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# Antimicrobial Activity of Cassava Seed Oil on Skin Pathogenic Microorganisms

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**Abstract**: An assessment of the antimicrobial activity of oil extracted from cassava (*Manihot esculenta*. Crantz) seeds was investigated using agar-well diffusion method against clinical isolates of *Staphylococcus aureus*, *Propionibacterium acnes*, *Escherichia coli*, *Pityrosporium ovale* and *Candida albicans* which were isolated from skin infections. The results of the investigation showed that cassava seed oil had inhibitory effect on the growth of all the test isolates. Significant differences ($p<0.05$) were observed in the degree of inhibition of the isolates, but non-significant variations were observed in inhibition among strains of the same species. The most pronounced inhibition as confirmed by the zones of inhibition around growing colonies was on *S. aureus*; *P. acnes* was moderately inhibited, while inhibition of growth of *E. coli* was mild. Growth inhibition by the oil was not significant ($p>0.05$) between *P. ovale* and *C. albicans*. The inhibitory ability of the oil decreases with a decrease in concentration of oil in the solvent, resulting in marked variation in the minimum inhibitory concentration. The implication of this observation is that the oil may be of medical and particularly dermatological importance

**Key words:** Cassava seed oil, *Manihot esculenta*, antimicrobial activity, skin infections, growth inhibition

## INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is an important staple crop in many tropical countries of the world. Cultivation of cassava is a good source of income for peasant farmers as well as commercial farmers because of the many domestic and industrial uses to which various parts of the plant is put into. The processed roots are consumed in various forms; cassava instant noodles have been produced from the roots, while syrups useful in the soft drinks industry is made from the roots. The roots, leaves and peels from the roots are also useful in the formulation of livestock feeds. Leaves of some hybrids is recently recognised as a source of protein (Nassar and Marques, 2006).

At maturity, cassava plant produces seeds in capsule; the seeds are similar in shape and size to those produced by Castor oil (*Ricinus cummunis*) seeds. When dry, the initially greenish seeds turn black in colour and explode to release the seeds. Cassava seed is rich in oil and some essential fatty acids (Popoola and Yangomodou, 2006). Recently, plant derived antibiotics and antimycotics are attracting the attention of cosmetic microbiologists and dermatologists because they are cheaper, safer, eco-friendly and within the each of the medical community.

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Oil from parts of plants has been shown to exhibit antifungal activity against a wide range of pathogenic fungi and bacteria species (Okeke *et al*., 2001; Pawar and Thaker, 2006). Oil of plant origin has also been used for various cosmetic purposes particularly in the formulation of skin and body care products depending on their source and constituents. Apart from a few domestic uses of cassava seed oil, local communities in Nigeria use the oil for the treatment of skin rashes and related skin infections caused by bacteria and fungi species. Although, there are no scientific justifications for this local practise, use of medicinal herbs and extracts from plant sources in the treatment of skin diseases is an age-long practice in many parts of the world (Irobi and Daramola, 1993). Treatment of skin rashes, boils, skin irritations, wounds, dermatitis and pyoderma with plant extracts is a common practise in Russia and Central Asia (Mamedov *et al*., 2005). This study was carried out to investigate the antimicrobial activity of cassava seed oil on some microorganisms that are normally commensals on the human skin but have been implicated in human skin infections.

## MATERIALS AND METHODS

### Collection of Cassava Seeds

Mature green capsules containing cassava seeds were obtained from experimental plots of International Fund for Agricultural Development (IFAD) farms in Ijebu-Ife, South West of Nigeria. Seeds collected were of Cassava Variety TMS 30572. They were dried in the sun for 4-5 days after which the seeds were removed from the capsules.

### Extraction of Oil

A known weight of the seeds were grounded into powder and dried in an air circulating oven at 50°C for 1 h. Oil was extracted from the dried grounded seeds with petroleum ether (boiling point 60-80°C) using a Soxhlet extractor. The solvent was distilled off at 80°C.

### Microorganisms

All the strains of bacteria and fungi species used in the study were clinical isolates. The bacteria species were obtained from the Department of Medical Microbiology and Parasitology, University Teaching Hospital, (UCH) Ibadan, while the fungi species were obtained from the Federal Medical Centre, (FMC) Abeokuta. The bacterial species included *Staphylococcus aureus*, *Escherichia coli* and *Propionibacterium acnes*. While the fungi species are *Candida albicans* and *Pityrosporum ovale*. Five strains of each species of organisms were used. Each of the strains has designated laboratory codes allotted to them; all the isolates were confirmed from source to be implicated in some kind of skin infection (Table 1). There were no information made available on the specific biochemical and characteristics that distinguish the strains. The identities of the isolates were however re-confirmed using standard morphological and biochemical methods (Cheesborough, 1991) and mycological diagnostic methods (Milne, 1980).

### Antimicrobial Assay

The antimicrobial activity of different concentrations of the oil was determined by modified agar-well diffusion method described by Perez *et al*. (1990) and Adeniyi *et al*. (1996).

In this method, nutrient agar plates were seeded with 0.2 mL of 24 h broth cultures of each isolate (Sabourand Dextrose Agar was used for *Pityrosporum ovale* and *Candida albicans* strains) The plates were allowed to dry for 1 h. A sterile 5 mm cork-borer was used to cut two wells of equidistance in each of the plates; 0.5 mL of the cassava seed oil was introduced into one of the two wells while the same amount of sterile oil was introduced into the second well as control. The plates were incubated at 37°C for 24 h (48 h for yeast species). The antimicrobial activity was evaluated by measuring the diameter of zones of inhibition (in mm). All the plates were made in triplicates and the experiments repeated thrice.

Table 1: Source of test microorganisms

| Species of microorganisms | Strain code | Sources |
|---|---|---|
| *Staphylococcus aureus* | 2431 | *UCH, isolated from skin rash |
| | 3411 | UCH, isolated from boil |
| | 3621 | UCH, isolated from skin rash |
| | 3001 | UCH, isolated from boil |
| | 1990 | UCH, isolated from unknown skin infection |
| *Escherichia coli* | 2333 | UCH, isolated from boil |
| | 1411 | UCH isolated from skin rash |
| | 1093 | UCH, isolated from skin rash |
| | 1114 | UCH, isolated from boil |
| | 1039 | UCH, isolated from infected skin (razor) rash |
| *Propionibacterium acnes* | 3143 | UCH, isolated from skin acne |
| | 2030 | UCH, isolated from skin rash |
| | 1190 | UCH, isolated from skin lesions associated with Dermatomycoses |
| | 1390 | UCH, isolated from skin acne |
| | 1102 | UCH, isolated from skin infection site on nails |
| *Pityrosporum ovale* | 2556 | **FMC, on skin infection (Pityriasis) |
| | 2035 | FMC, skin infection lesions |
| | 2441 | FMC skin (razor) rash |
| | 2099 | FMC, skin infection lesions/superficial mycoses |
| | 2016 | FMC, skin infection lesions |
| *Candida albicans* | 1001 | FMC, isolated from skin rash |
| | 1425 | FMC, isolated from infected skin surface (razor rash) |
| | 1346 | FMC, isolated from dry infected skin |
| | 1922 | FMC, isolated from skin associated with superficial granulation of skin |
| | 2140 | FMC, isolated from skin site infected with Tinea |

*UCH = University College Hospital, Ibadan; **FMC = Federal Medical Centre, Abeokuta

The Minimum Inhibitory Concentration (MIC) of the oil was determined by diluting cassava seed oil in petroleum ether or benzene to get concentrations of (v/v) 80, 40, 20, 10 and 5% of cassava seed oil in the solvent. The different concentrations of the cassava seed oil was incorporated into wells on agar plates and incubated as described in the method for antimicrobial activity assay. The lowest concentration of oil in solvent, which inhibited growth, was noted as the minimum inhibitory concentration.

## RESULTS

The results of this investigation showed that cassava seed oil had inhibitory effect on the growth of clinical isolates of *Staphylococcus aureus*, *Escherichia coli*, *Propionibacterium acnes*, *Pityrosporum ovale* and *Candida albicans* tested. Significant differences ($p<0.05$) were observed in the degree of inhibition of the isolates, but non-significant variations were observed in inhibition among strains of the same species. On bacterial species, the most pronounced inhibition as confirmed by the zones of inhibition around growing colonies was on *S. aureus*. *P. acnes* was moderately inhibited, while inhibition of growth of *E. coli* was mild. With the yeast species, 100% oil concentration proved effective in inhibiting growth of the test isolates. Growth inhibition by the oil was however not significant between *P. ovale* and *C. albicans* (Table 2).

The results also showed that the inhibitory ability of the oil decreases with a decrease in concentration of oil in the solvent, resulting in marked variation in the minimum inhibitory concentration. While 5% concentration inhibited growth of *S. aureus*, inhibition of *E. coli* was only effective at a much higher concentration of 80% oil in solvent. *P. acnes* and *P. ovale* were inhibited at 20% concentration and *C. albicans* at was inhibited at 10% concentration.

The type of solvent used for the dilution of cassava oil did not have a significant ($p>0.05$), effect on growth inhibition of bacteria isolates, however for the yeasts; MIC was at a lower concentration with oil diluted with benzene. A concentration of 10% for *P. ovale* and 5% for *C. albicans* with petroleum ether compared with 20 and 10%, respectively for *P. ovale* and *C. albicans* with benzene as the solvent (Table 2).

Table 2: Inhibitory effect of 100% cassava seed oil and different concentration of seed oil on growth of test organisms

| | | | Diameter of zones of inhibition (in mm) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Petroleum ether | | | | | | Benzene | | | | | |
| | | 100% cassava | Concentration (v/v)* | | | | | | Concentration (v/v) | | | | | |
| Microorganisms | Strain | Seed-oil | 80 | 40 | 20 | 10 | 5 | Control | 80 | 40 | 20 | 10 | 5 | Control |
| *Staphylococcus aureus* | 2341 | 13.01 | 11.12 | 8.03 | 5.04 | 3.14 | 2.01 | 0.00 | 10.21 | 7.48 | 4.21 | 3.42 | 3.03 | 0.00 |
| | 2311 | 12.05 | 10.20 | 8.50 | 5.50 | 3.01 | 2.00 | 0.00 | 10.20 | 8.25 | 6.44 | 3.81 | 2.78 | 0.00 |
| | 3621 | 11.03 | 11.08 | 10.04 | 8.55 | 5.21 | 4.98 | 0.00 | 10.15 | 8.11 | 5.82 | 4.34 | 4.04 | 0.00 |
| | 3001 | 12.04 | 11.50 | 10.08 | 8.10 | 4.95 | 3.21 | 0.00 | 10.48 | 8.42 | 5.91 | 4.80 | 3.88 | 0.00 |
| | 1990 | 12.30 | 10.15 | 10.53 | 5.20 | 4.15 | 2.91 | 0.00 | 10.85 | 7.74 | 6.23 | 4.20 | 2.96 | 0.00 |
| *Escherichia coli* | 2333 | 1.53 | 0.41 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.18 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | 1411 | 1.16 | 0.32 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.21 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | 1093 | 1.16 | 0.30 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.16 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | 1114 | 1.64 | 0.39 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.19 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | 1039 | 1.75 | 0.45 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.25 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Propionibacterium acnes* | 3143 | 4.52 | 3.72 | 3.01 | 2.80 | 0.00 | 0.00 | 0.00 | 2.95 | 1.15 | 1.09 | 0.00 | 0.00 | 0.00 |
| | 2030 | 3.67 | 3.02 | 2.03 | 2.05 | 0.00 | 0.00 | 0.00 | 1.01 | 0.92 | 0.43 | 0.00 | 0.00 | 0.00 |
| | 1190 | 2.65 | 2.96 | 2.03 | 1.95 | 0.00 | 0.00 | 0.00 | 1.45 | 0.67 | 0.35 | 0.00 | 0.00 | 0.00 |
| | 1390 | 4.44 | 3.50 | 2.43 | 1.65 | 0.00 | 0.00 | 0.00 | 1.53 | 0.72 | 0.48 | 0.00 | 0.00 | 0.00 |
| | 1102 | 3.87 | 2.87 | 2.25 | 1.23 | 0.00 | 0.00 | 0.00 | 1.21 | 0.65 | 0.44 | 0.00 | 0.00 | 0.00 |
| *Pityrosporum ovale* | 2556 | 8.01 | 6.05 | 2.44 | 1.17 | 0.00 | 0.00 | 0.00 | 6.21 | 5.41 | 2.40 | 1.89 | 0.00 | 0.00 |
| | 2035 | 8.52 | 5.05 | 3.21 | 1.21 | 0.00 | 0.00 | 0.00 | 6.14 | 5.72 | 2.53 | 2.00 | 0.00 | 0.00 |
| | 2441 | 9.04 | 6.53 | 5.25 | 1.51 | 0.00 | 0.00 | 0.00 | 6.27 | 4.01 | 2.47 | 1.47 | 0.00 | 0.00 |
| | 2099 | 9.52 | 6.24 | 4.22 | 1.67 | 0.00 | 0.00 | 0.00 | 5.21 | 4.50 | 2.54 | 1.22 | 0.00 | 0.00 |
| | 2016 | 7.89 | 5.41 | 3.15 | 1.15 | 0.00 | 0.00 | 0.00 | 5.15 | 3.84 | 2.67 | 1.67 | 0.00 | 0.00 |
| *Candida albicans* | 1001 | 9.50 | 8.75 | 8.04 | 5.36 | 3.01 | 0.00 | 0.00 | 8.01 | 7.53 | 5.28 | 3.41 | 1.08 | 0.00 |
| | 1425 | 9.71 | 8.99 | 7.22 | 4.51 | 2.95 | 0.00 | 0.00 | 7.22 | 7.10 | 5.21 | 3.50 | 1.01 | 0.00 |
| | 1346 | 8.04 | 7.52 | 7.21 | 5.22 | 2.73 | 0.00 | 0.00 | 7.14 | 6.94 | 5.01 | 3.21 | 1.15 | 0.00 |
| | 1922 | 9.05 | 8.41 | 6.48 | 5.75 | 2.81 | 0.00 | 0.00 | 8.21 | 7.56 | 5.01 | 3.45 | 1.21 | 0.00 |
| | 2140 | 8.08 | 7.34 | 6.23 | 5.53 | 2.56 | 0.00 | 0.00 | 7.47 | 7.32 | 5.11 | 3.01 | 1.31 | 0.00 |

*Concentration v/v = oil: solvent, Values are mean of 3 readings

## DISCUSSION

The antimicrobial activity of cassava seed oil as observed in this study appeared to be broad spectrum as both gram negative, gram positive as well as yeast species were sensitive to the oil. The test microorganisms were chosen because they are known primarily as commensals on the human skin and mostly occur as part of the normal skin flora, but they can be opportunistic as they have the ability to change from a commensal to a pathogenic role when the local or general resistance of host is lowered. The implication of the effect of the oil on growth inhibition is that the oil can help keep the pathogenic activities of these microorganisms in check.

*Staphylococcus aureus*, which is commonly found associated with boil and other secondary skin infections in the area of study, was most inhibited. The inhibition of *E. coli* by the oil was relatively low, while that of *P. acnes* was mild compared to *S. aureus* (Table 2).

Physico-chemical properties of cassava oil have been evaluated (Popoola and Yangomodou, 2006) the unsaturation property of the oil makes it useful in the formulation of liquid soap and hair shampoo. Ajiwe *et al*. (1993) provided formulations for the use of the oil in the preparation of liquid soap and hair shampoo. The inhibitory activity of the oil as observed in this study gives it added derma-therapeutic advantage. The results also suggest possible use of the oil as an aroma therapeutic agent.

Essential oils and vegetable oils from plant sources particularly Grapefruit oil (*Citrus paradise*), black pepper oil (*Piper nigrum*), Lemon oil (*Citrus limon*), Lime oil (*Citrus aurantifolia*), Sweet orange oil (*Citrus sinensis)*, Tea tree oil (*Melaleuca alternifolia*) are widely used in the formulation of some cosmetic products (Mori *et al*., 2002) particularly shaving oil, hair shampoo and body cream.

Previous works on the antimicrobial activities of several essential oil component indicated that cineole, citral geraniol, linalool and menthol were active against several yeast-like and filamentous fungus (Pattnaik *et al*., 1997). Reports have also shown that essential oils of plant sources cold be used as aroma therapeutic agent for the remedy of fungal diseases of man (Tampieri *et al*., 2005) and plants (Soylu *et al*., 2006).

Although this work is still at a preliminary stage, the result obtained gives some support to the traditional use of the oil to for the treatment of skin infections. As this study is the first report on the antimicrobial activity of oil extracted from cassava seeds, further works are ongoing on the nature of essential oil present in cassava seed and the phenolic components it contains in order to enable the cosmetic industry benefit from the potentials of cassava seed oil.

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