Original Research Article

ANTAGONISTIC POTENTIAL OF LOPHOPHORA WILLIAMSII, VINCA MINOR, AND HYDRASTIS CANADENSIS AGAINST DENTAL CARIES ASSOCIATED BACTERIA

ABSTRACT

Introduction: Dental caries is a bacterial illness that causes localized disintegration and loss of calcified components in the teeth. Different plant extracts have been used to assess the anti-bacterial activity against the pathogens known to induced dental caries. **Objective:**The aim of this research is to determine the antibacterial potential of *Lophophora williamsii*, *Vinca minor*, and *Hydrastis canadensis* against dental caries associated bacteria. **Methodology:**The antibacterial activity of aqueous and methanol extracts of *Lophophora williamsii* (LW), *Vinca minor* (VM), and *Hydrastis Canadensis* (HC) was studied. *Streptococcus mutans* (*S. mutans*), *Lactobacillus acidophilus* (*L. acidophilus*), *Pseudomonas aeruginosa* (*P. aeruginosa*), and *Staphylococcus aureus* were the bacteria employed (*S. aureus*). The disk diffusion method was used to determine the tendency of bacterial strains towards the two extracts.**Results:***S. pyogenes* and *S. aureus* were the most susceptible bacteria, whereas *P. aeruginosa* was the least susceptible. The methanol extracts of *Vinca minor* (VM) and *Hydrastis Canadensis* (HC) had the highest antibacterial activity against *S. pyogenes* and *S. aureus*, whereas the aqueous extract of *Lophophora williamsii* (LW) had the lowest antibacterial activity against (*L. acidophilus*) and (*L. acidophilus*) (*P. aeruginosa*). Methanolic extracts of almost all materials outperformed aqueous extracts in suppressing pathogenic bacteria growth but were less effective than ciprofloxacin extracts used as positive controls. All of the samples had saponins, according to phytochemical analysis. *Vinca minor* and *Hydrastis Canadensis* had alkaloids, while *Lophophora williamsii* contained flavonoids. Only *Vinca minor* and *Hydrastis Canadensis* contained tannins, respectively.**Conclusion:**Methanolic extracts of practically all materials suppressed pathogenic bacteria growth better than aqueous extracts, although not as well as ciprofloxacin extracts used as positive controls. Plant herbal blends have made major contributions to human health and well-being; as a result, plants have long been seen as a source of hope for novel therapeutic compounds.

Keywords: Lophophora Williamsii; Vinca Minor; Hydrastis Canadensis; Disk Diffusion Method.

Introduction:

Dental caries is a microbial infection that causes localized disintegration and loss of the teeth's calcified structures. *Streptococcus mutans* is the bacteria responsible for the production of tooth plaque and caries. The acid-producing *S. mutans* in the mouth damages

tooth structures when fermentable carbohydrates like sucrose, fructose, and glucose are present [1]. Plaque is a sticky substance that forms in the mouth from food waste, acid, microorganisms, and saliva. If plaque is not removed carefully and regularly, tooth decay will occur. Aside from being painful, chronic dental illness has been linked to diabetes, hypertension, heart disease, and multiple sclerosis. Heat, cold and sweet meals and drinks might aggravate the pain [2].Treatment frequently stops further infection of the tooth structure. Early decay treatment is less painful than late decay treatment. Dental caries can produce bad breath and tastes. In severe situations, the infection can spread to the soft tissues, resulting in edentulous mouth [3]. However, due to their considerable adverse effects, antibiotics like penicillin and erythromycin are rarely used clinically. Recent natural therapies using medicinal herbs as chemo-therapeutant reservoirs have helped reduce antibiotic adverse effects such hypersensitivity, supra-infections, and tooth discoloration. Medicine herbs have a proven antibacterial impact against mouth germs [4].According to a review of folkloric medical literature, *Psidium guajava* leaves are used to keep teeth clean, *Terminalia chebula* dried fruit is used as an anti-inflammation agent, *Achyranthes aspera* stem is used to treat toothaches, and *Mimusops elengi* stem is used to strengthen gums. Commercially available anti-anxiety medicines continue to be sought for, even though they exist. Synthetic chemical compounds have been shown to be ineffective in the prevention of dental caries when using natural materials [5]. Experiments with cariogenic bacteria have already revealed that the plant extracts of the two plants, *P. guajava* and *T. chebula*, exhibit antibacterial and antifungal properties. *A. aspera* and *M. elangai* anticariogenic activities have not been extensively studied; hence the study will focus on analyzing plant extracts in various solvents. This study therefore used infected patients *S. mutans* and *C. albicans* as the target organisms and used hexane extracts of *P. Guajava* leaves, driedfruit of *T. chebula*, roots of *A. aspera*, and sticks of *M. elengi* as the screening method for detecting these pathogens. Once the antibacterial properties of plant extracts have been tested in vitro against oral infections, in vivo trials for the treatment and prevention of dental caries can be conducted [6].Many people utilize herbal medicine that contains plant extracts, but there have been just a few studies into the differences in bioavailability between extracts and pure chemical compounds. It is vital to discover a reason for the pharmacological and therapeutic superiority of particular herbal extracts over isolated single elements because of their well-established, effective use as health promoting products or complementary treatments for various disorders [7]. Pharmacokinetic and physicochemical synergistic effects have been hypothesized, as well as better solubility and enhanced bioavailability. If, for example, polyphenols or saponins enhance the solubility and/or absorption of the extract's principal constituents via some form of pharmacokinetic impact, this enhances the extract's bioavailability and hence its efficacy over the isolated ingredients [8].In the Ranunculaceae buttercup family, *Hydrastis canadensis* also known as orangeroot or yellow puccoon is a perennial herb found in southeastern Canada and eastern United States. The broad, yellow-knotted rootstock makes it easy to spot. Under the soil, the purple and hairy stem joins to the yellow rhizome, which is yellow[9]. Clonal division occurs more frequently than asexual reproduction in Goldenseal; however, the rhizome-based method is the preferred method [10]. Between four and five years, a plant reaches sexual maturity, which is the moment at which it begins to produce flowers. As a colouring agent and a therapeutic cure for common maladies like wounds, digestive issues, ulcers, skin and eye ailments, and cancer it has been used by Native Americans for centuries. A nutritional

Comment [mb1]: REFERENCE

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supplement known as goldenseal has gained popularity in the United States and other countries.

Dentalcariesandperiodontaldiseasearetwooftheleadingcausesoftimeofffromworkandschool [11]. A variety of chemical and mechanical methods have been explored to manage the germs that cause caries and periodontal disease. There are, however, a number of medications on the market that can produce side effects[12]. This necessitates the creation of safe and effective alternatives from natural sources. Commercially known as "Goldenseal," the North American plant *Hydrastis canadensis* L. (Ranunculaceae) has long been used as an antiseptic to treat mouth problems [13].*Vinca minor* is a member of the dogbane family of flowering plants. Alkaloids in *Vinca minor* include *vincamine*, which is one of more than 50. Sometimes, the co-administration of other elements affects the bioavailability of pure compounds significantly, while in other situations, the so-called 'phytocomplex' may work as an enhancer of absorption for some phytochemicals [14]. In contrast to pure indole alkaloid, a standardized *Vinca minor*L. leaf dry extract has a better oral bioavailability of *vincamine*, as revealed in this research. As a point of reference, pure *vincamine* was used to characterize the alkaloid-enriched and standardized dry extract. The in vitro dissolution and in vivo bioavailability of the two products were then examined [15].

METHODOLOGY:

Microorganisms:

The antibacterial activity of aqueous and methanol extracts of *Lophophora williamsii* (LW), *Vinca minor* (VM), and *Hydrastis Canadensis* (HC) was studied. *Streptococcus mutans* (*S. mutans*), *Lactobacillus acidophilus* (*L. acidophilus*), *Pseudomonas aeruginosa* (*P. aeruginosa*), and *Staphylococcus aureus* (*S. aureus*) were used, which were isolated from caries- infected patients at the Department of Endodontics and Conservative Dentistry, in collaboration with The Institute of Molecular Biology and Biotechnology at The University ofLahore.

MediaUsed:

The transport medium used to keep clinical dental caries samples alive are thioglycolate broth (TGB) and brain heart infusion broth (BHI). Thioglycolate broth (TGB) comprised 15 g casein enzyme hydrolysates, 5 g yeast extract, 5.5 g dextrose, 2.5 g sodium chloride, 0.5 g L-cystine, and 0.5 g sodium thioglycolate with a pH of 7.1 at 25°C per litre of deionized water.Brain heart infusion broth (BHI) comprised 200 g calf brain infusion from, 250 g brain heart infusion from, 10 g protease peptone, 2 g dextrose, 5 g sodium chloride, 2.5 g disodium phosphate with a pH of 7.4 at 25°C per litre of deionized water. Nutrient agar (NA), blood agar (BA), and MacConkey agar are some of the growth mediums used to examine samples under aerobic conditions (MAC).Nutrient agar (NA) comprised 5 g Hi veg peptone, 1.5 g Hi veg extract, 1.5 g yeast extract, 5 g sodium chloride, and 15 g agar with a pH of 7.4 at 25°C per litre of deionized water. Blood agar (BA) is made by mixing 20 millilitres of sheep blood with 200 millilitres of nutritional agar media, as describedabove.Per litre of deionized water, MacConkey agar (MAC) contained: 17 g animal tissue peptic digest, 3 g Protease peptone, 10 g lactose, 1.5 g bile salts, 5 g sodium chloride, 0.03 g neutral red, and 15 g agar with a pH of 7.1 at25°C. Sabouraud's dextrose agar (SDA) comprised 40 g dextrose, 10 g peptone, and 20 g agar with a pH of 5.7 before autoclaving for the analysis of pathogenic fungi from dental caries samples.Brain heart infusion blood agar + 20% sucrose (BHIBA + 20% sucrose), thioglycolate agar (TGA), and trypticase yeast extract cystine sucrose bacitracin agar

Comment [mb5]: Mention a few

Comment [mb6]: Natural herbs are not alternatives but adjuncts to chemical means for oral hygiene

Comment [mb7]: Complete the statement

Comment [mb8]: Source of the bacterial strain ?

Comment [mb9]: Isolation storage transportation and culture method needs to be explained in brief

Comment [mb10]: Kindly mention the product details or company make

Comment [mb11]: Kindly confirm if sheep blood was used ?

Comment [mb12]: Kindly mention the department at which these medias were prepared or if the media is company based product only processed in the lab

(Schaeken *et al*. 1986), a medium for the selective isolation of *S. mutans* containing the following ingredients per litre of deionized water: 40 g trypticase soy agar (TSA).The medium was disinfected and cooled to 55°C before being used. Bacitracin 200 IE was added to the mix. BHI broth with agar was employed as a brain heart infusion agar for microaerophilic culture of *S. mutans*. TGB with 2 gm/lt agar served as thioglycolate agar (TGA) for *S. mutans* growing under microaerophilicconditions.BHIBA + 20% sucrose above given with H2 and N2 gas in the anaerobic jar was used to analyze the samples under anaerobic conditions. Antimicrobial susceptibility testing is performed using Mueller-Hinton agar (MHA), which contains the following ingredients per litre of deionized water: meat infusion 2.0, casein hydrolysates 17.5, starch 1.5, agar-agar 13.0, Mueller-Hinton broth (MHB), and brain heart infusion broth(BHI).

Collection and recovery of cariessample:

Patient's samples were taken under rigorous asepsis conditions. The patient was instructed to rinse the tooth with water before collecting dental caries samples and the tooth was then isolated with a rubber dam. The tooth was washed with 3 percent hydrogen peroxide and then decontaminated with a 2.5 percent sodium hypochlorite solution. A dental excavation instrument was used to remove food material from the chewingsurface.A physician retrieved the dental caries sample from the patient using an excavator under aseptic conditions and placed it in 2 ml TGB or BHI broth in appropriate sterile screw cap bottles. Before incubation, the clinical samples were well mixed with a magnetic stirrer. After that, the samples were streak plate injected onto the appropriate culture media under varied culture conditions (four separate media on aerobic, three separate media on microaerophilic, and one media on anaerobic culture conditions for each patientsample).

Identification of dental cariespathogen:

The pathogens' thorough colony morphology and biochemical characterization (Pallavi *etal*., 2022).

Plant materialscollection: Based on a literature review and interactions with herbal healers, three medicinal plants were chosen for antimicrobial assays based on their ethno medicinal and traditional applications against infectious illnesses. Leaves of *Lophophora williamsii* (LW), *Vinca minor* (VM), and *Hydrastis Canadensis* (HC) were gathered and identified with the help of plant taxonomist Prof. Dr. Ejaz Rasool of the University of Lahore.

Preparation of crudeextracts: Shade-dried and powdered plant parts were used for extraction; 100 g of dry powder was placed in an aspirator bottle with 300 mL hexane (1:3 W/V) and the mixture was agitated occasionally for 48 hours. The extract was then filtered. This technique was carried out three times, with all extracts being decanted and mixed at the end. The extracts were filtered before drying using Whatman filter paper no. 2 on a Buchner funnel, and the solvent was extracted for quantitative measurement by vacuum distillation in a rotary evaporator at 40°C; the extracts were then deposited in pre-weighed flasks before drying. Ethyl acetate, ethanol, and methanol were used to remove the residual plant residue in order.

Antimicrobial SusceptibilityAssay:

Disc diffusionassay: The disc-diffusion method was used to test antimicrobial activity. For each strain, 20 mL of sterile Mueller-Hinton agar (MHA) was produced in petri plates. The test cultures (108 CFU/mL bacteria in 100 uL solution) were swabbed on top of the solidified media and allowed to dry for 10 minutes. The tests were carried out at three different crude

Comment [mb13]: Reference

Comment [mb14]: Pictorial representation of each media prepared kindly attach

Comment [mb15]: Site of collection of sample?

Media used for transportation used ?

Comment [mb16]: Picture of this step ?

Comment [mb17]: 3% hydrogen peroxide directly on the tooth ? that's not the recommended dilution . there is blunder in selection of concentration without dilution . Have the patients consent been taken to introduce 3% H2O2 directly on tooth .

Kindly provide reference and ethical clearance for this procedure

Comment [mb18]: Sodium hypochlorite itself is antimicrobial agent.

When anti microbial agent is introduced how does the study expect to capture the targeted bacterias.

Comment [mb19]: Reference ?

Comment [mb20]: Precisely mention the name of the instrument used with pictorial reference.

Also this could have been easily avoided by asking to patient to brush before the procedure.

No where in the method is it mentioned for what treatment did the patient report to the department

Comment [mb21]: Kindly be specific tgb or bhi what was used . and if only one was used how did the single broth be sufficient for both aerobic as well as anaerobic bacteria

Comment [mb22]: After collection of sample how was the temperature maintained and time taken to reach the laboratory ?

Comment [mb23]: Name and make of the instrument

Comment [mb24]: Broth is not streaked directly on the culture plate .
At what dilution was the broth made before streaking ?

Comment [mb25]: Mention the material and method used precisely for each category with pictorial representation

Comment [mb26]: Picture of each colony in culture plate kindly attach

Comment [mb27]: Was a team trained to gather the leaves specifically
Kindly attach picture of each plant collected and the powder so obtained

Comment [mb28]: Is the expert one of the author with confounding interest in the study kindly specify

Comment [mb29]: Picture of the aspirator

Comment [mb30]: Filter used ?

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extract concentrations (200 mg crude extract diluted in 5% dimethyl sulfoxide (DMSO), 5 mg, and 2.5 mg per disc, respectively). The sterile 6 mm disc was impregnated with various extract concentrations. The loaded discs were placed on the surface of the medium and allowed to diffuse for 30 minutes at room temperature. The negative control was made with the same solvent as the positive control. Positive controls included penicillin and amphotericin-B (25 ug/disc). At 37°C, the plates were incubated for 24 hours. The experiment was done twice and the inhibition zone was measured in millimeters.

Minimum Inhibitory Concentration: The standard reference method (National Committee for Clinical Laboratory Standards, USA, 2002) was used to determine the minimum inhibitory concentration (MIC). Water + 2% dimethyl sulfoxide were used to dissolve the extracts (DMSO). The extract concentration ranged from 5 mg/mL to 0.075 mg/mL at first. The initial test concentration was diluted twice in a row. 5 uL of solution containing 108 CFU/mL of bacteria and fungus was injected into each well. Penicillin, an antibacterial drug, and Amphotericin-B, an antifungal agent, were used as positive controls in the tests. At 37°C, the bacteria plates were cultured for 24 hours. Following the incubation period, 5 uL of the tested broth was put on sterile MHA and BHI plates and incubated at the appropriate temperature. The MIC for bacteria was calculated as the lowest concentration of extracts that inhibited the test cultures' visible growth on the agar plate. A total of three replications were kept.

PhytochemicalAnalysis:

Phytochemical analysis of all the samples was determined as follows:

Test foralkaloids: 100 mg of powdered material was diluted in 5 mL methanol before being filtered. The filtrate was then combined with 5 mL of 1% aqueous HCI. In two test tubes, one millilitre of the mixture was taken separately. In one tube, a few drops of Dragendorff's reagent were applied, and the presence of an orange-red precipitate was considered positive. Mayer's reagent was added to the second tube, and the development of buff-colored precipitate was considered a positive test for the presence of alkaloids [16].

Test forsaponins: One gram of powdered material was cooked in distilled water for 10 minutes before being filtered. 3 mL distilled water was added to the filter and forcefully shaken for 5 minutes. The presence of saponins was confirmed by the formation of froth after shaking [16].

Test forflavonoids: Five hundred milligrams of sample were diluted in five millilitres of ethanol, warmed slightly, and filtered. A few pieces of magnesium chips and a few drops of concentrated HCl were added to the filtrate. The presence of flavonoids was confirmed by a pink, orange, or red to purple coloring [17].

Test fortannins: 500 mg of powdered material was combined with 10 ml of distilled water, filtered, and a few drops of 1 percent ferric chloride solution were added. The presence of tannins is indicated by the presence of a blue-black, green, or blue-green precipitate [17].

Antibiotic susceptibilitytesting

The antibiogram of the OTI isolates was determined using the disc diffusion method [18] and CLSI standards on Mueller Hinton agar [19]. Norfloxacin 10 mcg, nalidixic acid (DNA synthesis inhibitor) 30 mcg, ampicillin (Cell wall synthesis inhibitor) 10 mcg, tetracycline 30 mcg, chloramphenicol 10 meg, nitrofurantoin (Proteinsynthesisinhibitor)300mcg,andcephotaxime30mcgdosageswereusedtotreatOTI.

On the basis of the standard interpretation chart, the diameter of the zone of inhibition

Comment [mb32]: Why was amphotericin B an antifungal used when its not at all related to the title of the study including baceria

Comment [mb33]: Where are the plant extracts ?

Not a single plant extract is mentioned for Disc diffusion method but pictures are formulated below kindly justify

Comment [mb34]: Anaerobes would not grow within 24 hours . how did the study get growth ?

Comment [mb35]: Pictorial representation of each inhibition zone

Comment [mb36]: Why was fungal strain used when study title only speaks about bacterial involvement?

No where the material section includes fungal strain in the study

Comment [mb37]: Antifungal ? drug in the study kindly justify its use

Again how did anaerobic bacteria grow within 24 hours kindly justify the study is loosing its credibility by coming up with unmatched information

Comment [mb38]: Contradicting statement

Comment [mb39]: Pictorial justification please

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produced by each antibiotic disc was measured, recorded, and the isolates were classified as "resistant," "intermediate," or "sensitive."

Evaluation ofB-Lactamase: The iodometric approach was used to identify B-lactamase, and the acidometric method was used to confirm it [17].

Antibacterial activity of plantextracts:

The disc diffusion assay was used to determine the susceptibility of OTI isolates to the extracts [20]. Petri plates containing Mueller Hinton Agar medium were seeded with bacterial strains that had been cultured for 24 hours. By matching with 0.5 McFarland Nephlometer standards, the inoculum size was modified to achieve a final concentration of 108 cfu/ml. The sterile Whatman filter paper discs (5mm in diameter) were placed on the surface of the culture plates and incubated at 37°C for 24 hours, with the diameter of the Zone of Inhibition measured in mm. Control discs were made with acetone, ethanol, and autoclaved distilled water.

Result:

Because of their considerable biological ability, *Lophophora williamsii*, *Vinca minor*, and *Hydrastis Canadensis* have played a important role in traditional medicine. Different species of the genus these plants are active as anti-inflammatory, disinfectant, anti-diabetic, anti-malarial, anti-diuretic, antioxidant, anti-bacterial, and other chemicals. Medicinally active ingredients found in fruits, vegetables, and herbs can be used as dietary material to maintain dental caries among other things. Since plants play such an important role in traditional medicine, a study was designed to extract oils from various parts of *Lophophora williamsii*, *Vinca minor*, and *Hydrastis Canadensis* in dentistry.

Percentage (%) Yield of Essential and FixedOils:

The yield of *Lophophora williamsii*, *Vinca minor*, and *Hydrastis Canadensis* leaves was calculated and tabulated in Table 1. The leaves produced the most essential oil (0.08/100 g). Roots yielded the most fixed oil, followed by leaves, with yields of 4.2/100. The methanolic extract of shoots yielded the highest yield of the lot, ranging from 9.44 to 10.85 g/100g. The following is a list of percentage (%) yields of basic and fixed oils:

Table 1. Percentage Yield of Essential and Fixed oils of *Lophophora williamsii*, *Vinca minor*, and *Hydrastis Canadensis*.

Type of Oils	Part of Plant	Weight (g) of Oil	Percentage (%) Yield
Essential Oil	*Lophophora williamsii*	0.08 ± 0.001	0.04%
	Vinca minor	0.03 ± 0.002	0.03%
	Hydrastis Canadensis	0.04 ± 0.002	0.08%
Fixed Oil	*Lophophora williamsii*	3.2 ± 0.1	0.80%
	Vinca minor	2.6 ± 0.1	0.52%
	Hydrastis Canadensis	4.2 ± 0.2	0.60%

PHYTOCHEMICAL ANALYSIS:

The existence of flavonoids, alkaloids, glycosides, tannins, hormones, triglycerides, phenolics, reducing sugars, terpenoids, and amino acids was verified by phytochemical screening of aqueous and methanolic extracts of *Lophophora williamsii*, *Vinca minor*, and

Comment [mb41]: Anaerobic bacteria do not grow within 24 hours

Kindly provide photographs of any anaerobic growth within 24 hours

Comment [mb42]: How many petri plates showed contamination kindly specify

Comment [mb43]: Result section needs to include all data numbers percentages and ratio and significance values

Comment [mb44]: The study needs to focus on the anti bacterial properties and less on the yield

Hydrastis Canadensis leaves. Phytochemicals contained in various parts have a variety of pharmacological effects, and as a result, they are considered medicinally essential. The outcomes of phytochemical studies of *Lophophora williamsii*, *Vinca minor*, and *Hydrastis Canadensis* leaves in both aquous and methanol medium have been tabulated in (**Table2)**

Table 2. Different phytochemicals present in Leaves of Lophophora williamsii

Phytochemicals	**Aqueous extract**	**Methanolic extract**
Alkaloid	Present (++)	Present (+)
Flavonoids	Present (++)	Present (+)
Tannin	Present (+)	Present (+)
Saponin	Present (+++)	Present (+)
Highly present +++, slightly present ++, present +, absent -		

Table 3. Different phytochemicals present in Leaves of Vinca minor

Phytochemicals	**Aqueous extract**	**Methanolic extract**
Alkaloid	Present (+++)	Present (+)
Flavonoids	Present (+++)	Present (+)
Tannin	Present (+++)	Present (+)
Saponin	Present (++)	Present (+)
Highly present +++, slightly present ++, present +, absent -		

Table 4. Different phytochemicals present in Leaves of Hydrastis canadensis

Phytochemicals	**Aqueous extract**	**Methanolic extract**
Alkaloid	Present (+)	Present (+)
Flavonoids	Present (+)	Present (+)
Tannin	Present (++)	Present (+)
Saponin	Present (+)	Present (+)
Highly present +++, slightly present ++, present +, absent -		

Phytochemical analysis of *Lophophora williamsii*, *Vinca minor*, and *Hydrastis canadensis* in figure: 4 shows that highest value 74.59 % of alkaloids was observed in *Vinca minor* followed by *Lophophora williamsii (45.59) and Hydrastis canadensis*(33.26).Highest Levels of flavonoids was recorded recoded in *Vinca minor* (74.59%) followed by 56.35% and 55.58% in *Lophophora williamsii* and *Hydrastis canadensis* respectively.Highest levels of tannins were recorded in *Vinca minor* (65.26%) followed by *Hydrastis Canadensis (*64.29%) and *Lephophora williamsii* (47.259) respectively.The highest levels of saponins were noted in *Lephophora williamsii (75.29%)* followed by*Vinca minor (56.35),* and *Hydrastis Canadensis* (47.59) respectively.

Comment [mb45]: Pictorial representation to support growth in all extracts

Comment [mb46]: Kindly include numbers in the respective tables

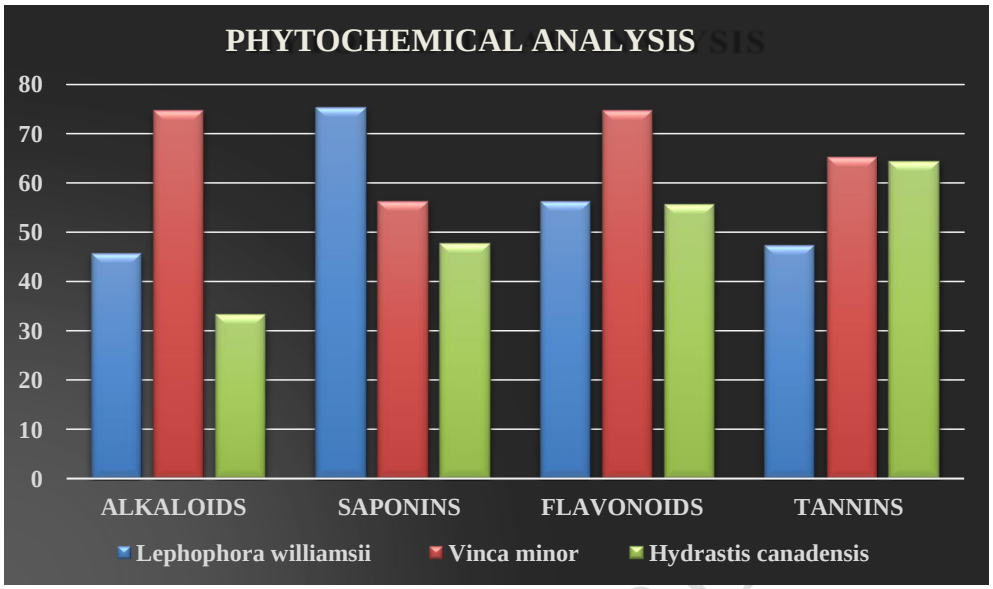

FIGURE: 1. PHYTOCHEMICAL ANALYSIS LOPHOPHORA WILLIAMSII, VINCA MINOR, AND HYDRASTIS CANADENSIS

Comment [mb47]: Figures need to be displayed

AntimicrobialAnalysis:

The results presented table 1 showed that antibacterial activity of Vinca *minor, Hydrastis canadensis, Lophophora williamsii* against resistant strains of bacteria (*Streptococcus pyrogenes, Staphylococcus aureus, Streptococcus mutans, Lactobacillus acidophilus)*, and gram negative bacterium (*Pseudomonas aeruginosa*).As Table-01 showed that standard drug (Ciprofloxacin) induced highest antibacterial activity against *Pseudomonas aeruginosa* with inhibition zone (41.6mm), followed by *streptococcus pyrogenes* (21.3mm) *Staphylococcus aureus* (36.6mm), *Lactobacillus acidophilus* (29.3mm) and *Streptococcus mutans* (21.6mm) respectively.Table-01 also showed that *Vinca minor* has significant antibacterial activity against gram positive (*Streptococcus pyrogenes, Staphylococcus aureus,Streptococcus mutans, Lactobacillus acidophilus*, and gram-negative bacterium (*Pseudomonas aeruginosa*) with zone of inhibition of (18.3mm, 22.3mm, 13mm, 14mm, and 20.3mm) respectively. Most significant antibacterial activity has been shown by the plant extract of *Hydrastis canadensis* against gram positive bacteria (*Streptococcus pyrogenes,Staphylococcus aureus,Streptococcus mutans, Lactobacillus acidophilus*) and gram-negative bacterium (*Pseudomonas aeruginosa*) with zone of inhibition of 20mm, 29.3mm, 19.3mm, 17.3mm, and 29.6mm respectively. On the other hand, very insignificant results of antibacterial activity have been shown by the plant extract of *Lophophora williamsii* against bacteria *Streptococcus pyrogenes*(2.6mm), *Staphylococcus aureus*(18mm), *Streptococcus mutans* (12mm), *Lactobacillus acidophilus* (00mm) and gram-negative bacterium *Pseudomonas aeruginosa* (11mm) respectively.

Comment [mb48]: S pyrogens in not mentioned in the micro organisms used section

Comment [mb49]: Kindly rearrange as P.aeruginosa followed by sa, la, sm, sp

Comment [mb50]: Is there contamination found during growth of psudomonas?

Comment [mb51]: Contradiction . pseudomonas shows 29.6mm and streptocossus shows 20mm

Table 5. Zone of inhibition of Lophophora williamsii

Plant	Inhibition of zone (mm)				
	S. Pyrogenes	S. Aureus	S. Mutans	L.Acidophillus	P.Aeruginosa
Lophophora williamsii	2.6mm	18mm	12mm	00mm	11mm
Standard (Ciprofloxacin)	21.3 mm	36.6 mm	21.6mm	29.3mm	41.6mm

Comment [mb52]: Figure showing no zone of inhibition

Comment [mb53]: Figure showing zone of inhibition

Comment [mb54]: Why was streptococcus pneumonia used in the study ?

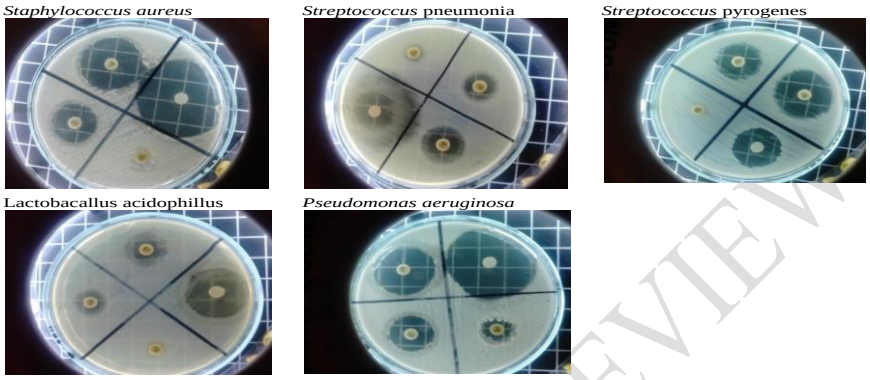

Fig 2: Inhibition zone of lophophora Willims

Table 6. Zone of inhibition of Vinca minor

Plant	Inhibition of zone (mm)				
	S. Pyrogenes	**S. Aureus**	**S. Mutans**	**L.Acidophillus**	**P. Aeruginosa**
Vinca minor	18.3mm	22.3mm	13mm	14mm	20.3mm
Standard (Ciprofloxacin)	21.3 mm	36.6 mm	21.6mm	29.3mm	41.6mm

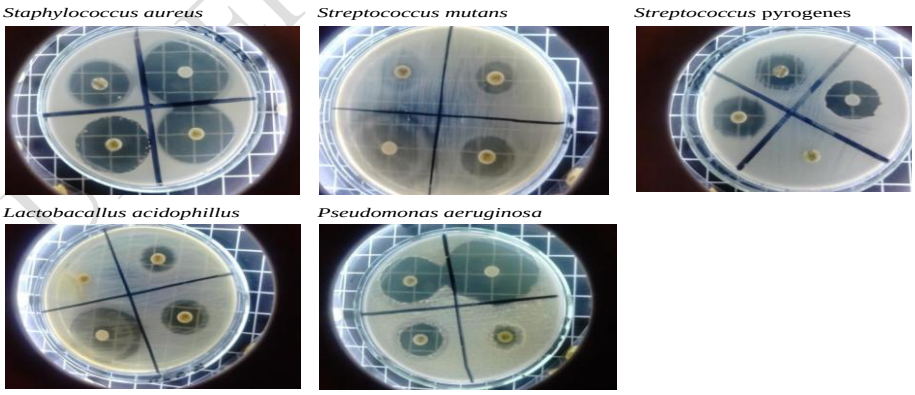

Fig 3: Inhibition of Vinca Minor

Table 7. Zone of inhibition of Hydrastis canadensis

Plant	Inhibition of zone (mm)				
	S. Pyrogenes	**S. Aureus**	**S. Mutans**	**L. Acidophillus**	**P. Aeruginosa**
Hydrastis canadensis	20mm	29.3mm	19.3mm	17.3mm	29.6mm
Standard (Ciprofloxacin)	21.3 mm	36.6 mm	21.6mm	29.3mm	41.6mm

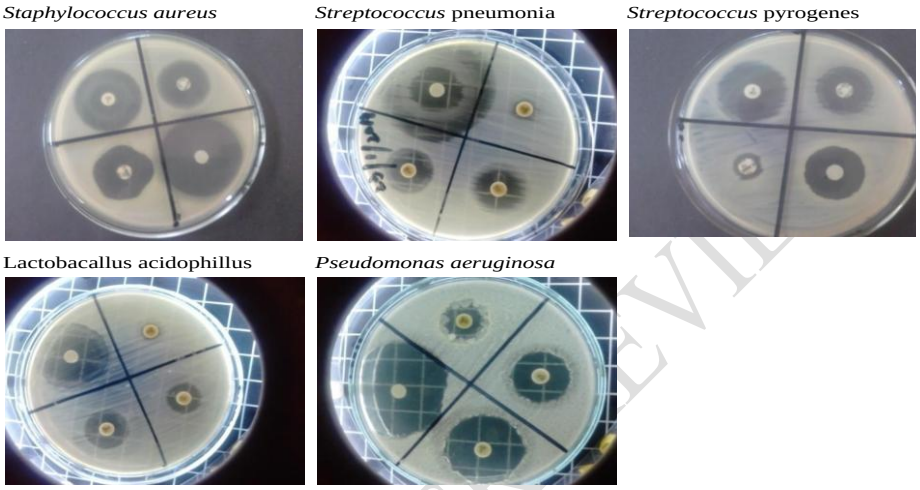

Fig 4: Inhibition zone of Hydrastis Canadensis

The antimicrobial capacity of *Lophophora williamsii*, *Vinca minor*, and *Hydrastis Canadensis* leaves was investigated using the diffusion assay method to determine MICs. The results of the MICs are shown in **Table 8**. The *Lophophora williamsii*, *Vinca minor*, and *Hydrastis Canadensis* were found to have substantial activity against bacterial strains such as *S. mutans*, *L. acidophilus*, *P. aeruginosa*, and *S. aureus* with MICs ranging from 15 to 34 milligrams of the regular drug methanol.

Table 8. Minimum inhibitory concentration (MIC's) of Lophophora williamsii, Vinca minor, and HydrastisCanadensis

Bacterial Strains	**MIC's(µg/ml)**		
	Lophophorawilliamsii	**Vinca minor**	**Hydrastis Canadensis**
S. mutans	>17.12	>26.9	>21.3
L. acidophilus	>23.45	>18.6	>15.4
P. aeruginosa	>15.6	>24.5	>21.1
S. aureus	>20.1	>19.8	>28.5
S. aureus	>19.1	>23.5	>21.5

Discussion:

Comment [mb55]: What is the diameter of petri dish used ?

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Comment [mb58]: Photo graph of the said MIC kindly attach ?

Comment [mb59]: What method was adopted to test the significance of the results

This century's most pressing public health issue is bacterial infection. Antibiotic resistance among bacteria is a major public health issue and finding new antibiotics with novel modes of action is essential to combating this problem. Plant herbal mixes have made significant contributions to human health and well-being; hence plants have traditionally given a source of hope for innovative medicinal molecules. For therapeutic treatments, the use of plant extracts with known antibacterial characteristics can be quite beneficial. Antibiotic susceptibility testing revealed that virtually all of the isolates examined were resistant to the majority of the antibiotics used in the study. It has been suggested that the antibiotic used has a direct relationship with the frequency and types of antibiotic resistant bacteria seen in humans [21].

Oral traditional medicine healers and patients utilise many herbs to treat oral bacterial diseases. The majority of medicinal herbs demonstrated an antibacterial activity in vitro, which supported their use in traditional medicine and dentistry, the authors write. Overall, gram- positive bacteria appear to be more susceptible to plant extract inhibitory effects than gram- negative bacteria, in line with earlier research. Gram-positive bacteria are susceptible due to their single-layer cell wall design, whereas gram-negative bacteria have numerous layers and a complexstructure.

Seventy-one percent of the five herbalists and 100 patients surveyed have dental issues, according to the results of this study. More than 75% of people who sought treatment for their ailments did so with the help of phytotherapy. According to our data, women (65 percent) and adults (58 percent) were more likely to used medicinal herbs than males and young people. The accessibility and low cost of medicinal herbs are the two most frequently cited grounds for their use.

Antimicrobial resistance can easily be passed between bacteria via transmissible elements/plasmids [22]. Antimicrobial resistance patterns are useful as a guide to empirical therapy and as an indicator of antimicrobial resistance determinants spread. Antibiotics with lactams are the most extensively used, and lactamases are the most common source of resistance to them. As a result, knowing extended spectrum lactamase detection is essential. [23] conducted investigations on ESBL generation in Enterobacteriaceae isolates from clinical specimens and found 9-50 percent ESBL producers.Thereisasignificantdiscrepancyinthecurrentfindings.Only42.4 percent of isolated uropathogens tested positive for lactams. In comparison to aqueous extract, the antimicrobial experiment demonstrated that acetone and ethanol extracts of the plant had broad spectrum action against the tested isolates. Pathogen susceptibility to solvent extract and aqueous extract differed. This suggests the involvement of multiple biologically significant active principles [24].

Traditional healers generally employ water as a solvent, but we found that plant extracts made in ethanol or acetone offered more consistent antibacterial action [25, 26]. These findings can be explained in polarity of the chemicals extracted, as well as their inherent bioactivity and capacity to dissolve or diffuse in the various mediums utilized in the test. In the current investigation, most of the plants had little inhibitory effect with aqueous extract, which could be attributed to the loss of some active compounds during the extraction process or a lack of solubility of active ingredients in aqueous solution[27].

Despite the fact that *S. aureus* demonstrated sensitivity to an ethanolic extract of *S.annua*in

Comment [mb60]: Can the same be said after Covid 19 pandemic encounter ?

Comment [mb61]: All the petri dishes showed zone of inhibition then how were bacterias resistant to antibiotics ?

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our investigation, no antibacterial activity against *S. aureus* and several human disease bacteria in their research. They found that alcoholic extracts (methanol and ethanol) of V. *minor* had significant antibacterial action against oral infections, but *V. minor* had little antimicrobial activity. According to our findings, some of the *vinca minor* extracts examined showed only modest suppression of gram-negativebacteriainourinvestigation.Inasimilarvein,Park*etal*[34] found that an isolated antibiotic from the root of V. *minor* had antibacterial action against gram-negative bacteria in the context of oral illnesses . Several researchers, including, investigated the cytotoxic effects of V. *minor* alkaloids on teeth. According to their findings, alkaloids were found to have an adverse effect on the growth and proliferation of these cell lines. In a similar vein, the studied extracts of V. *minor* leaves and flowers demonstrated significant oral inhibition in our investigation.

Table 01 shows the antibacterial activity of Vinca minor, Hydrastis canadensis, and Lophophora williamsii against resistant gram-positive bacteria (Streptococcus pyrogenes, Staphylococcus aureus, Streptococcus mutans, Lactobacillus acidophilus) and gram negative bacteria (Streptococcus mutans, Lactobacillus acidophilus) (Pseudomonas aeruginosa). According to Figure 2, Hydrastis canadensis extract has the highest antibacterial activity against Pseudomonas aeruginosa, with an inhibition zone of 29.6mm, followed by Streptococcus pyrogenes (20mm), Staphylococcus aureus (29.3mm), Lactobacillus acidophilus(17.3mm), and *Streptococcus mutans* (19.3mm). The susceptibility of the experimental organisms (*S. aureus, S. pyrogenes, L. acidophilus, S. mutans,* and *P. aeruginosa*) to certain regularly used antibiotics was also shown in Table 2. Cymbopogon citralus and caryophyllus aromaticum were shown to besensitivetoallofthebacterialstrains.*Alliumsativum*wasresistantto*S.pyrogenes*andL.*acidophilus*, but others were vulnerable. The measurement of the zone of inhibition in figure 3- 5 elucidates this.

Comment [V66]: Cymbopogon and caryophyllus ????????

In light of previous research showing that berberine-containing plants contain efflux pump inhibitors, they postulated that synergists present in H. canadensis aerial parts would serve as inhibitors of the norA efflux pump, which is the primary chromosomal efflux pump expressed by *S. aureus* [28]. It is clear from our research that extracts from aerial portions of *H. canadensis* contain norA efflux pump inhibitors, which is in line with earlier findings. Ethidium bromide, a norA substrate, fluoresces brightly when inserted inside bacterial cells because of its intercalation withDNA.

Comment [V67]: reference

This finding implies that other constituent(s) present in the extract from *H. canadensis* aerial parts synergistically improve the antibacterial activity of *S. mutans*, which is consistent with previous findings [29]. The presence of hydrastine and canadine in the leaf extracts does not contribute to this synergistic effect. The antibacterial activity of purified standards of these compounds, which showed no substantial antimicrobial activity (MIC values >30 g/mL), and did not increase the antimicrobial activity of *H.Canadensis*.

A common argument used by proponents of the usage of botanical dietary supplements is that their complexity results in increased efficacy as a result of synergistic interactions between various elements[30]. Extracts from the aerial sections of *Hydrastis* canadadensis include both the well-known antibacterial agent *V. minor* as well as additional chemicals that work together to enhance the antimicrobial action of *V. minor*, as

demonstrated in this study [31].

We further demonstrate that *Hydrastis canadensis* is categorized as endangered by the International Union for Conservation of Nature in much of its native habitat, making this discovery all the more intriguing [32]. According to our findings, the aerial components of this plant, which may be collected in a sustainable manner, are a good source of antibacterial chemicals that can be consumed. We further show that lesser dosages of V. minor are effective in vitro when given to the plant's complex phytochemical matrix in the leaves rather than theroots[33].

Lophophora williamsii extract was also found to directly stimulate the generation of nitric oxide by teeth; however, *Lophophora williamsii* was harmful to the cells that produced the nitric oxide. The presence of anti-oral disease effects in plant products has been known for a long time. Natural products, on the other hand, continue to be needed for the creation of new oral medications, which can be accomplished through screening. Many plant chemicals have yet to be studied at the molecular, cellular, and physiological levels in order to determine whether or not they have the potential to treat oral disorders inhumans[34].

Several results indicate of phytochemical activity in *Lophophora williamsii*, *Vinca minor*, and *Hydrastis Canadensis* were demonstrated that alkaloids level high in all these plants but saponins only high in *Lophophora williamsii*, and L. *williamsii* extracts were found to be cytotoxic to several murine and human oral illnesses in vitro. Our study showed that phytochemical analysis of *Lophophora williamsii*, *Vinca minor*, and *Hydrastis canadensis* shows that highest value of alkaloids, flavonoids, and tannins was observed in *Vinca minor*, was also.

This study shown that *Lephophora williamsii* extract was capable of boosting the immune system in order to combat oral illnesses. It will be required to investigate this differential dental activity by isolating and evaluating the bioactive chemicals found in peyote in order to fully understand it. Additionally, it has been claimed that *Lephophora williamsii* may have indirect (adjuvant) antibacterial characteristics due to its ability to activate lymphocyte and macrophage activities. The presence of a direct antibiotic effect of *Lephophora williamsii* against penicillin-resistant germs, which was connected with the activity of peyocactin and hordenine, has, on the other hand, been documented. Fresh *Lephophora williamsii* juice applied topically to wounds may help to avoid infections and promote healing in Huichol Indians, who use *Lephophora williamsii* juice to treat their wounds and promote healing in other cultures.

Conclusion:

In vitro investigation of the extracts against different bacterial strains can be concluded from this study. Methanolic extracts of almost all materials outperformed aqueous extracts in suppressing pathogenic bacteria growth but were less effective than ciprofloxacin extracts used as positive controls. Plant herbal mixes have made significant contributions to human health and well-being; hence plants have traditionally given a source of hope for innovative medicinal molecules.

Our investigation found that the majority of the medicinal plants had an antibacterial effect in vitro, which supported at least in part their usage in traditional medicine and dentistry, according to the authors. Overall, gram-positive bacteria appear to be more vulnerable to the inhibitory effects of plant extracts than gram-negative bacteria, which is consistent with

Comment [V68]: why was an endangered specie selected for the study kindly justify

Comment [V69]: did this study conduct any experiment on plant ?

plagiarism detected

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authors have only selected lines from different studies and copied pasted in the whole discussion section referring each statement as their own .

Comment [V71]: kindly justify this statement

Comment [V72]: lymphocyte activated in this study ?

Comment [V73]: when the study is conducted in parent country why are the authors concerned about people living in Huichol

previous research and findings. These encouraging findings suggest that these ethno medicines have significant antimicrobial potential and could be used as natural.

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COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

Comment [V75]: font style difference

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