World Journal of Pharmaceutical Science and Research
www.wjpsronline.com
Research Article
ISSN: 2583-6579
SJIF Impact Factor: 5.111
Year - 2026
Volume: 5; Issue: 1
Page: 64-80

TO EXTRACT AND EVALUATE THE ANTIMICROBIAL PROPERTY OF *BRASSICA OLERACEA VAR. BOTRYTIS*

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Article Received: 6 December 2025 | | ***Article Revised: 13 December 2025*** | | ***Article Accepted: 17 December 2025***

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DOI: https://doi.org/10.5281/zenodo.18108496

How to cite this Article: Pratik Hemant Kharade, Nayan C. Karjavkar, Tejal Mali, Mahek Naik, Vikas Yadav, Mrunali Yadav (2026) TO EXTRACT AND EVALUATE THE ANTIMICROBIAL PROPERTY OF BRASSICA OLERACEA VAR. BOTRYTIS. World Journal of Pharmaceutical Science and Research, 5(1), 64-80. https://doi.org/10.5281/zenodo.18108496

ABSTRACT

The present research involves extracting and assessing the antimicrobial activity of *Brassica oleracea* var. *botrytis* (cauliflower), a commonly consumed vegetable enriched with various beneficial phytochemicals. Cauliflower is a natural source of compounds such as glucosinolates, isothiocyanates, flavonoids, tannins, phenolic acids, and saponins, many of which are recognized for their antimicrobial and antioxidant effects. In this study, ethanolic extraction of cauliflower florets was carried out using the maceration technique, followed by preliminary phytochemical analysis to identify major secondary metabolites. The extract showed the presence of flavonoids, tannins, phenols, saponins, terpenoids, and some alkaloids, indicating a strong phytochemical composition. The antimicrobial potential of the extract was examined through the cup-plate diffusion method against *Escherichia coli* and *Staphylococcus aureus*. The ethanolic extract produced a notable zone of inhibition, signifying effective antibacterial activity. Subsequently, a herbal mouthwash containing varying concentrations of the extract was formulated and tested for its physical properties, pH, homogeneity, stability, and antimicrobial efficacy. Among all formulations, batch F4 demonstrated the best overall performance and superior antimicrobial action when compared with standard samples. Overall, the study indicates that cauliflower extract can serve as a natural and effective antimicrobial ingredient in herbal mouthwash formulations. Its safety profile, easy availability, and significant antimicrobial activity make it a promising eco-friendly substitute for conventional chemical-based oral hygiene products.

KEYWORDS: *Brassica oleracea var. botrytis*, cauliflower extract, antimicrobial activity, phytochemical screening, herbal mouthwash, Quercetin, glucosinolates, isothiocyanates, *E. coli*, natural oral care, bioactive compounds.

INTRODUCTION

Anatomy of Mouth Cavity

The oral cavity, enclosed by the lips, is divided into two main parts: the vestibule, located between the cheeks, lips, and teeth, and the oral cavity proper. The oral cavity proper is largely occupied by the tongue, bordered in the front and on the sides by the alveolar processes with teeth, and at the back by the isthmus of the fauces. Its roof is formed anteriorly by the hard palate and posteriorly by the soft palate, from which the uvula projects downward. The floor is made up of the mylohyoid muscles. Lining the inside of the mouth is the oral mucosa, a stratified squamous epithelial membrane. The submandibular and sublingual salivary glands secrete viscous, mucoid saliva that lubricates and maintains moisture within the cavity. (Lu, Xuan and Wang, 2019)

Beyond serving as the entry point for food and drink, the mouth is crucial for speech production and normal breathing. Teeth, the primary structures of the oral cavity, cut and grind food into smaller pieces to aid digestion. The tongue assists digestion by pressing food against the palates, forming it into a bolus for swallowing into the esophagus. It also provides the sense of taste through specialized papillae containing taste buds on its upper surface. In addition, the tongue is the key organ of articulation, shaping sounds into words by interacting with the teeth and palate. The palate itself separates the oral cavity from the nasal tract, ensuring both breathing and eating can occur at the same time.(*Mouth Anatomy*, 2011a)

The oral cavity is distinctive in its origin, as it develops from both ectodermal and endodermal tissues.

The tongue arises from multiple pharyngeal arches, with each arch contributing to different parts of the organ. Around the fifth week of embryonic development, small swellings called “lingual swellings” appear on either side of the first pharyngeal arch. These swellings enlarge and merge to form the anterior two-thirds of the tongue. The line of fusion is marked externally by the median sulcus. The posterior one-third of the tongue originates from the copula, which develops primarily from the second and third pharyngeal arches, with a minor contribution from the fourth arch. The junction between the anterior and posterior regions of the tongue is delineated by the V-shaped terminal sulcus.(ـ, n.d.)

The palate forms between the sixth and twelfth week from both primary and secondary embryonic structures. The primary palate develops from the embryonic frontonasal prominence and gives rise to the philtrum, upper incisor region, and the anterior part of the upper jaw. The secondary palate is derived from the fusion of paired maxillary prominences and makes up the remainder of the hard and soft palate. Initially, these outgrowths form as vertical palatal shelves, which later reposition horizontally above the tongue and fuse with the primary palate and nasal septum, resulting in the complete roof of the oral cavity.

The upper lip develops early in embryogenesis through the fusion of the maxillary, lateral nasal, and medial nasal prominences. These prominences are formed from migrating neural crest cells, mesoderm, and head ectoderm. Their growth and merging produce the upper facial structures. The lower lip is created by the fusion of the mandibular processes. (*Mouth Anatomy*, 2011b)

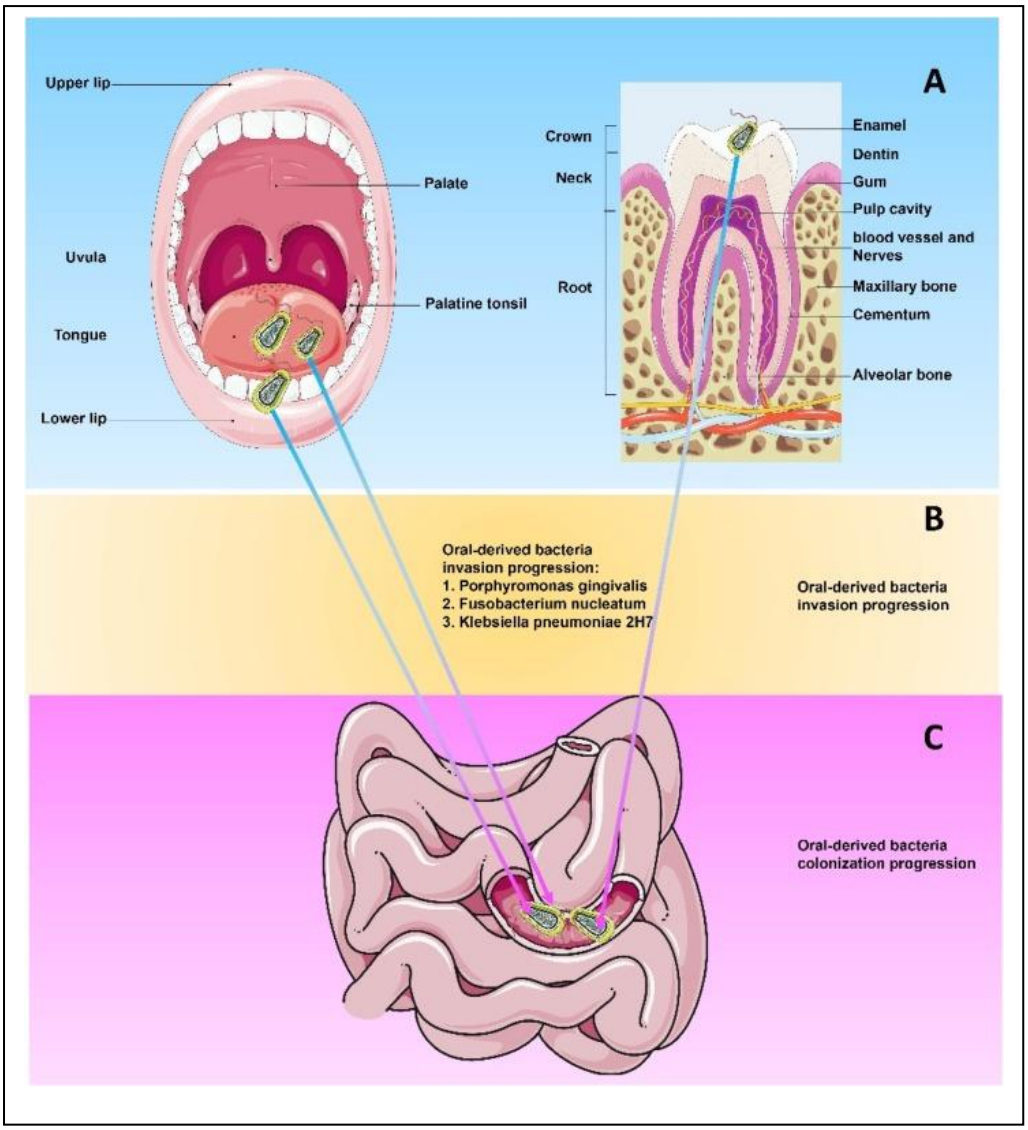

Fig. No. 1: Anatomy of Mouth Cavity

Dental Plaque Formation

Plaque buildup on teeth is unavoidable, as the bacteria in the mouth feed on the food we consume. While they prefer sugary and starchy foods, they can also draw nutrients from other sources.

Over time, these bacteria release acids that erode tooth enamel, leading to cavities—the earliest sign of tooth decay. Plaque can also extend below the gumline, damaging the bone that supports the teeth. Leaving food or drink residues on teeth accelerates this process. (Cai *et al.*, 2020)

Plaque formation depends on various factors, including diet, acid and moisture levels in the mouth, and bacterial activity. It commonly appears on the back molars, around dental work, and in hard-to-clean areas.(Valm, 2019a)

Though often invisible at first, plaque can be felt as a sticky film on teeth that lingers after meals. Good oral hygiene is essential to prevent its buildup. If not removed early, plaque hardens into tartar, which is far more difficult to eliminate. Addressing plaque while it is still soft helps protect against serious dental problems, saving discomfort and treatment later. (Valm, 2019b)

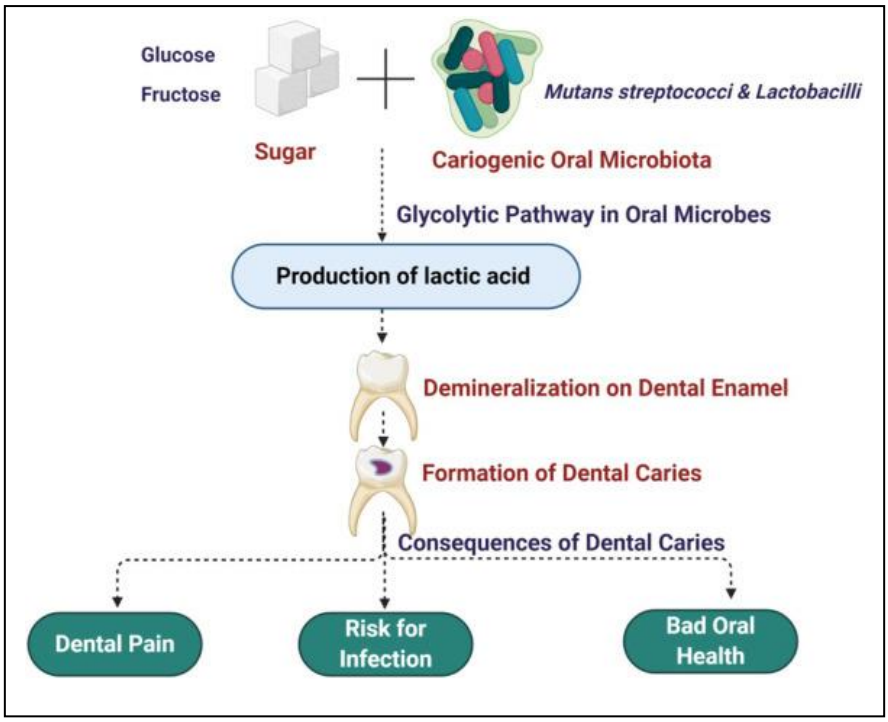

Fig. No. 2: Dental Plaque Formation.

Plant

Figure No. 3: ***Brassica oleracea var. botrytis.***

Taxonomy of Cauliflower

- Kingdom: Plantae
- Division: Magnoliophyta
- Class: Magnoliopsida
- Order: Capparales
- Family: Brassicaceae (mustard family)

- Genus: Brassica
- Species: B. oleracea
- Variety: botrytis

Cauliflower (Brassica oleracea var. botrytis L.), commonly called “Phoolgobhi,” is among the most familiar vegetables in India, first introduced in 1822 by Dr. Jemson, then in charge of the Botanical Garden at Saharanpur, Uttar Pradesh. It traces its origin to wild cabbage (*Brassica oleracea* var. *sylvestris*), with Cyprus considered its primary center of origin. Cauliflower is a monogenomic species belonging to the ‘C’ genome, with a chromosome number of n=9. Unlike other brassicaceous crops, it requires specific climatic conditions for the proper development of its economic part—the curd (Vanlalneihi et al., 2017).[4] Botanically, the edible portion is the prefloral fleshy apical meristem, flowering primordial, or immature inflorescence.(Topwal, Singh and Shanker, 2019)

The crop is highly sensitive to adverse environments, and factors such as extreme temperatures and drought often cause premature curd formation, reducing economic yield. Cauliflower is cultivated as a herbaceous annual for curd production and as a biennial for seed production. emphasized that wider variability within a crop enhances the chances of selecting superior genotypes. Wright pointed out that heritability consists of additive and non-additive components, of which the additive part primarily responds to selection. To assess the effectiveness of selection, estimating expected genetic advance is crucial. Burton and DeVane further highlighted that combining genetic coefficient of variation with heritability estimates provides a dependable measure of potential improvement through selection. They also notedthat expected genetic gain under a given system offers breeders practical guidance.(*A Study on Antioxidant and Antimicrobial activity of Organic and Inorganic Cauliflower*, no date)

Crop improvement is an ongoing process, and plant breeders continually work to develop high-yielding, adaptable, and superior varieties or hybrids. Their success largely depends on the genetic variability available in the crop and the strategic use of crop improvement techniques to achieve breeding goals.(Kaviyarasi and Subapriya, 2021)

Chemical Constituents

Cruciferous vegetables such as broccoli, kale, cauliflower, cabbage, and radish are well recognized for their numerous health benefits. Our research group focuses on the neuroprotective potential of broccoli sprouts (BrSps) during pregnancy. In rodent models of placental insufficiency and fetal inflammation, we observed that consuming BrSps in the final trimester significantly reduces pathological brain injury and related behavioral issues. The bioactive compound responsible is **sulforaphane (SFA)**, an isothiocyanate derived from the enzymatic hydrolysis of the glucosinolate **glucoraphanin (GRA)** by **myrosinase**. Glucosinolates, sulfur- and nitrogen-containing glycosides, are almost exclusively present in cruciferous vegetables. When plant tissues are disrupted, myrosinase converts glucosinolates into various products, primarily isothiocyanates (ITCs), which are nutritionally and pharmacologically important. The type of hydrolysis product formed depends on factors such as side-chain structure, pH, and the presence of myrosinase-associated proteins like epithiospecifier protein (ESP). Plants with ESP predominantly form nitriles and epithionitriles, which lack known health benefits.

Isothiocyanates, including SFA, exhibit diverse biological activities such as anticancer, antioxidant, anti-inflammatory, anti-diabetic, antimicrobial, antihypertensive, and neuroprotective effects, with strong emphasis on cancer chemoprevention. SFA has shown therapeutic promise in conditions like hypoxic-ischemic encephalopathy,

neurodegenerative disorders, hypertension, diabetes, autism spectrum disorder, and cancer. Its main mechanism involves activation of phase-II detoxification enzymes through the Nrf2 pathway and enhanced synthesis of glutathione, a key endogenous antioxidant. Kale is another rich source of glucoraphanin and SFA. A structurally similar compound, **sulforaphene (SFE)**—mainly found in radishes—shares similar benefits, including potent chemopreventive and antioxidant effects. Both SFA and SFE are among the most studied natural compounds for cancer prevention.

Although cruciferous seeds contain higher glucosinolate concentrations than sprouts, they also accumulate significant amounts of **erucic acid**, an antinutritional fatty acid linked to health risks such as myocardial lipidosis and cardiac lesions. Sprouting reduces erucic acid content, making sprouts safer for consumption. Therefore, when using cruciferous materials in health research, both beneficial and potentially harmful constituents must be considered.

While SFA and SFE are major bioactive compounds in broccoli, kale, and radish, additional phytochemicals, especially phenolics, contribute synergistically to health effects. Phenolics are potent antioxidants that scavenge free radicals, chelate metals, and modulate cellular signaling pathways, including phase-II enzyme activation. Studies indicate that whole broccoli sprouts are more effective at enhancing antioxidant gene expression compared to isolated glucoraphanin and myrosinase, likely due to these synergistic interactions.

Quercetin, a powerful antioxidant flavonoid and natural plant pigment present in broccoli, helps regulate key inflammatory processes. Broccoli extract influences multiple signaling pathways, including nuclear factor kappa B (NF-κB) and mitogen-activated protein kinases (MAPKs), both of which are essential for activating genes associated with inflammation. Through the modulation of these pathways, broccoli extract aids in managing and controlling inflammatory responses. The amount of Quercetin present in cauliflower is 29.3mg/100g. (Shrivastava *et al.*, no date)

Activities Shown by Brassica oleracea var. botrytis

Antiamnesic Effect

The antiamnesic activity of broccoli leaves (*Brassica oleracea var.*botrytis) was investigated through both **in vitro** and **in vivo** studies using amyloid-beta (Aβ)-induced neurotoxicity models. The chloroform fraction of broccoli leaves (CBL) demonstrated significant neuroprotection and strong acetylcholinesterase (AChE) inhibitory effects. The ability of CBL to alleviate Aβ1-42-induced cognitive impairment was assessed using behavioral tests such as the **Y-maze**, **passive avoidance**, and **Morris water maze**, which revealed improved learning and memory functions in treated groups. Post-behavioral analyses showed enhanced antioxidant activity (measured by SOD, GSH/total GSH ratio, and MDA levels) and AChE inhibition in brain tissue. Using Q-TOF UPLC-MS, **oxo-dihydroxy-octadecenoic acid (oxo-DHODE)** and **trihydroxy-octadecenoic acid (THODE)** were identified as major compounds. These findings suggest that CBL may serve as a natural candidate for managing Aβ1-42-related cognitive decline.

Antioxidant Effect

The antioxidant properties of *Brassica oleracea* var.botrytis extracts were evaluated. All extracts exhibited radical scavenging activity, with the highest activity recorded in extracts from **run 2**, obtained via supercritical CO_2 extraction. This method proved to be an effective alternative to conventional extraction, enabling the isolation of bioactive compounds with potential industrial and pharmacological applications.

Antiproliferative and Chemoprotective Activities

Biofortification of Cauliflower with selenium significantly enhanced phenolic content and antioxidant activity, particularly in seedlings, and exhibited strong antiproliferative effects. Additionally, extracts enriched with phenolics induced **Nrf2 signaling activation** in SH-SY5Y cells exposed to Aβ fragments, supporting neuroprotective roles.
Cauliflower sprout juice also showed renal protective effects in hypertensive models via **AMPK/SIRT1/PGC1α/PPARα/UCP2** pathway activation, suggesting therapeutic potential against organ damage.

Red and green kohlrabi extracts demonstrated **anti-inflammatory, antioxidant, and antidiabetic** properties, with red kohlrabi exhibiting higher total phenolic content and stronger bioactivity. Similar effects were observed in cabbage varieties, indicating their potential in reducing oxidative stress-related diseases such as cancer and cardiovascular disorders.

Histological studies confirmed the protective role of **red cabbage** against dyslipidemia and liver damage in cholesterol-fed rats, largely attributed to its antioxidant activity.

Antigenotoxic Effects

Hydroalcoholic extracts of *B. oleracea* leaves exhibited significant antigenotoxic properties by reducing DNA damage induced by doxorubicin in mice without showing genotoxic or clastogenic effects. These findings highlight the pharmacological relevance of broccoli extracts in cancer prevention.

Antimicrobial Activities

Cauliflower exhibits notable antimicrobial activity, mainly attributed to its abundance of bioactive constituents such as polyphenols and glucosinolates. Research indicates that it can suppress and eliminate various foodborne bacteria and fungi.

Investigations have revealed that cauliflower extracts are effective against several pathogenic microbes:
Bacillus subtilis: Ethanol extracts derived from cauliflower florets displayed strong antibacterial effects against this Gram-positive bacterium.

Candida albicans: Ethanol extracts from cauliflower flowers showed antifungal activity against this pathogenic yeast.
Escherichia coli: Certain strains, including *E. coli* O157:H7, were found to be inhibited by cauliflower extracts.(Kaviyarasi and Subapriya, 2021)

HERBAL MOUTH WASH

A herbal mouthwash is an antiseptic liquid preparation for oral hygiene made from plant extracts and essential oils like neem, clove, and peppermint.

Herbal mouthwashes can be used as an adjunct to various oral hygiene practices like tooth brushing, flossing. It's proven that they have effective anti-inflammatory, anti-plaque properties and hence can be used in supportive periodontal therapy. It does not contain alcohol, artificial preservatives, flavors or colors. Hence Herbal mouthwashes can be considered an alternative to chemical mouthwashes in sustaining oral hygiene, especially because of the added advantages provided by herbal preparations (Mcgrath *et al.*, 2023a)

Fig. No. 4: Marketed Herbal Mouthwash.

Types of Mouthwash

- **Fluoride mouthwash**

This type contains fluoride salts that help protect teeth from cavities. Since fluoride is also present in toothpaste and drinking water, its use should be monitored carefully, as excessive intake may harm overall health.

- **Antiseptic mouthwash**

The most common variety, usually containing alcohol, is used to prevent bacterial growth in individuals with oral infections. It is also effective for people with halitosis (bad breath). When combined with proper brushing and flossing, it helps control bacteria responsible for infections and unpleasant odor.

- **Cosmetic mouthwash**

This type has little to no therapeutic effect on oral health but temporarily freshens breath and masks bad odor.

- **Natural mouthwash**

Made from natural ingredients, this option offers similar benefits as other mouthwashes but is considered safer and alcohol-free. It is increasingly preferred by those looking for a gentle and more natural alternative.(Kshirsagar *et al.*, 2021a)

USES OF MOUTHWASH

The use of mouthwashes should be based on an accurate diagnosis of the oral condition and a proper understanding of the product. When choosing a mouthwash, several factors must be considered, including the patient's oral health status, risk of disease, effectiveness and safety of the formulation, and the individual's ability to maintain proper oral hygiene. Additional aspects such as the state of the teeth, gums, and oral mucosa, along with the potential benefits and side effects of the product, should also be evaluated. Mouthwashes are intended for short-term use and should never replace regular oral hygiene practices.(Kshirsagar *et al.*, 2021b)

They may be recommended in the following situations:

- Halitosis
- Mucositis
- Periodontal diseases
- Gum infections
- Xerostomia
- Cleaning of septic sockets
- Vincent's angina
- Plaque control
- Relief of tooth pain
- Fluoride delivery for caries prevention
- Reduction of inflammation (Mcgrath *et al.*, 2023a)

Advantages of Herbal Mouthwash

- Herbal mouthwashes are alcohol-free, non-staining, and non-irritating, making them preferable to chemical alternatives.
- They produce little to no side effects and are generally safer.
- Most herbal formulations are free from alcohol and sugar.
- They are mild and suitable even for sensitive mouths.
- Herbal mouthwashes possess natural antibacterial properties.
- They do not contain harsh chemicals or additives.
- Unlike chemical mouthwashes, they do not cause dry mouth.
- Their popularity and demand are steadily increasing.
- They help maintain oral health and prevent plaque buildup.(Ingale *et al.*, no date)

Disadvantages of mouthwash

- dental staining
- burning sensations from alcohol
- taste disturbances
- potential links to increased risks of certain cancers, prediabetes/diabetes
- sepsis(Ingale *et al.*, no date)

MATERIALS AND METHODS

Maceration Process

1. The Brassica oleracea var. italica florets were cleaned and cut into small pieces separated from their stems.
2. 500 grams of Brassica oleracea var. italica florets were weighted and then grind them to achieve the desired particle size for maceration.
3. Add 500ml of ethanol in ratio of 1:1 proportion as maceration solvent.
4. Put in this mixture in iodine flask for 7 days with occasional shaking
5. After 7days, filter the solution and evaporate the solvent (ethanol) using hot plate to get extract.
6. Extract was collected and stored in tightly closed container at 4°C.

Fig. No. 5: Extraction of Cauliflower using Maceration.

Fig. No. 6: Cauliflower Extract.

Phytochemical screening

Tests for Tannins and Phenols

1. **Ferric Chloride Test:** A small fraction of extract is dissolve in about 2ml of distilled water and filter.the filtrate is treated with the ferric chloride solution.Blue to bluish green colour indicates the presence of tannins and phenolic compounds.
2. **Lead Acetate Test:** A small fraction of extract is dissolve in about 2ml of distilled water and filter.the filtrate is treated with the ferric chloride solution.Brownish yellow ppt indicates the presence of tannins and phenolic compounds.

Test for Alkaloids

1. **Mayer's Test:** Extract was treated with Mayer's reagent. Formation of clear solution indicates the presence of alkaloids.
2. **Wagner's Test:** Extract was treated with Wagner's reagent. Formation of reddish colour precipitate indicates the presence of alkaloids.
3. **Hager's Test:** Extract was treated with Hager's reagent. Yellow colour ppt indicates the presence of alkaloids.

Test for Saponins

1. **Foam Test:** Extract was treated with 2ml of distilled water, small quantity of sodium carbonate and shaking. Foam formation indicates the presence of saponins.

Tests for Flavonoids

1. **Shinoda Test:** Extract was treated with 5ml 90% ethanol, conc. HCL and 0.5g magnesium turnings. Orange colour indicates the presence of Flavonoids.
2. **Sulphuric Acid Test:** Extract was treated with 66% sulphuric acid. Red colour indicates the presence of Flavonoids.
3. **Lead Acetate Test:** Extract was treated with lead acetate solution.Yellow coloured ppt indicates the presence of Flavonoids.
4. **Ferric Chloride Test:** Extact was treated with 5% ferric chloride solution.Brown colour ppt indicates the presence of Flavonoids.

Test for Terpenoids

1. **Sulfuric Acid Test:** Extract was treated with2ml of chloroform ,3ml of strong sulfuric acid solution.Reddish - brown hue of the interface indicates the presence of terpenoids (Statilko *et al.*, 2024)

Antimicrobial Property- Brassica oleracea var. botrytis extract

The anti-bacterial property is determined by the zone of inhibition formed by the microorganisms to a specific concentration of sample having known activity. There are different types of methods for microbiological assay of antibiotics like cup plate method and disc diffusion method. In cup plate method, anti-bacterial sample containing cylinder is diffused into agar layer containing the microorganisms. The zone is formed around the cylinder. The other method is disc diffusion method where zone of inhibition is measured around the antibiotic disc. The basic objective is to study various methods of microbiological assay**.**

Requirements

Organism: Escherichia coli, staphylococcus aureus

Chemicals: Nutrient agar medium

Apparatus: Borer, petri plate

Procedure: Cup-plate method Prepare nutrient agar inoculated with test organism, with a depth of 4-5mm and then allow it to solidify. Then with the help of sterile borer make cavity in one portion. Then fill cavity with standard solution. Then incubate the plate at 37°C for 24 hours. After incubation measure the zone of inhibition.(Balouiri, Sadiki and Ibnsouda, 2016)

Material and Method for development of formulation

Plant material

Among the most crucial substances employed in the current investigation to develop a Mouth Wash wa**s Brassica oleracea var. botrytis** extract

Glassware's

Beaker, glass rod, measuring cylinder, mortar and pestle, funnel.

Chemicals

Glycerin, Ethanol, Sorbitol Solution, Pepppermint Oil, Sodium Lauryl Sulphate, Sodium Benzoate, Sodium Bicarbonate, Brilliant Blue Colour, Purified Water.

Method of Preparation

The Mouth wash was formulated as follows:

The mouthwash was formulated by initially dissolving the necessary excipients, including humectants like glycerin and sorbitol solution, in purified water to create a uniform base. Ethanol was added as a solvent and preservative to support the stability and extraction of the herbal components. The herbal extract was then incorporated and thoroughly mixed to ensure a homogeneous solution. Surfactants, such as sodium lauryl sulphate, were included to improve foaming and cleansing properties. Sweeteners and flavoring agents, including peppermint oil, were added to enhance taste. Preservatives like sodium benzoate and stabilizers such as sodium bicarbonate were included to ensure microbial safety and maintain pH balance. Finally, coloring agents, such as brilliant blue, were added to achieve the desired appearance. The complete formulation was mixed uniformly, checked for clarity, and stored in sterile containers suitable for oral use. (Jagdale *et al.*, 2023)

Formulation Table

Table No. 1: Formulation Table.

Sr. No.	Ingredients	F1	F2	F3	F4	F5	Role
1.	Brassica oleracea var. botrytis extract	-	0.25gm	0.50gm	0.75gm	1.00gm	API, Antimicrobial
2.	Ethanol	10ml	10ml	10ml	10ml	10ml	Solvent
3.	Glycerin	5ml	5ml	5ml	5ml	5ml	Humectant
4.	Sorbitol Solution	10ml	10ml	10ml	10ml	10ml	Sweetener
5.	Peppermint Oil	0.05ml	0.05ml	0.05ml	0.05ml	0.05ml	Flavouring Agent
6.	Brilliant Blue Colour	Q.S	Q.S	Q.S	Q.S	Q.S	Aesthetic Appeal
7.	Sodium Lauryl Sulphate	0.2gm	0.2gm	0.2gm	0.2gm	0.2gm	Surfactant
8.	Sodium Benzoate	0.1gm	0.1gm	0.1gm	0.1gm	0.1gm	Preservative
9.	Sodium Bicarbonate	0.2gm	0.2gm	0.2gm	0.2gm	0.2gm	Mild Cleanser
10.	Purified Water	Q.S to 100ml	Q.S to 100ml	Q.S to 100ml	Q.S to 100ml	Q.S to 100ml	Vehicle
11.	Total	100ml	100ml	100ml	100ml	100ml	-

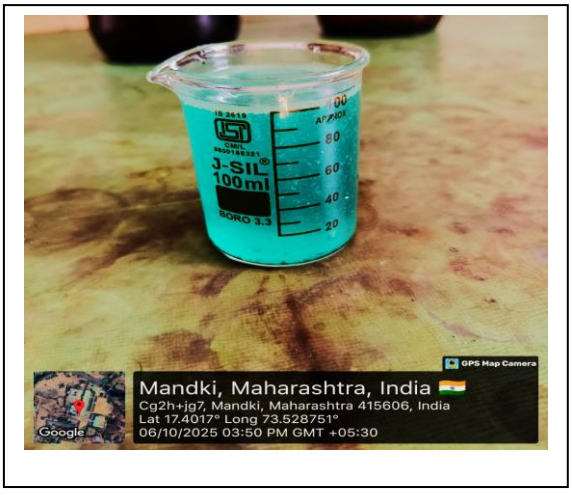

Fig. No. 7: Mouthwash of Brassica oleracea var. botrytis.

Evaluation Tests for Herbal Mouthwash

- **Colour and Odour:** The physical characteristics, such as colour and odour, of the herbal mouthwash can be assessed through visual inspection.
- **Appearance and homogeneity:** Appearance and homogeneity was evaluated by visual inspection**.**
- **pH:** The pH of the mouthwash can be determined using a pH meter, which should first be calibrated with standard buffer solutions. A measured portion of the mouthwash is diluted in distilled water, and the pH is recorded to assess its suitability for oral use.
- **Antimicrobial Test:** The mouthwash can be evaluated for antimicrobial contamination by applying it to agar plates, which are then incubated at 37°C for 24 hours. After incubation, the plates are examined for microbial growth and compared with control samples.
- **Stability Testing:** Stability testing is performed to ensure that the herbal mouthwash maintains its physical and chemical integrity under environmental conditions. This assessment helps determine the product's shelf life, safety, and overall stability over time.

RESULT AND DISCUSSION

PHYTOCHEMICAL SCREENINGS OF EXTRACT

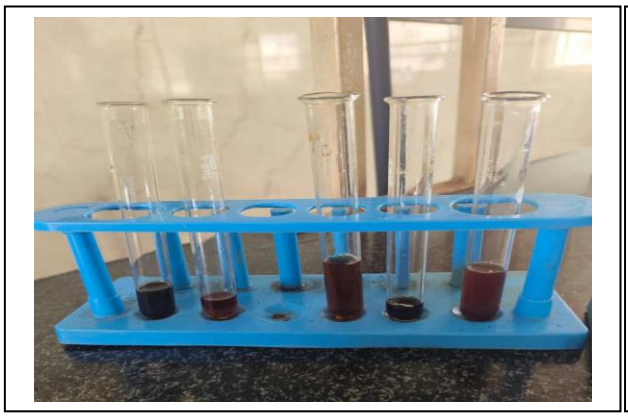

Fig. No. 8: Test for Alkaloids + Flavonoids.

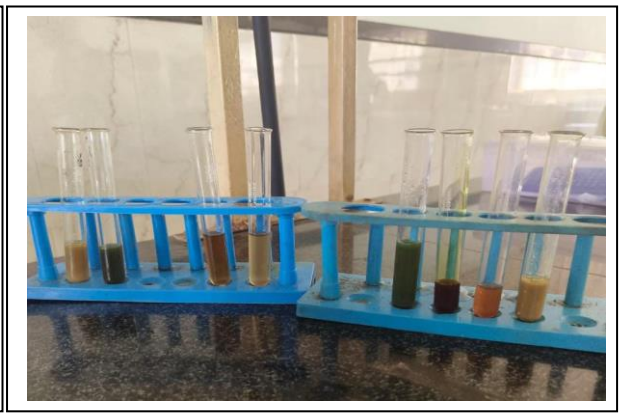

Fig. No. 9: Test for Terpenoids + Tannins.

Fig. No. 10: Test for Saponins.

Table No. 2: Phytochemical Screening Of Extract.

Sr. no.	Phytoconstituent	Test	Obsevation	Inference
1.	Tests for Tannins and Phenols	Lead acetate solution	Brownish yellow ppt appeared	+
		5% FeCl3 solution	Bluish black colour appeared	+
2.	Test for Alkaloids	Mayer's Test	Cream colour ppt appeared	+
		Wagner's Test	Reddish brown ppt	_
		Hager's Test	Yellow colour ppt appeared	+
3.	Test for Saponin	Foam Test	Persistent foam was observed	+
4.	Test for Flavonoids	Sulphuric Acid Test	Red colour appeared	+
		Ferric Chloride Test	Brown colour appeared	+
		Lead Acetate Test	Yellow coloured ppt appeared	+
		Shinoda Test	crimson red	_
5.	Test for Terpenoids	Sulfuric Acid Test	Reddish-Brown hue of the interface appeared	+

Organoleptic Evaluation

Sr. No	Evaluation Parameter	Result Obtained
1.	Colour	Sky Blue
2.	Odour	Pleasant odour
3.	Texture	Smooth texture

Appearance and Homogeneity

Sr. No.	Evaluation Parameter	Result Obtained
1.	Appearance	Good
2.	Homogeneity	Good

pH

The pH was determined using digital pH meter and the pH of the formulated Face wash was found to be **6.75**

Stability studies

The stability of hand wash was carried out by storing measured amount of hand wash in different temperature i.e. 25°C, 37°C, 40°C for 1 week. During stability studies no change in colour and no phase separation were observed in the formulation.

Anti-Microbial Property

Anti-bacterial property of herbal extract was determined by using herbal extract against E.coli.

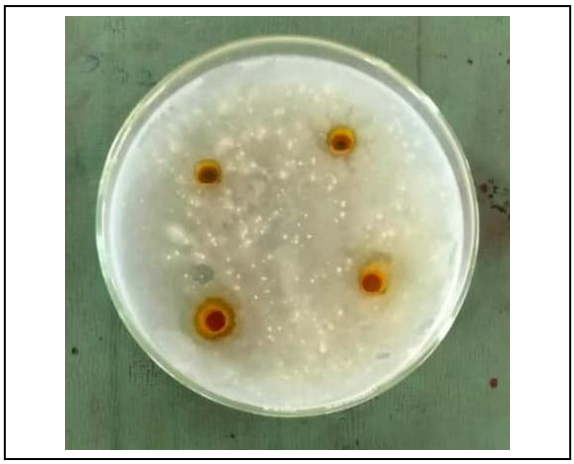

Fig. No. 11: Zone of inhibition of herbal extract against E.coli was found to be 18mm.

Anti-bacterial property of hand wash was determined by using prepared mouthwash in ethanol extract against E**.**coli.

Fig. No. 12: Zone of inhibition of mouthwash prepared by Cauliflower extract using ethanol as Solvent against E.coli was found to be 25mm.

Fig. No. 13: Zone of inhibition of solvent Ethanol against E.coli was found to be 13mm.

CONCLUSION

The herbal mouthwash formulated with *Brassica oleracea* var. *botrytis* (cauliflower) extract demonstrated notable antimicrobial potential, suggesting its suitability as a natural substitute for synthetic mouthwash products. The bioactive compounds present in cauliflower, including glucosinolates, flavonoids, phenolic acids, and isothiocyanates, are responsible for its strong antimicrobial and antioxidant effects, effectively reducing the growth of oral microorganisms associated with dental plaque, gingivitis, and halitosis. Since maintaining proper oral hygiene is essential to prevent microbial infections in the oral cavity, this herbal formulation not only cleanses the mouth but also offers protection

against harmful pathogens. The amount of Quercetin present in cauliflower is 29.3mg/100g which is best as antimicrobials.

The mouthwash was carefully developed to ensure compatibility with the sensitive oral mucosa, thereby minimizing any risk of irritation or unwanted side effects often linked to chemical-based formulations. Moreover, it provides a gentle, soothing, and refreshing effect, maintaining moisture and promoting a clean oral environment. Thus, it can be concluded that the mouthwash prepared from *Brassica oleracea* var. *botrytis* extract is a safer, more natural, and effective alternative compared to conventional mouthwashes. The presence of key phytoconstituents, particularly phenolic compounds and glucosinolates, plays a vital role in imparting antimicrobial activity. Therefore, *Brassica oleracea* var. *botrytis* holds great promise as a natural source for developing safe, eco-friendly, and efficient herbal mouthwash formulations that support oral health and hygiene.

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