Research Article

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Efficacy of Four Herbal Extracts on Streptococcus mutans: A Non-Randomised Trial

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ABSTRACT

Introduction: Dental caries results from acidogenic oral microbiota due to frequent sugar consumption. Streptococcus mutans is key contributor to dental caries. Ayurvedic medicinal plant extracts offer promising avenue for antimicrobial research against cariogenic pathogens.

Objective: To explore antimicrobial properties of extracts from indigenous medicinal plants against Streptococcus mutans, integrating traditional knowledge with scientific evidence.

Materials and Method: This non-randomised trial was conducted by department of Biotechnology, Kathmandu University from 2022 July to 2023 June following the tenets of Declaration of Helsinki. Convenience sampling method was utilised to collect four medicinal plants (Tejpatta, Vajradanti, Danti, and Ghotape) grown in different altitudes of Nepal. Their extracts were prepared using standard biochemical protocol. Phytochemical composition was analysed through Fourier Transform Infrared Spectroscopy and Liquid Chromatography - Mass Spectrometry. Antimicrobial activity against Streptococcus mutans was assessed through MIC, MBC, and zone of inhibition assays.

Result: All extracts showed antimicrobial activities against Streptococcus mutans. The dry extract of Vajradanti was most effective with highest zone of inhibition (31 mm) followed by dry extract of Danti (20 mm) and Ghotape (18 mm) at 100 mg/ml concentration. Danti was most effective, requiring lower concentrations (375 µg/ml for MIC and 750 µg/ml for MBC) to inhibit bacterial growth.

Conclusion: Phytochemical analysis of plant extracts revealed diverse compounds with potential therapeutic value. Danti (Baliospermum montanum) was found to be most potent against Streptococcus mutans, with lower MIC and MBC values. Vajradanti (Barleria prionitis) displayed significant inhibition zones, indicating potential of these indigenous plant extracts in addressing dental caries.

Keywords: Danti; ghotape; herbal extract; Streptococcus mutans; tejpatta; vajradanti.

INTRODUCTION

Dental caries (tooth decay) is major oral health problem affecting nearly 2.5 billion people globally.[1] Dental caries occurs when oral microbiota, which typically resides in oral cavity in homeostasis, shifts to acidogenic, aciduric, and cariogenic

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population due to frequent sugar consumption.[2] This shift can go unnoticed or result in visible tooth decay, indicating that caries process can occur independently of visible lesions.

Streptococcus mutans (*S. mutans*) produces acidic metabolic byproducts, which lead to demineralisation of inorganic and disintegration of organic contents of dental enamel, leading to dental caries. Several genes or operons of *Streptococcus mutans* are involved in process.[3] As global burden of dental caries continues to rise, there is growing need for innovative and sustainable approaches to address it.

In search for alternative ways to prevent caries, natural products have emerged as promising. Medicinal plants, with their diverse pharmacological properties and deep-rooted history in traditional medicine systems across various cultures, offer a promising avenue for antimicrobial research against cariogenic pathogens, particularly *Streptococcus mutans*.[4,5] While these practices are not founded on the strong scientific evidence, this study intends to bridge the gap between traditional beliefs and scientific proof. This study investigates effects of four medicinal plant extracts on *Streptococcus mutans*.

MATERIALS AND METHOD

This was an in vitro experimental study (non-randomised trial) done in Department of Biotechnology and Department of Microbiology of Kathmandu University. The study was done for a period of one year from 2022 July to 2023 June. This study was done following the tenets of Declaration of Helsinki.

Four indigenous medicinal plants traditionally used for oral cure were collected using convenience sampling technique from different locations of Nepal and coded as GP1, GP2, GP3, and GP4, corresponding to Tejpatta (*Cinnamomum tamala*), Vajradanti (*Barleria prionitis*), Danti (*Baliospermum montanum*), and Ghotape (*Centella asiatica*), respectively (Table 1).

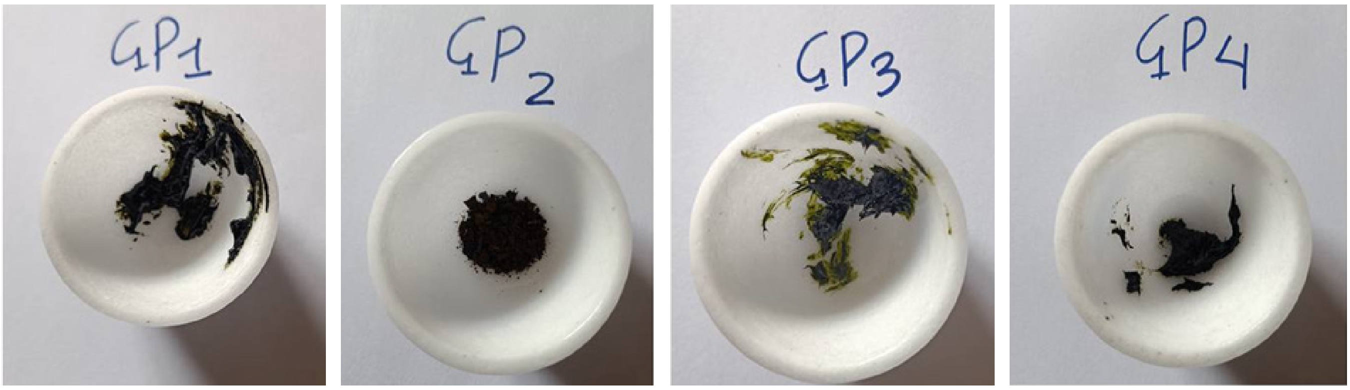
For the plant material preparation, the selected plants are collected, washed, and dried at room temperature in the shade. The dried plants were then finely powdered using a mechanical grinder (Figure 1).

Fifty grams of finely powdered plant samples were weighed into 1000 millilitre (ml) screw-capped reagent bottles of methanol. Maceration was carried out for four days at room temperature using methanol as the extraction solvent, maintaining a 1:10 ratio of plant material to the solvent.

During maceration, occasional shaking at 150 revolutions per minute was performed. The content of each bottle was filtered through muslin cloth and further filtered thrice using Sartorius grade 292 filter papers. The solvent was removed by rotatory evaporation under reduced pressure to obtain the crude extract. The concentrated crude

Table 1: Details of medicinal plants and sample collection sites.

S.N.	Sample code	Common name	Scientific name	Sample collection site	Altitude (metre)	Latitude	Longitude
1	GP1	Tejpatta	*Cinnamomum tamala*	Dhulikhel	1550	27°37’0”N	85°33’0”E
2	GP2	Vajradanti	*Barleria prionitis*	Gosaikunda	4421	28°5’3”N	85°24’31”E
3	GP3	Danti	*Baliospermum montanum*	Syangja	800	27°58'59.99" N	83° 46' 0.01" E
4	GP4	Ghotape	*Centella asiatica*	Syangja	800	27°59'59.4"N	83°46'58.0"E



GP1 GP2 GP3 GP4

Figure 1: Figure showing dry extract of different plant extract.

extracts were stored at -20°C until further use. Dry extracts were suspended in High-performance liquid chromatography (HPLC) grade methanol for most experiments. For antibacterial activity testing, the extract was dissolved in Dimethyl sulfoxide (DMSO).

The phytochemical composition of the plant extracts was determined using Fourier Transform Infrared (FTIR) Spectroscopy at Kathmandu University. Liquid Chromatography – Mass Spectrometry (LC-MS) was performed at Indian Institute of Technology (IIT) Mumbai, Maharashtra, India with reference number EXT.SAIF-Mumbai.REF.8659/2022-12-05/HRLCMS to separate, identify, and quantify known and unknown compounds in the plant extracts.

For the microbiological testing, the antimicrobial activity of the plant extracts were assessed against *Streptococcus mutans* ATCC 25175 purchased from Global Bioresource Centre, ATCC, USA. Both aerobic and anaerobic cultures were conducted at the Department of Microbiology, Dhulikhel Hospital, Kathmandu University Teaching Hospital. The aerobic culture was carried out using the blood agar method. Anaerobic culture was performed on sheep blood agar plates with vitamin K supplement. The qualitative estimation of antimicrobial potential of the plant extracts was determined using the agar well diffusion method. A concentrated stock solution of the plant extract (12,000 µg/mL) was prepared using 2% Dimethyl sulfoxide from Merck Life Science Pvt. Ltd. (India). In 96-well microplates by Tarsons (India), 150 µL of brain heart infusion (BHI) broth enriched with 1 µg/mL Vitamin K1 was dispensed. The plant extract underwent sequential two-fold dilution within the microplate, yielding final concentrations ranging from 6000 to 93.75 µg/mL. Bacterial strains (1-2×108 CFU/ml) were introduced, and incubation occurred in an anaerobic environment at 37°C for 48-72 hours within the Anaoxomat® III Jar System. Sterility control, growth control, and negative control with 2% DMSO were implemented. The microdilution procedure was conducted in triplicate, and observations were made to determine Minimum Inhibitory Concentration (MIC).

Following MIC, a 10 µL sample was taken from the 96-well microplate at the MIC concentration and two dilution steps above and below. These samples were evenly spread onto Mueller-Hinton Agar enriched with 1 µg/mL Vitamin K1. The culture plates were placed in the Anaoxomat® III Jar System at 37°C for 3-7 days. Minimum Bacterial Concentration (MBC) was determined based on the lowest concentration of the plant extracts that exhibited no discernible bacterial growth on the agar plates. These experiments were conducted in triplicate for reliability and consistency.

For the zone of inhibition, the agar plates were prepared with Mueller-Hinton and Anaerobic

Blood Agar, both enriched with 1 μg/mL Vitamin K1. Inoculated with microbial culture (1-2×108 CFU/mL), the plates were air-dried, and wells were created. Plant extract solutions with concentrations of 50 mg/mL, 70 mg/mL, and 100 mg/mL were introduced into the wells. These solutions were prepared by dissolving the plant extracts in DMSO. DMSO served as a negative control, while positive controls included Amoxicillin/ Clavulanic acid (AMC) and Tetracycline (TETR) antimicrobial susceptibility discs from Oxoid Ltd. (United Kingdom). The agar plates were incubated anaerobically at 37°C for 2-3 days. Tests were conducted in triplicates, and resulting inhibition zones were measured using the HiAntibiotic Zone Scale from Hi Media Laboratories Pvt. Ltd. (India).

Data collection was done in Microsoft Excel (version 2010, Microsoft Corporation, Redmond, Washington, USA) and descriptive analysis was performed using SPSS (version 21, IBM Corporation, Armonk, New York, USA).

RESULT

The phytochemical analysis of the four selected indigenous medicinal plant extracts revealed the presence of several chemical compounds, including alkenes, carbonyl compounds, and aromatic compounds, in all the extracts (Table 2). Additionally, Tejpatta exhibited alcohol and alkyl halides, Vajradanti contained aldehyde and carboxylic acid, Danti was found to possess nitrile, amides, and alkyl halides, while ghotape was found to possess tannins, steroids, terpenoids, cardiac glycosides. These findings indicated the diverse chemical composition of these traditional remedies, suggesting potential for therapeutic uses.

In terms of their antibacterial properties, the plant extracts were evaluated for minimal inhibitory concentration (MIC), minimal bacterial concentration (MBC), and zone of inhibition (ZOI) against *Streptococcus mutans*, a significant contributor to oral health issues (Table 3, Figure 2, Figure 3).

In the present study, all tested sample were effective in inhibiting *S. mutans* ATCC 25175 growth with MIC and MBC values ranging from 375 μg/ml to 750 μg/ml and 750 μg/ml to 3000 μg/ml respectively (Table 3). Among the four different plant extracts, GP3 exhibited the lowest MIC and MBC values of 375 μg/ml and 750 μg/ml, respectively (Figure 2). GP1, GP2 showed similar MIC and MBC value of 750μg/ml and 3000 μg/ml. The MIC and MBC values of GP4 were 750μg/ml and 1500 μg/ml, respectively.

Their antibacterial potency was quantitatively confirmed by an inhibition zone absence or presence all over the disc, loaded with the extract. All herbal products were found to be having variable antimicrobial activity against *S. mutans*. In this study, GP2 extract was reported to be the most significant against *S. mutans*. Mean zone of inhibition after 24 hours incubation of GP2 plant extract was 31 mm, 29 mm, 25 mm at a concentration of 100 mg/ml, 70 mg/ml, 50 mg/ml respectively. Similarly, GP3 plant extract showed 20 mm,14 mm, 12 mm ZOI at a concentration of 100 mg/ml, 70 mg/ml, 50 mg/ml respectively. Minimal zone of inhibition was shown by GP1 and GP4. Zone of inhibition of positive control AMC and TETR are 52 mm and 42 mm respectively. A bar diagram has been shown which clearly depicts the inhibitory effect of each herbal extract (Figure 2).

Table 2: Major phytochemical compounds found in plant extracts.

Plant	Scientific name	Compounds	Test done
GP1	*Cinnamomum tamala*	Alkaloids, flavonoids, alcohol and alkyl halides	FTIR, LC-MS
GP2	*Barleria prionitis*	Alkaloids, flavonoids,Aldehyde and carboxylic acid	FTIR, LC-MS
GP3	*Baliospermum montanum*	Alkaloids, flavonoids nitrile, amides, and alkyl halides	FTIR, LC-MS
GP4	*Centella asiatica*	Alkaloids, flavonoids, tannins, steroids, terpenoids, cardiac glycosides	FTIR, LC-MS

Table 3: Sensitivity of the plant extracts to the *Streptococcus mutans* ATCC 25175.

Extract ID	MIC	MBC	ZOI				
			100 mg/ml	70 mg/ml	50 mg/ml	AMC	TETR
GP1	750µg/ml	3000 µg/ml	16 mm	13 mm	13 mm	52 mm	42 mm
GP2	750µg/ml	3000 µg/ml	31 mm	29 mm	25 mm		
GP3	375µg/ml	750 µg/ml	20 mm	14 mm	12 mm		
GP4	750µg/ml	1500 µg/ml	18 mm	14 mm	12 mm		

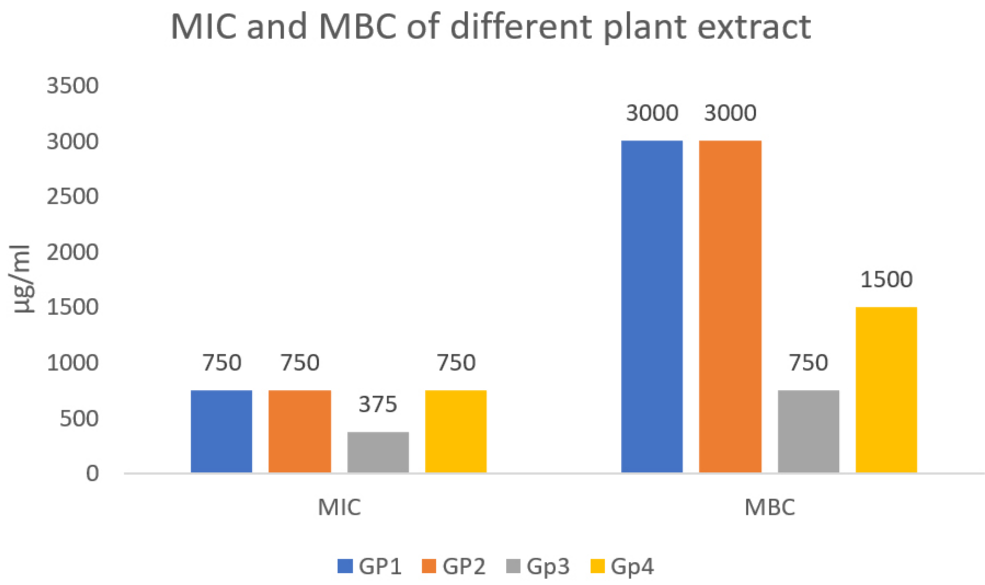


Figure 2: The minimal inhibitory concentration (MIC) and minimal bacterial concentration (MBC) of different plant extracts against *Streptococcus mutans*.

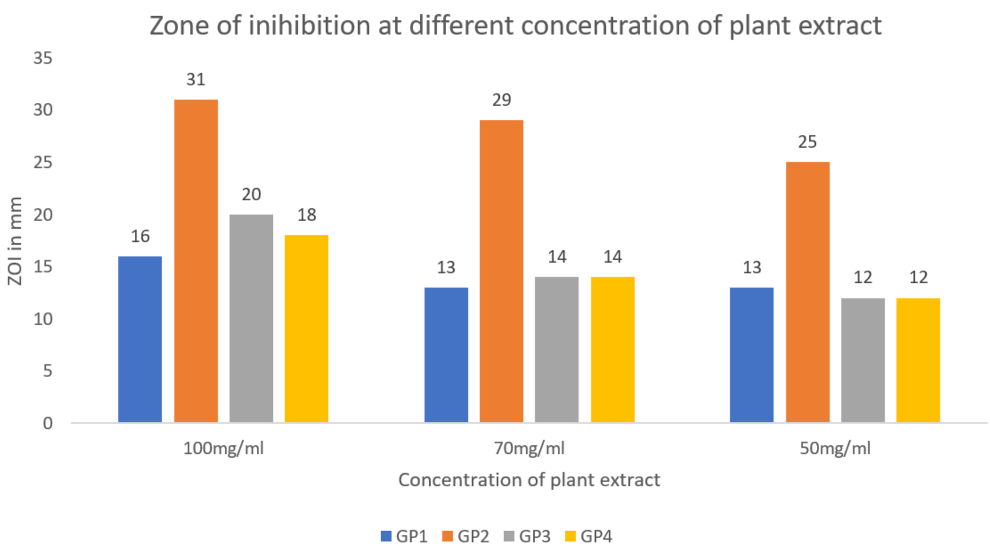


Figure 3: Zone of inhibition at different concentration of plant extract.(MBC) of different plant extracts against *Streptococcus mutans*.

DISCUSSION

Dental caries, commonly referred to as tooth decay or cavities, is a widespread oral health problem that affects individuals of all ages globally.[6] The primary aetiological agent responsible for the development of caries is the bacterium *Streptococcus mutans*.[7] This microorganism thrives in the oral cavity, specifically in dental plaque, and plays a pivotal role in the demineralisation of tooth enamel. While bacterial factors are the primary cause of caries, it is important to acknowledge the other contributory elements like genetics, dietary habits, oral hygiene practices, and host factors.[8,9]

Over the years, extensive efforts have been made to combat dental caries. Despite advancements in various fields of dentistry, including the exploration of caries vaccines,[10] the development of a universally successful and practical solution for caries prevention has remained elusive. This underlines the complexity of caries prevention and the multifactorial nature of this dental disease. Historical and anthropological studies have shown the significant impact of dietary habits on caries prevalence.[11] Communities that shifted from traditional diets to modern, high-sugar diets often experienced a surge in caries cases. Conversely, there have been instances where a return to traditional diets and the use of herbal remedies have led to a decrease in caries rates.[12] Medicinal plants, such as Tulsi, Neem, Vajradanti, Harro, Barro, Amla, Besar, Danti, Datiwan, Tejpatta, and Timur, have been traditionally employed in various cultures for their potential oral health benefits.[13,14] These plants possess antimicrobial properties, making them a natural choice for oral hygiene and caries prevention.

In traditional Indian medicine, various parts of the Vajradanti, including the stem, leaves, flowers, and roots, have been traditionally used to address diverse health issues, from catarrhal affections and glandular swellings to boils, fever, toothache, inflammation, and gastrointestinal disorders. The plant's bark serves as an expectorant for whooping cough, while the roots act as a tonic and diuretic.[15] In the context of Nepal, a country known for its diverse flora and indigenous knowledge of medicinal plants, there is a wealth of botanical resources spread across different ecological zones.[16-18] The country's geographical expanse is divided into three distinct regions: the high-altitude zone, the hilly terrain, and the terai plains. With each zone offering a distinct set of plant species adapted to its specific conditions, Nepal stands as a testament to the harmonious coexistence of nature and culture. For centuries, medicinal plants have played a crucial role in addressing oral health issues. Many of these plants, such as Tulsi, Neem, Vajradanti, Ghotape, and others, have been recommended by traditional healers and local Ayurveda experts. These plants contain secondary metabolites like tannins, terpenoids, alkaloids, and flavonoids, which have demonstrated antimicrobial properties in laboratory studies.[19]

Scientific studies have revealed that the leaves, stem, and roots of *Barleria prionitis (B. prionitis)* possess antibacterial and anti-inflammatory properties. Though number of application and reseraches has been done on *B. prionitis*, the unique feature of this plant available in Nepal is the high altitude and extremophilic weather condition. It is authors' belief that this species found in Nepal has more medicinal properties compared to the species found in other part of the world. Apart from this, other three plants used for toothache traditionally were chosen.

Cinnamomum tamala, commonly known as Indian bay leaf or Tejpatta, is a versatile and aromatic spice that holds a significant place in the culinary and medicinal traditions of the Indian subcontinent. This evergreen tree, native to the Indian subcontinent, is characterised by its glossy, elliptical leaves, which exude a warm and spicy fragrance when crushed. Beyond its culinary significance, *Cinnamomum tamala* also possesses various therapeutic properties, making it an essential ingredient in traditional

Ayurvedic and herbal medicine systems.[20] Another plant selected in this study is Danti, scientifically known as *Baliospermum montanum*, a versatile herb with a rich history in traditional healing, especially in Ayurveda. Extracting beneficial compounds from Danti involves methods such as cold pressing for oil, maceration for tinctures, and steam distillation for essential oils. It shows various therapeutic properties as it possesses alkaloids, flavonoids nitrile, amides, and alkyl halides.[21]

Ghotape, scientifically known as *Centella asiatica*, is a remarkable herb deeply rooted in the annals of Ayurveda, India's ancient system of natural medicine. This versatile plant, native to various parts of Asia, including India, has a rich history of traditional use. Its adaptogenic and healing properties have earned it the moniker "herb of longevity." Ayurvedic texts extol its potential to enhance cognitive function, facilitate wound healing, alleviate anxiety, and improve skin health. In recent years, *Centella asiatica* has garnered attention in dentistry for its antimicrobial properties, showing promise in combatting cariogenic organisms responsible for tooth decay.[22] The phytochemical analysis of the four indigenous medicinal plant extracts, uncovered a diverse array of major compounds. Alkenes, carbonyl compounds, and aromatic compounds were present in all extracts. Specifically, *Cinnamomum tamala* exhibited alcohol and alkyl halides, while *Barleria prionitis* contained aldehyde and carboxylic acid. *Baliospermum montanum* was found to possess nitrile, amides, and alkyl halides, and *Centella asiatica* showed the presence of alkaloids, flavonoids, tannins, steroids, terpenoids, and cardiac glycosides. Similar types of results were also obtained by Hassan et al., Sharma et al., Johnson et al., and Arumugam et al.[23-26]

Current study showed the potential antibacterial capabilities of the four herbal extracts against *S. mutans*, with *Baliospermum montanum* as the most effective, requiring lower concentrations (375 µg/ml for MIC and 750 µg/ml for MBC) to inhibit bacterial growth. The results of inhibition zone assays show *Barleria prionitis* is most effective against *S. mutans*. Despite these findings, the observed variability among the extracts necessitates exploration into influencing factors such as geographical origin and cultivation conditions.

A study on *Centella asiatica* by Vadlapudi et al. in 2012[27] reported no zone of inhibition against Streptococcus mutans. This differs from the findings in our study, where effectiveness against the cariogenic pathogen was observed. Notably, the results from current study align with the research conducted by Luthfi et al. in 2022, which also demonstrated similar efficacy against Streptococcus mutans.[22]

Baliospermum montanum was found to exhibit high sensitivity to Streptococcus mutans at a concentration of 125µg/ml.[28] It also possesses antibacterial and antifungal properties. In this research, it was observed that *Cinnamomum tamala* leaf displayed antibacterial efficacy against *S. mutans*. The potential antibacterial effects are likely attributed to its active components, particularly eugenol and tannins.[20]

In this study, *Barleria prionitis* exhibited significant antibacterial activity against *S. mutans*, likely attributed to its elevated levels of alkaloids, flavonoids, aldehyde, and carboxylic acid. A study done by Sawarkar et al. in 2016 [29] showed that the ethanolic extract of *B. prionitis* was highly effective against *S. mutans*, with a MIC ranging from 53.1 to 106.2 µg/ml and a ZOI measuring 25.9 mm at a concentration of 100mg/ml. In present investigation, the observed ZOI at 100 mg/ml is slightly higher than that reported in a previous study. This variance could potentially be attributed to the geographical location of the plant samples, characterised by high altitude and extremophilic weather conditions. Consistent with current findings, Patel et al. in 2015 study also revealed maximum inhibition zones against Gram-positive bacteria.[30]

This research is able to explore the chemical composition and antimicrobial potential of

Tejpatta, Vajradanti, Danti, and Ghotape which are abundantly available in Nepal. While the study provides promising results, several considerations and limitations must be acknowledged. The variability among extracts prompts further investigation into influencing factors, including geographical variations and cultivation conditions. The exclusive focus on *Streptococcus mutans* raises the need for broader studies encompassing a spectrum of antimicrobial effects against various pathogens. Additionally, the study does not delve into potential side effects or cytotoxicity, emphasising the importance of safety assessments before integrating these remedies into modern healthcare practices.

The research opens avenues for the integration of indigenous medicinal plants into oral health practices. The antimicrobial potency demonstrated against *Streptococcus mutans* suggests a potential role in addressing dental caries. However, the complex nature of oral health and the multifactorial causes of dental caries necessitate comprehensive studies to establish the safety, efficacy, and broader applications of these plant extracts.

CONCLUSION

Danti, *Baliospermum montanum* exhibited the highest effectiveness, requiring lower concentrations to hinder bacterial growth. In addition, Vajradanti, *Barleria prionitis* demonstrated higher inhibition zones across different concentrations, indicating its potency against *S. mutans*. So, from the findings of this study it can be concluded that *B. montanum* and *B. prionitis* can have the future application in dental caries control. This research can pave the way for new strategies to improve oral health and reduce dental caries worldwide

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Conflict of interest: None.

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