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Antibacterial Evaluation of Papaya Leaves Against Skin Infection Causing Bacterial Pathogens

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Abstract
Bacterial skin infections develop when bacteria enter through hair follicles or through small breaks in the skin that result from scrapes, punctures, surgery, burns, sunburn, animal or insect bites, wounds, and preexisting skin disorders. Herbal remedies for skin care with antibacterial and antifungal activities are prepared from a variety of plant parts such as leaves, stem, root, bark or fruit. Therefore aim of the present study was to investigate the antimicrobial activity of aqueous extract of Papaya leaves in combination with Coconut oil and Soya bean oil against skin infection causing bacterial pathogens *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Papaya leaves aqueous extract with Coconut oil and Soya bean oil was evaluated by agar well diffusion method. The result of this research revealed that aqueous extract of *Carica papaya* leaves as well as Coconut oil and Soya bean oil has inhibitory effect on skin infection causing bacterial pathogens. Antibiotic resistance profile showed that 60% *S. aureus* and 40% *P. aeruginosa* were found to be resistant to all the antibiotics tested. It is suggested that *Carica papaya* may be recommended as useful source to prepare natural bioactive products from which we can develop new antimicrobial drugs which will be cost effective.

Keywords
Antibacterial, Coconut oil, Papaya leaves, Soya bean oil.

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INTRODUCTION
The skin provides a remarkably good barrier against bacterial infections. Although many bacteria come in contact with or reside on the skin, they are normally unable to establish an infection. Bacterial skin infections develop when bacteria enter through hair follicles or through small breaks in the skin that result from scrapes, punctures, surgery, burns, sunburn, animal or insect bites, wounds, and preexisting skin disorders [1]. Most skin infections are caused by fungi, *Staphylococcus aureus* and *Streptococcus* species [2]. *S. aureus* is a major human pathogen, which will likely remain both common and serious due to the high environmental adaptability and development of resistance to antibiotics [3, 4]. Infections due to *S. aureus* isolates with resistance to vancomycin have been associated with multiple treatment failures [5]. At the same time, *P. aeruginosa* can be associated with refractory skin ulcer, bacterial biofilm formation and resistivity to the majority of antimicrobials [6, 7].

Herbal remedies for skin care with antibacterial and antifungal activities are prepared from a variety of plant parts such as leaves, stem, root, bark or fruit. These medicines are administered topically and may be applied in the form of cream, lotion, gel, soap, sap, solvent extract or ointment, and have been established to possess antimicrobial properties [2, 8-

15]. Gels, creams and soap formulations containing a variety of plant extracts have been used to treat various skin disorders caused by microbial infections [16-19].

Papaya (*Carica papaya* L.) is a member of Caricaceae Family. The papaya is especially susceptible to parasites, pests, and diseases. This fussy plant needs a lot of water but must have good drainage and it bears most fruit in light, porous, slightly acidic soils that are rich in organic matter. It has been used to treat digestive problems and intestinal worms as well as warts, sinusitis, eczema, cutaneous tubercules and hardness of the skin. Green fruits are used to treat high blood pressure, roundworm infection, dyspepsia, constipation, skin disease, and genitourinary disorders. The traditional use of *C. papaya* leaves in the treatment of various ailments including urinary tract infections [20]. The leaf of papaya contains beta carotene, calcium, carpaine, fats, flavonoids and vitamin C [21] as well as has phenolic compounds, such as protocatechuic acid, p-coumaric acid and caffeic acid [22].

In recent years, a large number of oils and their constituents have been investigated for their antimicrobial properties against bacteria and fungi [23]. The oils of medicinal plants have been used for the treatment of various ailments [24]. In the present study Coconut oil and Soya bean oil are used. The scientific name of coconut is *Cocosnucifera* L. Coconut oil is fatty oil that comes from the white pulp of the coconut referred to as the "tree of life" because of its many uses. Coconut oil has traditionally been used as a medicinal agent for cancer, diabetes, diarrhea, dry skin, and psoriasis and is used as an antibacterial, antifungal, and antiviral agent for the treatment of dermal infections. Evaluation of Cocosnucifera L. as an anti-infective agent is very important due to the increased prevalence of antibiotic-resistant infectious microorganisms. Coconut oil contains Median Chain Fatty Acid (MCFA). Fats with a chain length of 6 to 12 carbons are called medium chain triglycerides (MCTs). The antiviral, antibacterial, and antifungal properties of the medium chain fatty acids/triglycerides (MCTs) found in coconut oil have been known to researchers since the 1960s [25, 26]. Coconut oil has been shown to be as effective and safe as mineral oil when applied as moisturizers for mild to moderate xerosis [27].

Soybean oil or soya oil is vegetable oil extracted from the seeds of the soybean. It contains high concentrations of genistein, vitamin E, essential fatty acids (EFAs), and lecithin. Together, these chemical compounds can enable soybean oil benefits to include strong antioxidant protection [28]. Due to its various antioxidant constituents, topical application of soybean oil contributes antioxidant effects that help to protect the skin by neutralizing free radicals that are present in the environment [29]. In addition, soybean oil constituents- Vitamin E and EFAs- allow it to be easily infused into cosmetic formulas, as well as interact with the skin's surface [30]. As a result, skincare products often include soya oil skin benefits as a way to incorporate additional antioxidant protection. This can help to maximize the effect of personal care products such as anti-aging or brightening creams [31]. Overall, soya oil skin benefits are known to enhance the skin's overall quality and appearance.

The clinical effectiveness of many existing antibiotics is threatened by the emergence of multidrug-resistant pathogens rapidly [32]. According to World Health Organization, medicinal plants would be the very important resource to provide a variety of drugs. These plants used medically in various lands are resource of much potent agents [33]. Also, these plants should be searched to better understand their features, efficacy and safety. Therefore aim of the present study was to investigate the antimicrobial activity of aqueous extract of Papaya leaves in combination with Coconut oil and Soya bean oil against skin infection causing bacterial pathogens *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

MATERIALS AND METHODS

Preparation of Papaya Leaves Extract:

For the preparation of aqueous extract, leaves of papaya tree were rinsed with water. Dry powdered leaves were extracted with sterile distilled water and the aqueous extract was used. The aqueous extract was prepared by suspending 100grams of the finely blended leaves in 200ml of sterile distilled water. This was then agitated using the blender after which another 300ml of distilled water was added. The mixture was stirred every 3 minutes for 30 minutes and then allowed to stand for 24 hours. The extract was then decanted and filtered through a Whatman filter paper. The filtrate was then concentrated with the rotary evaporator at 45°C. This extract was then stored in the refrigerator at 4°C until use [34, 21].

Collection of Oil Samples:

Coconut oil and Soya bean oil were purchased from local market in Nagpur city.

Test organisms:

Skin infection causing bacterial pathogens such as *Staphylococcus aureus* (n=5) and *Pseudomonas aeruginosa* (n=5) were collected from pathology laboratory in Nagpur and were identified on the basis

of morphological, cultural and biochemical characteristics [35].

Antibiotic sensitivity test:

Antibiotic sensitivity test was performed by Kirby Bauer Disc Diffusion method [36]. Five different types of antibiotics were used in the study against *S. aureus* and *P. aeruginosa* each (Table 1). Skin infection causing bacterial pathogens were grown on nutrient agar at 37^{0}C for 24 hours and the colonies were suspended in sterile saline water equivalent to a 0.5 McFarland standard (1.5X108 CFU/ml). Hi-sensitivity agar plate was uniformly seeded by adding 100µl inoculated broth and was spread by means of spreader. The antibiotic discs were placed on each inoculated Hi-sensitivity agar plate. The plates were incubated at 37^{0}C for 18 hours. The diameter of the zone of inhibition was observed in mm and the isolates were classified as "resistant" or "sensitive" based on the standard interpretative chart according to Clinical and Laboratory Standards Institute (CLSI) guidelines [37].

Antibacterial activity of Papaya leaves and Edible Oils against skin infection causing bacterial pathogens:

Antibacterial activity of Papaya leaves and edible oils was performed by agar well diffusion technique. Skin infection causing bacterial pathogens were grown overnight on nutrient agar at 37^{0}C, and the colonies were suspended in sterile saline water equivalent to a 0.5 McFarland standard (1.5×108 CFU/ml). The suspension (100 µL) was spread over the Hi-Sensitivity agar. The wells of 6 mm diameter were cut into the agar medium with a sterilized cork borer. Then 20µl each of Papaya leaves extract, coconut oil and soya bean oil were added separately into the separate wells. The plates were incubated at 37^{0}C for 18 hours. The diameter of the zone of inhibition around each well was measured and recorded [36].

Synergistic activity of Papaya leaves with Edible oils against skin infection causing bacterial pathogens:

Equal quantity of Papaya leaves extract with Coconut oil and Soya bean oil was added in the wells on Hi-Sensitivity agar plates inoculated with the skin infection causing bacterial pathogens. The plates were incubated at 37^{0}C for 18 hours. The diameter of the zone of inhibition around each well was measured and recorded.

RESULTS AND DISCUSSION

A total of 10 skin infection causing bacterial pathogens were tested for the antimicrobial effect of Papaya leaves extract with Coconut oil and Soya bean oil. It was found that out of total *S. aureus* (n= 5) 60% were susceptible to Papaya leaves extract and Coconut oil each while 80% to Soya bean oil. However, when Papaya leaves extract was used in combination with Coconut oil and Soya bean oil, all *S. aureus* (100%) were found to be susceptible for each combination (Table 2). According to the previous research, papaya leaf extracts have phenolic compounds, such as protocatechuic acid, p-coumaric acid, 5,7-dimethoxycoumarin, caffeic acid, kaempferol, quercetin, and chlorogenic acid [38, 39]. The present findings are correlated with that of the previous research in which they reported that the papaya leaves aqueous extract showed inhibition on *S. aureus* with zone of inhibition of 24 mm [21]. On the other hand, when *P. aeruginosa* (n= 5) were tested 60% were susceptible to Papaya leaves extract while 80% to Coconut whereas 100% to Soya bean oil. However, when Papaya leaves extract was used in combination with Coconut oil and Soya bean oil, all *P. aeruginosa* (100%) were found to be susceptible for each combination (Table 3). These results have similarity with that of the results of the previous findings in which they evaluated the antimicrobial activity of papaya leaves aqueous extract against *Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa* [40]. It is reported that Gram-negative bacteria were more susceptible to the extracts of papaya leaf [41]. It is also reported that the acid components of coconut oil, monolaurin has been shown to have additional significance. Monolaurin is a monoglyceride derived from lauric acid. It comprises nearly 50% of coconut's fat content. Monolaurin displays antimicrobial activity by disintegrating the lipid membrane of lipid-coated bacteria including *Propionibacterium acnes*, *Staphylococcus aureus*, and *Staphylococcus epidermidis* [42, 43]. It was reported that Coconut oil in concentrations of 5% to 40% (*w/w*) exhibited bactericidal activity against *Pseudomonas aeruginosa, Escherichia coli, Proteus vulgaris*, and *Bacillus subtilis*. Cellular studies have also shown that monolaurin exhibits antiviral and antifungal activity [44]. Soya bean oil is a vegetable oil and it is reported that due to its healing and nurturing properties, vegetable oils are used in cosmetics and skincare products [45].

Antibiotic resistance profile of skin infection causing bacterial pathogens showed that 60% *S. aureus* were found to be resistant to all the antibiotics tested. Besides this, all *S. aureus* were resistant to Imipenem and Oxacillin (Table 4). On the other hand, 60% *P. aeruginosa* were found to be resistant to all the antibiotics tested. Besides this, all *P. aeruginosa* were resistant to Carbenicillin and Piperacillin/Tazobactam (Table 5). The papaya leaves extract has the compounds with antibacterial properties which can be utilized as antibacterial

agents in original drugs for the treatment of gastroenteritis, uretritis, otitis media, and wound infections [46].

Table 1: Antibiotics used in the study

Bacterial Pathogen	Antibiotics	Abbreviation	Concentration
	Amoxyclav	AC	30mcg
	Cephalexin	CF	30mcg
S. aureus	Imipenem	I	10mcg
	Oxacillin	OX	1mcg
	Trimethoprim	TR	30mcg
	Carbenicillin	CB	100mcg
	Co-trimoxazole	COT	25mcg
P. aeruginosa	Levofloxacin	LE	5mcg
	Piperacillin/ Tazobactam	PT	100mcg
	Sparfloxacin	SC	5mcg

Table 2: Antimicrobial activity of papaya leaves extract with edible oils against *Staphylococcus aureus*

Samples	*S.aureus* 1	*S.aureus* 2	*S.aureus* 3	*S.aureus* 4	*S.aureus* 5
Papaya leaves extract	R	22mm	27mm	R	16mm
Coconut oil	19mm	14mm	R	13mm	R
Soyabean oil	17mm	R	15mm	20mm	24mm
Papaya leaves extract + Coconut oil	22mm	22mm	28mm	17mm	19mm
Papaya leaves extract + Soyabean oil	20mm	24mm	18mm	16mm	12mm

Where R=Resistant

Table 3: Antimicrobial activity of papaya leaves extract with edible oils against *Pseudomonas aeruginosa*

Samples	*P.aeruginosa* 1	*P.aeruginosa* 2	*P.aeruginosa* 3	*P.aeruginosa* 4	*P.aeruginosa* 5
Papaya leaves extract	17mm	18mm	17mm	R	R
Coconut oil	13mm	19mm	18mm	20mm	R
Soyabean oil	17mm	20mm	21mm	15mm	17mm
Papaya leaves extract + Coconut oil	24mm	22mm	21mm	22mm	19mm
Papaya leaves extract + Soyabean oil	21mm	24mm	23mm	20mm	20mm

Table 4: Antibiotic resistance profile of *Staphylococcus aureus*

S.aureus	Amoxyclav	Cephalexin	Imipenem	Oxacillin	Trimethoprim
S. aureus 1	R	R	R	R	R
S. aureus 2	R	R	R	R	R
S. aureus 3	20mm	15mm	R	R	R
S. aureus 4	R	R	R	R	R
S. aureus 5	R	27mm	R	R	24 mm

Table 5: Antibiotics resistance profile of *Pseudomonas aeruginosa*

P. aeruginosa	**Carbenicillin**	**Co-Trimoxazole**	**Levofloxacin**	**Piperacillin /Tazobactam**	**Sparfloxacin**
P. aeruginosa 1	R	R	R	R	R
P. aeruginosa 2	R	22mm	24mm	R	20mm
P. aeruginosa 3	R	R	R	R	R
P. aeruginosa 4	R	20mm	16mm	R	19mm
P. aeruginosa 5	R	R	R	R	R

CONCLUSION

The result of this research revealed that aqueous extract of Papaya (*Carica papaya)* leaves has inhibitory effect on skin infection causing bacterial pathogens. The extract was active against both Gram-positive (*S. aureus*) and Gram-negative bacteria (*P. aeruginosa*) tested. This indicates a broad spectrum of activity. This study can be useful as a possibility of developing therapeutic substances that will be active against several drug-resistant organisms. It is suggested that Papaya leaves may be recommended as useful source to prepare natural bioactive products from which we can develop new antimicrobial drugs which will be cost effective. In the search for new pharmaceuticals, screening of such various natural organic compounds and identification of active agents must be considered as a fruitful approach. Coconut oil and Soya bean oils have showed the antimicrobial activity against skin infection causing bacterial pathogens. Future studies can add to current findings to allow for better understanding of these oils, with the potential to develop dermatological treatments and skin care products using these oils.

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