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Evaluation of Antifungal Activity of *Eugenia aromatica* L. Bail (Cloves) on *Malassezia restricta* and *Malassezia globossa* Associated with Dandruff

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Abstract

Study was conducted to determine the efficacy of *E. aromatica* seed extracts against the growth of *M. restricta* and *M. globossa* associated with dandruff (*in vitro*). Fungal pathogens were microscopically identified and isolated and their frequencies of occurrence were noted. The results showed that *M. globossa* was the most frequently occurring pathogen with 56.4% followed by *M. restricta* with 43.6%. *E. aromatica* powder was used to prepare ethanol, aqueous and hexane extracts. The result of the extracts yield of *E. aromatica* showed that ethanol yield more extract with 14.30%, followed by water with 13.85% and hexane with 13.82%. Varying concentrations of the extracts such as 10, 20,40,60 and 100 mg/ml were prepared using agar incorporation method. The effect extracts against the growth of test fungi is directly proportional to the increase in concentration. The test fungi were found to be completely inhibited by all the extract of *E. aromatica* at the highest concentration (100 mg/ml). Thin layer chromatography of the extracts was conducted and the profiles showed 16 fractions each with varying antifungal activity against the test fungal isolates. Among the thin layer chromatographic fractions 0.04Rf of hexane extract (EAH0.04) of *E. aromatica* was found to have higher antifungal activity. The result of the study showed that *E. aromatica* contains pharmacologically active compounds; hence extracts could be used in the management of dandruff. It is therefore suggested that, the active components should be isolated and purified for proper and adequate formulations for the management of dandruff.

Keywords: Antifungal; Dandruff; *M. restricta*; *M. globossa*; *Eugenia aromatica*

Introduction

E. aromatic belongs to the kingdom plantae. Division: Angiosperms. The class is unranked. Order: Myrtales. Family: Myetaceae. Genus: Syzygium. *E. aromatic* has about four synonymous names. The names are *Caryophyllus aromaticus L, E. aromatic* (L.) *E. caryophyllata*, *E. caryophyllus*. The specie is an indigenous to the Moluccas and southern Philippines, but currently cultivated in many tropical areas including Africa (e.g. Madagascar and United Republic of Tanzania), South America, Indonesia, Malaysia and Sri Lanka.

In British Columbia, flower of clove is widely used in the treatment of roundworms and tapeworms in pets and pigs whereas, flower bud of *E. aromatica* has been used as traditional medicine for the treatment of asthma and various allergic disorders; as vermifuge, antibacterial agent and in toothache in China, Japan and Korea [1]. The flower bud is a well known as remedy for headache, soar throat, dental and respiratory disorders, digestive system ailments, in traditional medicines of Australia and Asian countries. In addition, the clove is widely used as traditional medicine for treatment of dyspepsia, gastritis and diarrhea; as antipyretic, aphrodisiac, appetizer, expectorant, antiemetic, anxiolytic, myorelaxant, analgesic, decongestant, anti-inflammatory and hypnotic.

E. aromatic is been used as mosquito repellent, and to prevent premature ejaculation. In addition, *E. aromatic* oil is used in preparation of some toothpastes and Clovacaine solution, which is a local anesthetic used in oral ulceration and inflammation. Eugenol (or clove oil generally) is mixed with zinc oxide to form a temporary tooth cavity filling *E. aromatic* oil is use to anesthetize fish, and prolonged exposure to higher doses (the recommended dose is 400 mg/l) is considered a humane means of euthanasia [2].

There has been an increasing incidence of multiple resistances in human pathogenic microorganisms in recent years, largely due to indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases [3]. This has necessitated the search for new antimicrobial substances from various sources for instance, the medicinal plants. Search for new plant based antibacterial agents is a continuous process largely by screening many plants and plant product.

Recently it was reported by, that plant are potential sources of drugs. Studies of medicinal plants provide valuable materials with enormous therapeutic potential and heal many infectious diseases particularly emerging enteropathogens with multiple antimicrobial resistances nowadays; it is well known that plant

extracts are safer, contrary to the synthetic drugs which are associated with many side effects.

Currently, in developing countries including Nigeria, there is an increasing interest in the use of different types of plant extract for the control of pathogens because of the drawbacks of the synthetic antimicrobial agents in the control of microbial infectious diseases [4]. Findings of this study may provide promising extracts in the management of dandruff infection; that are cost effective and readily available. More so, the study may contribute to the existing knowledge of ethnobotany.

Materials and Methods

Collection and preparation of sample materials

E. aromatica seeds were used in the present study. *E. aromatic* seeds were purchase from Sokoto main market [5]. The samples collected were packed separately in clean sterilized polythene bags and brought to the herbarium of the department of biological sciences, Usmanu Danfodio university, Sokoto for identification and authentication. Voucher specimens of the samples were prepared and deposited in the same herbarium as directed by Kumar.

The fresh samples were washed with tap water and air dried under shade. Dried samples of plant material collected were milled into fine powder using high capacity grinding machine and subsequently stored separately in sterilized polythene bags until required for use.

For the isolation of fungal pathogens associated with dandruff, a sharp and sterile epilation forceps were used to detach the hair and scalp samples from twenty infected volunteers at Sahara area, Sokoto metropolis. The infected region was first washed with 70% ethanol followed by scraping with the sterile forceps, which was held at an angle of 90℃ with the head [6]. The specimen was then transferred into a dark sampling paper to prevent exposure to sunlight. Each sample was labeled accordingly. The samples were then taken to the laboratory for microbial analysis as described by Patil.

Extraction procedure

Two hundred (200) grams from powdered samples of E. aromatic was extracted in water. Sample was dispensed in 1.5 liters of distilled water; the solution was stirred, cupped with aluminum foils and kept for twenty four hours (24 hours). The resultant solutions was filtered using muslin cloth and each filtrate was separately evaporated to dryness using hot plate set at 40℃ to obtain crude extracts [7]. Similar method was used to extract the sample in 1.5 liter of ethanol and 1.5 liter of n-hexane. The crude extract of each sample was weighed and the percentage yield of each was calculated as follows:

Extract yield %=$W_1/W_2 \times 100$

W_1=Net weight of powder in grams after extraction and W_2=Total weight of wood powder in grams taken for extraction. All the crude extracts obtained were separately stored in the refrigerator until required.

Preparation of culture media

Sabouraund dextrose agar and Nutrient Broth Agar (NBA) were the media used for bioassay.

Sabouraund dextrose agar

Sixty five grams (65 g) of Sabouraund Dextrose Agar (SDA) was dissolved in 1000 ml of distilled water and 1 g chlorophenol antibiotic was added into it, in order to inhibit the growth of bacteria in the media. The mixture was then heated to ensure complete dissolution. The agar solution was then transferred to fourty 100 ml sized conical flasks, each containing 25 ml [8]. The conical flask was covered with cotton wool and cupped with aluminum foil to prevent the inlet and outlet of air during sterilization in the autoclave at 121℃ for 15 minutes. The pointer was allowed to drop back to 0℃ before the flask removed and then was allowed to cool to 45℃ at room temperature and their content was then each transferred into petridishes and allowed to solidify.

Nutrient broth media

Twenty five grams (25 g) of Nutrient Broth Agar (NBA) was weighed using weighing balance, after which the powder was dissolve in 1000 ml of sterilized distilled water. The mixture was stirred gently and heated to ensure homogenous mixture is obtained. 10 ml of the agar solution was then poured to twenty 20 ml sized test tube each [9]. The test tubes were covered with cotton wool and cupped with aluminum foil to prevent the inlet and outlet of air during sterilization in the autoclave at 121℃ for 15 minutes.

Inoculation procedure

Dandruff samples collected were introduced into test tube containing the nutrient broth agar using sterile forceps. The test tube was then labeled and stored at room temperature (25℃) for 14 days to revive some of the fungi that might be weak in the cause of taking the sample to the laboratory. The appearance of white suspension confirms the revival of fungi.

Incubation

Each of the dandruff suspension obtained from Nutrient Broth Agar (NBA) was then introduced directly into sterilized Sabouraund Dextrose Agar (SDA) in Petri dishes using sterilized inoculation needle. These were then incubated at a temperature of 25℃ for fourteen (14) days to obtain different growth of fungal colonies.

Sub culturing of isolates

The resultant fungi was purified by cutting the advancing edged of visibly seen isolates in the colonies and aseptically introduced each in fresh Sabouraund Dextrose Agar (SDA) media by using sterilized loop and inoculation needle. This was continued up to three times until pure cultures are obtained. The pure cultures of the isolates were maintained on Sabouraund Dextrose Agar (SDA) slope in Mccartney bottles at 4℃ in the dark.

Identification of fungi

Slide of the mycelium observed from different fungal isolate was prepared as follows: A drop of lacto phenol cotton blue solution was placed in the center of a grease free clear glass slides. A small portion of the unidentified fungi culture was cut out with inoculation needle; the portion was then placed in the lacto phenol cotton blue droplet on the slide and tease out with another needle. A cover slip was placed over the teased portion before the mounting on the stage of a binocular microscope for viewing and examination. The characteristics such as texture, structure of mycelia, fruiting bodies, colour and shape of the upper thallus as well as the production of pigment on the underside spore structure were noted and identified with the help of standard mycology atlas [10]. The identified isolates were *M. restricta and M. globossa*. Microscopic and colonial appearances were used as criteria for identification of fungal isolates as presented in Table 2.

Determination of frequency of occurrence

The frequency of the occurrence of fungi associated with dandruff was calculated using the formula reported as stated below.

% Frequency=Number isolated isolate/Total number of isolated fungi × 100

Preparation of concentration of plant extract

Stock solution was prepared using 10 g of the solid plant extracts dissolved in 100 mls of normal saline making a stock of 100 mg/ml. The concentration was prepared from the stock solution using dilution formula as follows.

$C_1V_1=C_2V_2$

Where,

C_1=present concentration V_1=Volume to use C_2=required concentration V_2=Required volume

10 mg/ml, 20 mg/ml, 40 mg/ml, 60 mg/ml, 100 mg/ml concentrations were used to test for the antifungal effect of the extracts of *E. aromatic* and *G. senegalensis* seeds. To prepare those concentrations a given amount of 3 ml, 6 ml, 12 ml, 18 ml and 30 ml of the stock solution of *E. aromatic and G. senegalensis* were drawn using string and each dissolved in a conical flask containing 30 ml of sterilized distilled water. The control plates contained 25 ml of the sterilized media.

Determination of *in vitro* activity of the extracts using agar incorporation

Five milliliters (5 ml) each of 10,20,40,60 and 100 mg/ml respectively, of the extracts was kept in different petri dishes and 20 ml of sterilized SDA solution was then added to each. The dishes were stirred carefully before the mixture solidifies. Five millimeter (5 mm) of the test organisms (*M. restrictaand, M. globosa*) was punched using cork borer and introduced at the center of the solidified extract incorporated plates using sterilized inoculating needle which was incubated at temperature of 20℃ for 20 days. For each of the test fungi (*M. restrictaand, M. globosa*) and the concentrations of the extracts were replicated three times. The experiment was terminated when the control of each test fills up the petri dishes. Radial growth (mm) was measured using a meter rule. The length of the diameter of the fungal growth was measured vertically and horizontally and the mean recorded in millimeter as described by Cheesebrough LM.

Thin layer chromatography

Preparation of thin layer chromatography plate: Slurry was prepared from 75 grams of silica gel, 100 cm^3 of methanol 50 cm^3 of chloroform and 25 cm^3 of water. TLC plate was formed and activated at 110℃ using oven for one hour.

TLC separation

Three grams (3 g) of each ethanol, hexane, and aqueous extracts was dissolved in their respective solvent, *i.e.* ethanol, n-hexane and water respectively to form a sample solution. The solvent system was made from hexane and methanol 4:1 (v/v). Capillary tube was used to spot a sample solution on the silica gel TLC plate at 1 cm from the edged of the plate and the drop is allowed to dry. The plate was placed in TLC (chromo tank) and allows ascend the TLC plate by capillary action. The plate was removed and the solvent front was marked then allowed to dry. The iodine was used as the visualizing agent to detect the spot. A meter rule was used to measure the distance moved by the solvent and distance moved by spot, from which the retention factor (R_f values) of the various spots was calculated.

R_f=Distance move by spot front/Distance move by solvent front

Testing *in vitro* activity of the chromatographic fractions

Each of the chromatographic fractions with different R_f was dissolves in 6 ml of distilled water. Each of the solution was transferred to six petri dishes each containing one milliliter (1 ml) and 25 ml of sterilized SDA solution was added to each. The dishes were stirred carefully before the mixture solidifies. Five millimeter (5 mm) of test organisms was punched using cork borer and introduced at the center of the solidified extract incorporated plates using sterilized inoculating needle which was incubated at temperature of 20℃. The experiment was terminated when the control of each test fills up the petri dishes. Radial growth (mm) was measured using a meter rule. The length of the diameter of the fungal growth was measured vertically and horizontally and the mean recorded in millimeter as described by Cheesebrough LM.

Data analysis

Values of obtained from extract yield of *E. aromatica* and the frequency of occurrence of *M. restricta* and *M. globosa* were express in percentages. The comparison of mean within the treatments was conducted using Analysis of Variance (ANOVA)

and the Duncan multiple range tests were used to separate the mean. P values of 0.05 were considered significant.

Results

Percentage yield of extracts

The result of the percentage yield of extract of *E. aromatic* is presented in Table 1. The result of the extract yield of *E. aromatica* showed that ethanol yield more extract with 14.30%, followed by water with 13.85% and hexane with 13.82%.

Table 1: Percentage yield of extracts.

Extracts	% extract yield
E.A (aqueous)	13.85
E.A (ethanol)	14.3
E.A (hexane)	13.82
Note: EA: *Eugenia Aromatica*	

Frequency of occurrence of fungi identified from dandruff

The result of percentage frequency of occurrence of *M. restricta* and *M. globossa* is presented in Table 2. The results of frequency of occurrence of *fungi* identified from dandruff are represented in Table 3. The result shows that *M. globossa* was the most frequently occurring pathogen with 56.4% followed by *M. restricta* with 43.6%.

Table 2: Frequency of occurrence of fungi identified from dandruff.

Isolates	% frequency
M. restricta	43.6
M. globossa	56.4

Antifungal activity of aqueous extract of *E. aromatica* on *M. restricta*

The results of the antifungal activity of aqueous extract of *E. aromatica* on the mycelial growth of *M. restrica* are presented in Table 3. The result showed that, complete growth inhibition of was recorded when *M. restricta was* treated with 100 mg/ml and the lowest percentage inhibition was recorded at 10 mg/ml, with 57.16%. It is worthy of note that, the growth of *M. restricta* significantly (P<0.05) reduced with increase in concentration.

Antifungal activity of hexane extract of *E. aromatica* on *M. restricta*

The results of the antifungal activity of hexane extract of *E. aromatica* on the mycelial growth of *M. restrica* are presented in Table 3. The result shows that complete growth inhibition of was recorded when *M. restricta* was treated with 100 mg/ml and lowest percentage inhibition was recorded at 10 mg/ml, with 63.78%. It is worthy of note that, the growth of *M. restricta* significantly (P<0.05) reduced with increase in concentration.

Antifungal activity of ethanol extract of *E. aromatica* on *M. restricta*

The results of the antifungal activity of aqueous extract of *E. aromatica* on the mycelial growth of *M. restrica* are presented in Table 3. The result shows that, complete growth inhibition of was recorded when *M. restricta* was treated with 100 mg/ml and lowest percentage inhibition was recorded at 10 mg/ml, with 60.22%. It is worthy of note that, the growth of *M. restricta* significantly (P<0.05) reduced with increase in concentration.

Table 3: Antifungal activity of extracts of *E. aromatica* on the mycelial growth of *M. restricta*.

Concentrations (mg/ml) of plant extract mean fungal growth (mm) ± SE
Aqueous extract
Control 90.00^{a} ± 0.00

10 38.56[b] ± 2.22
20 30.90[c] ± 1.65
40 26.47[d] ± 1.29
60 13.27[e] ± 1.54
100 0.00[f] ± 0.00
Ethanol extract
Control 90.00[a] ± 0.00
10 35.80[b] ± 1.79
20 28.50[c] ± 1.38
40 19.43[d] ± 0.43
60 9.43[e] ± 1.01
100 0.00[f] ± 0.00
Hexane extract
Control 90.00[a] ± 0.00
10 32.60[b] ± 1.15
20 26.57[c] ± 1.26
40 14.77[d] ± 0.52
60 6.63[e] ± 0.64
100 00.00[f] ± 0.00
Values are mean ± standard error (n=3) Means in a column with different superscripts are significantly different (P<0.05)

Antifungal activity of aqueous extract of *E. aromatica* on *M. globssa*

The results of the antifungal activity of ethanol extract of *E. aromatica* on the mycelial growth of *M. globossa* are presented in Table 4. The result shows that, complete growth inhibition of was recorded when *M. globossa* was treated with 100 mg/ml and lowest percentage inhibition was recorded at 10 mg/ml, with 58.82%. It is worthy of note that, the growth of *M. globossa* significantly (P<0.05) reduced with increase in concentration.

Antifungal activity of ethanol extract of *E. aromatica* on *M. globssa*

The results of the antifungal activity of ethanol extract of *E. aromatica* on the mycelial growth of *M. globossa* are presented in Table 4. The result shows that, complete growth inhibition of was recorded when *M. globossa* was treated with 100 mg/ml and lowest percentage inhibition was recorded at 10 mg/ml, with 57.26%. It is worthy of note that, the growth of *M. globossa* significantly (P<0.05) reduced with increase in concentration.

Antifungal activity of hexane extract of *E. aromatica* on*M.globssa*

The results of the antifungal activity of hexane extract of *E. aromatica* on the mycelial growth of *M. globossa* are presented in Table 4. The result shows that, complete growth inhibition of was recorded when *M. globossa* was treated with 100 mg/ml and lowest percentage inhibition was recorded at 10 mg/ml, with 64.70%. It is worthy of note that, the growth of *M. globossa* significantly (P<0.05) reduced with increase in concentration.

Table 4: Antifungal activity of extracts of *E. aromatica* on the mycelial growth of *M. globossa.*

Concentrations (mg/ml) of plant extract mean fungal growth (mm) ± SE
Aqueous extract
Control 90.00[a] ± 0.00
10 37.06[b] ± 2.24
20 29.13[c] ± 1.72
40 24.77[d] ± 2.15
60 14.67[e] ± 2.40
100 0.00[f] ± 0.00
Ethanol extract
Control 90.00[a] ± 0.00
10 38.47[b] ± 1.34
20 29.87[c] ± 0.15
40 19.83[d] ± 0.58
60 13.63[e] ± 0.88
100 0.00[f] ± 0.00
Hexane extract
Control 90.00[a] ± 0.00
10 31.77[b] ± 0.58
20 24.07[c] ± 0.76
40 14.77[d] ± 1.00
60 5.63[e] ± 0.40
100 00.00[f] ± 0.00
Values are mean ± standard error (n=3) Means in a column with different superscripts are significantly different (P<0.05)

Antifungal activity of thin layer chromatographic fractions of the extract *on M. globossa*

The results of the effect of chromatographic fractions of extracts on the mycelial growth of *M. globossa* are presented in Table 5. The results showed that, the highest percentage of growth inhibition was of *M. globossa* recorded at 0.4 R_f of *E. Aromatica*, Hexane extract (EAH0.04) with 65.86% and the lowest percentage inhibition was recorded at 0.91R_f *E. Aromatica*, Hexane extract (EAH0.91) with 34.86%. It is worthy of note that, the growth inhibition of *M. globossa* is significant (P<0.05) within the chromatographic fractions

Table 5: Antifungal activity of chromatographic fractions of the extract on *M. globossa* using Agar incorporation method.

Chromatographic spots	**Fungal growth inhibition (mm)**
Control	90.00[a] ± 0.00

EAH0.04	30.73^{q} ± 0.578
EAE0.13	34.20^{p} ± 0.850
EAA0.14	37.97^{o} ± 1.167
EAH0.17	42.40^{mn} ± 0.569
EAH0.24	45.37^{lm} ± 1.317
EAE0.26	36.87^{op} ± 0.549
EAA0.27	40.80^{n} ± 0.586
EAA0.35	42.57^{lm} ± 0.333
EAE0.45	41.57^{n} ± 0.639
EAA0.45	45.53^{l} ± 0.318
EAH0.5	49.80k ± 0.520
EAA0.54	51.20^{k} ± 3.859
EAE0.57	44.63^{lm} ± 0.669
EAH0.67	51.57^{k} ± 0.333
EAE0.89	57.13^{j} ± 1.073
EAH0.91	58.63^{hij} ± 1.424
EAA: Aqueous extract of *E. aromatic*; EAE: Ethanol Extract of *E. aromatic*; EAH: Hexane Extract of *E. aromatica* Values are mean ± standard error (n=3) Means in a column with different superscripts are significantly different (P<0.05)	

Antifungal activity of thin layer chromatographic fractions of the extracts on *M. restricta*

The results of the effect of chromatographic fractions of extracts on the mycelial growth of *M. restricta* are presented in Table 6. The results showed that, the highest percentage of growth inhibition of *M. restricta* was recorded at 0.4 R_f of *E. Aromatica*, Hexane extract (EAH0.04) with 65.37% and the lowest percentage inhibition was recorded at 0.91 R_f *E. Aromatica*, Hexane extract (EAH0.91) with 36.56% It is worthy of note that, the growth inhibition of *M. globossa* is significant (P<0.05) within the chromatographic fractions (Figure 1).

Table 6: Antifungal activity of chromatographic fractions of the extracts on *M. restricta* using agar incorporation method.

Chromatographic spots	**Fungal growth inhibition (mm)**
Control	90.00^{a} ± 0.00
EAH0.04	31.17^{t} ± 0.433
EAE0.13	34.23^{s} ± 0.991
EAA0.14	37.13^{r} ± 0.845
EAH0.17	42.83^{op} ± 0.318
EAH0.24	45.13^{n} ± 0.974

EAE0.26	$36.30^{r} \pm 0.265$
EAA0.27	$40.47^{q} \pm 0.524$
EAA0.35	$42.67^{p} \pm 0.233$
EAE0.45	$42.23^{pq} \pm 0.689$
EAA0.45	$46.00^{n} \pm 0.379$
EAH0.5	$49.47^{m} \pm 0.953$
EAA0.54	$50.27^{m} \pm 2.107$
EAE0.57	$44.63^{no} \pm 0.897$
EAH0.67	$51.97^{l} \pm 0.067$
EAE0.89	$55.53^{k} \pm 0.406$
EAH0.91	$57.10^{k} \pm 1.286$
EAA: Aqueous Extract of *E. aromatic*; EAE: Ethanol Extract of *E. aromatic*; EAH: Hexane Extract of *E. aromatica* Values are mean ± standard error (n=3) Means in a column with different superscripts are significantly different (P<0.05)	

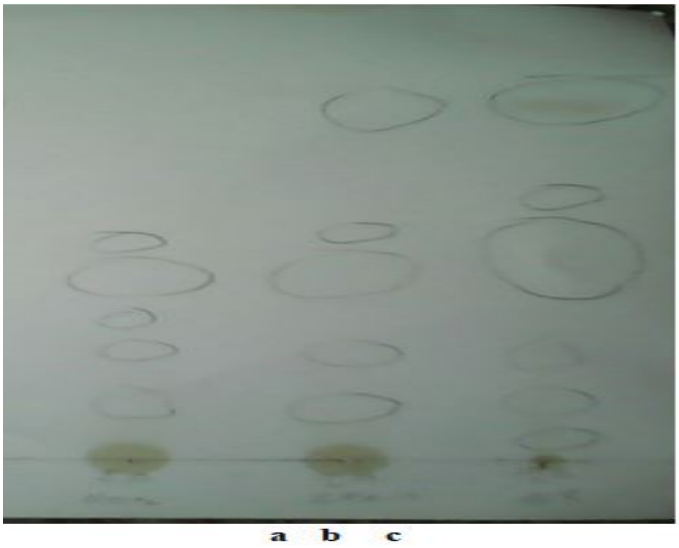

Figure 1: TLC fingerprint of *E. aromatica*. a) *E. aromatica* aqueous extract; b) *E. aromatica* ethanol extract; c) *E. aromatica* hexane extract.

Discussion

The antifungal effect of *E. aromatica* seeds indicated that *E. aromatica* seeds are very active against the tested fungal isolates especially at concentration of 100 mg/ml. These findings are in conformity with that of Iris. On extract of *Mimosa pudica* and that of who worked on the same plant against razor bump. The extracts of *E. aromatic* completely inhibited the growth of the tested fungal isolates at highest concentration of 100 mg/ml. This finding is in agreement with the work of who found *Fusarium* sp. and *Botrytis* sp. were sensitive and completely inhibited by 0.05% crude extract of *E. aromatica*. Other investigations that reported strong antifungal effect of *Eugenia aromatica* at a low concentration include.

The frequency of occurrence of *M. restricta* and *M. globossa* showed that both *M. restricta* and *M. globossa* appeared in all petri-dishes. This observation is in conformity with the work of who reported that *M. restricta* and *M. globossa* are the major causative agents of dandruff.

Reported that *Muntingia calabura* has higher amount extract yield from when methanol was used as a solvent, following by water, ethanol, chloroform, ether and citric acid. The result of the present study showed that *E. aromatica* yield higher amount of extract ethanol was used as a solvent followed by water and hexane. Other investigation that is in agreement this finding is the work of on *Terminalia arjuna*.

The result of thin layer chromatographic profiles of the extracts of *E. aromatica* and *G. senegalensis* showed 16 fractions this finding is in agreement with the work of who found 42 fractions on *Centella asiatica* causing different solvent system [11]. Antifungal activity of the chromatographic spots of the extract was conducted and the result revealed that the growths of the *M. restricta* and *M. globossa* were significantly reduced within all chromatographic fractions.

Conclusion

Based on the results of the present study, it can be concluded that the extracts of *E. aromatica* possess antifungal activity. Aqueous, ethanol and hexane extracts of *E. aromatica* showed promising activity against *M. restricta* and *M. globossa* growth.

E. aromatic was found to be concentration dependent. Therefore, the observed antifungal activity of both *E. aromatica* extract can be a positive attributes in the management of dandruff.

References

мϕ Vijayakumar R, Muthukumar C, Kumar T, Saravanamuthu R (2006) Characterization of *Malassezia furfur* and its control by using plant extracts. Indian J Dermatol 51:145-148

нϕ Lans C, Turner N, Khan T, Brauer G (2007) Ethno veterinary medicines used to treat endoparasites and stomach problems in pigs and pets in British Columbia, Canada. Vet Parasitol 148:325-340

оϕ Kim HM, Lee EH, Hong SH, Song HJ, Shin MK, et al. (1998) Effect of *Syzygium aromaticum* extract on immediate hypersensitivity in rats. J Ethnopharmacol 60:125-131

пϕ Kim EH, Ahn YJ (2003) Acaricidal activity of clove bud oil compounds against *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus (Acari: Pyroglyphidae)*. J Agric Food Chem 51:885-889

рϕ Domaracky M, Rehak P, Juhas S, Koppel (2007) Effects of selected plant essential oils on the growth and development of mouse preimplantation embryos *in vivo*. J Physiol Res 56:97–104

сϕ Olufolaji DB, Adeosun BO, Onasanya RO (2015) *In vitro* investigation on antifungal activity of some plant extracts against Pyricularia oryzae. Niger J Biotechnol 29:38-43

тϕ de Souza AM, de Oliveira CF, de Oliveira VB, Betim FC, Miguel OG, et al. (2018) Traditional uses, Phytochemistry, and antimicrobial activities of Eugenia species–a review. Planta Med 84:1232-1248

уϕ Stefanello ME, Cervi AC, Ito IY, Salvador MJ, Wisniewski A, et al. (2008) Chemical composition and antimicrobial activity of essential oils of *Eugenia chlorophylla* (Myrtaceae). J Essent Oil Res 20:75-78

фϕ Atanasova-Pancevska N, Bogdanov J, Kungulovski D (2017) *In vitro* antimicrobial activity and chemical composition of two essential oils and eugenol from flower buds of *9ʣƎŜyʎl· Ŏl·Ꞩè ƻLJKèffl·ǘl·*. Open Biol Sci J 3

млϕ Wankhede TB (2015) Evaluation of antioxidant and antimicrobial activity of the Indian clove *Syzygium aromaticum* L. Merr Perr Int Res J Sci Eng 3:166-172

ммϕ Wilson CL, Solar JM, El Ghaouth A, Wisniewski ME (1997) Rapid evaluation of plant extracts and essential oils for antifungal activity against *Botrytis cinerea*. Plant Disease 81:204-210

