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# Evaluation of anti-fungal and anti-bacterial properties of extract from *Cassia fistula* leaves

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## ABSTRACT

**Introduction:** Prolonged use of antibiotics and antifungals for treating oral infections may develop resistance as a consequence of which medicinal plants having antimicrobial properties are being used to explore natural alternatives that are almost equally effective along with being cost-effective to cater to patients having financial constraints.

**Objective**: The objective of this paper was to examine the presence of anti-bacterial and anti-fungal potential of the extract drawn from cassia fistula leaves. Ethanolic extract of cassia fistula leaves was evaluated for antimicrobial potential against bacterial and fungal strains responsible for causing denture-related stomatitis (DRS).

**Materials & Methods:** In this cross-sectional study, cassia fistula leaf extract was obtained using the Soxhlet apparatus. Anti-bacterial potential was examined utilizing the agar well diffusion method whereas anti-fungal ability was observed using the tube dilution method which aided in evaluating the Minimal Inhibitory Concentration (MIC) for the extract. The anti-bacterial and anti-fungal potential of cassia fistula leaf extract was tested contrary to staphylococcus aureus and candida albicans. The zone of inhibition (ZOI) of the extract for anti-bacterial activity was compared with 5µg/ml ampicillin while the MIC of the extract (4mg, 8mg, 16mg, 32mg, 64mg, 128mg) for anti-fungal potential was evaluated contrary to candida albicans.

**Results:** The outcomes exhibited the anti-bacterial potential of the extract against staphylococcus aureus which was confirmed by giving the ZOI to be 22mm whereas the anti-fungal potential of the extract in contrast to candida albicans was confirmed with the MIC to be 8mg/ml.

**Conclusions:** The research inferences from the analysis indicate that the ethanolic extract obtained from cassia fistula leaves shows potent antifungal and antibacterial properties. As a result, this plant holds the capacity as a natural medicinal resource for addressing diverse oral infections.

**Keywords:** Anti-Bacterial Agents; Antifungal Agents; *Candida albicans*; *Cassia fistula*; Denture; Senna Extract; *Staphylococcus aureus*; Stomatitis.

*The authors declared no conflict of interest. All authors contributed substantially to the planning of research, data collection, data analysis, and write-up of the article, and agreed to be accountable for all aspects of the work.*

## INTRODUCTION

Numerous plant extracts are widely used as antimicrobial agents.[1] Drugs derived from these extracts play an important part in the avoidance and management of infections.[2] In unindustrialized countries, conventional herbal medicines perform an imperative part in delivering basic healthcare.[3-4]

From 1981 to 2002, 61% of the drugs developed were based on herbal extracts and proved efficient in combating several infectious diseases.[5] According to WHO, these plant extracts with antimicrobial properties are used as traditional medicines by 81% of the world's population.[6]

Infectious diseases are responsible for the high mortality rate in developing countries and it is a common practice to prescribe antibiotics and antifungals to combat these diseases.[1,7]

However, over the preceding few years, the practice of antibiotics has become less popular attributable to drug resistance.[7,8] In contrast, investigations of drugs with antibacterial properties based on natural plant extracts have gained popularity.[9]

Conversely, fungal infections are also treated using antifungals but the extensive usage of these medications has likewise been known to develop resistance, especially in immunocompromised patients.[10] Some of the fungal strains have been documented to respond to extracts derived from natural plant sources.[11]

Pakistan possesses a considerable abundance of medicinal plants.[12] One such medicinal plant is *Cassia fistula* which belongs to the Caesalpiniaceae family and has both antibacterial and antifungal properties.[13,14] Antimicrobial properties of cassia fistula are attributed to the release of resultant metabolites such as tannins, terpenoids, and glycosides, along with alkaloids.[2] These metabolites attack the bacterial cell membranes and disrupt the protein synthesis mechanism.[15]

The current study sought to determine the in-vitro antibacterial and antifungal efficacy of the ethanolic extract derived from C*assia fistula* leaves to consider the possibility of using these plants for treating bacterial and fungal infections of the oral cavity associated with dental appliances.

## MATERIALS & METHODS

This cross-sectional analysis was performed from April 2019 to November 2020 in the G7 campus of Riphah International University, Islamabad. The pharmacognosy department at Riphah Institute of Pharmaceutical Sciences (RIPS) and the Herbarium of Pakistan, Quaid-i-Azam University, Islamabad, verified the collection of fresh and healthy leaves from the *Cassia fistula* plant under reference number 133567. Approval for the survey was granted by the Ethical Review Committee of Islamic International Dental College (IIDC), Riphah International University, Islamabad, Pakistan, as per the official letter with reference IIDC/IRC/2016/001/011.

### Formulation of Plant Extract

The withdrawal of extract from C*assia* leaves was conducted following a standardized protocol.[16] The dehydrated leaves were ground in a mechanical mill and the extract was obtained using a Soxhlet extractor using ethanol for 72 hours at 70°C. The extract was strained using filter paper whilst still warm. Then, it was reduced in vacuum conditions in reduced pressure employing a rotary flask evaporator (Eyela, Japan). After that, it was dried out in a desiccator. The ethanolic extract yielded a dark greenish firm filtrate which was kept in a sterile petri dish until further use. This crude extract was used to assess its potential antimicrobial properties.

### Preparation of Stock Solution

An empty epitube was weighed using an analytical balance. A weight of 0.1 gm of the dried extract was dissolved in 1 ml of DMSO (Dimethyl Sulfoxide) in an epitube to obtain a 10% stock solution. The stock was used for the preparation of all dilutions to be tested for antimicrobial activity.

### Refreshing of Test Organism Culture

Test organisms comprising bacterial strains including *Staphylococcus aureus* (ATCC 6538) and fungal strain containing *Candida albicans* (ATCC 60387) were obtained from RIPS and were confirmed by sub-culturing in suitable selective media.

### Antimicrobial Activity

In vitro, antibacterial and antifungal potential was observed for ethanolic extract. The antibacterial activity of the extract was investigated by the disc diffusion method whereas antifungal potential was by the tube dilution technique.

### Inoculation of Staphylococcus aureus

A total of 0.65 gms of nutrient broth (Oxoid, England) was dissolved in 50ml water in a beaker. This content was poured into a test tube and autoclaved. A sterile wire loop was used to scrape S*taphylococcus* and then dipped in a test tube containing nutrient broth. This broth was incubated for 24 hours at $37^0$ C in an incubator (Model B-53 Rmeco). The nutrient broth turned turbid the next day indicating that the strain had been produced.

### Agar Well Diffusion Method (Antibacterial Activity)

A total of 2.8 gms of nutrient agar (Oxoid, England) was dissolved in 100ml water in a conical flask and autoclaved. The nutrient agar plate was prepared by pouring the contents of the flask into a sterilized petri dish (94mm Via Delle, Italy). The contents were then incubated for 24 hours at 37°C in an incubator (Model B-53 Rmeco) to confirm their sterility.

A cotton swab was dipped in a test tube containing *Staphylococcus* and a lawn was formed on the nutrient agar plate. Three wells were made with an 8mm sterile brass borer (Figure 1). A total of 50 µl stock solution was taken in a sterile pipette and poured into one of the wells and 50 µl DMSO was taken as a negative control in another well.

Subsequently, 50ul of 5 ug/ml Ampicillin (positive control) was poured into the third well with the help of a sterile pipette and incubated for 24 hours at 37°C in an incubator (Model B-53 Rmeco). Afterward, the Zone of Inhibition (ZOI) was measured.

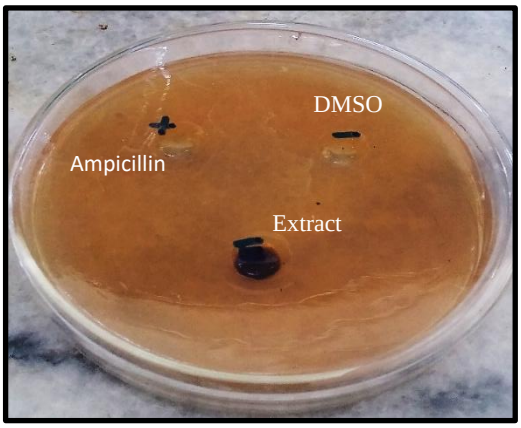


**Figure 1: Agar plate for anti-bacterial assay**

### Broth Tube Dilution Method (Antifungal Activity)

A total of 4 mg crude extract was placed in a test tube containing nutrient broth (Oxoid, England) under sterile conditions. *Candida* was transferred from the broth to a test tube containing the extract and a nutrient broth with the help of a sterile wire loop.

The same process was repeated for 4mg, 8mg, 16mg, 32mg, 64mg, and 128mg of the crude extract. All the tubes were vortexed to mix well and incubated at 27°C for 48 – 96 hrs in an incubator (Model B-53 Rmeco). The tubes were then observed for Minimum Inhibitory Concentration (MIC).

## RESULTS

### Antibacterial Assay

The antibacterial potential of the *cassia* extract was assessed against the bacterial strain *Staphylococcus aureus* (ATCC 6538), expressed by the ZOI diameters and MIC. The extract showed good antibacterial activity against *Staphylococcus*.

Ampicillin was used as a positive control for the control for the agar well diffusion assay, while DMSO was used as a negative control. The microbial strain was inhibited by the drug at 5ug/ml strength giving a ZOI of 21mm. Alternatively, the negative control DMSO showed no ZOI.

In comparison, the *Cassia* extract was used as a stock 10% w/v solution, and a ZOI at 22 mm was observed around the 8mm well (Figure 2).

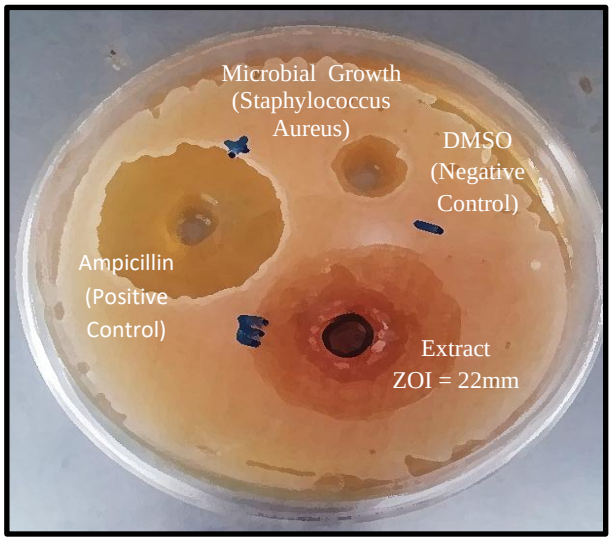


**Figure 2: Agar plate showing zones of inhibition**

The antibacterial assay was performed three times (Table 1) to obtain results for consistency and to calculate the mean ZOI. The positive control for comparison was Ampicillin; in all three plates, the *Cassia fistula* extract displayed larger zones of inhibition than the positive control; the mean ZOI of *Cassia fistula* was also greater than that of the positive control, Ampicillin.

**Table 1: Zone of Inhibition (ZOI) for Ampicillin (Positive Control) and *Cassia fistula* Extract**

| Plate No | ZOI Of Ampicillin (mm) | ZOI Of *Cassia Fistula* Extract (mm) |
|---|---|---|
| A | 21 | 22 |
| B | 21 | 21.5 |
| C | 20 | 21.66 |
| **Mean** | 20.66 ± 0.577 | 21.66 ± 0.288 |

**Antifungal Assay**

This assay was used to assess the antifungal activity and MIC. For the antifungal assay, the MIC was found to be 8mg/ml for each of the three times (Figure).


**Figure 3: Test tubes showing no growth from 8 mg/ml onwards.**

The antifungal assay was also performed three times to determine the MIC (Table 2). The mean was taken out which was used to calculate the standard deviation.

**Table 2: MIC of three different sets of test tubes.**

| Set A | Set B | Set C | Standard Deviation |
|---|---|---|---|
| 8 mg/ml | 8 mg/ml | 8 mg/ml | ± 0 |

**DISCUSSION**

The oral region delivers a favorable surrounding to usher in a diverse collection of fungal and bacterial strains.[17] Among the diverse mixture, S*taphylococcus aureus* and C*andida albicans* are accountable for a variety of oral diseases, the most common being angular cheilitis and acute dentoalveolar abscesses. Smith et al.,[18] reported a 63% isolation rate of *Staphylococcus aureus* from angular chelitis confirming it as the primary causative organism while Goldberg et al.,[19] found S*taphylococcus aureus* among the pathogens isolated from the oral surface of dentures. Moreover, in a report performed by Sumi et al.,[20] S*taphylococcus aureus* remained isolated in 30% while C*andida* was found in 77% of the residents. Similarly, in Japan, Senpuku et al.,[21] reported 5% S*taphylococcus aureus* and 40% C*andida* in the samples of elderly nursing home residents and 3% and 30% in non-residents respectively.

*Candida albicans*, along with other microorganisms, are responsible for causing DRS. A study by Dorko et al.,[22] reported C*andida* on 39.6% of denture surfaces in patients suffering from DRS. Denture surfaces have been reported to act as reservoirs for the colonization of both S*taphylococcus aureus* and *Candida albicans*.[23-25] The *Staphylococcus aureus* colonization rate of denture wearers has been reported to vary from 23% to 48%.[26,27] Moreover, oral microorganisms have been involved in bacterial endocarditis, aspiration pneumonia, gastrointestinal infections, along with chronic obstructive pulmonary disease.[28] Consequently, it remains imperative to control the growth of microbes on denture exteriors and in the oral cavity.

The results of the present suggest that *Cassia fistula* extract shows a significant ZOI (21.66 ± 0.28 mm) against 'gram-positive' *Staphylococcus aureus*. In comparison, Hamad et al.,[29] found *cassia* extract to have a ZOI of 20.24 + 1.00 mm against *Staphylococcus aureus*, which is quite comparable to the consequences of the current study. Durapandiyan et al.,[30] assessed six different *Cassia* extracts in which the ethyl acetate extract had a ZOI of 19mm. These results are also similar to those found in the current study. However, in an analysis directed by Seyyednejad et al.,[15] on antimicrobial properties of *Cassia* flowers, ZOI of only 7mm was revealed by ethanolic extract of *Cassia* contrary to *B. cereus* and *Staphylococcus aureus* whereas the highest potential was confirmed by ethanolic extract of *Cassia* flowers compared to E.coli. Nonetheless, the antibacterial potential of *Cassia* substantiates its historical use in treating different diseases a 3 That's where we need to start looking at anyway some changes yess an anti-bacterial agent.[29]

Bhalodia et al.,[2] reported noteworthy restrain of bacterial development against *Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli,* as well as *Pseudomonas aeruginosa*.

They also reported statistically substantial antifungal results compared to *Aspergillus niger*, *Aspergillus clavatus*, and *Candida albicans*.[2] These results are in accordance with the outcomes of the current study. Hamad et al.,[29] reported a MIC of 6.2 mg/ml against *Candida albicans* which is slightly lesser than the results of the present study (8 mg/ml). Conversely, Durapandiyan et al.,[30] calculated the MIC of bacterial and viral strains which was reported to be more than 5 mm. Since the exact MIC value was not reported, it might be that the results were similar to the present study.

An analysis accompanied by Sony et al.,[31] on the antifungal potential of *Cassia* extracts derived from leaves, barks, and seeds compared to fluconazole-resistant strains of *Candida* reported that *Cassia* seeds exhibited good inhibitory activity with MIC rate of 0.5 mg/mL, although MIC for ethanol extracts of bark and leaf was 1 mg/mL and 4 mg/mL correspondingly. Consequently, they concluded that ethanol extracts of *Cassia* seed displayed respectable potential ($p<0.05$) in contrast to bark and leaves. Correspondingly, an analysis by Irshad et al.,[32] stated that the MIC of the pulp and seed oils of cassia stretched amid 250-300 and 350-500 µg/mL concluding that functional complexes are seen in *Cassia* oil that are directed at the biosynthesis in candidal cell wall. In the present study, the extract used for performing antibacterial and antifungal assays was in crude form. Phytochemical studies performed for the crude extract of *Cassia* leaves suggest that the hydroalcoholic extract comprised of tannins, flavonoids, saponins, triterpenoids, steroids, glycosides, anthraquinones, reducing sugars, carbohydrates, proteins, in addition to amino acids are accountable for the antimicrobial characteristics of cassia fistula.[33,34]

## LIMITATIONS

The active component responsible for antibacterial and antifungal activity was not looked upon due to the limitation of time.

## CONCLUSION

The study presented strong anti-fungal and anti-bacterial properties of the ethanolic extracts of *Cassia fistula* leaves leading to the likelihood of considering this medicinal plant for treating oral infectious diseases.

## RECOMMENDATIONS

Future studies should be carried out to achieve greater advantages from this medicinal plant with fungicidal and bactericidal properties to bridge the gap of utilizing this plant for combating commonly occurring yet serious oral infectious diseases.

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