



**Comparative Analysis of Some Selected Natural Remedies on Dandruff Associated with  
*Malassezia globosa***

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**Abstract**

*A comparative analysis was carried out on the levels of effectiveness of some selected natural remedies in inhibiting the growth of Malassezia globosa associated with dandruff. The natural remedies used in this research include; Potash, Neem leaves and Cloves. Scalp samples (Hair flakes) were collected from infected individuals with dandruff. Agar incorporation method was employed to test the efficacies of the three selected natural remedies against the growth of Malassezia globosa. Three (3) species of Malassezia were obtained, these include; Malassezia globosa, Malassezia restricta and Malassezia furfur. Eleven (11) samples with 55% frequency of occurrence were positive for Malassezia globosa, five (5) samples with the percentage frequency of occurrence of (25%) were found to be positive for Malassezia furfur and 4 samples with the percentage frequency of occurrence of (20%) were found to be positive for Malassezia restricta. Catalase enzyme differential test between Malassezia globosa, Malassezia furfur and Malassezia restricta revealed catalase positive results with 11(55%) samples of Malassezia globosa and negative results with Malassezia furfur and Malassezia restricta. Potash was the most effective natural remedy in inhibiting the growth of Malassezia globosa than neem leaves and cloves extracts.*

**Keywords:** Analysis; Natural remedie; Malassezia globose; mean radial growth; Dandruff.

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**1.0 INTRODUCTION**

The Microbial origin of dandruff depends on the causal role of yeasts of the genus *Melassezia* (Saint-Leger 1990; Shuster 1984). *Malassezia* was named by Malassez in 1989 (Sei, 2003; Malassez, 1874). The genus was renamed as *Pityrosporum malasse* during the second half of the 20<sup>th</sup> century by Saboraud. Members of *Malassezia* were at one time classified into two

species, a lipid-dependent species (*Malassezia furfur*) and non-lipid dependent species (*Malassezia pachydermatis*). In the mid 1990s, studies of the morphological, ultra structural, physiologic and genomic differences in *Malassezia* led to the identifications of multiple lipid dependent species (including *Malassezia globosa*, *Malassezia restricta*, *Malassezia furfur*, *Malassezia obtuse*, *Malassezia slooffiae*,

*Malassezia sympodiatis*, *Malassezia japonica*, *Malassezia nana*, *Malassezia dermatitis* and *Malassezia yamatoensis*). The use of molecular markers is generally required to correctly differentiate between the various lipid dependent species using a molecular technique (terminal fragment length polymorphism) to eliminate any potential culture bias, *Malassezia globosa* and *Malassezia restricta* were identified as the predominant species present on the scalp of dandruff sufferers (Ashbee *et al.*, 2002; Gupta *et al.*, 2004; Schwartz, 2013).

Dandruff occurs in at least 40-50% of the general population, and in Seborrheic dermatitis 1-3% (Warner *et al.*, 2001; Ashbee, 2002). More than 50% of adults may be affected by these conditions which suggest a high socio-economic impact (Gupta *et al.*, 2003; Schwartz *et al.*, 2013).

Three factors are involved in dandruff etiology these include; *Malassezia*, sebum, and individual susceptibility. There are several potential ways for the treatment, one may treat the causes or one may treat the symptoms (Schwartz, 2013). Treatment of the symptoms involved calming the inflammation with anti-inflammatory steroidal agents, minimizing cell proliferation with anti-proliferative, or by simply grooming away the resultant flakes (Schwartz, 2013; Dikshit *et al.*, 2012). Treating the causes mean inhibition of the fungi with anti-fungal agents or by suppressing the secretion of sebum. The use of non-medicated shampoos to simply wash away the flakes is not very effective; they can be simply generated too quickly. The uses of Anti-proliferative therapies have also been poorly effective due to their minimal efficacies. Suppression of Sebum has also proved to be very difficult with few topical treatments mostly natural plants e.g. tea tree available at this time (Dikshit *et al.*, 2012; Schwartz *et al.*, 2013). Treatment with anti-inflammatory steroidal agents can be effective in short-term but cannot be used for long-term prophylaxis due to limiting adverse effects.

Lemon Juice, Neem extract, Potash and Cloves have been used as antifungal therapy since decades and proven in recent studies on fungal inhibition to be effective in controlling dandruff causing fungal growth with minimal side effect.

However, *Malassezia* fungi are capable of growing back in the scalp once the above remedies are not constantly used (Kekuda, *et al.*, 2007). The use of natural remedies can cause less side effects compared to the chemical constituents present in shampoos and creams (Duvauchelle, 2010).

About 40-50% of the world population is suffering from dandruff, especially the low class individuals which differ among ages. This makes it one of the most important infections of the body and a socio-economic burden. To get rid of this problem individuals have been using different types of ointments and creams both of natural and synthetic origin, using anything that will remove the flakes from the scalp regardless of whether it will be effective or not or even worth the money spent on it. This necessitated the need for a thorough investigation on some readily available, cost effective natural remedies that have been shown in so many literatures and hear says to be effective on dandruff but have not been used in practice.

## **2.0 MATERIALS AND METHODS**

### **2.1.0 Sample Collection**

#### **2.1.1 Collection of the Hair flakes.**

Sample of dandruff were collected from the scalp of infected individuals. A total number of twenty (20) samples were obtained from infected individuals. Infected areas were first cleaned with surgical spirit. Hair flakes were collected using sterilized forceps held at angle of 90° to pluck from the scalp. The collected samples were put on a clean piece of paper (5cm square), the papers were folded to enclose the specimens (Cheesebrough, 2000). The collected samples were labeled and transferred to Microbiology Laboratory of Usmanu Danfodiyo University, Sokoto for the culture of associate organisms.

#### **2.1.2 Collection and Identification of Plant Material**

Neem leaves were obtained around Usmanu Danfodiyo University Sokoto permanent site. Authentication of the plant was carried out in the Herbarium of the Usmanu Danfodiyo University Sokoto. The plant leaves were packed in a sterile polythene bag and were air dried under shed and

pulverized into a fine powder using Mortar and pestle (Kumar *et al.*, 2013).

### 2.1.3 Collection of Cloves and Potash

Cloves and potash were purchased from Sokoto central Market. The samples obtained were taken to Herbarium of Usmanu Danfodiyo University Sokoto for identification. Voucher specimen were prepared and deposited in the herbarium in accordance with the procedure of Kumar *et al.*, (2013).

## 2.2 Sample Analysis

### 2.2.1 Inoculation Procedure

Small amount of the samples collected was introduced into the test tubes containing Nutrient Broth agar with the use of a sterile forceps. The test tubes were labeled and incubated at room temperature (25°C) for 14 days to revive some fungi that might be denatured in the cause of taking the sample of white suspension confirms the revival of the fungi (Cheesebrough, 2000).

### 2.2.2 Incubation

Each of the dandruff suspension obtained from the nutrient broth agar was then introduced directly on sterilized sabouroud dextrose agar in Petri dishes using sterilized inoculation needle. The Petri dishes were then labeled and incubated at room temperature (25°C) for 3 days and then up to 2 weeks to obtain different growth of fungal colonies (Cheesebrough, 2000; Kindo *et al.*, 2004).

### 2.2.3 Purification of the Isolates

The resultant fungal colonies were purified by cutting the advancing edge of visibly seen isolates in the colonies and aseptically introduced each in fresh sabouroud dextrose agar media by using sterile loop and inoculation needle, until pure culture were obtained. The pure culture of the isolates obtained was maintained on sabouroud dextrose agar slope in McCartney bottles (Cheesebrough, 2000).

## 2.3 Identification of the Isolated Fungi

### 2.3.1 Direct Microscopy

Slide of the mycelium observed from different fungal isolates was prepared as follows: A drop of 10% potassium hydroxide was introduced onto a slide containing the sample and covered with a cover slip. Samples were then heated over flame to remove bubbles. The slides were viewed using x10 and x40 objectives. A globose *blastoconidia* and *pseudomycelia* coupled with mycelia in the form of “spaghetti” was observed (Crespo *et al.*, 2000; Kindo *et al.*, 2004).

### 2.3.2 Biochemical Tests

#### Catalase Test

Catalase test was carried out in accordance with the procedures of (Cheesebrough, 2000; Khosviel *et al.*, 2009). 3ml of 3% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) solution was poured into a test tube. Several colonies of the isolated fungal colonies were immersed into the test tube using sterile glass rod, the active bubbling was observed, signifying the release of oxygen from the hydrogen peroxide indicated positive test for catalase production.

### 2.4 Preparation of Samples (Remedies used for Antifungal test)

The fresh samples of the Neem leaves collected were washed with tap water and then shed dried under natural conditions. Dried materials were grinded into fine powder using motor and pestle. The powdered Neem leaves prepared was then stored in sterilized polythene bag until required for extraction (Crespo *et al.*, 2000).

The sample of cloves (*Euglena aromatica*) was grinded into fine powdered using sterilized mortar and pestle. The fine powdered material was stored in a sterilized polythene bags until required for extraction (Crespo *et al.*, 2000).

### 2.5 Extraction of the Plant Materials (Neem leaves and Cloves)

100grams from each powdered of neem leaves and cloves were extracted separately with distilled water. The extract was obtained by 1.5liter of distilled water in 2000ml beaker. The solution was stirred, cupped with aluminium foil and keep for 24hours. The resultant solution was

filtered using clean cloth (Muslim cloth). And each filtrate was separately poured inside a stainless steel bowl and evaporated to dryness using a hot plate set at 40 and crude extract was obtained. The crude extract of each plant (neem leaves and cloves) was grinded and weighed in the refrigerator until require for use (Cheesebrough, 2000).The Percentage yield was calculated as follows:

Extract yield in %= $w_1/w_2 \times 100$

Where  $W_1$ = net weight of powder in grams after extraction.

$W_2$ =Total weight of wood powder in grams taken for extraction.

## 2.6 Preparation of the Natural Remedies at different Concentration.

### Preparation of Potash

Stock solution was prepared using 10grams of the powdered potassium carbonate, which was dissolved in 100mls of distilled water making a stock of 100mg/ml. The concentration was prepared from the stock solution using the formula as follows;  $C_1V_1=C_2V_2$

Where  $C_1$ =Present concentration,  $V_2$ =Volume to use,  $C_2$ =required concentration,  $V_2$ =required volume.

10mg/ml, 20mg/ml, 40mg/ml, 60mg/ml, 100mg/ml concentration was used to test for antifungal effect of potassium carbonate. To prepare these concentrations, a given amount of 3mls, 6mls, 12mls, 18mls and 30mls of the stock solution of potash were drawn using syringe and dissolved in a conical flask containing 30mls of sterilized distilled water. The control plate contains 25mls of sterilized media (Cheesebrough, 2000).

### Extraction of Neem Leaves

Stock solution was prepared using 10grams of the powdered crude extract of neem leaf and dissolved in 100mls of distilled water making a stock of 100mg/ml. The solution was heated using a hot oven to obtain homogenous mixtures. Using a conical flask the concentration

was prepared from the stock solution using the formula as follows as stated by Cheesebrough (2000).

$$C_1V_1=C_2V_2$$

Where  $C_1$ =present concentration,  $V_1$ =volume to use,  $C_2$ =required concentration,  $V_2$ =required volume.

10mg/ml, 20mg/ml, 40mg/ml, 60mg/ml, 100mg/ml, was used to test for antifungal effect of neem leaves extract. To prepare these concentrations a given amount of 3mls, 6mls, 12mls, 18mls and 30mls of the stock solution of neem were drawn using syringe and dissolved in a conical flask containing 30mls of distilled water. The control plates contain 25mls of sterilized media (Cheesebrough, 2000).

## 2.7 Extraction of Cloves

Stock solution was prepared using 10grams of the powdered crude extract of cloves leaves dissolved in 100mls of distilled water making a stock of 100mg/ml. The solution was heated using a hot oven to obtain a homogenous mixture. Using a conical flask the concentration was prepared from the stock solution using the 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.

10mg/ml, 20mg/ml, 40mg/ml, 60mg/ml, 100mg/ml was used to test for antifungal effect of cloves extract. To prepare these concentrations a given amount of 3mls, 6mls, 12mls, 18mls and 30mls of the cloves were drawn using a syringe and dissolved in a conical flask containing 30mls of distilled water. The control plates contain 25mls of sterilized media (Cheesebrough, 2000)

## 2.8 Antifungal Test

The antifungal activity the three natural remedies was carried out using agar incorporation method. The media was poured aseptically into the Petri dishes using three replicate plates each. The extract in the test tubes were incorporated inside the media contained in the Petridishes. The sample of *Malassezia globosa* was inoculated inside the Petri dishes and the Petri dishes were incubated at 20°-25°C for 24 hours. The result was obtained by measuring the mean radial growth (Oyeleka and Manga 2008).

### 3.0 RESULTS

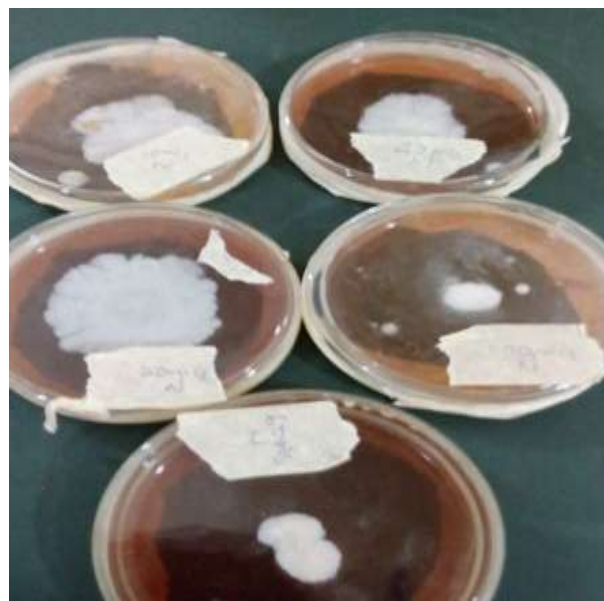
The results obtained in this research indicated that out of twenty skin samples scrapped from individuals with dandruff, three species of *Malassezia* were obtained, these include; *Malassezia globosa*, *Malasseziarestricta* and *Malassezia furfur*.

Table 1 shows the results for identification of *Malassezia globosa* from the Dandruff Patients. From the results obtained, out of the twenty (20) samples analyzed 11 samples (55%) were found to be positive for *Malassezia globosa*, 5 samples with the percentage frequency of occurrence of (25%) were found to be positive for *Malassezia furfur* and 4 samples with the percentage frequency of occurrence of (20%) were found to be positive for *Malassezia restricta*. Table 2 indicated the results of Catalase enzyme differential test between *Malassezia globosa*, *Malassezia furfur* and *Malassezia restricta*. Catalase enzyme differential test carried out between the three species of *Malassezia* shows catalase positive with 11(55%) samples of *Malassezia globosa* and negative results with *Malassezia furfur* and *Malassezia restricta*.

Plate 1 shows the Mean radial growth of *Malassezia globosa* at 10mg/ml, 20 mg/ml, 40 mg/ml, 60 mg/ml, and 100 mg/ml concentrations of neem leave extract. The Mean radial growth of *Malassezia globosa* at 10mg/ml, 20 mg/ml, 40 mg/ml, 60 mg/ml, and 100 mg/ml concentrations of cloves is shown in Plate 2.

Table 3 shows the efficacy of three natural remedies on the growth of *Malassezia globosa*. From the results obtained the mean radial

growth of *Malassezia globosa* at the highest concentration of 100mg/ml of potash was 3.3mm with 96% of effectiveness and at a concentration of 10mg/ml of potash which was the least concentration, the mean radial growth of *Malassezia globosa* recorded was 55 mm with 36% percentage of effectiveness. At concentration of 100mg/ml of neem leaves extract, the mean radial growth of *Malassezia globosa* was 18mm with 90% percentage of effectiveness, while at concentration of 10mg/ml of Neem leaves extract the mean radial growth of *Malassezia globosa* was with 57mm and percentage of effectiveness was 29%. Furthermore, at concentration of 100mg/ml of Cloves extract the mean radial growth of *Malassezia globosa* was 13mm with the level of effectiveness of 84% and at the lowest concentration of 10mg/ml of Cloves extract the mean radial growth recorded was 56mm and level of effectiveness recorded was 30%.



**PLATE 1:** Mean radial growth of *Malassezia globosa* at 10mg/ml, 20 mg/ml, 40 mg/ml, 60 mg/ml, and 100 mg/ml concentrations of neem leave extract.



**PLATE 2:** Mean radial growth of *Malassezia globosa* at 10mg/ml, 20 mg/ml, 40 mg/ml, 60 mg/ml, and 100 mg/ml concentrations of cloves.

**Table 1:** Results for identification of *Malassezia globosa* from the Dandruff Patients

Fungal pathogen	Sample positive (%)	Sample negative (%)	Total (%)
<i>Malassezia globosa</i>	11 (55)	-	11 (55)
<i>Malassezia restricta</i>	4 (20)	-	4 (20)
<i>Malassezia furfur</i>	5 (25)	-	5 (25)
Total			20 (100)

**Table 2:** The Catalase Enzyme Production Test of *Malassezia spp.*

Fungal Pathogen	Catalase Positive(%)	Total (%)
<i>Malassezia globosa</i>	11 (5)	11 (55)
<i>Malassezia furfur</i>	-	
<i>Malassezia restricta</i>	-	

Key: - = Catalase negative

**Table 3:** Mean radial growth recorded against three natural remedies on *Malassezia globosa*.

Natural Remedies	Technique	Concentrations	Mean Radial Growth (mm)	Rate	Percentage of effectiveness (%)
Potash extract	Pour plating	100	3.3	High	96
		60	18		78
		40	30		63
		20	45		44

		10	51		36
Neem leave extract	Pour plating	100	8	Medium	90
		60	13		83
		40	23		71
		20	28		65
		10	57		29
Cloves extract ( <i>Euglena aromatica</i> )	Pour plating	100	13	Low	84
		60	22		73
		40	28		65
		20	55		31
		10	56		30

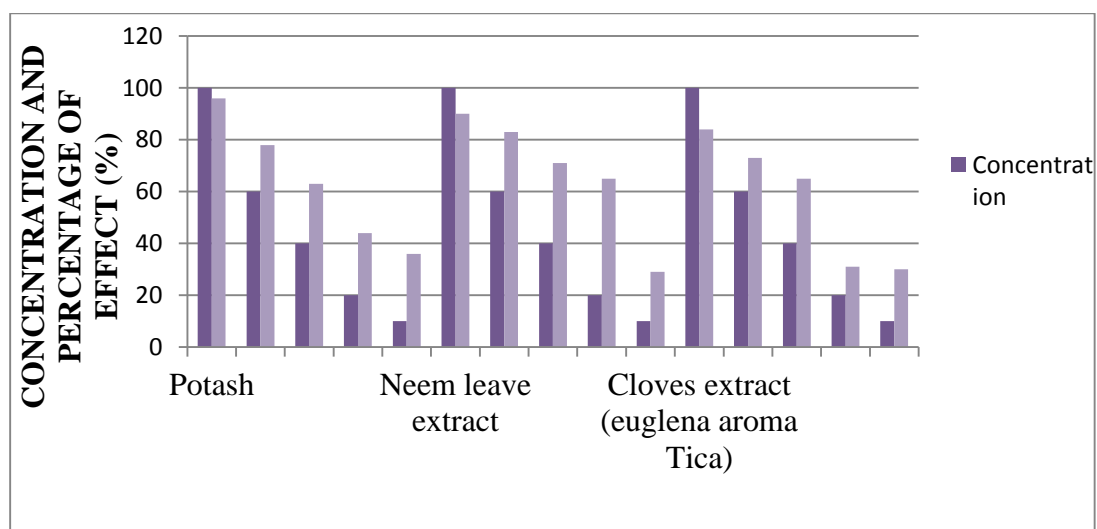


Figure 1: Percentage of effectiveness at various concentrations of three natural remedies on the growth of *Malassezia globosa*.

#### 4.0 DISCUSSION

From the results obtained it was observed that, *Malassezia globosa* was the predominant species isolated. This finding agrees with the finding of authors (Khosravi *et al.*, 2009; Dikshit *et al.*, 2012; Shuaibu *et al.*, 2013), who independently worked on dandruff caused by different *Malassezia* species. The results of catalase production test obtained indicated 75% of the samples collected are positive. For

*Malassezia globosa* which is catalase positive and others *Malassezia restricta* and *Malassezia furfur* catalase negative. This result corresponds with the work of Kindo *et al* (2004) and Shuaibu *et al.* (2013) whose work both included the isolation of *Malassezia*.

The findings of this research revealed that each remedy had its own characteristic level in inhibiting the growth of *Malassezia*. Potash extract was found to be highly stable and

capable in inhibiting the growth of *Malassezia globosa* for a long time frame of 3 days with the least mean radial growth of 3.3mm and a maximum radial growth of 51mm respectively. This agrees with the findings of (Mecteau *et al.*, 2000; Ordonez *et al.*, 2009) of potash compound being an excellent antimicrobial agent. The technique used for the antifungal test with natural remedies has been found to be effective in controlling the growth of *Malassezia* fungi (Kumar, 2013).

Neem leaves extract was capable of inhibiting the growth of *Malassezia spp* and was effective within the time frame of two days, though the results proved not to be stable over long time frame. This finding agrees with the work of (Agrawal 2001; Biswas *et al.*, 2002). Neem also showed the least mean radial growth at highest concentration (100mg/ml) measuring about 8.3mm mean radial growth and shows poorest mean radial growth at lower concentration of (10mg/ml). This goes in line with the work of Kumar (2013), who investigated that Neem possesses antifungal property.

Cloves showed least effect in inhibiting the growth of *Malassezia globosa*, among the three natural remedies used in this research, the inhibitory action was slow compared with the work of other remedies used, because it took cloves 3 days before any reasonable effect was noticed on the growth of *Malassezia globosa*. Cloves had a poorest mean radial growth on *Malassezia globosa* at lowest concentration. This work disagrees with the work of Hammer *et al.*, (2014) who showed cloves to be effective antifungal remedies. However, this may be possible because they used essential oil from cloves and also work on different organism (*Fusarium* species).

From the graph of multiple bar chart, the highest concentration (100mg/ml) of the all three natural remedies show highest percentage of effectiveness in all three natural remedies used with potash having the highest percentage (Fig 1).

## 5.0 CONCLUSION

From the results of this research, it could be concluded that potash (potassium carbonate) is the most effective and has the highest stability in inhibiting the growth of *Malassezia globosa* than neem leaves extract and cloves extract. The neem extract is also capable of inhibiting the growth of *Malassezia globosa* though not at highest rate. Cloves had the least effect in controlling/inhibiting the growth of *Malassezia globosa* and had the lowest effectiveness among all the natural remedies used in this research. Potash and neem leaves are two readily available domestic commodities, economically viable and easily obtainable in this locale. With adequate research and publications to back their importance, there will be improved awareness and also scientific backing in the use of the two in safeguarding the health of the scalp and at the same time saving cost.

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