Antimicrobial, Cytotoxic and Phytochemical analysis of *Otostegia limbata*Leaves ethanolic extract against oral pathogens

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Abstract: Otostegia limbata (Benth.) Boiss Family Phytochemical, cytotoxic potential, antifungal, and antibacterial herbal remedies have all been employed previously with the essential understudied ethnomedicinal plant known as Lamiacae. The objective of the current work was to assess with the HPLC analysis, cytotoxic activity, phytochemical analysis, preliminary antibacterial and antifungal activities against oral pathogen of the *O. limbata* leaf extracts. In various countries since centuries, several diseases are cured related to *O. limbata*. It diverted our attention to one of its potent species to be unveiled in this research. The focus of this study was to examine systematically its biological activities and seek out its chemical constituents. Qualitative phytochemical analysis removed phenols, glycosides, flavonoids, alkaloids, quinone, carbohydrates, amino acid, terpenes and coumarins are presence while tannins, saponins and sterols are absence. Quantitative phytochemical screening of the *O. limbata* leaves parts for isolation of active phtometabolites namely alkaloids, sterol, flavonoids, tannins and phenols. The HPLC analysis showed that the samples contained eight identified phenolic compounds, of which Gallic acid, Catechol, Hydroxybenzoic acid, Caffeic acid were most abundant. The antibacterial activity of ethanolic extract displayed the highest inhibition region against *Streptococcus mitis* that was 35 ± 0.1 (ZOI±SD) and the lowest inhibition region against *S .aureus* that was 14 ± 0.3(ZOI±SD) in 200mg/ml ± SD.The antifungal activity resulted that ethanol shows the maximum inhibition zone against *A.fumigatus*that was 29 ± 0.1 (ZOI±SD) and the minimum inhibition zone against *Aspergillus flavus* that was 19 ± 0.4 (ZOI±SD) in 200mg/ml ± SD. The ethanolic extract of research plant were exposed to cytotoxic assay at concentrations is 50µg/ml, 100µg/ml and 150µg/ml and their results were calculated that

indicates that at 50µg/ml is 60%, 100µg/ml is 70%and 150µg/ml is 80%.The current study suggested that, after the isolation of individual components, O. limbata be investigated for assessing biological activity.The mixture and various combinations of these compounds may indicate a truly potent agent that is novel in its ability to combat a wide range of bacteria and oral pathogens.

Key word: Antibacterial, Antifungal, Cytotoxic, Phytochemicals and HPLC, *Otostegia limbata.*

1. Introduction

Pakistan has received a priceless gift from nature in the form of medicinal plants. Since ancient civilizations, medicinal plants have occupied a permanent position for treating a variety of diseases[1]. Natural materials and their preparations make a significant contribution to solving practical issues for people, animals, agricultural, veterinary, food goods, cosmetics, and other industries [2].The family Lamiaceae includes one of the well-known genera Otostegia, which is geographically widespread around the world. There are roughly 4000 species and 220 genera in this group of flowering plants.Otostegia limbata, also known as Rydingia limbata (Benth.) Scheen & V. A. Albert and Ballota limbata is a significant medicinal plant of this genus [3].The common names "Spin aghzai," "Chiti booti," "Chitti jharri," "Spin azghay," and "Bui" are used to identify certain plant species..The plant's distinctive features include a cluster of pale yellow flowers, oblong leaves with a thick pointed tip, pointy bracts, and a tiny petiole. Plant species contain a variety of chemical components, including the acids ballotenic and ballodiolic, limbatolide A, B, C, and D[4].The Otostegia limbata is commonly used as an ethnomedicine in Pakistan for a variety of ailments, including jaundice, cancer, scabies, boils, goitre, ulcer, cuts, wounds, dental issues, and animal diseases [5]. Traditional healers use fresh leaf infusion to treat conditions including acidity, hypertension, depression, ulcer, jaundice, gum disease, and ocular infection in Pakistan's Azad Jammu & Kashmir, KPK, Punjab, and Himalayan regions[6].

To meet our everyday basic needs, nature has bestowed upon us a wealth of valuable treasures. Plants are among the most crucial sources. Plants have been used for therapeutic purposes for a very long time. These ancient medicinal plants are essential to complementary medicine since they are used to cure a variety of ailments. The Indian subcontinent is home to a rich trove of various plant species with a variety of practicable medicinal characteristics. Herbal medicines also play a major role for gums and oral problems. Herbal medications have unique recommendations and a long history of respectability. Herbal medicine, which was once used to

treat heart conditions like heart failure, plays a vital part in the management and treatment of disorders like digitalis, which contains cardiac glycosides[7]. Even in this cutting-edge, technological age, doctors still recommend a variety of medications with botanical origins. Up to 10% of local communities around the world employ medicinal plants to treat various illnesses, yet only 1% of these plants have been identified by scientists. The Alkaloids, tannins, and flavonoids, among other secondary metabolites, are widely distributed in plants with antibacterial characteristics. Because medicinal plants are less poisonous and have less negative effects, they are utilised to treat a variety of ailments [8]. The development of caries and periodontal illnesses is significantly influenced by germs on the tooth surface, according to the movie Dental Plaque [9]. Mutans streptococci have the capacity to produce extracellular polysaccharides from sucrose, mostly water-insoluble glucan, using the glucosyl transferase enzyme, which allows them to colonise the tooth surface and start the production of plaque [10]. This sucrose-dependent adherence and accumulation of cariogenic streptococci is important to the establishment of a pathogenic plaque. The microbial composition of the plaque surrounding the gingival margin and subgingival area may change from being dominated by streptococcus to being more Actinomyces species and more capnophilic and necessary anaerobic bacteria, including Porphyromonas gingivalis [11]. These microbes appear to play a role in periodontal disease and root caries, respectively. Therefore, antimicrobial treatments for certain oral pathogens, especially those that might alter plaque production, could be very effective in preventing dental caries and periodontal disorders. The plant's ethanolic extract has numerous pharmacological properties, including anti-inflammatory, anaesthetic, and cytostatic effects in addition to antibacterial activity. Streptococcus mutans is another bacterium that it is antibacterial for[12]. There is, however, little information available regarding its antibacterial efficacy against other oral pathogens or its impact on dental plaque formation in vitro.

Over the past few decades, there has been a noticeable growth of bacteria that are resistant to antibiotics. Antibiotic overuse and abuse are the primary causes of the rising prevalence of resistant microorganisms worldwide. It's interesting to note that traditional medicine, including herbal medicine, has long been used in developing nations for healthcare (World Health Organization, 2002), and numerous studies have confirmed its efficacy in controlling a variety of infectious diseases.Plant extracts made from the leaves, stems, and roots serve as a valuable resource for the discovery of powerful and innovative antibacterial and biofilm medications [13,

14].Otostegia limbata is a spiny, 40–60 cm tall shrub with many branches (Fig. 1). It is known as "spin azghay" locally in (Lower Dir) and thrives in dry environments.It is widely grown in Kashmir and throughout Pakistan. O. limbata is useful for treating wounds and is effective against ophthalmia, gum, and skin problems. This study focuses on the crude methanolic extracts, water, and hexane fractions of aerial portions (leaves) from J. regia and O. limbata's anti-pseudomonal activity against P. aeruginosa planktonic and biofilm forms in vitro [15, 16, 17].

Fig 1:*Otostegia limbata*(Benth.) Boiss

2. Materials and methods

2.1. Collection and authentication of plants

The plant sample was collected from district (Mansehra) during session June 2021 and Identified by Prof. Dr Ghulam Mujtaba Shah, Chairman, Department of Botany, Hazara University Mansehra KP, Pakistan. After identification the voucher Number (15060) was assigned to the plant species and specimen were deposited in the Herbarium of Hazara University (HUP) for permanent record. The plant materials were washed with tap water, separated and dried in shade for 15 days. These materials were used afterward for phyto-

chemical, and biological activities of *in-vitro* biological screening i-e antimicrobial activities against oral pathogen. The plant material was powdered with the help of electrical grinder. The Whatman filter paper was used after the muslin cloth to filter the extracts. Rotary evaporation will be used at 40°C to remove extra solvent from the filtrate. Until further examination, the extract was kept in a container of amber colour.

2.2. Extraction of plants material

Plant extracts were prepared using microwave extraction technology, according to a previously reported procedure. The microwave's power setting was set at 9000 W. There are three basic stages to this process. In the first step, 750 mL of ethanol and 100 g of each plant powder were added to separate beakers in a 1000 mL container. The microwave was on for 2 minutes, then off for 30 seconds while the beakers were in it.Five times these procedures were carried out. The same process will be used to complete two additional cycles. The muslin cloth will be used to filter the extracts first, and then Whatman filter paper. Rotary evaporation at 40°C was used to remove extra solvent from the filtrate. The extracts will be kept till further analysis in a container of amber colour[11].

2.3. Phytochemical Analysis of *Otostegia limbata*

2.3.1. Qualitative analysis

Different protocolsused to detect the presence or absence of different classes of phytochemicals **(**Phlobatannins, Cardiac glycosides, Quinones, Steroids, Saponins, Coumarins, Tannins and Terpenoids). Presence of these chemicals was detected by production of different colours [12].

2.3.2. Quantitative analysis

The spectrometere was used in quantitative phytochemical analyses of total alkaloid contents, total saponins, total flavonoids contents, total tannins contents and total phenolic contents by using standered protocol methods [13].

2.4. Test organisms

The Six bacterial strains (*Streptococcus mutans*(ATCC 25175), *Streptococcus mitis*(ATCC 23175), *Staphylococcus aureus*(ATCC-6538), *Pseudomonas aeruginosa*(ATCC-15442, *Bacillus subtilis*(ATCC6633), *and Escherichia coli* (ATCC-25922), associated with dental infections were used for antibacterial analyses. Similarly *Aspergillus flavus*(FCBP-0064), *Aspergillus fumigatus*(FCBP- 66), *Aspergillus niger*(FCBP-0198), *Fusarium solani (*ATCC

36031) *and Candida albicans (*ATCC 26081) were used to detect antifungal activity. Cephradine 50µg was used as positive control for antibacterial activity and same quantity of fluconazole used as positive control against fungal strains. DMSO was used negative control against bacterial and fungal strains.The Department of Microbiology at Hazara University in Mansehra, KPK, Pakistan and the Department of Biotechnology at the University of Science and Technology in Bannu provided all the microorganisms.Throughout the study, stock cultures of bacteria and fungi were kept in their proper growth medium at 4 °C. Both antibacterial and antifungal studies were conducted using the agar well diffusion method [14].

3. Antimicrobial activity

Anti-microbiological action 100 mg/ml, 150 mg/ml, and 200 mg/ml of crude extract were the chosen concentrations. Standard antibiotics were employed as the drug of choice for the positive control for various bacterial and fungal infections, and DMSO was utilised for the negative control.Drugsthat were in powder form had been accurately weighed and dissolved in the proper dilutions to the necessary 200 mg/mL concentration. The antibacterial assays were evaluated using the agar-well diffusion method. Agar Mueller-Hinton was employed to prepare the media [15].

4. Cytotoxic activity

The cytotoxic activity was done by following standard protocol method [16].

4.1 Required media

Brine shrimp eggs, sea salt, distilled water, a tray or container with partitions, plant extract, test tubes, micro tips, and a magnifying glass.

4.2 Stock solution preparation

The 20 mg of Plant extract were dissolved in 2 ml of ethanol to create the stock solution.

4.3 Method

Following techniques allowed for the determination of the plant's potential for cytotoxicity [16]. Brine shrimp eggs were first placed in a plastic container or tray with a perforated partition and 3.8 grammes of sea salt was first dissolved in 1000 ml of distilled water. This media was then added, and the container was placed at a temperature of 34–36 oC for one day to hatch the brine shrimp eggs. As they emerged, the shrimp went to the opposite side of the container. Following the creation of three concentrations 100 mg/ml, 500 mg/ml, and 1000 mg/ml stock solutions were added in accordance with these concentrations and the test tubes

were left for the remainder of the day to allow the ethanol to evaporate. Next, 2 mg of sea salt was added to the test tubes to make the total volume 5 mg, and ten newly hatched brine shrimp were then placed inside the test tubes using a micro-pipette, and the tubes were then left The following day, using a microscope, the number of alive and dead brine shrimp in each test tube was determined.

5. High performance liquid chromatography (HPLC) analyses

In 20 ml of methanol (62.5%) and 5 ml of HCl were used to extract the ground plant material (6M). The extract was sonicated for 15 minutes and then refluxed in a water bath for two hours following nitrogen purging [17]. Before injecting into HPLC, filter the extract twice via a 0.2 m Millex-HV membrane filter. The Shimadzu LC-20AT HPLC system includes a column oven, an auto-sampler, and a diode array detector (SPD-M20A). Utilized was an analytical column with a guard column (KJO-4282, Phenomenex): Purospher Star RP-18 endcapped 5 m 100 A° (250 x 4.60 mm, Merck, Germany). The composition gradient programme was used with just minor alterations with the mobile phase consisting of (A) 0.1% acetic acid and (B) methanol [18]. The flow rate was 0.8 mL per minute. By contrasting the retention times and UV-Vis spectra of chromatographic peaks with those of genuine reference standards at 280 nm, phenolic chemicals were identified [19].

Statistical analyses

Data tabulated and analyzed by using statistic software statistic 8.1.

Result

4.1. Qualitative phytochemical analysis of*Otostegia limbate*

Phytochemical analysis revealed that the crude extract of *Otostegia limbate* included many different types of compounds such as phenolic and glycosides, as well as flavonoids and alkaloids, as well as quinones, carbohydrates, amino acid, terpenoids and coumarins. However, the tannins, saponins and sterols test results for the crude extract showed no change in colour (Table No 4.1)

Table No 1: Qualitative phytochemical analyses of ethanolic extract of *Otostegia limbata*

S.No	Constituents	Present (+)	Absent (-)
1	Phenols	+	
2	Glycosides	+	
3	Tannins		-
4	Flavonoids	+	
5	Alkaloids	+	
6	Saponins		-
7	Quinones	+	
8	Sterols		-
9	Carbohydrates	+	
10	Amino acids	+	
11	Terpenoids	+	
12	Coumarins	+	

Key = Negative sign (-) indicate absence, positive sign (+) indicate presence

4.2. Quantitative analysis of *O. limbata*

Quantitative phytochemical screening of the leaves parts of the *O. limbate* for isolation of active phtometabolites namely alkaloids, sterol, flavonoids, tannins and phenols in (Table-4.2).The results revealed the bioactive constituents in leaves are alkaloids were in the range of (16.66 ± 1.33 mg/g) and sterol (14.68 ± 0.66 mg/g), flavonoids (11.5±0.33 mg/g), tannins is (14.30 ±0.10 mg/g) and phenols is (56.73±0.25 mg/g).

Table 2: Quantitative analysis of*Otostegia limbata*. All values are mean ± SEM of three determinations. All values are expressed in mg/g.

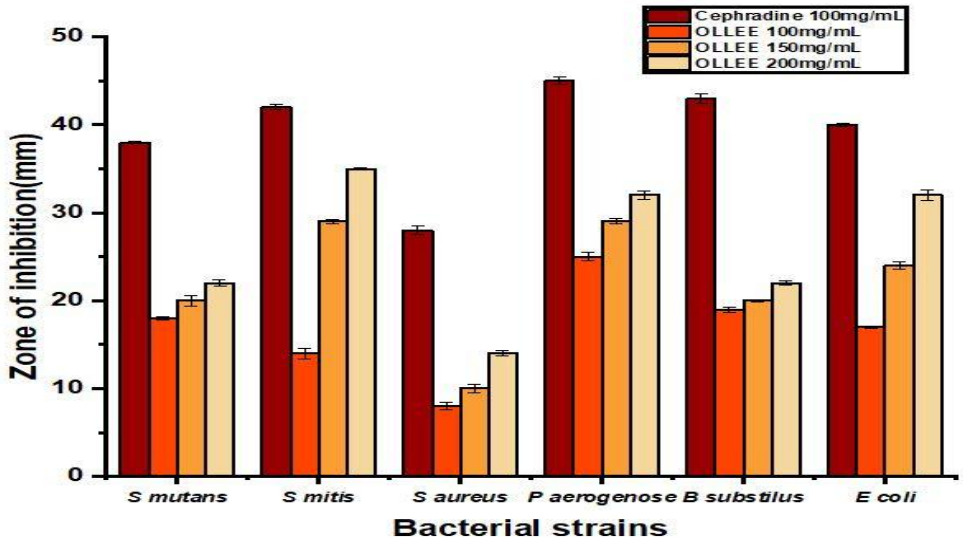
S.No	Extract	Alkaloids	Sterol	Flavonoids	Tannins	Phenol
1	OLL	16.66 ± 1.33	14.68 ± 0.66	11.5±0.33	14.30 ±0.10	56.73±0.25

Key = OLL= Otostegia limbata Leaves, ND= Not detected

Table 3: Antibacterial activity of *Otostegia limbata*

Tests Microorganism	**Antibiotics ZOI(mm) ± SD**	**Leaves ZOI(mm)Means ± SD**		
Bacterial strains	**Cephradine 100mg/ml ZOI(mm)**	**OLLEE 100mg/ml± SD**	**OLLEE 150mg/ml ±SD**	**OLLEE 200mg/ml ± SD**
S. mutans	38±0.1	18±0.2	20±0.6	22±0.4
S .mitis	42±0.3	14±0.6	29±0.2	35±0.1
S .aureus	28±0.5	8±0.4	10±0.5	14±0.3
P.aerogenose	45±0.4	25±0.5	29±0.3	32±0.5
B.substilus	43±0.6	19±0.3	20±0.1	22±0.2
E .coli	40±0.2	17±0.1	24±0.4	32±0.6

Key=*S.mutans*=*Streptococcus mutans*, ***S.mitis*=***Streptococcus mitis*,***S .aureus*=***Staphylococcus aureus*, ***P.aerogenose*=***Pseudomonas aeruginosa*, ***B.substilus*=***Bacillus subtilis*, ***E .coli*=***Escherichia coli*and **OLLEE=**Otostegia limbata leaf ethanolic extracts**,** **SD =**Standard deviations**)**



Key= PSLEE (Otostegia limbata leaves ethanolic extract)

Fig 2, Graphical representation of antibacterial activity of *Otostegia limbata*

Antibacterial activity of *Otostegia limbata*

Figure 2 displays the Otostegia limbata ethanolic extract's antibacterial activity. Streptococcus mitis showed a maximum inhibition zone of 35 0.1, while S. aureus showed a minimum inhibition zone of 14 0.3 in 200 mg/ml standard deviation. Antibiotics had a maximum zone of inhibition of 45 0.4 against P. aerogenose and a minimum zone of inhibition of 28 0.5 against S. aureus in 100 mg/ml SD. Table 3 of the results shows the results for the plant extract and antibiotic in terms of standard and mean deviation values. When used as a negative control, DMSO does not inhibit bacterial strain development (Table 3).

Table 4; Antifungal activity of ethanolic extracts of *Otostegia limbata*

Tests Microorganism	**Antibiotics ZOI(mm) ± SD**	**Leaves ZOI(mm) Means ± SD**		
Fungal strains	**Fluconazole100mg/ml ZOI(mm)**	**OLLEE 100mg/ml± SD**	**OLLEE 150mg/ml ±SD**	**OLLEE 200mg/ml ± SD**
F.flavus	35.33 ±0.5	10 ±0.2	12 ±0.6	19 ±0.4
A.fumigatus	32.33 ±0.3	12 ±0.6	15 ±0.2	29 ±0.1
C.albicans	36.66 ±0.4	13 ±0.4	15 ±0.5	19 ±0.3
A.niger	29.66 ±0.2	16 ±0.5	20 ±0.3	21 ±0.5
F.solani	30.33 ±0.1	12 ±0.3	15 ±0.1	19 ±0.2

Key=*F.flavus* *=Aspergillus flavus,* ***A.fumigatus*** *=Aspergillus fumigatus,****C.albicans****=Candida albicans,* ***A.niger*** *=Aspergillus niger,* ***F.solani s****=Fusarium solani*and **OLLEE=**Otostegia limbata Leaf ethanolic extracts**,**
S.D =Standard deviations**)**

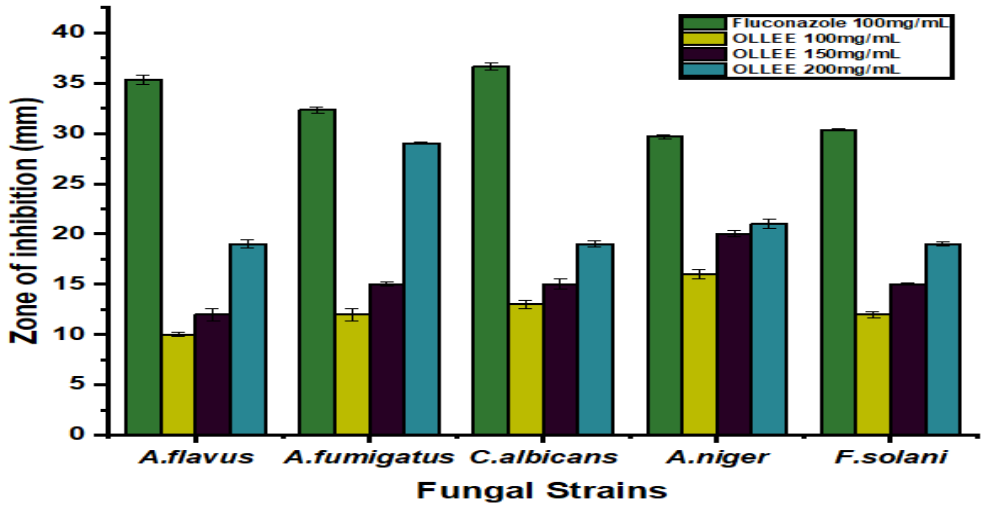


Fig 3, Graphical representation of antifungal activity of *Otostegia limbata*

Antifungal activity of *Otostegia limbata*

The greatest inhibition zone against Aspergillus fumigatus was 29 0.1, and the minimum inhibition zone against Aspergillus flavus was 19 0.4, according to an ethanol extract of the antifungal activity. Antibiotics had a maximum zone of inhibition of 36.66 0.4 against C.albicans and a minimum zone of inhibition of 29.66 0.2 against A. niger in (table 4) of the results shows the results for the plant extract and antibiotic in terms of standard and mean deviation values. DMSO is utilised as a negative control and exhibits no growth suppression or resistance to fungi. (Table4).

Table 5: Cytotoxic activity of *Otostegia limbata*

Concentrations µg/ml	Total no Shrimps	Living Shrimps ± SD	Dead Shrimps ± SD	Death %
OLLEE 50 µg/ml	10	4± 0.2	6± 0.3	60
OLLEE 100 µg/ml	10	3± 0.3	7± 0.1	70
OLLEE 150 µg/ml	10	2± 0.1	8± 0.2	80

Key=OLLEE=Otostegia limbata Leaf ethanolic extracts**, SD =**Standard deviations**)**

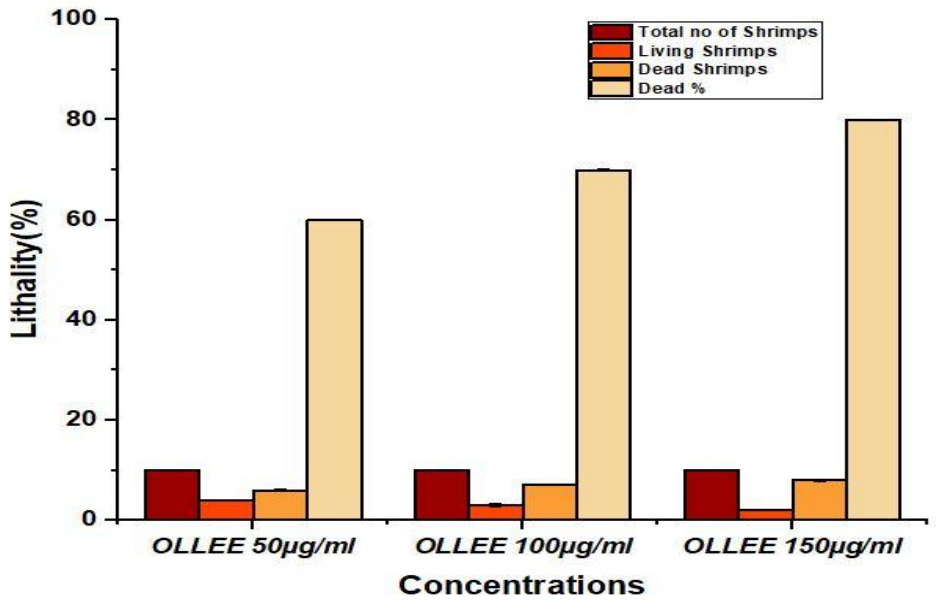


Fig 4, Graphical representation of cytotoxic activity of *Otostegia limbata*

Cytotoxic Brine shrimps assay

The cytotoxic activity of *O. limbate*extracts at various doses (50, 100 and 150µg/ml) was conducted. It was shown that *O. limbate*extracts had a cytotoxic impact on brine shrimps when evaluated for 72 hours under controlled conditions. Results after 24 hours show that brine shrimp mortality is inversely related to extract concentrations. *The O. limbate* reported a maximum lethality of 80 % at 150 µg/ml, as seen in the (Fig 4). The highest mortality was reported in 80 % at 150 µg/ml(Fig 4). The bioactive components in both plants make them more cytotoxic than plant extracts.

Table 6: Phenolic composition (mg g^{-1} dry weight) of *Otostegia limbata*

S.No	Compounds	Retention time	*O. limbata*
1	Pyrogallol	12.557	n.d
2	Gallic acid	15.192	12.11 ± 0.25
3	Catechol	18.145	3.01 ± 0.07
4	Hydroxybenzoic acid	19.136	n.d
5	Chlorogenic acid	23.59	n.d
6	Caffeic acid	24.756	7.57 ± 0.92
7	Coumaric acid	30.039	n.d
8	Ferulic acid	31.835	n.d

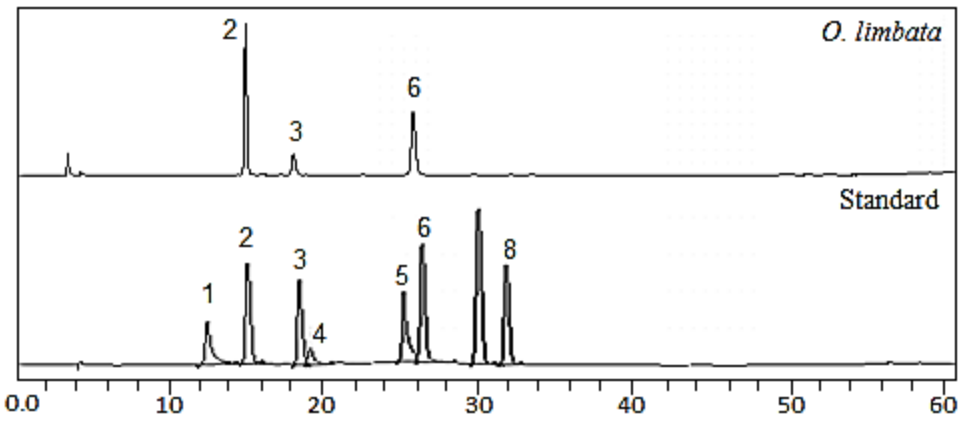


Fig 5, Graphical representation of Quantitative analysis of *Otostegia limbata*

Figure 2: HPLC chromatograms of standard compounds *i.e.* Pyrogallol (1), Gallic acid (2), Catechol (3), Hydroxybenzoic acid (4), Chlorogenic acid (5), Caffeic acid (6), Coumaric acid (7), and Ferulic acid (8) and *Otostegia limbate* extract.

High performance liquid chromatography (HPLC) analyses

The HPLC analysis showed that the samples compound is contained eight identified phenolic compounds, of which Gallic acid, Catechol, Hydroxybenzoic acid, Caffeic acid were most abundant.The Gallic acidis 12.11 ± 0.25, Catechol is 3.01 ± 0.07 and Caffeic acid 7.57 ± 0.92. The highest compound isGallic acidis 12.11 ± 0.25 while the lowest compound is Catechol is 3.01 ± 0.07

Discussion

The qualitative and quantitative phytochemicals analysis of ethanolic extract was used for detection of phytochemicals. Most of the phytochemicals (alkaloids, terpenes, coumarins, saponins, cardiac glycosides, phlobatannins, flavonoids, quinone, steroids and tannins) were qualitatively and quantitatively detected. Ethanol observed as a best solvent used for the extraction of different phytochemicals. Our results are agreed with the findings of they compare different solvent for phytochemical extraction and found that ethanol is the best solvent for extraction of different phytochemical [20]. Presence of these biologically active compounds shows the medicinal value of *Otostegia limbata*as these phytochemicals have different medicinal properties. Otostegia limbata's phytochemical analysis found that it contains phenols, glycosides, flavonoids, alkaloids, quinones, carbohydrates, amino acids, terpenoids, and coumarins but not tannins, saponins, or sterols, which are thought to be the phytochemicals that give plants their antimicrobial properties[21]. Numerous biological processes, including antibacterial, antioxidant,

and inflammatory ones, have been connected to flavonoids. They are also known to be able to suppress cell growth and regulate enzymatic activity. They are well recognised to act as a plant's defence mechanism against encroaching diseases[22].Tannins bind to proline-rich proteins to create complexes that prevent the creation of proteins in cells. It is recognised that the combined effects of tannins, flavonoids, alkaloids, and saponins can stop pathogen growth[23].Alkaloids are renowned for their anaesthetic, anti-inflammatory, and cardioprotective effects[24]. Tannins are significant phenolic substances that are well known for their antibacterial properties. The capacity of tannins to precipitate proteins, block the availability of the substrate to the bacterial cells, directly attack the microbial cells, and restrict the uptake of iron by microorganisms is what gives tannins their specific ability to combat dangerous bacterial diseases[25]. Coumarin's ability to suppress anticholinesterase is what makes them beneficial for treating Alzheimer's disease[46]. Phlobatanins used to cure treating swelling, new wounds, and lymphatic diseases [26].These chemicals are found in P. stewartii, according to phytochemical tests, which suggests that this plant is used to treat a variety of illnesses.The*Otostegia limbata*ethanolic extract show the significant activity againstall selected oral bacteria strains Table 3, (*Streptococcus mutans, Streptococcus mitis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis, and Escherichia coli),* this might be due to the antibacterial compound present in our plant are well extracted by ethanol and thus inhibiting the growth of selected bacteria. Our findings are similar to that codded [27].Using the disc diffusion method, the antibacterial activity of three medicinal plant extracts, including Azadirachta indica, Melia azedarach, and Spilanthes acmella, was examined against Streptococcus mutans and Staphylococcus aureus.

In order to combat the bacteria that cause denture plaque, plant extracts may be a safe substitute for dangerous medications and chemicals. Similar outcomes were categorized as well [28]. They discovered that the two plants' ethanolic extracts had a growth-inhibiting impact on all four strains of periodontal pathobionts.Tannerella forsythia was the target of C. zeylanicum's highest antibacterial activity, whereas Aggregatibacter actinomycetemcomitans was the target of C. zeylanicum's lowest antibacterial activity among all the groups under study (MIC = 12.5 3.25 mg/mL, MBC = 75 8.23 mg/mL, respectively).Candida albicans is significantly inhibited by ethanol extract, although Aspergillus flavus is the fungus that is most resistant to our plant. This action demonstrates the antifungal constituents' presence in *O. limbata* and their absorption by the fungi strains. If A. flavus activity is lower than usual, it may be because this strain is resistant

to the component found in the chosen plant. Our findings concur with the findings [28].They discovered that S. marianum extract works well against Candida species at 400-800 g/mL doses. As a result, it was discovered that the plant's fruit sections might be a natural source of antifungal and antibacterial agents. The ethanolic extract of*O. limbata*showed zone of inhibition against *Aspergillus flavus, Aspergillus fumigatus, Aspergillus Niger, Fusarium solani and Candida albicans*.

Conclusions

By using qualitative phytochemical screening, it was discovered that Otostegia limbata possesses active secondary metabolites such as alkaloids, terpenes, coumarins, saponins, cardiac glycosides, phlobatannins, flavonoids, quinone, steroids, and tannins. According to the results of the antimicrobial assay, Otostegia limbata plant ethanol extract is a useful tool for testing new antimicrobial medications for the treatment of oral microorganism-related disorders. From the data taken together, it can be inferred that HPLC is a flexible, repeatable chromatographic method for the quantification of medicinal products. Regarding the quantitative and qualitative estimation of active compounds, it has a wide range of applications in various sectors. At 150 g/ml of concentration, the plant likewise exhibits the highest potential for cytotoxic activity. The high performance liquid chromatography potential of Otostegia limbata yields impressive results.

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