opportunities

The combination of two under-utilised medical plants allows novelty as it may lead to potential identification of new compounds with antioxidant, antimicrobial or anti-inflammatory properties. Potential discovery of unique phytochemicals or synergistic interactions and value addition by transforming tradition knowledge into standardized scientifically validated products. Formulating into an oral product for example toothpaste, syrup, mouthwash or tablets targeting a specific indication like oral antimicrobial or metabolic support and promoting cultivation and conservation of these plants could support local economies and biodiversity.

CHAPTER 3

MATERIALS AND METHODS

3.1 Sample collection and preparation

The leaves and barks of *Flacourtia indica* and *Gardenia jovis-tonantis* were obtained from Zimuto rural district of Masvingo province in Zimbabwe and authenticated at the National Herbarium and Botanic garden in Harare. The leaves and barks were washed three times using distilled water and dried in a shadow for a week. The dried barks and leaves were ground into a fine powder utilizing mortar and pestle.

3.2 Extraction of sample

Extraction was carried out using the maceration method. Twenty five (25) g of each powdered plant extract material were soaked in 250ml of 70% ethanol. The extraction mixtures were placed in sealed containers to prevent solvent evaporation and the mixtures left to stand 4 days with occasional shaking to improve extraction efficiency. After extraction the mixtures were filtered using filter paper in order to obtain the filtrates. The solvent was then evaporated using gentle heating in order to obtain concentrated extracts. The macerates were transferred into air tight containers and labelled as follows: *G. jovis-tonantis* leaves (A), *G. jovis-tonantis* barks (B), *F. indica* 1:5 (C), and *F. indica* 1:10 (D).

3.3 Phytochemical determination

3.3.1 Flavonoid / Shinoda's test

Three (3) ml of the plant extract was placed in a test tube, followed by the addition of 10 drops of dilute HCI. Subsequently, magnesium ribbon was introduced, which dissolved upon the addition

of a few drops of concentrated HCI. The experiments were done in triplicates for each plant extract, Hyacinthe *et al*., (2023).

3.3.2 Alkaloids / Wagner's reagent test

One (1) ml of the plant extract was placed in a test tube and dissolved in 2ml of 1% HCI. Following this, 3 drops of Wagner's reagent were added to the solution. The experiments were done in triplicates for each plant extract.

3.3.3 Phenolic / Ferric chloride test

One (1) ml of the plant extract was dissolved in 1ml of sterile distilled water within a test tube, to which 3 drops of 1% FeCI3 were added. The experiments were done in triplicates for each plant extract.

3.3.4 Saponins / Foam test

One (1) ml of the plant extract was dissolved in 2ml of distilled water in a test tube. The mixture was vigorously shaken for 30 seconds in a sterile test tube. The experiments were done in triplicates for each plant extract.

3.3.5 Terpenoids / Salkowski's test

Five (5) ml of the plant extract was placed in a test tube and dissolved in 2ml of chloroform along with 2 drops of concentrated H_2SO_4. The mixture was thoroughly shaken and allowed to stand. The experiments were done in triplicates for each plant extract.

3.4 Antimicrobial Evaluation

3.4.1 Agar well diffusion

Mueller-Hinton agar was prepared according to the manufacturers instruction, it was then autoclaved at 121^0C for 15 minutes, then cooled to approximately 45-50^0C before pouring into sterile petri dishes. The inoculum was standardized by transferring 4 well isolated colonies of *Streptococcus mutans* from a fresh agar plate into a tube containing sterile saline. The turbidity was adjusted to match the 0.5 McFarland standards. The standardized was used within 20 minutes of preparation in order to maintain viability and consistent inoculum density. A sterile cotton swab was immersed in the standardized inoculum suspension then pressed and rotated against the inside of the tube, just above the fluid level, to eliminate excess inoculum. The standardized inoculum was then applied to the entire surface of the Mueller-Hinton agar plate three times while rotating the plate at 60^0 each time to ensure even distribution in the inoculum. The inoculated plates were left to stand for 5 minutes at room temperature for the surface to dry before creating wells. Using a sterile cork borer, uniform diameter well of 5mm were created in the inoculated agar and using a sterile pipette a fixed volume of plant extract was added into each well. The test was done with the aid of a positive and negative control. The plates were left to stand for 30 minutes to enable pre-diffusion of the plant extract. The plates were sealed with paraffin, labelled, inverted and incubated at 37^0C for 24 hours. Using a ruler, the diameter of the inhibition zones were measured from the bottom of the plate under reflected light. The same procedure was carried out with *Candida albicans* using Potato Dextrose Agar. All tests were done in triplicates to ensure uniform results were obtained.

3.4.2 Minimum inhibition concentration

Preparations of bacterial inoculum were achieved by selecting 4 well-isolated colonies of *Streptococcus mutans* from a fresh culture on a non-selective agar plate. The colonies were then suspended in sterile saline and adjusted to 0.5 McFarland Standard and within 15 minutes of standardization the suspension was dilute in CAMHB to achieve the final target inoculum concentration. The dilution of approximately 5×10^5 CFU/ml was the goal for each test well. Using

a multichannel pipette 100µL of sterile CAMHB was added to all wells of the 96 well microtiter plate and 100µL of the plant extract was added to the wells in column 1 and a 2 fold serial dilution was performed by using the multichannel pipette set to 100µL to mix the contents of column 1 by pipetting up and down 6 times. 100µL from column 1 was transferred to column 2 and the process sequentially repeat from column 2 to column 10. Column 11 was used as a positive test while column 12 was used as a negative test. 100µL of diluted bacterial inoculum was added in the wells in column 1 through column 11. The plate was covered with the lid and incubated at 37^0C for 20 hours in ambient air. After incubation the plate were examined for bacterial growth. The wells were visually inspected from the lowest concentration to the highest concentration. The same procedure was carried out with *Candida albicans* using Potato Dextrose Agar. All tests were done in triplicates to ensure uniform results were obtained.

3.5 Formulation of toothpaste

Fifteen (15) ml of distilled water, 20g of sorbitol solution, and 20g of glycerin were weighed and transferred into a beaker and the mixture was stirred continuously for 5 minutes and 0.5g of sodium benzoate were added and stirred until completely dissolved. 1g of carboxymethyl cellulose was added gradually to avoid lump formation and the mixture was stirred for 15 minutes until a gel formed. 30g of calcium carbonate was gradually added and mixed until a smooth paste formed and 1.5g Sodium lauryl sulfate was added and mixed gently to avoid excessive foam formation. 3ml of each plant extract were added one by one with continuous stirring followed by the addition 0.2g of saccharin sodium and mixing thoroughly until it's all evenly distributed. The pH was checked and adjusted to the target of 6.5 – 8.0 range. The mixture was stirred for 10 minutes to ensure smooth consistency uniform texture and no lumps. The paste was left to stand for 30 minutes in order to remove trapped air bubbles and the paste was then transferred into an airtight container and sealed immediately to prevent contamination, Senthilkumar *et al*., (2022).

3.6 Evaluation of formulated product

3.6.1 Physical examination (colour, odour, taste, and smoothness)

The toothpaste's colour was evaluated visually, while the odour was checked by smelling the product and the flavour was tested manually. The paste's smoothness was confirmed by rubbing it between fingers. All tests were done in triplicates to ensure uniform results were obtained.

3.6.2 pH

Ten (10) g of the toothpaste was measured and placed into a 50ml beaker, followed by the addition of 10 ml of distilled water, which was mixed thoroughly. A pH meter was employed to determine the pH of the suspension. All tests were done in triplicates to ensure uniform results were obtained, (Senthilkumar *et al*., 2022).

3.6.3 Formability

Two (2) g of the toothpaste was weighed and mixed with 5ml of water in a test tube. The mixture was mixed by shaking and the total volume of foam was calculated. All tests were done in triplicates to ensure uniform results were obtained, (Senthilkumar *et al*., 2022).

3.6.4 Spreadability

Two (2) g of the toothpaste were measured and positioned between two glass slides, which were then moved in opposite directions. After 5 minutes the amount of toothpaste spread was measured using a ruler Senthilkumar *et al*., (2022). All tests were done in triplicates to ensure uniform results were obtained.

3.7 Microbiological Evaluation

The AWD method was used to conduct in-vitro anti-bacterial and anti-fungal research of the prepared toothpaste against oral pathogenic bacterial strain *S.mutans* and fungi strain *C.albicans*. Mueller-Hinton agar was prepared according to the manufacturers instruction, it was then autoclaved at 121^0C for 15 minutes, then cooled to approximately 45-50^0C before pouring into sterile petri dishes. The inoculum was standardized by transferring 4 well isolated colonies of *Streptococcus mutans* from a fresh agar plate into a tube containing sterile saline. The turbidity was adjusted to match the 0.5 McFarland standards. The standardized was used within 20 minutes of preparation in order to maintain viability and consistent inoculum density. A sterile cotton swab was dipped into the standardized inoculum suspension then pressed and rotated against the inside of the tube above the fluid level to remove excess inoculum. The standardized inoculum was swabbed on the entire surface of the Mueller-Hinton agar plate three times while rotating the plate at 60^0 each time to ensure even distribution in the inoculum. The inoculated plates were left to stand for 5 minutes at room temperature for the surface to dry before creating wells. Using a sterile cork borer, uniform diameter well of 5mm were created in the inoculated agar and using a sterile pipette a fixed volume of toothpaste was added into each well. The test was done with the aid of a positive and negative control. The plates were left to stand for 30 minutes to enable pre-diffusion of the plant extract. The plates were wrapped in paraffin, labelled, inverted and incubated at 37^0C for 24 hours. Using a ruler, the diameter of the inhibition zones were measured from the bottom of the plate under reflected light. The same procedure was carried out with *Candida albicans* using Potato Dextrose Agar. All tests were done in triplicates to ensure uniform results were obtained.

3.8 Statistical analysis

Statistical analysis was performed utilizing the Statistical package for the Social Sciences (SPSS) IBM 20 and Microsoft Excel. The average zone of inhibition diameter in (mm) obtained was subjected to a normality test using Shapiro-Wilk and Two-way ANOVA was conducted to determine the effect of the extracts and concentration on the measured response variable (ZOI). One-way ANOVA was implanted to determine whether significant differences existed among the groups for MIC. Turkey's post-hoc test was applied to determine specific group differences.

CHAPTER 4

RESULTS

4.1 Phytochemical results

Qualitative phytochemical screening showed that both *Flacourtia indica* and *Gardenia jovis-tonantis* extracts contained alkaloids, flavonoids, phenolics, saponins, and terpenoids (Table 1).

However *F. indica* showed a higher presence of phenolic compounds (+++) compared to *G. jovis-tonantis* (+).

Table 1: Qualitative phytochemical constituents of plant extracts

Phytochemical	*F.indica*	*G. jovis-tonantis*
Alkaloids	++	++
Flavonoids	++	++
Phenolic	+++	+
Saponins	++	++
Terpenoids	++	++

4.2 Antimicrobial activity of plant extracts Against *Streptococcus mutans*

The antimicrobial activity of the plant extracts against *Streptococcus mutans* increased with increasing extract concentration. At 100% concentration *G. jovis-tonantis* bark extract showed the highest activity a with a mean inhibition zone of 23.67 ± 1.53 mm, followed closely by *F. indica* (1:5) extract with 22.33 ± 2.52 mm. Lower inhibition zone were observed at lower concentrations for all extracts.

Table 2: Mean zone of inhibition (mm) against *S. mutans*

Test agent	mean ± STD in mm			
	100% conc	75% conc	50% conc	25% conc
Flacourtia indica bark 1:5	22.33 ± 2.52	15.33 ± 0.58	10.33 ± 0.58	9.00 ± 1.00
Flacourtia indica bark 1:10	18.67 ± 1.15	12.67 ± 2.52	10.00 ± 0.00	6.00 ± 1.00
Gardenia jovis-tonantis leaves 1:10	19.33 ± 1.15	13.33 ± 1.53	10.00 ± 0.00	8.67 ± 1.15

Gardenia jovis-tonantis bark 1:10	23.67 ± 1.53	18.33 ± 1.53	10.33 ± 0.58	6.67 ± 0.58
Amoxicillin (+ve)	24.67 ± 1.15	21.33 ± 1.53	17.33 ± 0.58	16.67 ± 1.53
Water (-ve)	0.00	0.00	0.00	0.00

4.3 Antimicrobial activity of plant extracts against *Candida albicans*

The antimicrobial activity of the plant extracts against *Candida albicans* increased with increasing extract concentration (Table 3). At 100% concentration *F. indica* (1:5) extract showed the highest activity with a mean inhibition zone of 20.33 ± 0.58 mm, followed closely by *F. indica* (1:10) extract with 17.67 ± 0.58 mm. Lower inhibition zones were observed at lower concentrations for all extracts.

Table 3: Mean zone of inhibition (mm) against *C. albicans*

Test agent	mean ± STD in mm			
	100% conc	75% conc	50% conc	25% conc
Flacourtia indica bark 1:5	20.33 ± 0.58	15.33 ± 2.52	13.00 ± 2.00	10.67 ± 0.58
Flacourtia indica bark 1:10	17.67 ± 0.58	14.67 ± 0.58	13.00 ± 1.00	10.00 ± 0.00
Gardenia jovis-tonantis leaves 1:10	16.33 ± 1.15	12.33 ± 1.15	8.00 ± 1.00	6.67 ± 0.58
Gardenia jovis-tonantis bark 1:10	14.67 ± 0.58	12.33 ± 0.58	9.33 ± 1.15	7.00 ± 0.00
Fluconazole (+ve)	22.67 ± 0.58	20.33 ± 1.15	18.67 ± 0.52	16.33 ± 0.58
Water (-ve)	0.00	0.00	0.00	0.00

4.4 Comparative Antimicrobial Activity at 75% Concentration

The antimicrobial activity of the plant extracts at 75% concentration against Streptococcus mutans and Candida albicans varied by plant extract. The combined plant extracts showed the highest activity with a mean inhibition of 20.33 ± 0.58 mm against *C. albicans* and 21.67 ± 0.58 mm against *S. mutans*. The positive controls recorded a mean inhibition of 23.67 ± 0.52 mm for both microbes.

Table 4: Activity of extracts against oral pathogens at 75% (Mean ± SD, mm)

Microorganism	*F. indica* (1:5)	*F. indica* (1:10)	Gardenia jovis-tonantis Bark	Gardenia jovis-tonantis Leaves	Combined	Control
C. albicans	15.33 ± 2.52	14.67 ± 0.58	12.33 ± 0.58	12.33 ± 1.15	20.33 ± 0.58	20.33 ± 1.15
S. mutans	15.33 ± 2.52	12.67 ± 2.52	18.33 ± 1.53	13.33 ± 1.53	21.67 ± 0.58	21.33 ± 1.53

4.5 Minimum inhibition concentration (MIC)

The combined extracts showed the lowest MIC value against *Streptococcus mutans* with a mean MIC value of 1.56 ± 0.00 mg/ml, followed by *G. jovis-tonantis* bark extract with 2.08 ± 0.90 mg/ml. The combined extracts showed the lowest MIC value against *Candida albicans* with a mean MIC value of 1.30 ± 0.45 mg/ml, similar to *F. indica* (1:5) extract with 1.30 ± 0.45 mg/ml.

Table 5: MIC values (mg/mL)

Microorganism	Extract	Mean MIC ± SD
S. mutans	Bark	2.08 ± 0.90
	Leaves	2.61 ± 0.90
	F. indica (1:10)	3.13 ± 0.00
	F. indica (1:5)	2.61 ± 0.91
	Combined	1.56 ± 0.00
	Control	1.04 ± 0.45

Microorganism	Extract	Mean MIC ± SD
C. albicans	Leaves	2.08 ± 0.91
	Bark	2.61 ± 0.91
	F. indica (1:10)	2.08 ± 0.91
	F. indica (1:5)	1.30 ± 0.45
	Combined	1.30 ± 0.45
	Control	1.04 ± 0.45

4.6 Formulation of Herbal Toothpaste

4.6.1 Composition and Development

The herbal toothpaste was formulated using optimized concentration of plant extracts along with standard excipients such as abrasives, humectants and binders. The formulated toothpaste showed good physicochemical properties.

Table 6: Physicochemical properties

Parameter	Observation
Colour	Dark pink
Odour	Mild Herbal
Taste	Slightly bitter
pH	6.9 - 7.6
Texture	Smooth and uniform

Foaming ability	Good

4.7 Antimicrobial Activity of Formulated Toothpaste

The formulated herbal toothpaste showed a mean inhibition zone of 23.33 ± 0.58 mm against *Streptococcus mutans* and 20.67 ± 1.15 mm against *Candida albicans*.

Table 7: Activity of herbal toothpaste

Microorganism	Mean ZOI ± SD (mm)
S. mutans	23.33 ± 0.58
C. albicans	20.67 ± 1.15

4.8 Stability Studies

The formulated toothpaste showed no significant changes during the study period.

Table 8: Stability over 4 weeks

Parameter	Week 1	Week 2	Week 3	Week 4
Colour	Stable	Stable	Stable	Stable
Odour	Stable	Stable	Stable	Stable
pH	7.2	7.1	6.9	6.9
Texture	Stable	Stable	Stable	Stable



Figure 1: Formulated toothpaste



Figure 2: Before and after brushing with formulated toothpaste

4.9 Statistical Analysis

Table 9: ANOVA: Two-Factor with Replication plants extract against S.mutans

Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F critical***
Sample	70.0625	3	23.35417	14.94667	2.95E-06	2.90112
Columns	1274.896	3	424.9653	271.9778	7.68E-23	2.90112
Interaction	83.02083	9	9.224537	5.903704	7.69E-05	2.188766
Within	50	32	1.5625			
Total	1477.979	47				

Table 10: ANOVA: Two-Factor with Replication plant extract against C.albicans

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F critical*
Sample	144	3	48	38.4	1.02E-10	2.90112
Columns	519.1667	3	173.0556	138.4444	2.1E-18	2.90112
Interaction	12.83333	9	1.425926	1.140741	0.364377	2.188766
Within	40	32	1.25			
Total	716	47				

CHAPTER 5:

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS.

5.1 Discussion

This project was conducted to evaluate the phytochemical composition and oral care potential of *Flacourtia indica* and *Gardenia jovis-tonantis* and to determine their suitability in the formulation of herbal toothpaste.

5.1.1 Phytochemical screening interpretation

The phytochemical screening results showed that both *F. indica* and *G. jovis-tonantis* contained important secondary metabolites such as alkaloids, flavonoids, phenolics, saponins, and terpernoids. Similar findings have been reported in studies involving medicinal plants extracts against common human pathogens and oral pathogens (Koperuncholan & Kulandaivel, 2022; Erammam & Gayathri, 2013; Ngbolua *et al*., 2015). These compounds are widely known for their anti-inflammatory, antioxidant, and antimicrobial properties. Phenolic compounds and flavonoids, in particular, have been associated with antioxidant activities because they neutralize free radicals that contribute to tissue damage and antibacterial and antifungal activities because they can damage microbial cell walls and interfere with microbial metabolism (Ahmad *et al*., 2020; Chatterjee *et al*., 2015). Alkaloids compounds have been associated with antimicrobial activity because they interfere with DNA replication, and terpenoids have been associated with antimicrobial activities because they disrupt microbial cell members (Chatterjee *et al*., 2015). Saponins compounds have been associated with surfactant properties. Further qualitative and quantitative tests should be carried out in order to have a better understanding of all phytochemical properties present in the plant extract.

5.1.2 Antimicrobial Susceptibility of *F.indica* and *G.jovis-tonantis*

The plant extracts demonstrated antimicrobial activity against both *Streptococcus mutans* and *Candida albicans*. The increase in inhibition zones with increasing concentration suggests that the antimicrobial effect was concentration dependent. Similar findings have been reported in studies involving medicinal plants against oral pathogens and common human pathogens (Eramma and Gayathri, 2013; Chatterjee *et al*., 2016). Among the tested extracts *G.jovis-tonantis* barks showed the highest antimicrobial activity against *S.mutans* with an average zone of inhibition of 23.67 ± 1.53mm and *F.indica* showed the highest antimicrobial activity against *C.albicans* with an average zone of inhibition of 20.33 ± 0.58mm. The strong activity of these extracts may be due to higher concentrations of active phytochemicals such as phenolic, alkaloids and flavonoids. Previous studies have shown that bark extracts often contain higher levels of antimicrobial compounds compared to leaves (Koperuncholan, and Kulandaivel, 2022). The combined extracts showed greater antimicrobial activity than individual extracts, suggesting a possible synergistic effect between the phytochemicals present in the two plants. The combined extracts showed average zones of inhibition of 21.67 ± 0.58mm and 20.33 ± 0.58mm respectively against *S.mutans* and *C.albicans*. Synergistic interactions among plant compounds may improve antimicrobial effectiveness by targeting different microbial pathways simultaneously (Sultana, 2025; Abdalla 2014).

5.1.3 Minimum inhibition concentration

The MIC results further confirmed the effectiveness of the combined extracts, as lower MIC values indicate stronger antimicrobial activity. The combined extract showed the lowest MIC values of 1.56 ± 0.00mg/ml and 1.30 ± 0.45mg/ml against *C.albicans* and *S.mutans* respectively. The combined extract showed the lowest MIC values against both microorganisms, supporting its potential use in herbal oral care products. Among the tested *F.indica* (1:5) recorded the lowest MIC value for both plant extracts with an average of 1.30 ± 0.45mg/ml against *C. albicans* this concur with the studies done (Chingwaru *et al*., 2020). *G.jovis-tonantis* leaves recorded the lowest MIC value against *S.mutans* with an average of 2.10 ± 0.90mg/ml.

5.1.4 Properties of formulated herbal toothpaste

The formulated herbal toothpaste demonstrated positive physicochemical properties. The formulated toothpaste demonstrated strong antimicrobial activity against both *Streptococcus mutans* and *Candida albicans*. This is important because *S. mutans* is one of the main bacteria associated with dental caries, while *C.albicans* contributes to oral fungal infections (Oluwasina *et al*., 2023). The herbal toothpaste showed an average zone of inhibition of 23mm against *S.* mutans and 21mm against *C.*albicans. The toothpaste also remained physically stable throughout the four-week study period, suggesting that the formulation was suitable for short-term storage. During the study period the formulated herbal toothpaste showed no microbial contamination and no phase separation or degradation.

5.1.5 Statistically Analysis

The statistical analysis confirmed that extract concentration significantly influenced antimicrobial activity. The significantly interaction effects observed against *S.mutans* and *C. albicans* indicate that the effectiveness of the extracts depended on both extract type and concentration. Two-way ANOVA showed that both extract and concentration independently influenced the response variable with a significant combined interaction effect. One-way ANOVA showed that the differences observed between the groups were not statistically significant as the group means were considered statistically similar.

5.2 Conclusions

The study demonstrated that *Flacourtia indica* and *Gardenia jovis-tonantis* contain important phytochemical with antimicrobial properties. The plant extracts showed activity against *Streptococcus mutans* and *Candida albicans*, with antimicrobial activity increasing as extract concentration increased. The combined extracts showed strongest antimicrobial activity and the lowest MIC value indicating possible synergistic effects between the plant extracts. The formulated herbal toothpaste possessed acceptable physicochemical properties, remained stable during storage, and showed strong antimicrobial activity against oral pathogens. These findings

suggest that the plant extracts have potential for use in the development of herbal oral healthcare products.

5.3 Recommendations

1. Further studies should isolate and identify the specific bioactive compounds responsible for the antimicrobial activity.
2. Toxicity and safety studies should be conducted before clinical application of the formulated toothpaste.
3. Longer-term stability studies should be carried out to determine the shelf-life of the herbal toothpaste.
4. Addition oral microorganisms should be tested to evaluate the broad-spectrum activity of the extracts.
5. Clinical studies involving human participants should be conducted to confirm the effectiveness of the herbal toothpaste under practical conditions.
6. Future research should investigate the synergistic interactions between the plant extracts in more detail.

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APPENDICES

Appendix A ***F.*indica, Governor's plum, Munhunguru**

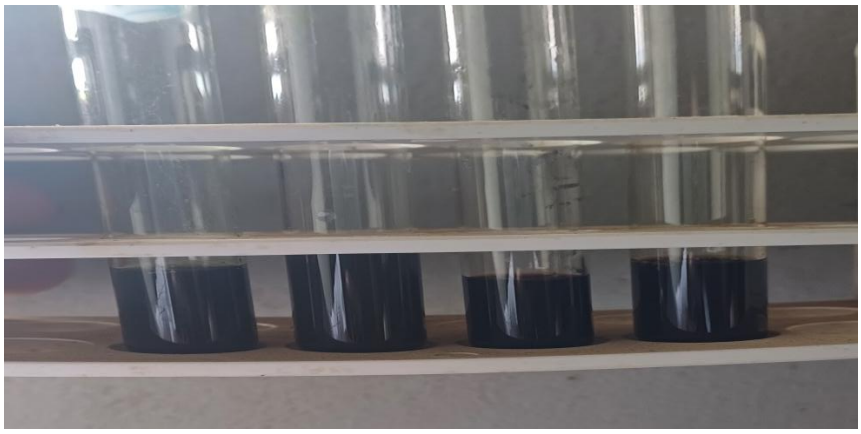


Appendix B ***G.jovis*-tonantis, Powder-bark gardenia, Mutarara**

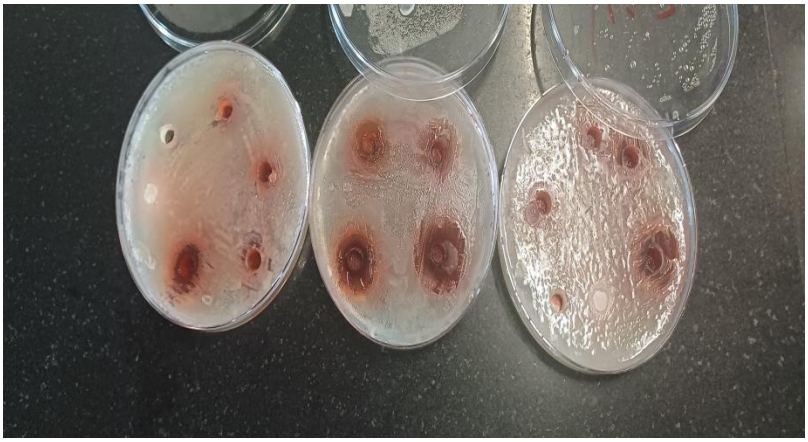


Appendix C **Phytochemical tests**





Appendix D **Zone of inhibition for *F.indica* 1:5 extract**

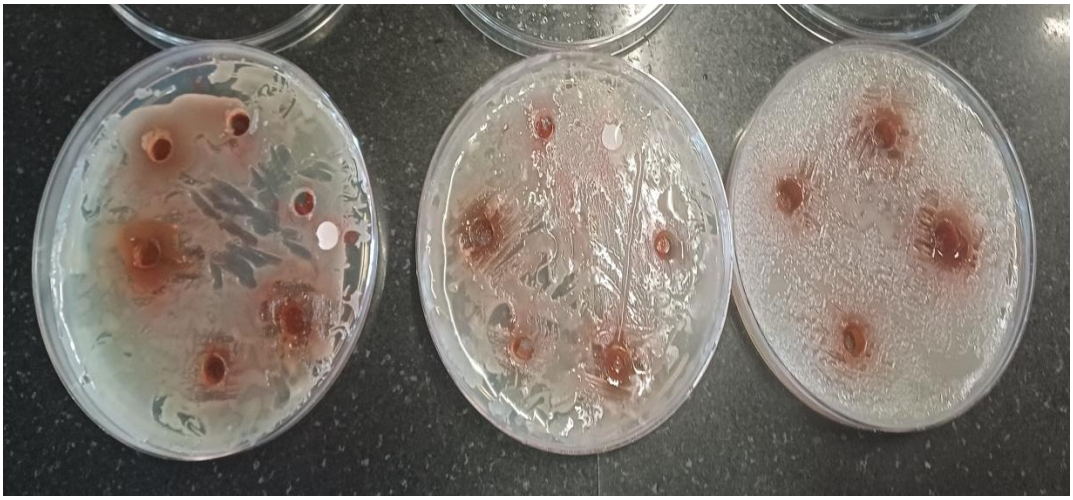




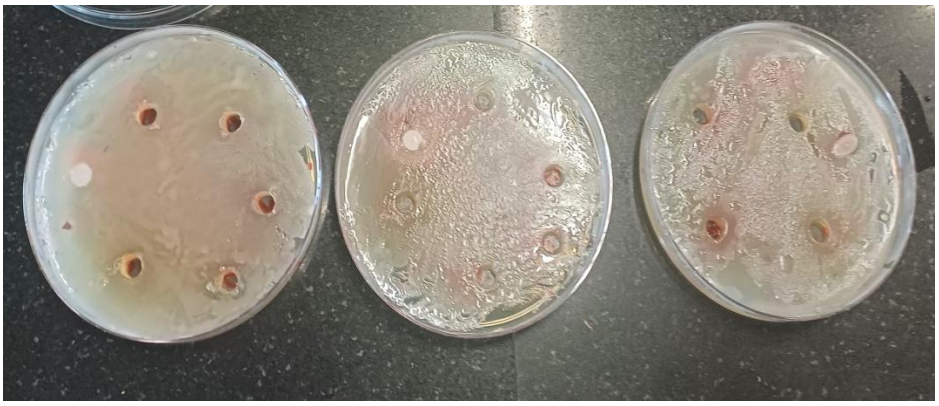
Appendix E Zone of inhibition of *G.jovis-tonantis* leaves



Appendix F Zone of inhibition of *F.indica* 1:10



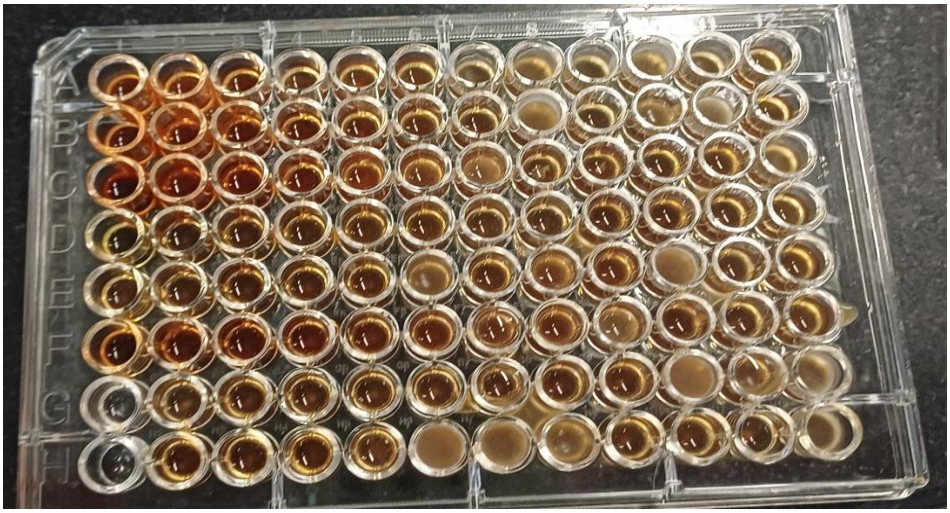
Appendix G **Zone of inhibition of *G.jovis-tonantis* Bark**



Appendix H **Minimum inhibition concentration against *C.albicans***



Appendix I **Minimum inhibition concentration against *S.mutans***



Appendix J Normality test

Tests of Normality[c]

	Extract	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	Df	Sig.	Statistic	Df	Sig.
Zone	Antibiotic	.265	6	.200*	.799	6	.058
	F.indica	.183	15	.188	.940	15	.383
	G.jovis-tonantis	.230	16	.024	.907	16	.104

Appendix K One way ANOVA for MIC

ANOVA Single Factor for *C.albicans*

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F critical*
Between Groups	4.272493	4	1.068123	2.166667	0.146552	3.47805
Within Groups	4.9298	10	0.49298			
Total	9.202293	14				

ANOVA Single Factor for *S.mutans*

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F critical*
Between Groups	3.850173	4	0.962543	1.676613	0.231065	3.47805
Within Groups	5.741	10	0.5741			
Total	9.591173	14				