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Antimicrobial activity (MIC) of essential oils against isolated clinical pathogens

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ABSTRACT

Studies were carried out on isolation and identification of bacteria and fungi from the clinical specimen and assay the antimicrobial activity (MIC) of essential oil against the clinical isolates. In this study isolated bacterial colonies such as *Bacillus subtilis, Escherichia coli, Klebsiella pneumonia, Salmonella typhi, Staphylococcus aureus, Streptococcus pyogenes* and *Pseudomonas aeruginosa*. The KOH and lacto phenol mounting results the isolated fungal colonies were confirmed as *Aspergillus flavus, Aspergillus niger, Candida albicans* and *Trichoderma viridae*. The present investigation antibacterial activity (MIC) of essential oil was analyzed against isolated bacteria. The maximum activity was observed in coconut oil and then compared with other essential oil such as peanut oil and sesame oil. The results were compared with standard antibiotics.

KEYWORDS

Bacteria;
Fungi;
Coconut oil;
Sesame oil;
Peanut oil;
Antimicrobial activity (MIC).

INTRODUCTION

The demand for safer and more natural foods has been increasing since consumers have become more concerned over the presence of chemical residues in their food stuff. Spices and herbal oils offer a promising alternative to the chemical preservatives used in the food products. The antimicrobial activity of herbal oils is well documented[4] and reviewed[7,9,10,15]. Coconut provides food, drink, medicine, health, shelter, aesthetics and wealth. Coconut oil is excellent as a skin moisturizer and softener. A study shows that extra virgin coconut oil is as effective and safe as mineral oil when used as a moisturizer, with absence of adverse reactions[1]. In India and Sri Lanka, coconut oil is commonly used for styling hair, and cooling or soothing the head. People of Tamil Nadu and other coastal areas such as Kerala, Karnataka, Maharashtra and Goa bathe in warm water after applying coconut oil all over the body and leaving it as for an hour to keep body, skin, and hair healthy.

Sesame oil (also known as "gingelly oil" or "til oil") is an edible vegetable oil derived from sesame seeds. It is reputed to penetrate the skin easily, and is used in India for oil massage and also is one of the few oils recommended for use in oil pulling. Sesame oil is used in the manufacture of Ayurvedic drugs. Peanut is an organic oil derived from peanuts, noted to have the slight aroma and taste of its parent legume. In the UK it is marketed as 'Groundnut Oil'. It was used as the original source of fuel for the diesel engine. Peanut oil is most commonly used when frying foods, particularly French fries and chicken. In this present study was iso-

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lation and identification of bacteria and fungi from the clinical specimen and assay the antimicrobial activity of essential oil against the clinical isolates.

EXPERIMENTAL

Study Materials

The samples were collected from Government Medical College, Hospital at Thanjavur. The collected specimens were stored on specific aseptic container (Plate-I), for further study.

Collection of seeds

Coconut (kopra), Sesame seeds and Peanut seeds were obtained from local market at Thanjavur. The seeds were air dried at normal temperature. They were stored in double- layered paper bags and further study.

Preparation of essential oil

The dried seeds materials (250 g) were macerated in grinder and the 100% pure essential oils were collected. Dispensed into dark bottles, and stored at 27ºC until used.

Isolation of Bacteria and Fungi

The nutrient and Potato Dextrose agar (PDA) medium was prepared and it was poured onto the sterile petri-plates. After solidification, the selected clinical specimens were spread on the medium. Then the plates were incubated at 37°C for 24 hours for the isolation of bacteria. The PDA plates were incubated at 27°C for 72 hours for the isolation of fungi.

Identification of Bacteria and Fungi

The seven major isolated bacteria identified based on morphological and Biochemical characteristics. The four isolated fungi identified based on KOH and lacto phenol cotton blue staining techniques[2].

Assay of Antimicrobial activity

Minimum Inhibitory Concentration (MIC)

The dilution test was performed to determine minimum inhibitory concentration (MICS) using the standard procedure as described by Jargensen *et al*.[11].The nutrient broth and Potato dextrose broth was prepared and the oil such as coconut oil, sesame oil and peanut oil was mixed with medium separately in the concentration of 20%, 40%, 60% and 80%. After that 20µ of bacterial and fungal culture were inoculated into each tube separately. Similarly both broth concentrated (50µg/ml) with commercial antibiotic such as ampicilin and Amphotericin-B added separately and the test organisms inoculated, which were, maintained as a positive control. Another one tube filled with nutrient broth and test organisms were inoculated maintained as a negative control. All the tubes were incubated at 37ºC for 24 hrs. After the incubation measure the OD of the each tube at 630 nm.

RESULTS AND DISCUSSION

In this present study the isolated bacterial colonies such as IB1, IB2, IB3, IB4, IB5, IB6 and IB7 culture, morphological and biochemical characteristics results were compared with Bergey's manual of systematic bacteriology. Based on the comparison the isolated colonies were identified as *Bacillus subtilis, Escherichia coli, Klebsiella pneumonia, Salmonella typhi, Staphylococcus aureus, Streptococcus pyogenes* and *Pseudomonas aeruginosa* respectively. The investigated results were tabulated in TABLE 1. Similarly Fewins *et al.*,[6] made studies on types of bacteria that can be isolated from intestinal or fecal material of swine such efforts, using selective plate media, have led to the enumeration of only particular bacterial groups, namely, *lactobacilli, Streptococci, Bacteroides, Escherichia coli,* and *Clostridium perfringens*. In other work on

TABLE 1 : Identification of isolated bacterial colonies

S. No.	Morphological and Biochemical Charastriston	Isolated Bacterial Colony						
		IB1	IB2	IB3	IB4	IB5	IB6	IB7
1	Gram standing	'+' Rod	'-' Rod	'-' Rod	'-' Rod	'+' Coccus	'+' Coccus	'-' Rod
2	Motility Test	'+'	'–'	'–'	'–'	'+'	'–'	'–'
3	Indole Test	'+'	'+'	'–'	'–'	'–'	'–'	'–'
4	Methyl RedTest	'–'	'+'	'–'	'+'	'+'	'+'	'–'
5	Voges Proskauer Test	'–'	'–'	'±'	'–'	'±'	'–'	'–'
6	Citrate Utilization Test	'–'	'–'	'+'	'+'	'–'	'–'	'+'
7	Trip Sugar Iron Test	'–'	AG	AG	A	A	A	'–'
8	Ureas Hydrolysis Test	'–'	'–'	'+'	'–'	'–'	'–'	'–'
9	Oxidase Test	'–'	'–'	'–'	'–'	'–'	'–'	'+'
10	Catalase Test	'+'	'+'	'+'	'+'	'+'	'–'	'+'
11	Carbohydrate	'+'	A±	AG	A±	'–'	'–'	'–'
12	Hydrogen Sulfide Production	'–'	'–'	'–'	'+'	'+'	'–'	'+'

"+" – Positive, "–" – Negative, "AG" – Acid/Gas, "A" – Acid, "±" – Variable

swine microflora, gram-negative anaerobes comprising species of *Bacteroides, Veillonella, Fusobacterium,* and *Peptostreptococcus elsdenii* have been isolated from different segments along the intestinal tract. Also, a new species of Bacteroides, *B. multiacidus,* has recently been isolated from pig feces and described[13].

The present study isolated fungal colonies IF1, IF2, IF3 and IF4 were identified by KOH and lacto phenol cotton blue staining techniques. Among the KOH and lacto phenol mounting results the isolated fungal colonies were confirmed as *Aspergillus flavus, Aspergillus niger, Candida albicans* and *Trichoderma viridae.* The results were presented in TABLE 2. Cohn[3] and Pierard *et al.*,[14] reported that superficial fungal infection are among the most common skin diseases, affecting millions of people throughout the world. These infections are commonly caused by dermatophytes, yeasts and nondermatophyte molds and occur in both healthy and immune compromised people. Similar results also reported Drake *et al.*,[5] isolated Dermatophytes, specifically *Trichophyton, Epidermophyton* and *Microsporum* species are responsible for most superficial fungal infections. The estimated lifetime risk of acquiring a dermatophyte infection is between 10 and 20 percent results is similar as the investigated reviews. In this present study fungal clinical specimens the identified isolates were *Aspergillus flavus, Aspergillus niger, Candida albicans* and *Trichoderma viridae.*

TABLE 2 : Identification of Fungal Colony

S. No.	Isolated Colony	Colony Morphology and Microscopy Observation
1.	IF 1	White to Green
2.	IF2	White to Black
3.	IF3	Cream coloured, Smooth, Party, Yeasty, Odour
4.	IF4	White to pink colony

Minimum Inhibitory Concentration

The TABLE 3 results revealed the minimum inhibitory concentration of essential oil against bacteria. Among this study minimum inhibitory concentration was observed at 80% concentration for all the organisms using coconut oil (78%). At the same time minimum inhibitory concentration was noted at above 80% concentration using sesame oil and peanut oil for all the clinical isolates. The minimum inhibitory concentration results were compared with standard antibiotics (Ampicillin).Review for some of the rationale for the use of coconut oil as a food that will serve as the raw material to provide potentially useful levels of antimicrobial activity in the individual. The lauric acid in coconut oil is used by the body to make the same disease-fighting fatty acid derivative monolaurin that babies make from the lauric acid they get from their mothers= milk. Recognition of the antimicrobial activity of the monoglyceride of lauric acid (monolaurin) has been reported since 1966. The seminal work can be credited to Jon Kabara. This early research was directed at the virucidal effects because of possible problems related to food preservation. The early worked by Hierholzer and Kabara[8] that showed virucidal effects of monolaurin.

TABLE 3 : Antibacterial Activity Minimum Inhibitory Concentration

S.No.	Bacterial Isolates	Control	Minimum Inhibitory Concentration (%)											
			Coconut Oil				Sesame Oil				Peanut Oil			
			20	40	60	80	20	40	60	80	20	40	60	80
1	*Bacillus subtilis*	0.14	++	+	+	-	++	++	+	+	++	++	+	-
2	*Escherichia coli*	0.14	++	++	-	-	++	++	++	+	++	++	+	-
3	*Klebsiella pneumonia*	0.14	++	++	-	-	++	++	+	+	++	++	+	+
4	*Salmonella typhi*	0.14	++	+	+	-	++	++	++	+	++	++	+	+
5	*Staphylococcus aureus*	0.15	++	+	+	-	++	++	+	+	++	++	+	-
6	*Streptococcus pyogenes*	0.15	++	++	-	-	++	++	++	+	++	++	+	+
7	*Pseudomonas aeruginosa*	0.14	++	+	+	-	++	++	+	+	++	++	+	-

"++" - High Growth, "+" - Moderate Growth, "-" - No Growth

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The TABLE 4 results revealed the minimum inhibitory concentration of essential oil against fungi. Among this study minimum inhibitory concentration was noted at 80% concentration for all the organisms using peanut oil (79%). When compared than other essential oil Coconut oil (68%) and Sesame oil (38%). At the same time low antimicrobial activity observed in sesames oil. The minimum inhibitory concentration results were compared with standard antibiotics. (Amphotericin-B). Vaijayanthimal *et al.*,[16] reported that *Trichophyton rubrum* and *Trichophyton mentagrophytes* were isolated from the patients with dermatophytosis and identified. Minimum inhibitory concentration and minimum fungicidal concentration of seven edible and non edible (unrefined) oils were tested against *T. tubrum* and *T. mentagrophytes*. Through all the oils exhibited varied antifungal activities, oils of *Azadirachta indica*, *Pongamia pinnata* and *Seamum indicum* showed maximum inhibitory activities. Kabara[12] and others have reported that certain fatty acids (e.g., medium-chain saturates) and their derivatives (e.g., monoglycerides) can have adverse effects on various microorganisms, those microorganisms that are inactivated include bacteria, yeast, fungi, and enveloped viruses.

TABLE 4 : Antifungal Activity

S. No.	Fungal Isolation	Minimum Inhibitory Concentration (%)								
		Coconut Oil			Sesame Oil			Peanut Oil		
		20	40	80	20	40	80	20	40	80
1	*Aspergillus flavues*	++	+	-	++	+	+	++	+	-
2	*Aspergillus niger*	++	+	+	++	+	+	++	-	-
3	*Candida albicans*	++	+	-	++	+	-	++	+	-
4	*Trichoroderma viridae*	++	+	-	++	+	+	++	-	-

"++" - High Growth; "+" - Moderate Growth; "-" - No Growth

CONCLUSION

Finally concluded the coconut oil has more antimicrobial activity which is highly recommended to skin disease patient for curing and also the pure coconut oil daily apply to body that inhibit the growth of pathogenic microbes.

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