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IN-VITRO ANTIMICROBIAL ACTIVITY OF EDIBLE OILS AGAINST HUMAN PATHOGENS CAUSING SKIN INFECTIONS

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ABSTRACT: In recent years, a large number of oils and their constituents have been investigated for their antimicrobial properties against bacteria and fungi. The present investigation evaluated the antimicrobial potential of edible medicinal oils of *Cocos nucifera, Helianthus annus, Brassica juncea, Ricinus communis, Arachis hypogea, Glycine max, Gossypium hirsutum* and *Sesamum indicum* extracted from different solvents. There is a basis for the traditional use of these plants for local health remedies. Oils of these plants were screened for their antimicrobial activity against human pathogenic bacteria and fungi, causing skin diseases. The antimicrobial activity of these oils was investigated against *Escherichia coli, Trichophyton rubrum* and *Candida albicans* by agar well diffusion method, *Helianthus annus* seed oil showed maximum antimicrobial activity against tested bacteria and fungi with MIC values ranging from 0.62 to 40 mg/mL using inhibitory zone estimation. These results support the edible medicinal oils can be used to cure skin diseases.

INTRODUCTION: Edible oils extracted from plant sources are important in foods and various other industries (*e.g.* cosmetics, pharmaceuticals, lubricants). They are key components of the diet and also provide characteristic flavors and textures to foods. The chemical and physical properties of edible oils depend primarily on composition (and hence on biological origin) and temperature [1]. Seed oils are an important source of fatty acids for human nutrition and hydrocarbon chains for industrial products [2]. Many naturally occurring compounds found in edible and medicinal plants, herbs, and spices have been shown to possess anti-microbial functions and could serve as a source of antimicrobial agents against bacteria and fungi [3-5].

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In recent years, a large number of oils and their constituents have been investigated for their antimicrobial properties against bacteria and fungi [6]. The oils of medicinal plants have been used for the treatment of various ailments since men learned the art of extraction [7]. The vegetable oils are important for agriculture and food industry. India is fortunate in having a wide range of oilseeds crops grown in its different agro-climatic zones. Sunflower, Soyabean Groundnut, mustard, sesame, safflower, linseed, and castor are the major traditionally cultivated oilseeds [8].

Many components naturally present in vegetable oils have been shown to have beneficial properties [9]. Many of the fatty acids and other compounds present in vegetable oils have long been known to benefit our health. There is great potential for developing functional vegetable oils [10-12]. Due to their healing and nurturing properties, vegetable oils have been extracted from various plants for many years for use in cosmetics and body and skincare products.

These plants are everlasting, easily available, and century-old tested source for healing various skin ailments [11]. Therefore, this research work explored the antimicrobial properties of the edible oil against human pathogens, causing skin diseases, to minimize the overuse of antibiotics in the treatment of infectious diseases for which little data exist.

MATERIALS AND METHODS:

Collection of Plant Material: For the present study edible oil seeds were selected, growing around Gulbarga University, Gulbarga, Karnataka, India, were collected **Table 1**.

TABLE 1: EDIBLE OIL PLANTS THEIR BOTANICAL NAME, COMMON NAME, FAMILY AND PLANT PART USED

Botanical name	Common name	Family	Part used
Cocos nucifera	Coconut	Arecaceae	Endosperm
Helianthus annus	Sunflower	Asteraceae	Seeds
Brassica juncea	brown mustard	Brassicaceae	Seeds
Ricinus communis	castor	Euphorbiaceae	Seeds
Arachis hypogea	peanut	Fabaceae	Seeds
Glycine max	soybean	Fabaceae	Seeds
Gossypium hirsutum	cotton	Malvaceae	Seeds
Sesamum indicum	sesame	Pedaliaceae	Seeds

Preparation of Plant Extract: The collected seeds were initially rinsed with distilled water to remove soil and other contaminants and dried in the laboratory for a week. The dried seeds were ground to semi-powdered state and about 250g powdered plant part was extracted successive method, *i.e.*, hexane, petroleum ether, chloroform, ethyl acetate, methanol and aqueous in a soxhlet extractor for 48 h. The fractions obtained were combined into calibrated flasks, evaporated to dryness and weighed to determine the extraction's efficiency. The oils were stored in a sealed glass vial (bijoux bottle) in a refrigerator at 4 ºC until required. The oils were solubilized in DMF (Dimethyl formamide) to a final concentration of 5 mg/ml. These all oils of the above plants were screened for their antimicrobial activity.

Media Preparation: Antimicrobial susceptibility was tested on solid Agar-agar media (gm/l: beef extract, 3g; peptone, 5g; sodium chloride, 5g; agar, 20g) and for fungus PDA(gm/l: potato, 250g; dextrose, 20g; agar, 20g) was used for developing surface colony growth. The minimum inhibitory concentration (MIC) values were determined by agar well diffusion method.

Culture Maintenance and Preparation of Inoculum: The isolate of *Escherichia coli*, *Trichophyton rubrum* and *Candida albicans* used for the present study were obtained from Microbiology Department, Gulbarga University, Gulbarga. Karnataka, India, The fungal cultures were maintained on Sabouraud Dextrose Agar (SDA) medium supplemented with Chloramphenicol (50 mg/ml) and Streptomycin sulfate (500 µg/ml) and subcultured on Potato Dextrose Agar (PDA) every 15 days to prevent pleomorphic transformations. The fungal lawn was prepared using 5 days old culture strain. The fungal strains were suspended in a saline solution (0.85% NaCl) and adjusted to a turbidity of 0.5 Mac Farland standards (108 CFU/ml) and used for antimicrobial assay tests. Bacterial cultures were grown in nutrient broth (Himedia, M002) at 37 ºC and maintained on nutrient agar slants at 4 ºC.

Antimicrobial Screening: Oils were screened for their antimicrobial activity against tested organisms by agar well diffusion method [13]. Inoculum (1ml) was spread over the potato dextrose agar medium using a sterilized glass spreader.

Using flamed sterile borer, wells of 4 mm diameter were punctured in the culture medium. About 20 µl of 5 mg/ml of solubilized oils were added to the wells. The plates thus prepared were left for the diffusion of extracts into media for one hour in the refrigerator and incubated for 48 h at 28 ºC. The test was performed in triplicate. The diameter zone of inhibition was measured and expressed in millimeters. DMF was used as a negative control. Standard antibiotics Ketoconazole used as a positive control (1000µg/ml). The same method was followed for testing antibacterial activity using nutrient agar medium incubated at 37 ºC for 18 h. Streptomycin sulfate used as a positive control for bacteria.

Determination of Minimum Inhibitory Concentrations (MIC) using Agar Well Diffusion Method: The minimum inhibition concentration MICs were determined as the lowest concentration of oil inhibiting the visible growth of each organism on the culture plates. The MIC values were determined by agar well diffusion method. Required concentrations of serially diluted seed oils (0.6, 1.2, 2.5, 5, 10, 20 and 40 mg/ml) were added to the wells. The least concentration of each oils showing a clear of inhibition was taken as the MIC.

Statistical Analysis: Each experiment has three replicates, and three determinations were conducted. Means and standard deviation were recorded.

RESULTS AND DISCUSSION: The results obtained in the evaluation of the antimicrobial activity of the edible oils against *E. coli*, *T. rubrum*, and *C. albicans* summarized in **Table 2**. The oil obtained from the seeds of *H. annus* petroleum ether extract exhibited a maximum activity, 0.62 mg/ml of extract displaying strong activity with MIC values summarized in **Table 3** against *E. coli* and *C. albicans*, respectively, while *T. rubrum* appears resistant with minimum activity as compared to *E. coli* and *C. albicans.*

TABLE 2: ANTIMICROBIAL ACTIVITY OF EDIBLE OILS OF MEDICINAL PLANTS

S. no.	Botanical name	Test organisms	Zone of Inhibition					
			1	2	3	4	5	6
1	*Cocos nucifera*	*E. coli*	16.66±0.28	14.66±0.28	14.16±0.28	13.16±0.28	15.16±0.28	9.33±0.28
		T. rubrum	14.5±0.5	12.83±0.28	12.16±0.28	10.5±0.5	13.33±0.28	7.33±0.28
		C. albicans	9.16±0.28	8.33±0.28	8.66±0.28	8.16±0.28	9.66±0.28	8.33±0.57
2	*Helianthus annus*	*E. coli*	16.66±0.57	17.16±0.28	13.33±0.57	12.16±0.28	14.66±0.57	5.66±0.57
		T. rubrum	13.66±0.28	14.16±0.28	11.33±0.57	10.33±0.57	12.16±0.28	4.16±0.28
		C. albicans	14.66±0.57	15.33±0.57	11.83±0.28	10.83±0.28	13.16±0.28	5.5±0.5
3	*Brassica juncea*	*E. coli*	15.66±0.28	16.16±0.28	14.83±0.28	12.16±0.28	15.33±0.57	7.5±0.5
		T. rubrum	13.16±0.28	14.16±0.28	12.16±0.28	10.83±0.28	12.66±0.57	5.83±0.28
		C. albicans	14.66±0.57	15.16±0.28	13.5±0.5	11.66±0.57	14.16±0.28	6.33±0.28
4	*Ricinus communis*	*E. coli*	16.83±0.28	14±0	12.66±0.57	11.33±0.57	15.16±0.28	6.16±0.28
		T. rubrum	14.66±0.28	10.66±0.28	10.16±0.28	9.16±0.28	12.16±0.28	4.16±0.28
		C. albicans	15.33±0.28	12.16±0.28	11.16±0.28	9.33±0.57	13.16±0.28	6.83±0.28
5	*Arachis hypogea*	*E. coli*	13.33±0.57	12.66±0.28	14.66±0.28	11.66±0.57	14.16±0.28	8.33±0.28
		T. rubrum	11.16±0.28	9.5±0.5	12.83±0.28	8.66±0.57	11.66±0.57	6.16±0.28
		C. albicans	12.16±0.28	11.16±0.28	13.83±0.28	9.66±0.57	13±0	6.83±0.28
6	*Glycine max*	*E. coli*	15.16±0.28	13.83±0.76	15.66±0.57	12.16±0.28	10.16±0.28	8.66±0.57
		T. rubrum	12.83±0.28	12.16±0.28	13.83±0.28	9.66±0.57	8.66±0.57	4.66±0.57
		C. albicans	13.66±0.57	13±0	15.16±0.28	11.16±0.28	9.33±0.57	6.66±0.57
7	*Gossypium hirsutum*	*E. coli*	12.33±0.57	11.16±0.28	9.33±0.57	8.33±0.57	15.16±0.28	8.5±0.5
		T. rubrum	10±0.	8.33±0.28	7.5±0.86	6.33±0.57	13.16±0.28	5.66±0.57
		C. albicans	10.16±0.28	9.5±0.5	8.66±0.57	7.66±0.57	14.16±0.28	6.16±0.28
8	*Sesamum indicum*	*E. coli*	15.66±0.28	13.66±0.57	11.66±0.57	9.83±0.28	9.33±0.57	8.33±0.57
		T. rubrum	13.33±0.28	11.5±1.32	9.33±0.57	9±0	8.16±0.28	6.33±0.57
		C. albicans	14.83±0.28	12.83±0.76	12±0	10.5±0.5	9.16±1.04	7.66±0.57

TABLE 3: MINIMUM INHIBITORY CONCENTRATION OF *HELIANTHUS ANNUS* SEEDS OIL

S. no.	Botanical name and part used	Family	Test organisms	Zone of inhibition						
				40 mg/ml	20 mg/ml	10 mg/ml	5 mg/ml	2.5 mg/ml	1.25 mg/ml	0.62 mg/ml
1	Helianthus annus (seeds)	Asteraceae	*E. coli*	14.83 ±0.28	11.16 ±0.28	16.33 ±0.57	15.83 ±0.57	12.16 ±0.28	14.66 ±0.28	17.5 ±0.5
			T. rubrum	9.83 ±0.28	11.16 ±0.28	11.66 ±0.28	9.33 ±0.28	9.83 ±0.28	9.16 ±0.28	7.16 ±0.28
			C. albicans	12.83 ±0.28	11.5 ±0.5	13.83 ±0.28	12.16 ±0.28	15.83 ±0.76	11.83 ±0.28	14.83 ±0.28
2	Positive control		Streptomycin sulphate (Bacteria)					30.0 ± 0.0		
			Ketoconazole (Fungi)					24.0 ± 0.0		
3	Negative control		DMF					NA		

In present findings, *H. annus* exhibited maximum activity against tested pathogens followed by *Ricinus communis, Cocos nucifera, Brassica juncea, Sesamum indicum, Gossypium hirsutum, Glycine max, and Arachis hypogea* **Table 2**. Sunflower seed oil could be used as an antimicrobial agent [1] and was effective on some microorganisms such as *Staphylococcus aureus, Bacillus subtilis, E. coli and C. albicans* which are commonly involved in urinary tract infection [14]. The sesame seed oil has been used as healing oil for thousands of years and also enjoyed by humans since the dawn of civilization [15]. Skin infections are widely encountered in the tropics with lots of orthodox remedies involving the use of systemic antibiotics, the problems of drug resistance and reported allergies also abound.

In the present investigation, coconut oil and castor seed oil hexane extract shows maximum activity against *E. coli* and *C. albicans* **Table 2**, similar results were reported by Amit [16], where they studied the antifungal activity of lemongrass oil, coconut oil, almond oil and clove oil against *Candida* species isolated from bloodstream infection and they revealed that lemongrass showed highest anticandidal activity against *C. tropicalis* followed by *C. tropicalis* and Clove oil showed maximum activity against *C. albicans*, *C. tropicalis* and *C. guilliermondii*. In the case of almond oil maximum activity was reported against *C. guilliermondii* and Coconut oil showed maximum activity against *C. albicans*.

The antimicrobial activity of coconut oil had been attributed to the carboxylic acid – monolaurin metabolized to lauric acid in the body [17].

Coconut oil has been confirmed to possess antimicrobial, antiviral, and antiprotozoal activities [18-20]. Phytochemical studies indicated that lauric acid, which is its major fatty acid component, was highly responsible for the activities of the oil [21]. The castor oil extracted from the seed have been used in small doses in clinical setting for numerous medical conditions such as, liver and gallbladder disturbances, abscesses, headaches, appendicitis, epilepsy, hemorrhoids, constipation, diarrhea, intestinal obstructions, skin diseases, hyperactivity in children and to avert threatened abortion in pregnant women [22-24].

Although much has been documented on the uses of castor oil, there is no report on its antimicrobial activity [25]. The active compound in the oil previously identified to be monolaurin could have had an enhanced penetration due to the presence of surface-active emulsifying agents used in formulating the cream since emulsification of oils generally increases their absorptivity [26]. The choice of an anionic (sodium lauryl sulfate) and cationic (cetrimide) emulsifying agents were to avoid incompatibility with the selected preservatives notably phenolics and carboxylic acids [27].

In our finding, hexane and methanol extracts of *Ricinus communis* seed oil showed maximum antimicrobial activity against *E. coli*, *C. albicans*, and *T. rubrum* as compared to aqueous extract **Table 2**.

Kushwah [28] reported the antimicrobial activity of *Ricinus communis* seed against *E. coli, B. subtilis, B. cereus, S. aureus, C. glabrata,* and *C. albicans* and revealed methanolic seed extract with strongest antibacterial activity against *E. coli* (15mm). Similar studies reported by Abhishek [29], from roots of *Ricinus communis*, where hexane and methanol extracts showed maximum antimicrobial activity (200mg/ml) against *E. coli, S. aureus, Pseudomonas aeruginosa, Salmonella typhimurium, Proteus vulgaris, B. subtilis, C. albicans,* and *Aspergillus niger*. Interestingly, the aqueous extract has shown no significant antimicrobial properties, unlike the present finding and the findings of kushwah [28].

The results revealed that all the extracts are potent antimicrobials against all the microorganisms studied. Among the different solvents extracts studied Hexane and Methanol showed a high degree of inhibition followed by Petroleum ether, Chloroform, Ethyl acetate, and water extract.

Activity index of the extracts validate the possibilities of using the oil as a therapeutic agent. The continuous research for more reliable antibiotics becomes a worthwhile and noble mission. This study was, therefore, set up to ascertain the antimicrobial properties of edible oils. There is the need to further conduct *in-vivo* studies with the extracts to confirm the present *in-vitro* findings as the diameter of the zone of inhibition is

not only affected by the sensitivity of the microorganisms alone, but also the concentration of the extract is very important. Further studies should be undertaken to elucidate the exact mechanism of action by which extracts exert their antimicrobial effect.

CONCLUSION: The present investigation of these edible oil plants contain potential antimicrobial components that may be useful to cure infectious skin diseases.

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CONFLICT OF INTEREST: Nil

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