

Effect of *Garcinia Imberti Bourd* Against *Candida* Isolates in Rootcanal Infection

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ABSTRACT

Background: Many research works are being carried out in preparing intracanal medicaments using herbal plants since it is safe as well as containing a better therapeutic value. The present study was designed to evaluate the anticandidal properties of the leaves of *Garcinia imberti* bourd in associated with the root canal infection. **Method:** Totally 94 root canal infected samples were collected and their previous history also recorded. Samples yielded *Candida* isolates were screened through drug resistant study. Multi drug-resistant *Candida albicans* was isolated and characterized by 18S rDNA sequencing. An antifungal activity of the extracts of the leaves of *G.imberti* bourd was performed by disc diffusion method. Using Silica-60 column active fraction of the methanol extract was partially purified and was analyzed by Gas Chromatography–Mass Spectrophotometry (GC-MS). Prepare intracanal medicament with methanol extract of *G.imberti* bourd and was tested with multidrug-resistant *C.albicans* (Ca-09) under *in-vitro* conditions using decoronated teeth. Efficacy also compared with chlorohexidine. **Result:** A total of 48 *Candida* sp. were isolated and screened for antibiotic susceptible test. Among them isolate Ca -09 showed multiple antibiotic resistant. Crude methanol fraction of *G. imberti* bourd leaf extract,

as well as the partially purified compound of this extract, showed better anticandidal activity. 0.01g of methanol extract with 2% methyl cellulose gel showed significant reduction (63%) of *C.albicans* in *in-vitro* intracanal treatment. **Conclusion:** Methanol extract of *Garcinia imberti* bourd leaf contains glycerine, which can be used in preparing the suitable intracanal medicament for the treatment of root canal infection.

Key words: *Candida albicans*, Chlorohexidine, *Garcinia imberti bourd*, Glycerine, Intracanal medicament, Root canal infection.

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INTRODUCTION

Root canal infection is the most significant oral health problem, and it develops due to the presence of microbial communities within the root canal.¹ As the root canal system act as reservoir² which results in a death of dental pulp, this may occur due to dental caries thermal damage, mechanical trauma tooth wear from grinding and micro leakage of dental restoration or tooth fracture.³ Usually, these are favoring to facilitate the growth of opportunistic pathogens like *Candida albicans*, *C. tropicalis* and *C. dubliniensis*.⁴ These species are primarily influencing the oral bacterial diversity as well antibiotic resistance. Although *Candida* species are sensitive to intracanal medicaments, their virulence factor enables them to adhere and penetrate into the root canal.⁵ Sometimes the growth of the microorganism is enhanced by procedural errors of experts during treatment.² An aim of the treatment is to prevent the infection as well as the side effects resulted from the irrigants, NaOCl, chlorohexidine (CHX) and calcium hydroxide.⁶ As the solutions applied in intracanal medicaments often causing toxicity, tissue necrosis and reducing the hardness and structural stability of the dentin within the root canal.⁷ Many research works are being carried out to focus the treatment using herbal extracts.⁸ In this series, the present study was conducted to evaluate the inhibitory effect of leaves of *Garcinia imberti* bourd towards the species of *Candida* isolated from root canal infection.

MATERIALS AND METHODS

Infected root canal samples were collected from local Dental hospitals of Kanyakumari district after getting the ethical clearance from the ethical committee, Govt. medical college, Kanyakumari District, Tami Nadu, India. By using the dried paper points samples were drawn from the root canal infected cases.⁹ Simultaneously their clinical history was also noted.

Culturing and Identification of Isolates

Transfer the samples into Amies transport medium and were brought into the laboratory within a hour. For the isolation, it was streaked primarily on Sabouraud's Dextrose Agar (SDA), after confirming the growth; the colonies were differentiated by streaking it over the Hichrom *Candida* differential agar. Morphologically distinct colonies were identified by the biochemical test as well as Germ tube test. Then the isolates were subjected to antifungal sensitive assay with standard disc diffusion method using Himedia disc such as, fluconazole (10 mcg), ketoconazole (10 mcg), itaconazole (30 mcg) and amphotericin B (100 Units).

Collection of plant materials

Leaves of *Garcinia imberti* bourd (local name: Mania Kanji) were collected from Ponmudi forest, Kerala, India and was identified by Dr. Ramasubbu, Department of Biology, Gandhigram University, Dindigul. The voucher specimen was stored in the same department. Leaves were dried under the shadow and then powdered.

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Preparation of crude extract

30 g of the dried leaf powder was extracted with 300 ml of Hexane in Soxhlet apparatus.¹⁰ After 12 hours, Hexane was evaporated by using rotary evaporator at 40°C. Similar procedure was performed to prepare methanol, ethyl acetate, and petroleum ether extracts. Eventually, the portion of the extracts was stored in DMSO (1 g/ml) for further use.⁴

Anticandidal activity

Disc diffusion method was performed to screen anticandidal activity. 50 µl of different *Candida* isolates were swabbed over the SDA agar. Discs with 2.0 mg concentrations of various crude extracts of *G. imberti* bourd were placed. Plates were incubated at 37°C for 2 days. After incubation, the inhibitory zone was measured and recorded.

Column chromatography

Crude methanol extracts were subjected into the scheme of column chromatography using silica gel (60-120 mesh) and were eluted with the solvent such as chloroform, ethyl acetate and methanol (100:0, 90:10, 80:20, 70:30, 60:40 ... 0:100).¹¹ After that, the active fraction was subjected into Thin Layer Chromatography (Preparative -TLC) and their activity was evaluated.

Gas Chromatography – Mass Spectrophotometry (GC-MS) analysis

The active fraction F21 was analyzed by GC-MS method.¹² In this, initial temperature programmed for the oven was from 60°C (2 min) to 300°C for 6 min, samples to be injected in the split mode with the ratio of 10:1 percentage, and the active constituents of the extract was expressed as a percentage by peak area.

In-vitro intracanal medicament efficacy of plant extracts against *C. albicans* using decoronated teeth

Freshly extracted thirty single-rooted mandibular premolar teeth were taken. Teeth were decoronated using the diamond disc at the level of CEJ. Canals with the size of F3 were made using the protaper NiTi system. Teeth were irrigated with 5.25% NaOCl and EDTA for 5 min and then rinsed with physiological saline (0.9% NaCl). The outer surface of the root was coated with two coats of nail varnish. Apical foramen sealed with type-2 glass ionomer cement and the teeth were transferred to sterile glass tubes. After autoclaving the tubes containing roots were infected with *C. albicans* (Ca-09) at 10³CFU/ml and incubated the tubes at 37°C for 21 days, every three days, it was replenished with fresh *C. albicans* Ca-09. The experiment was performed with three different groups. In group-I, normal saline with one gram of 2% methyl cellulose was used as an intracanal medicament. In group-II, 0.01 g of a methanol extract with 2% methyl cellulose was applied. In group-III, 20 µl of 2% chloro-hexidine with 2% methyl cellulose combination was employed. Finally, the canals were irrigated, and dentinal shavings were made by using paesoreamer size No 4. Aliquots of saline along with dentinal shavings were drawn from the root canal and plated on SDA. After incubation, the growth of *C. albicans* and the anti-candida activity were measured.¹³

RESULTS AND DISCUSSION

Out of 94 samples, *Candida* spp. resulted from 48 patients (51.06%), from which 58.33% *C. albicans* (n=28), 27.08% *C. tropicalis* (n=13) and 14.6% *C. krusei* (n=7) (Table 1). Among these, 4 isolates (14.28) out of 28 *C. albicans* was yielded from the patient with diabetes. It seems that the root canal infection is commonly associated with diabetes. In a diabetic patient, glucose provides the carbon source for the betterment of the growth of *C. albicans*. This finding was almost compatible with the previous study results.^{14,15} isolated *Candida albicans* in root canals of teeth with apical periodontitis. The growth of *C. albicans* on Hichrome *Candida* differential agar was examined (Figure 1a) and the microscopic

examination of *C. albicans* has been appeared in blue colour with lactophenol cotton blue staining (Figure 1b). This study showed that there was no *Candida* species yielded from patients who have been affected by the urinary tract infection and cardiac infection. Hence those cases were not focused in this study. In terms of the resistance study, all the isolates were analyzed with the agents like Fluconazole, Ketoconazole, Itaconazole and Amphotericin B (Figure 1c). In the present study, single drug resistance were observed in four *C. albicans* strains isolated from diabetic endodontic cases, whereas strain Ca-09 alone shows multidrug resistance. It might be due to the patients could have received the repeated course of antifungal medication.^{16,17} Remaining all other *Candida* isolates were found to be sensitive to above antifungal agents. Secluded multidrug resistance isolate (Ca-09) was further characterized by 18S rDNA sequencing study and also reported (Figure 1d), it was 99% sequence similar to *C. albicans* (gi-347810985).¹⁸ identified *C. albicans* from dental biofilm, however,¹⁹ used species specific DNA probes for rapid identification of *Candida* species.

Anticandidal activity of the leaves of *Garcinia imberti* bourd was performed with solvents like methanol, ethyl acetate, petroleum ether, and hexane. However, highest anti-candidal activity has resulted from the methanol extract (21 mm; 2 mg/disc). It is due to the active constituents of leaves could have been eluted with methanol (Figure 2). However, the hexane extract showed the less inhibitory zone (<7 mm) and the moderate inhibitory zone (17 mm) resulted from the ethyl acetate extract. Usually, the members of *Garcinia* species were found to be potentially active against *Candida albicans*.²⁰ The methanolic extract of *Garcinia indica* had been reported to have specific inhibition towards *C. albicans*.²¹ The results of the present study are approximately similar with the report, which has been showed by the previous study.²² Interestingly, the preparation of crude extract of *Garcinia* using methanol seems to be good activity against *C. albicans*. Comparatively, inhibition towards the *C. albicans* was relatively high when the isolate has been tested with partially purified compound (10 µg/ml: 26 mm), which could have been achieved by the presence of active compounds. The active compounds of the F21 fraction eluted through Silica column (60-120 mesh size) was further characterized by GC-MS method (Figure 3), which shows the compounds such as, Decozene, 3,7,11,15 tetra methyl-12- Hexa decen 1-01, 3 Butoxy 1,1,1,5,5,5-hexa methyl 3-(trimethyl siloxy) trisiloxane (Table 2). Apart from this, Glycerine was also identified in the sample, since this can be recommended as active ingredients in making herbal intracanal medicament.²³

According to *in-vitro* study, saline (0.9% NaCl) with 2% methyl cellulose could be used as the control¹³. The activities of methanolic extract of *G. imberti* bourd and control intracanal medicament CHX were evaluated after irrigation. The results were reported as colony forming unit

Table 1: Screening of *Candida* isolates with clinical history and drug resistant profile

Isolates (n=94)	% of Positive	Clinical history				Drug Resistance	
		Diabetic	Cardiac	UTI	RTI	Single drug	Multi drug
<i>C. albicans</i>	29.78 (n=28)	4 (14.28)	-	-	-	4	1
<i>C. tropicalis</i>	13.82 (n=13)	-	-	-	-	-	-
<i>C. krusei</i>	7.44 (n=7)	-	-	-	-	-	-

UTI – Urinary tract infection; RTI – Respiratory tract infection

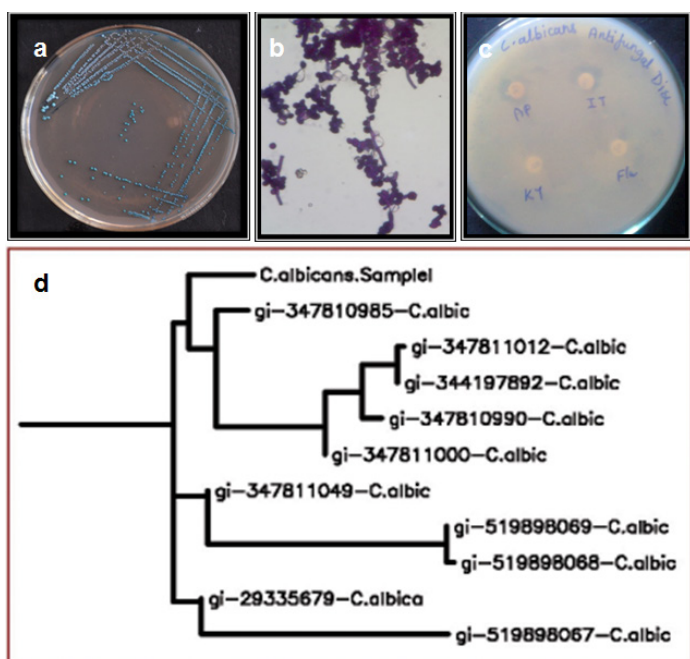


Figure 1: Characterization of drug-resistant *Candida albicans* from the root canal infected sample. a) *C. albicans* (Ca-09) growth on Sabouraud's Dextrose Agar, b) microscopic image of *C. albicans* (Ca-09) after lactophenol cotton blue (100 X), c) drug resistant analysis of *C. albicans* (Ca-09) against antifungal drugs (Himedia-disc), d) Phylogenetic tree of *C. albicans* (Ca-09).

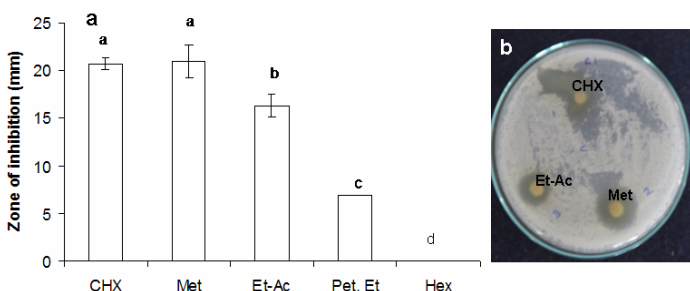


Figure 2: Anti-candidal activity of *Garcinia imberti* board leaf extracts against multidrug resistant *C. albicans* (Ca-09). a) CHX- chlorohexidine (Control), Met – methanol fraction, Et-Ac – ethyl acetate fraction, Pet. Et – Petroleum ether fraction, and Hex – hexane fraction. b) Plate showing anti candidal activity of the *Garcinia imberti* board leaf extract. Bar showing different superscript has significantly different ($P < 0.05$) Tukey HSD test.

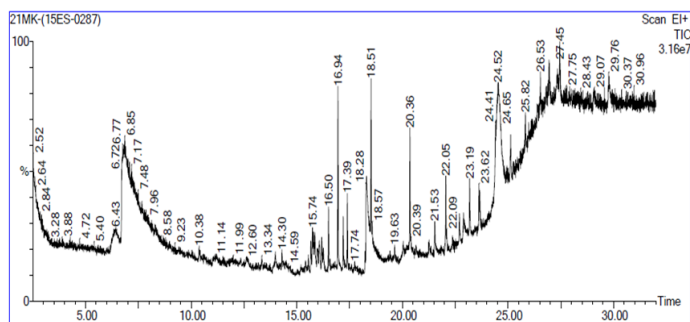


Figure 3: GC-MS spectrum of partially purified compound from methanol extract (F21 fraction of column chromatography) of *Garcinia imberti* board leaf.

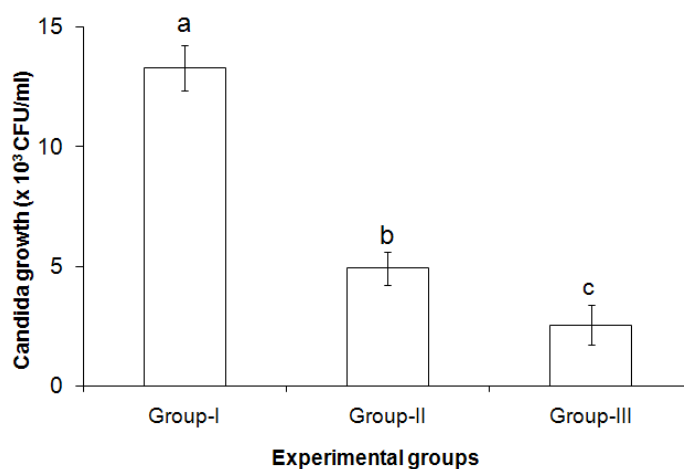


Figure 4: Intracanal medicament activity of methanol extract of *Garcinia imberti* board leaf in decoranated teeth. GROUP I (control; Neutral gel) normal saline with 1 g of 2% methyl cellulose; GROUP-II, 0.01 g of a methanolic extract with 2% methyl cellulose gel; GROUP-III: 20µl of 2% chlorohexidine with 2% methyl cellulose gel. After 21 days of incubation the viable count was enumerated by standard plate count method on SDA. Bar showing different superscript has significantly different ($P < 0.05$) Tukey HSD test.

Table 2: Bioactive compounds detected by GC-MS method from the F21 fraction of methanol extract of *G. imberti* board

S.No.	Rt value	Area (%)	Compounds
1	6.85	35.541	Glycerine
2	16.94	5.98	3,7,11,15-Tetramethyl-2-Hexadecen-1-oL
3	18.300	8.66	N-Hexadecanoic acid
4	18.51	3.987	1-Decosene
5	20.36	4.574	1-Decosene
6	24.41	3.34	3,7,11,15 Tetra methyl -12 Hexadecen 1-oL
7	24.54	30.43	3-Butoxy 1,1,1,5,5,5 Hexamethyl -3-(Trimethyl Siloxy) Trisiloxane

(Figure 4). The extract showed better inhibitory action towards *C. albicans*, overall 63% of the *C. albicans* were reduced, besides, chlorohexidine was more effective (81% inhibition) than the methanol extract. Similar findings were reported by previous works done.¹³ Although many experiments have been carried out in detecting the efficacy of herbal extracts against the microbes in associated with root canal infection,²⁴ the efficacy of the *G. imberti* board towards *C. albicans* in associated with root canal infection is highlighted first through this study.

CONCLUSION

As the methanolic extract of *Garcinia imberti* board was found superior in activity against *C. albicans*. It seems to be the potential candidate for an anticandidal agent. GC-MS analysis of the methanol extract showed glycerine, one of the major compound, which could be used as an active ingredient in making suitable herbal intracanal medicament for the root canal treatment.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATION USED

CHX: Chlorohexidine; **NaOCl:** Sodium hypochlorite; **GCMS:** Gas Chromatography Mass Spectroscopy; **SDA:** Sabourad Dextrose Agar.

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