ORIGINAL ARTICLE
DOI:10.33897/fujd.v5i2.480

Formulation of Dispersible Tablet Based on Extract from Cassia Fistula Leaves and Evaluation of its Anti-Fungal Effect on Heat Cure Acrylic Resin

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Received: 27 Mar 2025 / Revised: 08 Jul 2025 / Accepted: 24 Jul 2025 / Published online: 30 Jul 2025

ABSTRACT

Objective: The objective of this research paper was to investigate the antifungal outcome of dispersible tablets based on cassia fistula leaf extract on acrylic resin infected with candida albicans.

Materials and Methods: This cross-sectional analysis was piloted at the G7 campus of Riphah International University from January 2017–January 2019._For this, the ethanolic extract of cassia fistula leaves was obtained and the excipients for the tablet along with the extract obtained were mixed. This mixture was then proceeded through a sieve to acquire granules which were dried and compressed. Afterward, acrylic discs were prepared and inoculated with candida albicans. These discs were then soaked overnight in three different tablet solutions including the placebo tablet solution. The discs were removed after 24 hours and inoculated into a specific media, Sabouraud Dextrose Agar.

Results: The formulated tablet based on cassia fistula leaf extract was found to be equally effective when compared to the commercially available tablet (Poligrip) showing no growth of candida albicans on the SDA plates

Conclusion: The study concluded that the formulated denture cleansing tablets based on extract obtained from cassia fistula leaves could be used as a prophylactic denture hygiene measure against candida albicans and staphylococcus aureus. Denture cleansing tablets based on natural sources can be equally effective as compared to chemical-based formulations in addition to being not only non-toxic, and non-irritant, but also cost-effective.

Keywords: Acrylic resin, Antibacterial, Antifungal, Candida Albicans, Cassia fistula, Dental, Denture, Senna extract, Staphylococcus Aureus, Stomatitis.

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DOI:10.33897/fujd.v5i2.480

How to cite this Article:

Hasan U, Ehsan A, Moeen F, Afzaal H. Formulation Of Dispersible Tablet Based on Extract from Cassia Fistula Leaves and Evaluation of its Anti-Fungal Effect on Heat Cure Acrylic Resin. Found Univ J Dent. 2025;5(2):39-45

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INTRODUCTION

Dental surgeons frequently encounter patients with bacterial and fungal diseases of the oral mucosa in association with dental appliances as the denture support functions as a substrate for microbe attachment and biofilm development leading to denture-related stomatitis (DRS) and fungal infections caused as a result of *candida albicans.*[1] Clinicians prescribe antifungals, oral hygiene instructions, and prosthesis adjustments to treat the condition. However, there is a likelihood of *candida* re-colonization after cessation of therapy, making re-treatment common. This exposure of patients to antimicrobials makes them susceptible to adverse effects notably medication resistance.[1]

In contrast, natural herbal alternatives to combat disease have low toxicity levels, reduced effects on the environment, and increased acceptance by the general population which can be used as an alternative to antimicrobial therapy.[2] Dentures are commonly used by the elderly population and owing to their dwindling motor coordination skills, mechanical cleaning is not a viable option. However, denture soaking agents are preferable attributable to their easy handling.[3-4] Commercially available chemical cleansers contain a variety of active agents with favorable results against DRS.[5] Some of the commonly known chemical cleansers include sodium hypochlorite, alkaline peroxide, and glutaraldehyde.[6]

Although chemical cleansers possess antimicrobial activity against denture biofilms they also have toxic effects and their extensive use influences the composition and integrity of acrylic-based dentures.[1,7] The active ingredient in these cleansers leads to several allergic reactions like tissue damage, and the Food and Drug Administration (FDA) warned manufacturers to consider the use of alternate ingredients.[8] Consequently, extensive research is being employed around the world to explore the benefits of natural over synthetic medicines.[9]

Cassia fistula is a plant whose leaf extract has been used as an antibacterial and antifungal agent.[10-11] However, there is no reported literature on the practice of *cassia* leaf extract as a denture cleaner. Therefore, the research paper aimed to establish the anti-fungal potential of the extract of the leaves of *Cassia Fistula* in vitro and to formulate a dispersible tablet based on the extract to be used as a denture cleanser.

MATERIALS AND METHODS

This cross-sectional analysis was piloted at the G7 campus of Riphah International University. Fresh and healthy leaves of the plant cassia fistula were collected and verified by the pharmacognosy department at Riphah Institute of Pharmaceutical Sciences (RIPS) and by the Herbarium of Pakistan, Quaid-I-Azam University, Islamabad vide number 133567. The investigation was endorsed by the Ethical Review Committee of Islamic International Dental College (IIDC), Riphah International University, Islamabad, Pakistan vide letter: IIDC/IRC/2016/001/011.

Formulation of Dispersible Tablets:

To prepare the tablet, Primogel was used at a concentration of 0.9 gm. A mix constituting 7.8 gm of sodium bicarbonate, 3.5 gm citric acid, and 5.2 gm of sodium carbonate was used with Primogel. Methylparaben (0.2 gm) and propylparaben (0.1 gm) were added as antimicrobials while 0.2 gm of sodium lauryl sulfate (SLS) and 0.9 gm of magnesium stearate were added as wetting agent and glidant. All the excipients were blended to reduce particle size. This blend was passed through a sieve. Following this, cassia extract, sodium bicarbonate, citric acid, sodium carbonate, methylparaben, propylparaben, SLS, and primogel were blended and wetted with ethanol (99.9%). The mixture was again passed through a sieve and the extruded particles were desiccated in a dry oven (Sanfa, DHG-9101A 108L) at 37°C for 6 hours.[12] Magnesium stearate was then mixed with the dried granules which were compressed in a single-stroke punch machine (Rogen Pharma, Rawat, Pakistan) to achieve the final form of tablets.

Evaluation of Dispersible Tablets:

Tablets were assessed for mass, width, disintegration, rigidity, and friability. Twenty, randomly selected tablets were individually weighed using an analytical balance (Shimadzu ATX224).[13] Six randomly selected tablets were examined for thickness using a vernier caliper while another six were tested for fragmentation period. Distilled water was used as a fragmentation mode and they were placed inside six chambers of the disintegration apparatus, as recommended by United States Pharmacopic (USP). The average breakdown time was then calculated utilizing the disintegration machine. Also, another six randomly selected tablets were tested

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for hardness using a Monsanto hardness tester. Friability testing was done using a Roche friabilator. Ten tablets were assessed and exposed to a mutual effect of attrition and shock in a plastic compartment. The friabilator was functioned for 100 revolutions and the tablets were then cleaned and reweighed.

Preparation of Teflon Moulds and Heat Cure Acrylic Disks:

Teflon moulds were fabricated according to ISO 1567:1999 with 25.4 mm thickness, 4 mm depth, and 32 mm diameter. Moulds were then flasked with type II gypsum. Heat cure acrylic powder (Meadway Heatcure Supercure) was weighed on a digital weighing scale with an accuracy of 0.1 mg (UniBloc Analytical Balances ATY 224, Shimadzu, Japan). Methyl-methacrylate monomer (Meadway Universal Heatcure) was gauged with a pipette (370710-10, PYREX VISTA, USA). The heat cure fluid was then positioned into a blending container and the powder was scattered on top of the liquid for 30 seconds. The mix was packed at a doughy stage into Teflon moulds. The containers were then placed in a hydraulic bench press (Dental Hydraulic Flask Press, BISON, Intensive Industries, India) at 80 bars of compression for 25 minutes. The containers were then submerged in water (enveloped by 7 cm of water) and treated employing an electrically regulated water bath. After curing, finishing of the acrylic disks was done with an acrylic trimmer. Polishing was completed with pumice slurry atop a lathe polishing buff.[14]

Antifungal Activity of Dispersible Tablets on Heat Cure Acrylic Resin Specimens:

Three acrylic discs were disinfected in an autoclave (SA 230 Taiwan) at 121°C for 15 minutes.

The discs were inoculated with candida which was adjusted to 0.5% McFarland standard as per WHO recommendations, and were incubated for 1 hour at 37°C in an incubator (Model B-53).[15] The discs were then rinsed with distilled water for 15-30 seconds. Three different discs were prepared:

Disc 1: Immersed in placebo tablet overnight (negative control).

Disc 2: Immersed in herbal tablet overnight.

Disc 3: Immersed in a commercial tablet (Poligrip) overnight (positive control).

The discs were removed from tablet solutions the next day and inoculated into a specific media, sabouraud dextrose agar (Oxoid, England), and incubated for 24 hours at 37°C in an incubator (Model B-53 Rmeco). The experiment was conducted in triplicate to confirm the results. Colony forming units (CFU) were measured for all tablets using a CFU counter.

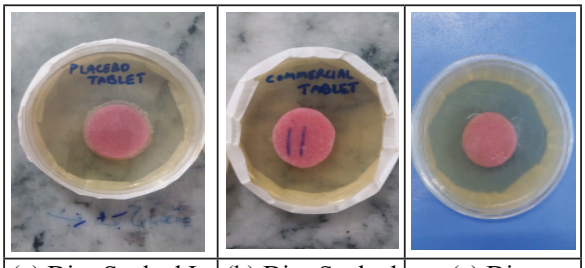
RESULTS

Post Formulation Studies:

For the weight variation test, the mean mass was determined. The mean mass of the tablets remained 380.03 $\pm$ 0.81 mg, the mean thickness was found to be 6.65 + 0.51 mg, the mean time for tablets to disintegrate was found to be four minutes and the mean hardness was found to be 8.25 $\pm$ 0.52 kg/cm^2. The friability was calculated to be 0.7% which lies within the official limits, which is less than 1% according to USP standards.

Antifungal Activity of Dispersible Tablets on Heat Cure Acrylic Resin Specimens:

Three different tablets were tested for antifungal activity. The negative control presented a CFU greater than 600 while the positive control exhibited no CFU. In comparison to the commercial tablets, the herbal tablets were spot zero showing no microbial growth as well. Consequently, no CFU was found on the agar plate after an incubation period of 24 hours at 28°C (Figure-1). The procedure was repeated three times to confirm the results.



(a) Disc Soaked In Placebo Tablet	(b) Disc Soaked In Effervescent Mixture	(c) Disc Soaked In Herbal Tablet

Figure 1: Antifungal Activity of (a) Placebo Primogel tablet; (b) Effervescent mixture and (c) Herbal tablet

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DISCUSSION

Removable acrylic dentures act as reservoirs for the growth of oral microflora.[1] Lack of denture hygiene measures precedes the development of a microbial biofilm atop the fitting side. This biofilm acquires nutrients from human saliva and proliferates as well as matures while enhancing its adherence with the rough denture surfaces.[1,16] Furthermore, as the denture exterior maintains a proximal connection through the mucosa, the biofilm proliferation leads to inflammation of the mucosa, which is a characteristic feature of DRS.[16] The main etiological agent in dental plaque that leads to DRS is *candida*.[17] Increased microbial load leads to various denture-related problems for patients. Consequently, it is of paramount importance for the patient to maintain the hygiene of the denture to minimize the incidence of DRS.[18]

The use of tablets is a highly widespread method of cleaning dentures in technologically advanced countries.[19] Nevertheless, denture cleaning tablets are associated with a high cost if they are to be used regularly. In a developing country like Pakistan, with a significant proportion of the country living below the poverty line, such a cost is not economically feasible for the general population. A systematic survey also reported using toothpaste as the most recommended method of cleaning dentures in developing countries. However, it must be noted that regular use of this method of denture cleaning leads to disruption of the physical properties of the denture surface.[4] For elderly patients, amount and effortless accessibility are significant features when choosing a denture cleaner. The elderly denture-wearing population with financial dependence on others finds it hard to afford denture cleaners so there is a dire need to explore economical denture cleaning tablets, which also have a reduced influence on the physical properties of dentures.[20]

The leaf extract of *cassia* has antiseptic, antifungal, and anti-inflammatory properties in addition to having no reported side effects.[21-23] It has also been used to treat several bacterial and fungal diseases and is cost-effective to prepare tablets using it. Hexane, ethyl acetate, chloroform, methanol, and water extracts of *cassia* flowers have been evaluated for their antifungal and antibacterial activities with significant results for all the extracts. [24-26] In a study by Bhalodia et al., it was reported that *cassia* extract exhibited an extraordinary restrain of bacterial growth against *Staphylococcus Aureus, Streptococcus Pyogenes, Escherichia Coli,* and *Pseudomonas Aeruginosa*. They also reported significant antifungal results against *Aspergillus Niger*, *Aspergillus Clavatus,* and *Candida Albicans*.[24]

The present study identified the antibacterial and antifungal properties of tablets formulated from *cassia* leaf extract with the capability to disinfect and maintain the hygiene of heat-cured acrylic dentures. The active component responsible for antibacterial and antifungal activity was not investigated due to restraint of time. Nonetheless, these antimicrobial characteristics are because of the existence of tannins, terpenoids, glycosides, and alkaloids.[24] Both the *cassia* herbal tablet and the commercial tablet were found to have equally effective antifungal properties in the present study with the herbal tablet having no allergic or toxic effects. The commercial tablet weighed 2.72 gm, in comparison to 0.38 gm of the herbal tablet demonstrating that the commercial tablet weighed seven times more than the herbal one. In the present study, only four tablets were used to obtain the desired results, illustrating the strong antifungal properties of the herbal *cassia* extract.

Consistent with Felton et al., an ultimate denture cleaner must be bactericidal, fungicidal, as well as cost-effective which was found to be in the formulated herbal tablet used in the present study.[18] The non-toxic characteristics of the tablet were not examined in the current analysis which was seen by Jothy et al., who found no side effects when a solitary amount of 5000 mg/kg of methanolic extract of cassia was dispensed in mice.[27] Hence, it may be safely anticipated that the tablets have no toxic effects. Several other formulations of natural-based denture cleansers have also been reported; Saraya et al. formulated an herbal denture cleansing solution containing four medicinal plants which also demonstrated an anti-candidal effect.[28] Similarly, Pooja et al. used cashew leaf and aloe vera for denture cleansing and compared it with commercial denture cleansing tablets demonstrating a statistically significant reduction in the candidal count for both the tablets.[29] Conversely, in an analysis piloted by Khan et al. comparing the effectiveness of two herbal extracts and two commercially obtainable denture cleaners hostile to *candida*, the commercial denture cleaning

tablet was reported to be the most effective.[30]

The CFU count was only after the acrylic plates had been soaked in the tablets. Future studies should be done with CFU measurements taken before and after disinfection. Clinical trials are recommended for optimizing tablet performance and to show evidence of biocompatibility, which should eventually lead to the introduction of the plant-based denture cleansing tablet.

CONCLUSION

This study showed that formulated tablets from ethanolic extracts of cassia fistula leaves have strong antifungal activity. Consequently, this plant can be considered a biological medicinal basis for treating various oral infectious states. The formulated tablet can additionally also be used as a prophylactic measure to enhance denture hygiene, consequently reducing the chances of DRS as it was as useful as the commercially available denture cleaning tablet.

DISCLAIMER

None.

CONFLICT OF INTEREST

None to declare.

ETHICAL STATEMENT

The ethical approval is provided by the Ethical Review Committee of Islamic International Dental College (IIDC), Riphah International University, Islamabad, Pakistan vide letter: IIDC/IRC/2016/001/011.

FUNDING DISCLOSURE

The author received no financial support for the research, authorship, and/or publication of this article.

AUTHORS CONTRIBUTION

Conception and design of the study: U. Hasan, A. Ehsan
Acquisition of data: F. Moeen, A. Ehsan
Analysis and interpretation of data: A. F. Moeen, H. Afzaal
Drafting of the manuscript: U. Hasan
Critical review of the manuscript: A. Ehsan
Approval of the final version of the manuscript to be published: U. Hasan, A. Ehsan, F. Moeen, H. Afzaal

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