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In vitro anti-cancer and antimicrobial activities of *Abelmoschus esculentus* root extracts

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Abstract

This study explored the potential of *Abelmoschus esculentus* for treating oral squamous cell carcinoma and associated risk factors. The extract, obtained via soxhlet extraction, was formulated into a carbopol 940 gel for topical and oral mucosal delivery. Phytochemical screening revealed alkaloids, flavonoids, carbohydrates, and phenolic compounds. The Folin-Ciocalteu method showed high total phenolic content (R^2=0.9953). The extract exhibited promising *in vitro* anti-cancer activity against a squamous cell carcinoma cell line, achieving a 52.9% inhibition rate at the highest concentration 100 µg/mL. Additionally, the extract demonstrated potential antibacterial activity against *Helicobacter pylori* ATCC 43501 compared to clarithromycin. The extract-loaded gel formulation with 1.5% carbopol was developed, exhibiting optimal viscosity (1245 cps) and a suitable pH (6.45) for topical use.

Introduction

Oral cancer is a significant global health concern, ranking among the most common malignancies affecting the head and neck region. It primarily arises from the squamous epithelium of the oral mucosa. It is often associated with risk factors such as tobacco use, alcohol consumption, human papillomavirus (HPV) infection, and prolonged exposure to carcinogenic substances. Despite advancements in conventional treatments such as surgery, radiation, and chemotherapy, these approaches often lead to severe adverse effects, including mucositis, and compromised immune function (Badwelan et al., 2023)

Plant-based formulations have gained significant attention in cancer research due to their bioactive compounds, which exhibit potent anti-cancer and antimicrobial properties. Herbal gels, enriched with phytochemicals, offer a promising therapeutic approach by delivering active agents directly to the affected site, enhancing bioavailability, and minimizing systemic side effects (Shrihastini et al., 2021). These formulations not only target cancerous cells through apoptosis and inhibition of cell proliferation but also help combat secondary infections commonly associated with oral malignancies (Asma et al., 2022).

This study focuses on the *in vitro* anti-cancer and antimicrobial activity of a plant-based gel on the squamous epithelium, highlighting its potential as a novel adjunct therapy for oral cancer treatment (Bajpai et al., 2024). By integrating natural compounds with proven medicinal properties, this gel could be an effective, non-invasive alternative for managing oral cancer while preventing microbial infections that can complicate disease progression (Popovici et al., 2022).

The medicinal properties of *Abelmoschus esculentus* are attributed to its rich array of bioactive substances, such as polyphenols, polysaccharides, and flavonoids (Liu et al., 2021). Numerous biological properties of these substances, including immunomodulatory, anti-inflammatory, and antioxidant effects (Kwok et al., 2025). *A. esculentus*, also known as lady's finger, gumbo, or okra, or bhindi in South Asia. Traditionally, parts of the plant are believed to have medicinal properties, including antioxidant, antispasmodic, demulcent, diaphoretic, diuretic, emollient, and stimulant effects. *A. esculentus* is a source of protein, vitamins C and A, iron, calcium, and dietary fibers. It contains large quantities of glycans responsible for the viscosity and gum-like consistency of its aqueous suspension (Seifu, 2017).

Materials and Methods

Chemicals

Dimethyl sulfoxide and MTT (3-(4,5-dimethylthiazol-2-yl)2-5-diphenyltetrazolium bromide) were procured from Sigma Aldrich India. MCF7 cancer cell line was purchased from the National Centre for Cell Sciences, Pune. Carbapol 940 and tri-ethanolamine were obtained from the central store of Appasaheb Birnale College of Pharmacy, Sangli. All chemicals were of analytical grade.

Collection and authentication of plant

A. esculentus was collected in January from Sangli District. It was authenticated by a local botanist. The sample was provided in a herbarium sheet. Further dried root parts were grinded into a fine powder using a pulverizer.

Preparation of extract

A soxhlet apparatus was used to 500 g of root parts powder using 300 to 400 mL of ethanol. The solvent was then evaporated under decreased pressure using a rotary evaporator (Labline PBU 6D, India) to produce the extract. Finally, the yield was recorded (Kothari et al., 2012).

Total phenolic contents

Standard solutions of gallic acid in ethanol, such as 10, 20, 40, 60, 80, and 100 µg/mL were prepared. Folin-Ciocalteu reagent (2.5 mL) (diluted 1:10) was mixed with 0.5 mL of ethanolic extract or standard in the test tube. Let the mixture react for 5 min at room temperature. Sodium carbonate (7.5% w/v, 2.0 mL) solution was added (Kamal et al., 2025) and then placed in the dark. The absorbance was measured with a UV-Vis spectrophotometer at 765 nm. To determine the sample's total phenolic content, the gallic acid standard curve was used. The results were expressed as mg GAE/g of extract (Olugbami et al., 2015).

Total flavonoid contents

A standard quercetin or rutin solution in methanol was made with concentrations between 10 and 100 µg/mL. One mL of the standard solution or ethanolic extract was taken. Five minutes of incubation were required after adding sodium nitrate (0.3 mL, 5%). Another 5 min was incubated after adding aluminum chloride (0.3 mL, 10%). To the mixture, add 2.4 mL of distilled water and 2 mL of 1M sodium hydroxide. After thoroughly mixing, let it rest at room temperature for 15 min. A UV-Vis spectrophotometer was used to measure the absorbance at 415 nm. The TFC was expressed as mg of quercetin equivalent (QE) or rutin equivalent (RE) per gram of extract (mg QE/g or mg RE/g) (Saeed et al, 2012).

Preparation and evaluation of carbopol gel of extract

Carbopol 940 (1-2%) was dissolved in distilled water.

Box 1: MTT assay

Principle

The MTT assay is a colorimetric assay frequently used to evaluate cell viability and proliferation.

Requirements

A. esculentus root extract; CO_2 incubator; Dimethyl sulfoxide (DMSO); 5-Fluorouracil; Human oral squamous carcinoma cancer cell line OECM-1; MTT (**3-**(4,5-dimethylthiazol-2-yl)-2,5 -diphenyltetra-zolium bromide); microplate ELISA reader

Procedure

Step 1: The human oral squamous carcinoma cancer cell line OECM-1 was plated into a 96-well plate with a flat bottom at a density of 1.0×10^4 cells/well

Step 2: Grown at 37°C in a humidified 5% CO_2 incubator

Step 3: On the following day, the cells were subjected to different doses of the different solvent extracts at 10, 20, 40, 80, and 160 µg/mL.

Step 4: As control~~s~~, well-containing cells treated with DMSO (0.5%) as opposed to a test chemical was used.

Step 5: 5-Fluorouracil was employed as the norm.

Step 6: Following a 24-hour treatment with the test chemical, 10 µL of the MTT solution (0.5 mg/mL) was added to the wells, and for 4 hours, the plates were incubated at 37°C.

Step 7: After the supernatant was removed, 100 µL of DMSO was used to dissolve the violet formazan crystals and a microplate ELISA reader (Benspera E21, India) was used to quantify the amount of adsorption at 570 nm.

Step 8: Utilizing the dosage response inhibition curve, the IC_{50} values were determined

Reference

Patil and Wadkar, 2024

Stir continuously and let it swell for 4-6 hours for proper gel formation. Ethanol extract (5%) was added using stirring continuously at 2,000 rpm using a homogenizer. The pH was adjusted using triethanolamine (TEA) (pH should be around 5.5–6.5). It was mixed until a smooth, uniform gel cream was formed (Khan et al., 2022).

Antibacterial activity

Incubated *H. pylori* in brain heart infusion (BHI) broth with 10% fetal bovine serum and then incubated for 48 hours at 37°C under microaerophilic conditions (5%O_2, 10%CO_2, and 85%N_2). Spread the bacterial suspension uniformly over Mueller-Hinton agar (MHA) that had been prepared with 5% sheep blood. Using a sterile cork borer, create wells (6 mm diameter) in the agar. Add 50–100 µL of the test compound at different concentrations into the wells. The standard antibiotics (clarithromycin) were considered as positive controls and the solvent as negative control. Plates were incubated under microaerophilic conditions at 37°C for 48–72 hours. The zone of inhibition (in mm) around each well was measured. The result of control was compared with test extract and standard antibiotic to determine antibacterial efficacy.

Statistical analysis

Data were shown as mean ± SD. For data analysis, three duplicates of the results were made. The differences between each circumstance were identified using a one-way analysis of variance (ANOVA). The graph was shown using Graph Pad Prism 6.01 software (USA). A p -value of less than 0.05 was deemed significant.

Results

%Yield of extract

The percentage yield of the *A. esculentus* ethanol extract was 18%.

Total phenolic contents

The ethanol extract contained 97.6 mg GAE/g dry weight of phenolic compounds, indicating a substantial presence of phenolic constituents that might contribute to the observed biological activities of the extract.

Total flavonoid contents

The flavonoid content of the extract was calculated to be approximately 78.3 mg quercetin equivalents (QE) per gram of dry weight (mg/g dry weight).

Squamous cancer cell line inhibition analysis

Table I depicts the percentage inhibition of the standard drug (5-fluorouracil) and *A. esculentus* extract against a squamous cancer cell line. The maximum concentration (100%) of 5-fluorouracil resulted in a 78.7% inhibition rate with a strong correlation coefficient (R^2 = 0.9853). In comparison, the *A. esculentus* extract achieved a maximum inhibition of 52.94% at the same concentration, with a good correlation coefficient (R^2 = 0.9608).

Antibacterial activity of test compound against H. pylori

Strong antibacterial activity was shown by the 26 mm zone of inhibition displayed by the standard antibiotic clarithromycin (Figure 1). With a zone of inhibition of 18 mm, the test extract at 500 µg/mL showed significant antibacterial activity against *H. pylori*. The absence of an inhibitory zone in the negative control (solvent) demonstrated that the extract's active ingredient was the cause of the antibacterial action.

The extract displayed a zone of inhibition superior to the standard antibiotic clarithromycin, indicating promising *in vitro* antibacterial activity.

Prepared carbopol 940 gel

The results revealed that the gel with 1% concentration had a viscosity of 585cP, which does not have a gelling

Table I

Effects of extracts against squamous cancer cell lines

Sample	Concentration (µg/mL)	Absorbance (mean ± SD)	%Inhibition	IC_{50} (µg/mL)
Control				-
5-Fluorouracil	20	0.74 ± 0.02	43.5	32.2
	40	0.63 ± 0.03	52.0	
	60	0.55 ± 0.01	57.9	
	80	0.44 ± 0.03	66.6	
	100	0.28 ± 0.05	78.7	
Extract	20	1.12 ± 0.01	7.2	95.7
	40	1.06 ± 0.02	18.9	
	60	0.92 ± 0.01	30.0	
	80	0.87 ± 0.02	33.4	
	100	0.62 ± 0.03	52.9	

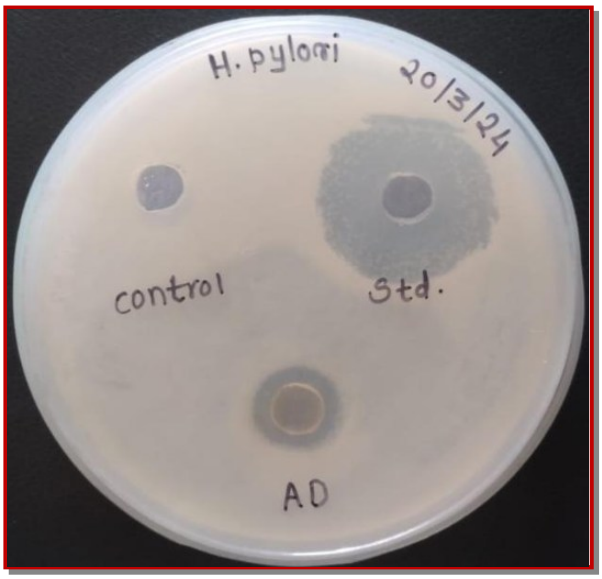

Figure 1: Zone of inhibition of standard (500 µg/mL; clarithromycin) and extract (500 µg/mL) against *H. pylori*

consistency, whereas gel with 2%carbopol concentration had a viscosity of 2375cP, which resembled a hard stiff gel, with 1.5%carbopol showed optimum viscosity of 1245cP (Table II). All three gels had acceptable pH values ranging from 6.8 to 7.4, but the one with 1.5%carbopol concentration had a pH of 7.3, which was near to skin pH, therefore it was chosen and further tested.

Table II

Effects of carbopol against viscosity

%Concentration	pH	Viscosity (cP)
1.0	6.80	585
1.5	6.45	1245
2.0	7.40	2375

Discussion

The present study evaluated the therapeutic potential of *A. esculentus* root extract in the treatment of oral squamous cell carcinoma and associated microbial infections, particularly *H. pylori*. The extract demonstrated significant phytochemical richness, particularly in phenolics and flavonoids, which are known for their antioxidant and therapeutic benefits. Suggesting the extract's ability to neutralize reactive oxygen species, a major contributor to cancer progression and cellular damage (Tosun et al., 2025).

The anti-cancer activity observed *in vitro* against oral squamous cell carcinoma cells showed a maximum inhibition rate of 52.9% at full concentration, which, although lower than the standard chemotherapeutic agent 5-fluorouracil (78.7%), remains promising for a natural extract. This supports the hypothesis that *A. esculentus*'s bioactive compounds may suppress tumor cell proliferation and could serve as a complementary therapy with reduced toxicity (Angellotti et al., 2023).

The antibacterial assay further demonstrated the extract's efficacy, particularly against *H. pylori*, a known risk factor in gastrointestinal and possibly oral carcinogenesis. The extract exhibited a larger zone of inhibition compared to clarithromycin, indicating strong antibacterial properties and supporting its use in managing secondary infections in oral cancer patients (Elbestawy et al., 2023).

Formulation of the extract into a carbopol 940 gel optimized at 1.5% concentration resulted in a stable product with suitable viscosity (1245 cP) and pH (7.3), aligning with mucosal application requirements. This gel offers a non-invasive, localized delivery method that enhances bioavailability while minimizing systemic side effects commonly associated with conventional treatments (Suzilla et al., 2020).

Overall, these findings highlight the potential of *A. esculentus* as a multifaceted agent in oral cancer therapy due to its antioxidant, anti-cancer, and antimicrobial properties. Triterpenoids and phenolic compounds are key classes of phytochemicals widely studied for their anti-cancer properties. Both act through multiple molecular mechanisms to inhibit cancer progression. Phenolic compounds, such as flavonoids and phenolic acids, exert strong antioxidant activity, neutralizing reactive oxygen species (ROS) that cause DNA damage and contribute to cancer initiation (Abdel-Razek et al., 2023). In addition, they regulate signaling pathways involved in cell proliferation, apoptosis, angiogenesis, and metastasis. Triterpenoids, on the other hand, possess a unique ability to modulate various oncogenic and tumor-suppressor pathways (Yadav et al., 2010). They inhibit nuclear factor-kappa B (NF-κB), a transcription factor often overexpressed in cancers, and induce apoptosis via mitochondrial disruption and caspase activation. Their anti-inflammatory activity also contributes to suppressing tumor-promoting inflammation. The combination or co-existence of these compounds in plant extracts, such as that of *A. esculentus,* may offer synergistic effects. Phenolics provide oxidative protection and support immune function, while triterpenoids actively interfere with cancer cell signaling and survival.

Limitations of this study are as follows: a) to identify phyto-constituents using thin-layer chromatography, b) study using animal model, and c) several doses of extracts to examine the antimicrobial effect.

Conclusion

This study highlights the potential of *A. esculentus*

extract in treating oral squamous cell carcinoma. The extract, rich in phenolics and flavonoids, showed notable antioxidants, antibacterial (against *H. pylori*), and moderate anti-cancer activity. A 1.5% carbopol-based gel was successfully formulated with suitable pH and viscosity for topical use.

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Ethical Issue

Cell lines derived from the expansion of primary cell cultures *in vitro* were not relevant material, as all the original cells were divided and so the cell line had been created outside of the human body. The storage and use of cell lines created from primary human tissue, for research purposes, did not require ethical approval.

Conflict of Interest

Authors declare no conflict of interest.

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