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Parametric Studies of Carrot Seed Oil Extract for the Production of Medicated Soap

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***Abstract*— The research is to investigate the possible use of Carrot seed oil extract in the production of medicated soap. The oil extraction from Carrot seed was achieved through Soxlet extraction method. Carrot seed contains high carotene, a major pigment of carrot and enhancer of LOPS microbial activity as reported by Hayashi *et al.* (2013). Some physicochemical properties of the oil were analysed and they were found within specifications except for its boiling point which was found to be 134°C, below the specified boiling point range of 150°C to 300°C for essential oils as reported by Ranken *et al* (1997). The soap produced gave a pH of 7.4, foam height (4.8 cm), alcohol insoluble (6.0%), moisture content (9%). The antifungal activity of the soap fortified with Carrot seed oily extract on Trichophyton rubrum shows a very good sensitivity of 24.1 mm a bit below that of the Standard soap (26.6 mm) and above that of the Control soap (8.0 mm). From the analysis of the soap produced, it can be concluded that a highly effective medicated soap can be produced from Carrot seed extract.**

***Keywords*—Carotene, Carrot seed, Carrot seed oily extract, Medicated soap, Trichophyton rubrum.**

I. INTRODUCTION

Carrot (*Daucus Carota*) is a biannual root plant and one of the most commonly used vegetables as food for humans. Its taproot which is the consumable part of carrot is regarded as a very nutritious and healthy food supplement due to its high vitamins and fiber content ([Gonny *et al.*, 2004] and [Özcan and Chalchat, 2007]).

Carrot is an erect vegetable plant that flowers every two years. The plant produces the edible root and a leafy top within the first year. If left in the ground for another year its flower and seeds are produced (Negi & Roy, 2000).

Oil from carrot seed is an essential oil that is extracted from the seeds of carrot and should not be confused with the cheaper macerated “Carrot oil” made when people infuse the carrot material in a base oil (Staniszewska & Kula, 2001).

The main chemical constituents of the oil extract include β-bisabolene, camphene, β-pinene, sabinene, myrcene, γ-terpinene, limonene, α-pinene, Geranyl acetate and carotene (Staniszewska & Kula, 2001).

Scientists Asahina and Tsukamoto in 1925 were the 1st to isolate Carotene pigment. It is one of the major components found in oil from carrot seed comprising approximately 66% of this essential oil. This sesquiterpene alcohol is thought to be formed in carrot seeds during the vegetation period. Additional studies have indicated that carotene may be involved in allelopathic interactions expressing activity as an antifungal, herbicidal and insecticidal agent (Staniszewska & Kula, 2001).

Oliveira *et al.* (2008) investigated the antimicrobial activity of Hezelnut oil containing carotene against Gram positive (such as Staphylococcus aureus), Gram negative (such as Escherichia coli) and fungi (such as Candida albicans) and the results obtained revealed a high antimicrobial activity against Gram positive bacteria. Also Hayashi *et al*. (2013) investigated the effect of carrot oil extract on the bactericidal activity of a bovine lactoperoxidase system (LPOS) and the result obtained showed that LPOS antimicrobial activity increased from 1.4 to 3.8 log units by addition of 20 fold diluted carrot extract containing carotene. This research is focused on production of medicated soap from carrot seed oily extract for the treatment of fungal (Trichophyton rubrum) skin infection.

II. MATERIALS AND METHODS

A. *Materials Acquisition and Pre-treatment*

Carrot seeds used were purchased locally from local farmers in Farin Gada, Jos, Plateau State. They were cleaned to remove all foreign matter such as dust, dirt, stones, chaff. Immature and broken seeds were discarded as well. Prior to a chemical analysis, 300 g of carrot seed were ground to pass a 0.5 mm screen. The analytical grade n-hexane solvent produced by Bauchi Surgical Laboratory was used.

B. *Oil Extraction*

150g of crushed carrot seed was placed inside a thimble made from thick filter paper, which is loaded into the main chamber of the soxhlet extractor.

250ml of n-hexane was poured into a distillation flask and the soxhlet extractor was placed onto this flask which was equipped with a condenser. The solvent was heated to reflux at constant temperature of 68°C and the vapour travelled up a distillation arm into the chamber housing the thimble of solid where the vapour is condensed and the warm solvent flow under gravity and percolate through the bed of the crushed carrot seed to extract the oil. When the solvent chamber is almost full, the chamber is automatically emptied by a siphon side arm, with the solvent running back to the distillation flask. The cycle was repeated for 8 hours. After the extraction, the extract was separated from the solvent via a rotary evaporator (Jensen, 2007).

C. Oil Characterization

The following physicochemical properties of the carrot seed oily extract determined include: colour, density, specific gravity, viscosity, amount of oil yield, boiling point, refractive index, acid value, iodine value and saponification value.

Boiling Point: The boiling point of the oil was determined by heating the oil in a beaker on a heating mantle. The oil was observed carefully in the presence of a thermometer, immediately the oil started circulating and bubbling, the temperature on the thermometer was read and recorded as the boiling point of the oil.

Density: The density of the carrot seed oil was determined by taking the weight of sample and divides it by its corresponding volume (TSE, 1971).

$$Density = \frac{Mass}{Volume} \quad (1)$$

Specific Gravity: The specific gravity was calculated by dividing the oil density by the density of water (1000 kg/m^3) (TSE, 1971).

$$Specific\ gravity = density\ of\ oil / density\ of\ water \quad (2)$$

Determination of Saponification Value: Five (5)g of the oil sample was weighed into a conical flask. 50 ml of 0.5N KOH was added to the sample, a reflux condenser was attached to the flask and heated for 30 minutes for perfect dissolution of the sample. It was allowed to cool then 1 ml of phenolphthalein indicator solution was added and the content was titrated with 0.5N HCl to an end point. The same process was repeated using blank sample (Saad *et al.*, 2007).

The saponification value was calculated using the formula;

$$Saponification\ Value = 56.1 \times \frac{N}{weight\ of\ sample}(V_2 - V_1) \quad (3)$$

Where; 56.1 = Molecular weight of KOH
N = Normality of the KOH
V_1= Titre value for sample
V_2= Titre value for blank

Detrmination of Acid Value: Five (5)g the oil sample was weighed into clean conical flask and mixture of 25 ml diethyl ether and 25 ml ethanol was added and used to dissolve the oil in the mixed neutral solvent. 1 ml of phenolphthalein added and the solution was carefully titrated with 0.1N KOH until a pink colour which persists for 15 seconds was obtained (Saad *et al.*, 2007)

The acid value is calculated as thus;

$$Acid\ value = 56.1 \times N \times \frac{V}{M} \quad (4)$$

Where; 56.1 = molecular weight of KOH
N = normality of KOH
V = volume of KOH used
M = mass of the sample

D. Soap Production

Two kinds of soap (Test soap (TS) and control soap (CS)) were produced following the same steps only that carrot seed oily extract was incorporated into TS while CS was not fortified with the extract. A commercial Antiseptic soap (Funbact A) regarded as standard soap (SS) was purchased from a commercial store.

The boiling process was used during the soap preparation. 48g of palm kernel oil was place in the 500cm^3 beaker and 18g of the carrot seed oily extract was added. 8.8g of Sodium hydroxide (NaOH) in 20cm^3 of water was added to the mixture of oils and extract in the beaker. The mixture was heated for an hour in a water bath, maintaining the temperature in the range of 80 – 90°C with frequent stirring at a time intervals. 5mL of distilled water was added occasionally to prevent the content of the flask from becoming solid due to evaporation of water and alcohol during heating. After one hour of heating, 100cm^3 of a saturated solution of sodium chloride was added to the hot mixture and allowed to cool. The addition of the salt solution precipitates the soap out of solution ("salting out"). The soap float on the surface of the solution; it was filtered and place in the mould to dry.

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E. Soap Characterization

pH of soap: Buffer solution was used to calibrate the pH meter using pH values between 4.0 and 7.0, it was thereafter dipped directly into the soap sample while the reading was taken immediately (Moulay, 2005 & Hassan et al., 2010).

Soap Moisture Content: The moisture content in soap was determined by weighing Ten (10)g of the soap before heating and later reweighed after open heating for about 30minutes. The difference between the two weights gives the moisture content expressed in percentage (Moulay, 2005 & Hassan et al., 2010).

Free Acid Content: To determine the free acid content, Six (6)g of the produced soap sample was taken and dissolved in $70cm^3$ hot neutral alcohol and titrated against 2M H_2SO_4 using phenolphthalein indicator (Moulay, 2005 & Hassan et al., 2010).

$$\text{Free acid content} = \frac{3.1MV}{W} \quad (5)$$

where:
M – Molarity of H_2SO_4 solution, $mol{\cdot}L^{-1}$;
V – Volume of H_2SO_4 solution used in titration, ml;
W – weight of the soap sample, g.

Foam Height: To determine foam height, Two (2)g of produced soap was dissolved in a one liter volumetric flask and, 50ml of the solution was introduce into a measuring cylinder such that it followed the walls of the column to avoid foaming. 200ml of the solution was taken in a conical flask and poured into a funnel. The measuring cylinder was then put directly beneath the funnel while the level (height) of the foam generated was read from the cylinder immediately the funnel outlet was opened (Moulay, 2005& Hassan et al., 2010).

Alcohol Insoluble: Five (5)g of produced soap sample was measured and dissolved in 50ml of hot alcohol and transferred to already weighed filter paper; the residue on the filter paper was dried in oven at 105^0C for 30minutes, desiccator was used for cooling and weighed again (Moulay, 2005 & Hassan et al., 2010).

Antifungal Sensitivity Analysis: The Test microorganism used in this study was Trichophyton rubrum and the isolate was obtained from Abubakar Tafawa Balewa University Teaching Hospital Bauchi. 0.1 g/mL of the three soap samples (TS, CS and SS) were prepared. Various dilutions (10^{-1}, 10^{-2}, 10^{-3} and 10^{-4}) were also prepared for the three soap samples. Potato Dextrose Agar (P.D.A.) media was also prepared for 9 plates (three plates for each test samples).

From each prepared concentrations, 20 µL soap solution was aseptically transferred into a 5 mm bored well in a solidified P.D.A. plate which has been inoculated with the test isolate via pour plate technique. Plates were kept for 30 min before incubation at 37^oC for 24 h. the extent of susceptibility was recorded as clear zones of inhibition around the wells.

III. Results And Discussion

Table I
Physicochemical PROPERTIES Of Oil EXTRACT FROM Carrot Seed

Parameter Determined	Value
Colour	yellowish-brown
Density (Kg/m^3)	918
Specific Density	0.918
Amount of Oil Yield (%)	23.4
Boiling Point (^OC)	134
Acidity	2.5806
Saponification Value	173.91

Table II
Physicochemical PROPERTIES Of The TEST Soap(FROM CARROT SEED OIL) PRODUCED

Parameter Determined	Value
pH	7.4
Foam Height (cm)	4.8
Alcohol Insoluble (%)	6
Moisture Content (%)	9
Free Acidity	0.41

Table III
Antifungal Activity Of The Test Soap (Ts) From Carrot Seed Oil On Trychophyton Rubrum Fungus

S/N	Diameter of zone of inhibition (mm)	Dilutions			
	0.1 mg/mL of soap	10^{-1}	10^{-2}	10^{-3}	10^{-4}
1	24	23.4	16.8	9.2	6.4
2	24.2	23	17	8.8	6.2
3	24	23.2	17	9	6.4
Mean	24.1	23.3	16.9	9	6.3

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Table IV
Antifungal ACTIVITY Of The Control Soap (CS) On Trychophyton Rubrum FUNGUS

S/N	Diameter of zone of inhibition (mm)	Dilutions			
	0.1 mg/mL of soap	10^{-1}	10^{-2}	10^{-3}	10^{-4}
1	8	7.7	7.6	7.2	6.4
2	7.9	7.8	7	6.8	6.2
3	8	7.6	7.2	6.6	6.2
Mean	8	7.7	7.3	6.9	6.3

Table V
Antifungal ACTIVITY Of The Standard Soap (SS) On Trychophyton Rubrum FUNGUS

S/N	Diameter of zone of inhibition (mm)	Dilutions			
	0.1 mg/mL of soap	10^{-1}	10^{-2}	10^{-3}	10^{-4}
1	24.7	23.8	17.5	11	7.2
2	24.6	23.6	17.6	10.8	7
3	24.5	23.8	17.6	11	7.4
Mean	24.6	23.7	17.6	10.9	7.2

From Table I, it shows the quality standard of the extracted oil produced. The extracted oil was yellowish-brown in colour; this may be due to the presence of carotene pigment in the carrot seed oil. Ranken *et al.* (1997) reported that most oil extracted from oil seed are either green or orange in colour due to the presence of chlorophyll or carotene in the seed. The characteristic yellowish-red of much natural fat is caused by the presence of polyene carotenoid. The percentage oil yield obtained was 23.4% which is a bit lower than that of Caper seed oil (39%) as reported by Akgül and Özcan (1999). The specific gravity obtained was 0.918, lower than that of caper seed oil (1.084-1.1045) as reported by Akgül and Özcan (1999). Ranken *et al.* (1997) reported that the boiling points of most essential oils ranges from 150°C to 300°C, when they are distilled with water. The boiling point obtained was 134°C; this below specification boiling point obtained may be due to the presence of some traces of hexane used as extraction solvent which boils at a temperature range from 68°C to 69°C.

Table II shows the quality criteria of the Test soap produced. The pH value of the medicated soap produced is 7.4, a bit above Neutral (7.0), this indicated low amount of unspecified and unsaponificable matter due to incomplete alkaline hydrolysis. The foam height was found to be 4.8 cm; this could be trace to the type of oil (palm kernel) whose major fatty acid is lauric acid and which is known for its "foamability" (Ong *et al*., 1990; Olonisakin *et al*., 2005; Aigbodion *et at*., 2004). The value of alcohol insoluble which measures the amount of non-soap ingredient or contaminant was found to be 6% which is lower as compare to alcohol insoluble of Borax soap (20%) as reported by Popescu et al (2011), this may be due to absence of fillers and other minor constituents such as whitening agent. The moisture content was found to be 9% which conform to the work done by Kuntom et al (1999), they have studied soaps with Coconut oil, glycerine, olive oil, palm kernel oil and Canola oil and the result showed that moisture content was between 9 – 16%. The free acid content was found to be 0.41 which is between 0.06% in the Camey soap and 0.88% in the Dermafex soap as reported by Popescu *et al* (2011). The values determined were within the limits set by standards as spotted in the official journal of the European Union.

Tables III, IV and V showed the inhibitory activity of samples soaps against the test isolate. The inhibitory activity of Carrot seed oily extract fortified soap against Trichophyton rubrum was significant over CS. The sensitivity values (mm) for Trichophyton rubrum to TS ranged between mean values of 6.3 mm to 24.1 mm as compare to its low sensitivity to CS (6.3 mm to 8.0 mm). Also, the activity of TS and SS decreased progressively with an increasing dilution.

IV. CONCLUSIONS

The prospect of the production of medicated soap from oil extracted from carrot seed to treat fungal infections such as Trichophyton rubrum was found to be promising. From the analyses carried out, the efficacy and potency of the medicated soap produced was found to be good when compared to a standard medicated soap in treatment of fungal infections. This will go a long way in minimizing the cost of additives to provide antimicrobial property to the common soap despite achieving the same goal of prevention and cure to fungal infections.

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