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In-vitro antimicrobial activity of *Madhuca indica* and *Cassia fistula* leaves against food-borne pathogens

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Abstract
The antimicrobial activity of ethanol extract of leaves of *Madhuca indica and Cassia fistula was* tested against food-borne pathogens including two species of Gram-negative bacteria (*E. coli O157:H7* and *Salmonella enteriditis)* and two species of Gram-positive bacteria (*Listeria monocytogenes and methicillin–resistant Staphylococcus aureus*) using disc diffusion method. In addition, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of plant extracts were determined. In the disc diffusion method, both the plants extracts were effective in reducing the bacterial growth of tested food-pathogens. The zone inhibition of ranged from 16 to 19 mm for *Madhuca* and 12 to 13 mm for *Cassia* plant extracts. *Madhuca indica* extracts were effective in reducing bacteria growth compared to *Cassia fistula* extracts ($p<0.05$). The MIC of *Madhuca* and *Cassia* plant extracts ranged from 0.6 to 0.8% and 0.9 to 1.1%, respectively whereas MBC of *Madhuca* and *Cassia* plant extracts ranged from 1.5 to 1.7% and 2.5 to 2.6%, respectively. The results of the study suggest that the extracts from leaves of both *Cassia fistula* and *Madhuca indica* plants could be potentially used as natural food preservatives to control and prevent food-borne pathogens. However, further studies on the toxicities and organoleptic properties of food containing plant extracts are needed before recommending their usage.

Keywords: *Madhuca indica, Cassia fistula,* food-borne pathogens, antimicrobial activity

Introduction
Incidence of food-borne illness cases have been increasing in recent years due to consumption of foods contaminated with pathogens. The presence of various microorganisms including pathogens in foods results in food spoilage, reduction in food quality and food-borne illness [1]. Most of the food-borne illnesses are associated with bacterial contamination with *Salmonella* Enteriditis, *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Staphylococcus aureus* [2-4]. Food-borne illness and food spoilage are traditionally prevented by the use of chemical preservatives [5]. However, their use has been associated with accumulation of chemical residues in foods and food chain, development of antimicrobial resistance and harmful side effects on human health [6, 7]. Hence, there is a focus on development of naturally occurring plant compounds as food preservatives that are potentially effective, safe, easily degradable and economical [8, 9].

Plants have been acting as valuable and indispensable sources of natural bioactive compounds that have potential for improving human health since ancient times [10, 11]. Plants in tropical and sub-tropical areas of the world have numerous medicinal herbs and plants with antimicrobial, antiviral, antifungal, anti-inflammatory, anti-oxidant and anti-cancer properties. Various parts of plants such as flowers, leaves, barks, stems, fruits and roots extracts are used for pharmacological purposes.

Plants act as backbone of traditional medicines and around 25% of prescribed drugs in the world are of plant origin [12]. The antimicrobial and other pharmacological properties of plants are due to phytochemicals synthesized in the secondary metabolism [13, 14]. The secondary metabolites in plants with antimicrobial properties include tannins, alkaloids, phenolic compounds and flavonoids [15, 16]. According to the World Health Organization [17], medicinal plants are best source of bioactive compounds with the ability to combat disease, antimicrobial, antiviral and antifungal activities [18].

Cassia fistula (Common name: Golden shower) plant has been used for treatment of skin diseases, liver troubles, tuberculoses glands, hematemesis, pruritus, leucoderma and diabetes

mellitus [19]. In addition to these aforementioned uses, extracts of these plants are recommended as a pest and disease control agents in India [20, 21]. The extracts of *Cassia fistula* showed significant antimicrobial activity against treatment of some diseases as broad-spectrum antimicrobial agents [22]. *Madhuca indica* (common name: Mahua) plants parts are used as stimulants, demulcents, emollients and astringents. The bark is used for treatment of itching, swellings, fractures, snake bites and diabetes mellitus. The plant extracts are found to possess antimicrobial activity [23]. Hence, the proposed study aimed at investigating the antimicrobial effect of ethanolic extracts of leaves of *Madhuca indica* and *Cassia fistula* against food-borne pathogens namely *E. coli* O157:H7, *Salmonella* Enteriditis, *Listeria monocytogenes* and methicillin–resistant *Staphylococcus aureus.*

Materials and Methods

Preparation of plant leaves extracts

Madhuca indica and *Cassia fistula* leaves were collected from the Veterinary College and Research Institute, Orathanadu campus. The taxonomic identities of plants were confirmed by Botanical Survey of Coimbatore, Tamil Nadu. The collected leaves were first washed under running tap water and air-dried in shade at room temperature. The dried leaves were ground to fine powder using a blender. A 40 g of ground leaves were extracted with 100 ml of ethanol using the Soxhlet apparatus. The extraction was performed for 3 days and the collected extract solutions were evaporated using a vacuum rotary evaporator for obtaining a viscous mass [24]. The extracts were tested for antimicrobial activity against food-borne pathogens namely *E. coli* O157:H7, *Salmonella* Enteriditis, *Listeria monocytogenes* and methicillin –resistant *Staphylococcus aureus*.

Inoculums preparation

E. coli O157:H7 (ATCC 43888), *Salmonella* Enteriditis (Egg isolate), *Listeria monocytogenes* (ATCC 13932) and methicillin–resistant *Staphylococcus aureus* (milk isolate) were cultured separately in 10 ml of nutrient broth 37 °C for 24 h. After 24 hours, the cultures were sedimented by centrifugation (4 ºC, 8000 X *g* for 10 min), washed thrice and resuspended in 10 ml of sterile phosphate buffered saline (PBS). The bacterial population in each culture was determined by plating 0.1-ml portions of appropriately diluted culture on nutrient agar plates with incubation at 37 °C for 24 h [25]. A 100 µl of the appropriately diluted suspension was used as the inoculum (~ 5 log Colony forming unit (CFU) for determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of plant extracts.

Determination of antimicrobial zone of inhibition of plant extracts using disc diffusion method

The antimicrobial effect of plant extracts using disc diffusion method was performed in Mueller Hinton Agar (MHA) plates as described previously [26]. The MHA plates were lawn cultured with each bacterial pathogen at 6.0 log10 CFU/mL concentration. The inoculated MHA plates were bored with four wells of 6 mm with the help of sterile cork-borer (6 mm). The treatment included 50 µl of *Madhuca indica*, 50 µl of *Cassia fistula* extracts, Tetracycline disc (30 mcg, positive control) and 50 µl of ethanol (reagent control). The treated MHA plates were allowed to diffuse at room temperature for about 30 minutes and incubated for 24 hours at 37 °C. After incubation, the zone of inhibition indicated by a clear zone around the well corresponding to the antimicrobial activity of tested plant extracts were measured in mm.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of plant extracts

The MIC and MBC of ethanol extracts of *Madhuca indica* and *Cassia fistula* leaves against *E. coli* O157:H7, *Salmonella* Enteriditis, *Listeria monocytogenes* and methicillin–resistant *Staphylococcus aureus* were determined by the broth dilution assay as described previously [25, 27]. Nutrient broth tubes containing ethanol extracts of *Madhuca indica* and *Cassia fistula* in the range of 0 to 2.5% (vol/vol) in increments of 0.1% were inoculated separately with each bacterial pathogen at 6.0 log10 CFU/mL and incubated at 37 °C for 24 h. Control samples included nutrient broth samples inoculated with each pathogen. Following incubation, the samples were serially diluted (1:10) in PBS and appropriate dilutions were plated on nutrient agar plates. The plates were incubated at 37 °C for 24 h. The lowest concentration of the plant extracts treatment that inhibited visible growth of the pathogen after incubation was taken as the MIC of the treatment. The lowest concentration of the treatment that prevented growth of the organism after subculture on nutrient agar plants following serial dilution and plating was taken as the MBC. Duplicate samples were included for each treatment and the experiment was replicated 3 times.

Results and Discussion

The ethanol extracts from leaves of *Cassia fistula* and *Madhuca indica* were tested for antimicrobial activity against food-borne pathogens including two species of Gram-negative bacteria (*E. coli* O157:H7 and *Salmonella* Enteriditis) and two species of Gram-positive bacteria (*Listeria monocytogenes* and methicillin–resistant *Staphylococcus aureus*) using disc diffusion method (Table 1 and Figure 1). Both the plants extracts were effective in reducing the bacterial growth of tested food-pathogens. The zone inhibition of ranged from 16 to 19 mm for *Madhuca* and 12 to 13 mm for *Cassia* plant extracts. *Madhuca indica* extracts were effective in reducing bacteria growth compared to *Cassia fistula* extracts ($p<0.05$). The antibacterial effect of *Madhuca* extracts were more effective against Gram-positive bacteria compared to Gram-negative bacteria ($p<0.05$) whereas *Cassia* extracts were equal in bacterial action on both Gram-positive and Gram-negative bacteria.

Table 1: Antimicrobial zone of inhibition of plant extracts using disc diffusion method

Microorganisms	**Zone of inhibition with standard error (mm)**			
	Madhuca indica extracts	***Cassia fistula extracts***	**Ethanol (reagent control)**	**Chloromphenicol (positive control)**
E. coli O157:H7	16.33 ± 1.8	13.00 ± 0.58	0 ± 0	32.67 ± 0.58
Salmonella Enteriditis	17.67 ± 0.33	13.33 ± 0.33	0 ± 0	34.00 ± 1.00
Methicillin-resistant *Staphylococcus aureus*	19.33 ± 1.45	12.00 ± 1.15	0 ± 0	30.33 ± 0.88
Listeria monocytogenes	18.00 ± 0.58	11.17 ± 0.6	0 ± 0	30.00 ± 0.58

Fig 1: Microbial activity of ethanolic fraction of *Madhuca indica* and *Cassia fistula* leaves against methicillin-resistant *Staphylococcus aureus and E. coli O157:H7*

The MIC and MBC of plant extracts were determined to evaluate their bacteriostatic and bactericidal properties and results is provided in the Table 2. The MIC of *Madhuca* and *Cassia* plant extracts ranged from 0.6 to 0.8% and 0.9 to 1.1%, respectively whereas MBC of *Madhuca* and *Cassia* plant extracts ranged from 1.5 to 1.7% and 2.5 to 2.6%, respectively.

Table 2: MIC and MBC of plant extracts *E. coli* O157:H7, *Listeria monocytogenes*, *Salmonella* Enteriditis and methicillin–resistant *Staphylococcus aureus*

Microorganism	*Madhuca indica extracts*		*Cassia fistula extracts*	
	MIC %	**MBC %**	**MIC %**	**MBC %**
E. coli O157:H7	0.8%	1.5%	1.1%	2.5%
Salmonella Enteriditis	0.6%	1.5%	0.9%	2.5%
Methicillin –resistant *Staphylococcus aureus*	0.7%	1.7%	1.0%	2.6%
Listeria monocytogenes	0.8%	1.7%	1.0%	2.6%

The *in vitro* antimicrobial study using tetracycline as positive control clearly indicated that both the *Madhuca indica* and *Cassia fistula* leaves extracts showed promising antibacterial activity against *E. coli* O157:H7, *Listeria monocytogenes*, *Salmonella enteriditis* and methicillin–resistant *Staphylococcus aureus. Madhuca indica* and *Cassia fistula* plants extracts were previously reported to have antimicrobial activity against wide range microorganisms. However, study investigating the efficacy of ethanol extracts of leaves of *Madhuca indica* and *Cassia fistula* involving all the tested food-borne pathogens namely *E. coli* O157:H7, *Salmonella* Enteriditis, *Listeria monocytogenes* and methicillin–resistant *Staphylococcus aureus* have not been reported. Hence, antimicrobial activity of *Madhuca indica* and *Cassia fistula* plant extracts were tested against food-borne pathogens were determined in this study.

A study by Purnima [28] found that the leaves of *Madhuca Longifolia* extract were effective in inhibiting *Escherichia coli* and *Staphylococcus aureus*. Jyothi and Seshagiri [29] studied the antibacterial effect of saponins extracted from *Madhuca longifolia*, *Celastrus paniculatus* and *Semecarpus anacardium* on *Streptococcus mutans*, *Streptococcus mitis*, *Streptococcus salivarius*, *Staphylococcus aureus* and *Lactobacillus acidophilus.* The authors suggested that the antimicrobial activity of *Madhuca longifolia* might be due to the presence of complex triterpenoid saponins, oleanane type triterpenoid glycosides or atypical pentacyclic triterpenoid saponin. Another study by Chiratan [30] recorded that *Madhuca longifolia* fruit seeds were shown to have antimicrobial activity against *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa and E. coli*. Antibacterial activities of *Cassia fistula* leaves extracted with petroleum ether, chloroform, ethanol, methanol and water were tested against *E. coli*, *E. coli* O157:H7, *Salmonella* typhimurium, *Shigella sonnei*, *Bacillus subtilis*, *Bacillus licheniformis*, *Staphylococcus aureus* and *Staphylococcus epidermidis*. All the five extracts showed antibacterial activity against test bacterial species however maximum antibacterial activity was observed for ethanol [31] (Panda *et al*., 2011). Another study by Seyyednejad *et al.* [32] determined the antibacterial effect of methanolic and ethanolic extracts of *Cassia fistula* on *Bacillus cereus*, *S. aureus*, *S. epidermidis*, *S. typhi*, *K. pneumoniae*, *E. coli*, *P. aeruginosa* and *P. mirabilis*.

Plants and herbs have been used in foods ever since ancient times as flavoring agents and food preservatives [33] due to their antimicrobial effect against food-borne pathogens and spoilage microorganisms [34, 35]. The use of natural products for extension and preservation of foods are well documented [36, 37]. The amount of the plant extracts added to food should be monitored with sensory evaluation and have advantage over chemical preservatives as they generally are regarded as safe (GRAS) and food grade by Food and Drug Administration [38].

Conclusion

The results of the present study suggest that the extracts from leaves of both *Cassia fistula* and *Madhuca indica* plants could be potentially used as natural food preservatives to control and prevent food-borne pathogens thereby reducing food poisoning cases. In addition, plant extracts could be potentially used in the food industries as flavor and aroma enhancers. Further, in depth studies on the toxicities and organoleptic properties of food containing plant extracts are needed before recommending their usage.

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