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Formulation and Evaluation of *Orchis laxiflora L.* Antibacterial Mouthwash

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Abstract

Mouthwashes are aqueous topical medicines that clean the interior of the mouth by washing and gargling. There are a variety of mouthwashes with a variety of active ingredients available over the counter and by prescription that can be used as an adjunct to help with the multifactorial management of complex oral conditions like halitosis, gingivitis, periodontal disease, oral mucositis, and even xerostomia, among others. Clove is the most commonly applied directly to the gums for toothache, pain control during work and other dental related issues. We have developed a mouthwash with some common food materials and herbs and which can replace costly chemicals like alcohol, coloring agents and preservatives making our mouthwash economically more viable than commercial mouthwash. The anti-inflammatory and anti-infectious properties of Neem make it a powerful treatment for gum disease.

The results of this study indicate that the existence of compounds such as glycosides, terpenoids, flavenoids and saponins that can be responsible for the anti-bacterial efficacy of extracts and poly herbal mouthwash rinse against these microorganisms that are known to be mostly responsible for oral cavity bacterial infection.

Key-words: *Orchis laxiflora L, Streptococcus mutans*, *virus, bacterium, fungus, Prion, protozoa*

Introduction

A pathogen (from the Greek pathos, which means "suffering, passion," and genes, which means "creator of illness") is a bacterium that causes disease in another creature (the "host")[1].The oral cavity, which is home to a wide variety of microorganisms, encourages the formation of unique microbial communities on the mucosa and teeth[7]. The human mouth is home to between 700 and 1000 microbial species. Oral illnesses including dental caries, periodontal disease, and oral cancer are strongly linked to oral bacteria' incidence and progression. Oral bacteria can enter the bloodstream through a damaged oral mucosa, causing an increase in systemic antibody levels and raising the risk of cardiovascular disease[3].

Mouthwashes are aqueous topical medicines that clean the interior of the mouth by washing and gargling[2]. There are a variety of mouthwashes with a variety of active ingredients available over the counter and by prescription that can be used as an adjunct to help with the multifactorial management of complex oral conditions like halitosis, gingivitis, periodontal disease, oral mucositis, and even xerostomia, among others.To cure infections, decrease inflammation, relieve discomfort, and minimize halitosis, or to administer fluoride locally for caries prevention, a mouthwash may be suggested.

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Mouthwash is an aqueous solution that is commonly used for deodorizing, refreshing, and antimicrobial qualities, as well as plaque management. Alcohol, glycerin, synthetic sweetness, surface active agents, flavoring agents, coloring agents, and other ingredients may be present.

Antimicrobial agents have become a major source of worry across the world. Multi-drug resistant bacteria are those that are resistant to more than two types of antibiotics (MDR). The global spread of MDR bacteria has steadily raised morbidity and death rates, as well as treatment costs, limiting the efficacy of current medicines and causing considerable treatment failure

Orchislaxiflora L. is a BULB that may reach a height of 0.8 m. (2ft 7in). It is cold resistant and hardy to zone 5 (UK)[3]. From May through June, it is in bloom. Insects pollinate the species, which is hermaphrodite (has both male and female parts). Light (sandy), medium (loamy), and heavy (clay) soils are all suitable. Acid, neutral, and basic (alkaline) soils are all suitable pH levels. It is unable to thrive in the shade. It loves soil that is damp or humid.

Fig. 1: Root of *OrchisLaxiflora L.* (Buzidan)

Scientific classification

Kingdom: Plantae
Clade: Tracheophytes
Clade: Angiosperms
Clade: Monocots
Order: Asparagales
Family: Orchidaceae
Subfamily: Orchidoideae
Genus:*Orchis*
Species: ***A. laxiflora***

Material and Methods

Collection of plant material

Root of *Orchislaxiflora L*[4]. werecollected from rural area of Bhopal (M.P), India in the months of January, 2021.

Fig. 2: Collection of plant material (Root)

Preparation of plant material for study

The roots used for the research were properly cleaned under running tap water and then rinsed in distilled water before being allowed to dry. The components from these plants were then shade dried for 3 to 4 weeks without being contaminated. An electric grinder was used to ground dried plant materials[5]. The dried plant material was stored in an airtight container until it was needed again.

Extraction procedure

For the production of hydroalcoholic extract from shade dried and powdered drug following technique was used.

Extraction by maceration process

45.5 gm dried powdered *Orchislaxiflora L.* was extracted for 48 hours with hydroalcoholic solvent (methanol: water; 75:25), filtered, and dried at 40ºC using a vacuum evaporator.

Fig. 3: Extraction by Maceration Method

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Determination of percentage yield

The percentage yield of each extract was calculated by using following formula:

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of Powder drug taken}} \times 100$$

The crude extract produced following the maceration extraction procedure was concentrated on a water bath, fully evaporating the solvent to get the real extraction yield. To measure the standard extraction efficiency for a given plant, various sections of the same plant, or different solvents employed, obtaining the percentage yield of extraction is a very significant phenomena in phytochemical extraction. Table 3.1 shows the yield of extract produced from samples using hydroalcoholic as the solvent[6].

Table 1: % Yield of *Orchislaxiflora L.* extract

S. No.	Solvent	% Yield
1.	Hydroalcoholic	5.41

Phytochemical Screening of Extracts

The chemical tests were performed for testing different chemical groups present in extracts

A. Alkaloids
B. Glycosides
C. Flavenoids
D. Phenolics
E. Proteins
F. Carbohydrates
G. Saponins
H. Diterpins

Table 2: Result of Phytochemical screening of *OrchisLaxiflora L.*

S. No.	Constituents	Hydroalcoholic extract
1.	**Alkaloids** Hager's test Mayer's test Wagner's test	 -ve -ve -ve
2.	**Glycosides** Legal's test	 -ve
3.	**Flavonoids** Lead acetate Alkaline test	 +ve +ve
4.	**Phenolics** Ferric Chloride Test	 +ve
5.	**Proteins** Xanthoproteic test	 -ve
6.	**Carbohydrates** Fehling's test	 -ve
7.	**Saponins** Froth Test	 +ve
8.	**Diterpins** Copper acetate test	 -ve

Quantitative study of Bioactive constituents

Total Phenolic content estimation

Principle: The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method[7].

Preparation of Standard: 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 5- 25µg/ml was prepared in methanol

Preparation of Extract: 10mg of dried extract of plant material was extracted with 10 ml methanol and filter. 2 ml (1mg/ml) of this extract was for the estimation of Phenol.

Procedure: 1 ml Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) sodium carbonate were combined with 2 ml of each extract or standard. The mixture was vortexed for 15 seconds before being left at 40°C for 15 minutes to develop color. A spectrophotometer was used to measure the absorbance at 765 nm.

Table 3: Preparation of calibration curve of Gallic acid

S. No.	Concentration (µg/ml)	Absorbance
0	0	0
1	5	0.194
2	10	0.422
3	15	0.637
4	20	0.848
5	25	1.035

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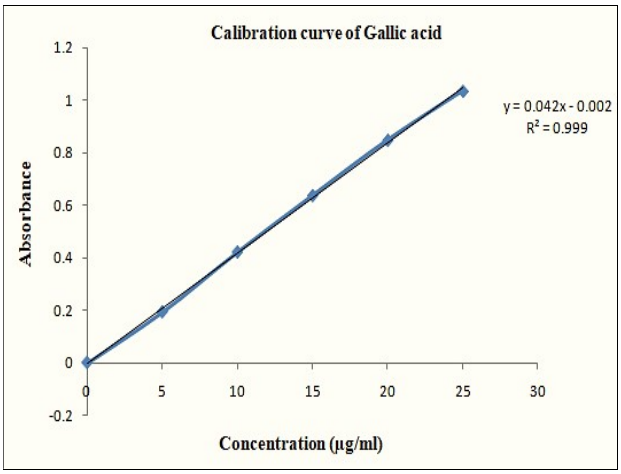
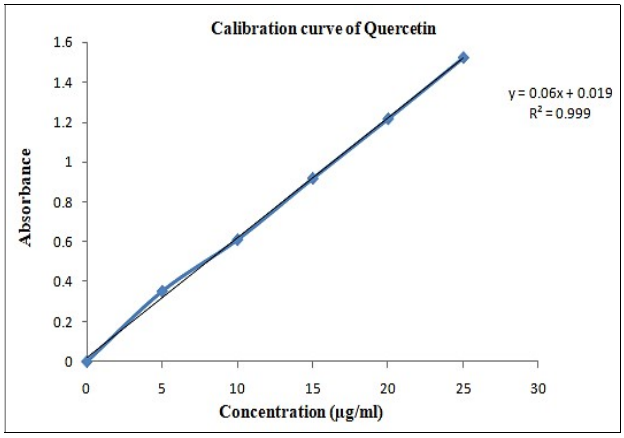


Graph 1: Graph of calibration curve of Gallic acid



Graph 2: Graph of Estimation of calibration curve of Quercetin

Total flavonoids content estimation

Principle: Determination of total flavonoids content was based on aluminium chloride method[75].

Preparation of standard: 10 mg quercetin was dissolved in 10 ml of methanol, and various aliquots of 5- 25µg/ml were prepared in methanol.

Preparation of extract: 10 mg of dried extract plant material was extracted with 10 ml of methanol and filter. 3 ml (1mg/ml) of this extract was for the estimation of flavonoid[8].

Procedure: 1 ml of 2 percent AlCl3 methanolic solution was added to 3 ml of extract or standard and allowed to remain at room temperature for 15 minutes before measuring absorbance at 420 nm.

Table 4: Preparation of calibration curve of Quercetin

S. No.	Concentration (µg/ml)	Absorbance
0	0	0
1	5	0.352
2	10	0.61
3	15	0.917
4	20	1.215
5	25	1.521

Results of evaluation of prepared mouthwash formulations

Results of Colour and Odour

Visual observation against a black and white backdrop was used to assess the color of the formulations. Precipitation was an issue with some formulations during storage, which was solved by extending the stirring duration during the formulation process.

Table 5: Total phenolic and total flavonoid content of *OrchislaxifloraL* .extract

S. No.	Total Phenol content	Total flavonoid content
1.	0.684 mg/100mg	0.966 mg/100mg

Table 6: Colour and Odour of **formulations**

Formulation code	Colour	Odour
F1	Light green	Mint odour
F2	Light brown	Mint odour
F3	Reddish brown	Mint odour
F4	Light green	Mint odour
F5	Light brown	Mint odour
F6	Light green	Mint odour

pH Determination

The developed formulations were evaluated for pH by using digital pH meter. The pH of formulations F1, F2, F3, F4, F5 and F6 were found 6.5, 6.8, 6.7, 6.8 and 6.5 respectively.

Table 7: pH Determination

S. No.	Formulation	pH
1.	F1	6.5
2.	F2	6.8
3.	F3	6.7
4.	F4	6.8
5.	F5	6.8
6.	F6	6.5

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Viscosity study

Viscosity of formulation was determined before and after gelation by using Brookfield's viscometer in the small volume adaptor and the angular velocity was determined at 10 rpm[9].

Table 8: Viscosity of mouthwash formulation

Formulation code	Viscosity (cps)
F1	485
F2	492
F3	510
F4	476
F5	489
F6	595

Table 9: Antimicrobial activity of standard drugand mouth washagainst*Streptococcus mutans*

S. No.	Name of drug	Zone of Inhibition (nm)		
		30 µg/ml	**20 µg/ml**	**10 µg/ml**
1	Ofloxacin	17±0.94	15±0.47	12±0.5
		100%	**50%**	**25%**
2.	F1	9±0.5	8±0.94	6±0.47
3.	F2	10±0.47	7±0.5	6±0.74
4.	F3	15±0.57	13±0.74	11±0.86
5.	F4	11±0.74	6±0.86	6±0.5
6.	F5	12±0.47	8±0.86	7±0.57
7.	F6	12±0.57	9±0.47	7±0.5

Conclusion

Herbal remedies from nature are well-researched and shown to be effective and safe natural treatments for a wide range of ailments[10]. The goal of this study was to demonstrate preliminary chemical screening, mouthwash composition, and antimicrobial efficacy.The dried plant material was thoroughly cleaned under running water before being ground using an electric grinder. The powder was extracted using a hydroalcoholic solvent and the maceration technique. Organoleptic measurement, percentage yield, phytochemical screening, quantitative calculation of total phenols and flavonoid contents, formulation and assessment of mouth wash, and antimicrobial studies were all used to evaluate *Orchislaxiflora L.*

Table 3.1 shows that the hydroalcoholic extract of *Orchislaxiflora L.* yielded a similar percentage yield of 5.41 percent.

A number of phytochemicals were evaluated in the hydroalcoholic extract. Flavonoids, phenolics, and saponins were found in the hydroalcoholic extract, as shown in table 3.2. In hydroalcoholic extract, phytochemical screening revealed no alkaloids, glycosides, protein, carbohydrate, or diterpenes.

When hydroalcoholic extract of *Orchislaxiflora L.* was incubated with *Streptococcus mutans*, a zone of inhibition was detected, but when formulation F3 was incubated with *Streptococcus mutans*, the greatest zone of inhibition was found. The findings of zone of inhibition show that *Orchislaxiflora L.* mouth wash formulations F1, F2, F4, F5, and F6 were ineffective against *Streptococcus mutans*.

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