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In-Vitro Antimicrobial Screening of Medicinal Plants against Different *Enterococcus* and *Candida* Isolates

S.R. Sri Ramkumar*

Research Scholar, Department of Microbiology, Bharathiyar University, Coimbatore, India

*Corresponding Author Email Id: sriram.microbio@gmail.com

Abstract

Aim

- Aim of the present study was to screen the active herbal extracts against *Enterococcus* sps, and *Candida* isolates in root canal infection.

- **Materials and methods:** In this study, crude extract of selected plants were prepared separately from the solvents like Ethyl acetate, Methanol, Ether, Acetone and Hexane. All the extracts of each plant were subjected into antimicrobial assay by disc diffusion method with 6mm disc carries 500 µg/ml concentration of extract. After incubation their efficacy has been observed and measured by mm.

- **Results:** Out of eight plants used, the Ethyl acetate extract of *Mania Kanji* (Garcina imberti bourd) exhibited maximum inhibitory effect on above culture yielded root canal isolates, whereas, *Elephantobus scaber* was found to be less active against such isolates. **Conclusion:** In this study, it is the first report that the *Garcina imberti bourd* indeed exhibit a potent activity on both *Enterococus* and *Candida* isolates of root canal infection.

Introduction

The medicinal plants are being traditionally used for treating infectious and non-infectious disease, as it is safe as well as less cost (Dahanukar, *et al*. 2000). The medicinal plants containing the chemical constituents are capable of inducing physiological action of human body (Xavier and Rajeswari 2013). Plants and plant products are widely applied for the treatment of wound healing, helminthic and some inflammatory diseases (Sunita, 2011). Hence, the people are highly fervent on using such medicinal plants in order to extent their healthy life with rejoice as it is more effective and less toxic drugs (Supreetha, *et al.,* 2011).

But chemically synthesized drugs have some potential to diminish the growth of normal flora of human body (Kanso Iwaki, *et al.,* 2006), thereby which also responsible for impaired defense mechanism to the host. The continuous usage of such antimicrobial agents causes the alteration of physiological and metabolic activities of microbes to become resistant (Jarvinen *et al.,* 1993). This condition may leads towards immunity of feeble in host, which results in favoring the growth of opportunistic microorganisms (Neelam Mittal and Jyoti Jain, 2013). Therefore, Eradication of such infectious organisms is a challenging task. In oral cavity, opportunistic infections like periodontitics and endodontitics are very common problem caused by *Enterococcus* species and *Candida* species. If not properly treated which ends in extraction of teeth. So the treatment of endodontic infection is seems to be an object of challenge among the dental professionals. To overcome this problem, various researches have been carried out towards root canal infections. The present study is a trial to find out the suitable plants for formulating herbal irrigant to treat endodontic infections.

Materials and Methods

Herbal plants, *Achyranthes aespera, Solanum Xanthocarpum solanum nigrum, Psidium guajava, Garcinia imberti bourd, Elephantobus scaber, Coleus aromaticus* and *Mentha pipertia (Figure-)* were collected from in and around Kanyakumari District, Tamil Nadu, and the plants were identified by Dr. R. Ramasubu (Botanist), Gandhigram rural institute, Department of Biology. Gandhigram Deemed University, Dindugal. Such voucher specimen as stored in the same department.

Microbial cultures

Root canal pathogens *Enterococcus faecalis, E. faecium, Candida albicans, C. crusei,* and *C. tropicalis* have been isolated and identified through molecular study.

Extraction method

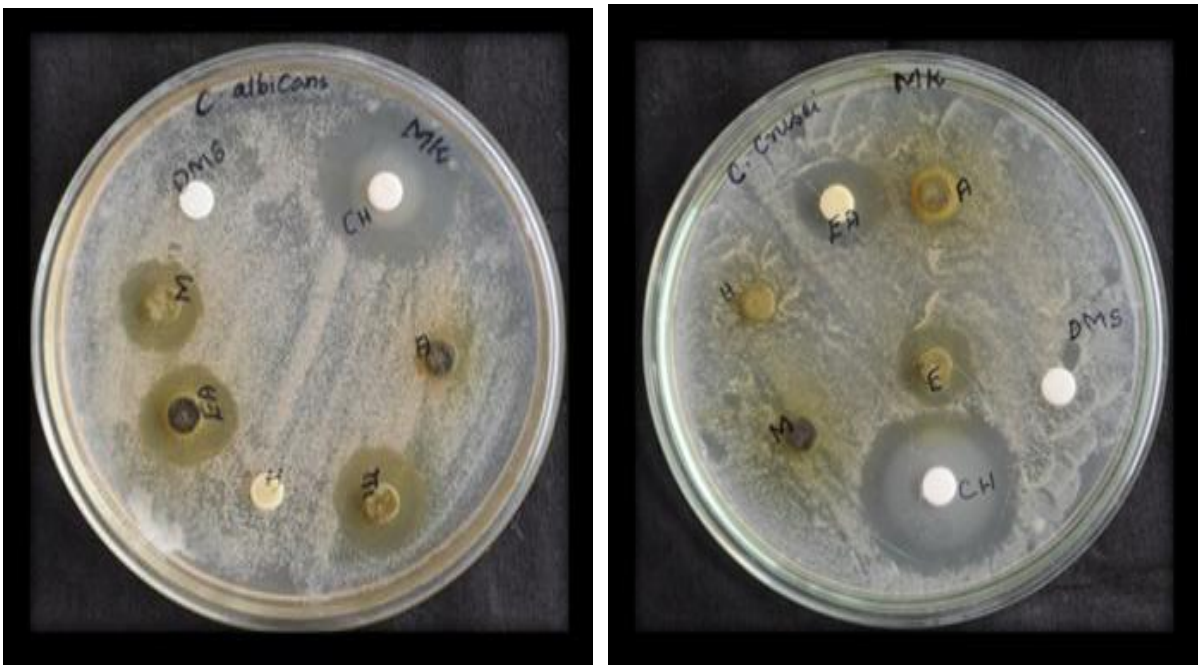
Each shadow dried powdered plant leaves were subjected into to fractionation method of extract preparation by using soxhlet extractor. In this, crude extracts were prepared by dissolving 100 g of each plant powder into 200 ml of methanol initially and the experimental set up was run for 3 days. Then the methanol solvent was evaporated by using rotatory evaporator, followed by the sequential fractionation was performed by using the same volume of hexane, ethyl acetate, ether, acetone. Allow the solvents to be evaporated at 40°C using rotatory evaporator. Then the extracts were filtered twice by using Whatman's no 1 filter paper. Eventually the dried or pasty extracts were stored for further use.

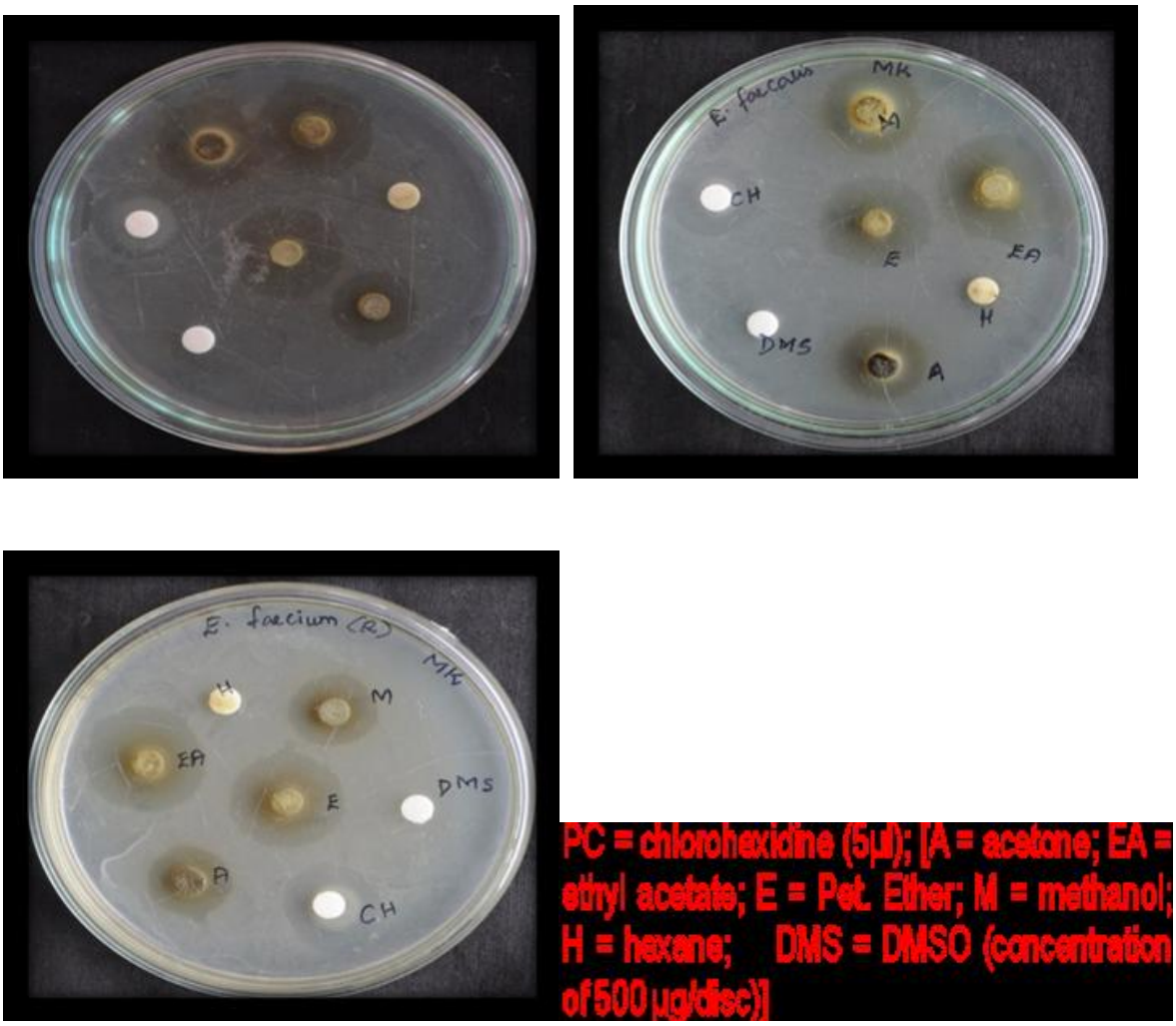
Disk diffusion method

Disc diffusion method was used to evaluate the antimicrobial activity in which 0.1 ml of diluted inoculums of 10^8 CFU/ml of 24 hours isolates were swabbed on Muller Hinton Agar plates, followed by 6 mm disc carries known concentration (50µl/500 µg) of each extracts were placed. Chlorohexidin (5 µl) is used as positive control and (50 µl/$DMSO_4$) used as negative control for evaluating the efficacy. The zones of inhibition were measured after 24 hours incubation at 37^0C.

Results

In this study activity of the traditionally used medicinal plants and chlorohexidine (positive control) on Enterococcus and Candida isolates were measured and tabulated in Table 1. Out of 8 plants used, Garciniagimberti bourd has been found to have highest inhibitory effect on the isolates this is the first report that the plant has such ability towards the pathogen, whereas Elephentobus scaber showed minimum inhibitory activity. No other plant extracts were produced significance results. Therefore, the Garciniagimberti bourd has been high lightened further to test its efficacy on yielded isolates. In these, ethyl acetate extract of Garciniagimberti bourd (500 µg) exhibited maximum inhibitory activity followed by methanol extracts has been shown to have significant effect in comparison to ether and acetone extracts of the same plant. But the hexane extract of the above plant merely not produced affordable result. Despite apparently the ethyl acetate extract of above plants showed maximum activity against both isolates. The activity is found to be less in *Candida* species in comparison to *Enterococcus* species.





a) *Candida albicans*
b) *C. crusei*
c) *C. tropicalis*
d) *E. faecalis*
e) *E. faeciu*

Table : 1 Inhibitory effect of different plant extract in different solvent

Plants		Ethyl acetate (500 µg) / 50µl			Methanol (500 µg) / 50µl			Acetone (500 µg) / 50µl			Ether (500 µg) / 50µl			chloroforms (500 µg) / 50µl			Control + (5 µl)	- (50 µl)
Achyranthes aespera	*E.faecalis*	-	-	-	++	++	++	+	+	+	-	-	-	+	-	-	+++	-
	E.faecum	-	-	-	++	++	++	-	-	-	-	-	-	-	-	-	+++	-
	C.albicans	+	+	+	+	+	+	-	-	-	-	-	-	+	+	+	+++	-
	C.tropicali	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+++	-
	C.krusei	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+++	-
Solamnum xanthocarpum	*E.faecalis*	+	+	+	++	++	+-	+	_	+	+	+	++	-	-	-	+++	-
	E.faecum	+	+	+	++	++	+-	+	-	+	++	+	+	-	-	+	+++	-
	C.albicans	+	+	+	+	+	+	-	+	-	+	-	+	-	-	-	+++	-
	C.tropicali	-	-	-	+	-	+	-	-	-	-	-	+	-	+	-	+++	-
	C.krusei	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+++	-
Solanum Nigrum	*E.faecalis*	+	+	+	++	-	+	+	+	-	+	+	+	-	-	+	+++	-
	E.faecum	+	+	+	-	+	+	-	-	-	-	+	-	+	-	-	+++	-
	C.albicans	+	+	+	+	+	++	+	+	-	+	-	+	-	+	-	+++	-
	C.tropicali	-	-	-	-	-	+	+	+	-	+	+	+	-	-	-	+++	-
	C.krusei	-	-	-	+	+	-	-	-	+	-	-	+	-	-	-	+++	-
Psidium gujava	*E.faecalis*	++	-	-	-	-	-	-	+	-	-	-	-	+	-	-	+++	-
	E.faecum	++	+	+	-	-	-	-	-	-	+	-	-	-	-	-	+++	-
	C.albicans	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	+++	-
	C.tropicali	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+++	-
	C.krusei	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+++	-
Garcinia gymberti bourd	*E.faecalis*	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	-	-	-	+++	-
	E.faecum	+++	+++	+++	+++	+++	+++	-	-	-	+++	+++	+++	-	-	-	+++	-
	C.albicans	+++	+++	+++	+++	+++	+++	+	+	+	+++	+++	+++	-	-	-	+++	-
	C.tropicali	+++	+++	+++	+++	+++	+++	-	-	-	-	-	-	-	-	-	+++	-
	C.krusei	+++	+++	+++	-	-	-	+	+	+	+++	+++	+++	-	-	-	+++	-
Elephantobus scaber	*E.faecalis*	+	+	+	+	-	-	-	+	-	-	+	-	+	+	+	+++	-
	E.faecum	-	-	-	-	-	-	-	-	-	-	+	-	+	+	-	+++	-
	C.albicans	+	-	-	+	-	-	+	-	-	+	-	-	+	+	+	+++	-
	C.Tropicali	-	+	-	-	+	-	-	-	+	-	-	+	-	+	+	+++	-
	C.krusei	-	-	-	-	-	-	-	+	-	+	-	-	+	-	+	+++	-
Coleus aromaticus	*E.faecalis*	+	+	+	++	++	++	-	-	-	-	-	-	-	-	-	+++	-
	E.faecum	-	-	-	+++	+++	+++	-	-	-	-	-	-	-	-	-	+++	-
	C.albicans	++	++	++	-	-	-	+	+	+	-	-	-	-	-	-	+++	-
	C.tropicali	-	-	-	+	+	+	-	-	-	-	-	-	+	+	+	+++	-
	C.krusei	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+++	-
Mentha pipertia	*E.faecalis*	++	+	+	-	-	-	+	-	-	-	-	-	-	+	-	+++	-
	E.faecum	+	-	+	-	+	-	-	-	-	-	+	-	-	-	-	+++	-
	C.albicans	-	+	+	-	-	-	+	-	-	-	-	+	-	-	+	+++	-
	C.tropicali	+	+	+	-	-	-	-	-	+	+	-	-	-	+	-	+++	-
	C.krusei	-	-	+	-	-	-	-	-	-	-	+	-	+	-	-	+++	-

Notes : PC – Positive Control, Chlorohexidine (2 %), Zone of inhibition – < 6, - Indicate –, 7 to 9 +, 10 - 14 ++, more than 14 +++, NC – $DMSO_4$

Table-2: Antimicrobial activity of different extracts of *Garcinia imbertibourd*

Groups	Bacteria		Fungi		
	E. faecalis	*E. faecum*	*C. albicans*	*C. tropicali*	*C. krusei*
Chlorohexidine (2%)	+++	+++	+++	+++	+++
Ethyl acetate extract of *G. Imberti*	+++	+++	+++	+++	+++
Methanol extract of *G. Imberti*	+++	+++	+++	+++	-
Acetone extract of *G. Imberti*	+++	-	+	-	+
Petroleum ether extract of *G. Imberti*	+++	+++	+++	-	+++
Chloroform extract of *G. Imberti*	-	-	-	-	-

[-= Zone of inhibition (<6), +=Zone of inhibition (7 to 9), ++=Zone of inhibition (10-14), +++=Zone of inhibition (14<)]

Figure-1: Identification of *Sta*

Results and Discussion

The antimicrobial activity of selected medicinal plants (*Achyranthes aespera, Solanum Xanthocarpum solanum nigrum, Psidium guajava, Garcinia gimberti bourd, Elephantobus scaber, Coleus aromaticus, Mentha pipertia*) on *Enterococcus* and *Candida* isolates were tabulated in Table 1. Among the eight medicinal plants, *Garcinia gimbertibourd* has been found to have highest inhibitory effect against oral pathogens (Fig. 1). Medicinal and aromatic plants are used as large scale in medicine against drug resistant bacteria, which are considered one of the most important reasons for lack success of treatment in infectious disease using chemical

drugs (Ayman *et al.,* 2014). Based on the traditional value the present task was carried out on various medicinal plants.

In the present study, ethylacetate, methanol, acetone, ether, hexane was used for the extraction of antimicrobial agents. Among the solvent, ethyl acetate extract, followed by methanol showed promising antimicrobial activity against oral pathogens. Intrinsically the methanolic extract of *Achyranthes aespera* and *Coleus aromaticus* were identically produced the result against the above bacterial isolates. This result was in accordance the observation made previously. Acetone could not be a suitable solvent in extracting polar compounds like phenols due to its nonpolar entity, and it is understood that methanol and ethylacetate extracts contain higher polar compounds than water. Many studies have confirmed that also in other plant species polar solvents produce a higher yield of phenolic concentration compared with the non-polar ones (Trabelsi *et.al.,* 2009).

Methanol, ether extract of *Solanum santhacarpum* showed moderate activity on oral pathogens when compared to other extract. In the present study, hexane also showed significant activity against oral pathogens. This result was similar to that of previous report on various solvent on the extraction of antimicrobial compounds from medicinal plants. The hexane extract of *Garcinia gimberti bourd* highly coincided with previous results with other medicinal plants. The suitable solvent for extracting active compounds should be selected carefully because the extracted compound will be based on the type of solvents used (Zarnowski and Suzuki 2004).

A polar solvent will isolate polar compound and non-polar solvent will extract non-polar compound thus different solvents will yield different extracts and extract composition. The highest yield is commonly achieved by using methanol or ethanol and their mixture with water. Generally, ethanol and water are widely used solvents due to their low toxicity and high extraction yield and in advances their polarity can be modulated by mix them at selected ratio (Franco *et al.,* 2008). Methanolic extact of *Psidium gujava* and *Solanum nigrum* were exihibited moderate and unsatisfactory results on isolates respectively. But it is agreed with the similar studies (Chetia *et al.,* 2014). In *Elephantobus scaber*, the ethyl acetate extract showed moderate antimicrobial activity against various bacteria (Avanri and Neela, 2005).

In India, species of *Garcinia* grow extensively in semiwild state, in the Konkan region of Maharashtra, Goa, coastal areas of Karnataka and Kerala, and evergreen forests of Assam, Khasi, Jantia hills, Nagaland, West Bengal, and Gujarat. Some species like *Garcinia cambogia*, *G. indica*, and *G. cowa* are cultivated in certain parts of India. *G. pedunculata*, *G. kydia*, *G. cowa*, and *G. lanceaefolia* are the most important species in north eastern parts of India (Utpala and Nandakishore, 2014). *Garcinia* sp. was included under the list of endangered species of medicinal plants of South India (Rajashakaran and Ganeshan, 2002). In recent years, researchers obtained more than 120 caged xanthones from plants of the *Garcinia* species, and most of them exhibited various potentially useful biological activities, such as anti-HIV-1, antibacterial, anticancer, anti-inflammatory, and neurotrophic activities (Anantachoke *et al.,* 2012). The ethyl acetate extract of *Garcinia gimberti bourd* showed maximum inhibitory action on *Enterococcus* sp. than that of *Candida* sp. The candidate and the extract of *Garcinia gimberti bourd* may have great application in the treatment of endodontic infection.

Conclusion

From this study it can be concluded that *Garciniagimberti bourd* indeed exhibits a potent activity on both Enterococcus and Candida species. But more research needs to be done to evaluate the cytotoxicity and safety problems of this plant extract prior to recommend as an endodontic irrigant or intracanal medicament.

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