Journal home page:
http://www.iajpr.com/index.php/en/

INDO AMERICAN JOURNAL OF PHARMACEUTICAL RESEARCH

COMPARATIVE STUDY OF ANTI DANDRUFF ACTIVITY OF *SYZYGIUM AROMATICUM* AND *ZINGIBER OFFICINALE*

S. Mohanapriya, C. Mercy Bastine, R. Caroline Jeba*

**Department of Industrial Biotechnology, Dr. MGR Educational and Research Institute, University, Maduravoyal, Chennai-600 095*

ARTICLE INFO

Article history
Received 01/06/2013
Available online
30/06/2-13

Keywords
Dandruff,
malassezia furfur,
Syzygium aromaticum

ABSTRACT

To study the inhibitory effect of *Malassezia furfur* by using the plants *Syzygium aromaticum* and *Zingiber officinale*.The antidandruff activity of hexane, ethyl acetate and methanol extracts of *Zingiber officinalae* and *Syzygium aromaticum* was studied by agar well diffusion and broth dilution assay. The Minimum inhibitory concentration (MIC) of the methanol extract of *S.aromaticum* was studied as 100µg/ml and IC_{50} as 850µg/ml. Partial purification through TLC and bio-autography were also studied. Out of the three extracts methanol extract, showed good activity comparatively. MIC activity was good in *S.aromaticum* when compared to *Z.officinale*. In *S.aromaticum* R_f value of 0.153 was the active compound which showed good activity against dandruff.Comparing the inhibitory activities of the two plants *S.aromaticum* showed Minimum inhibitory activity against *M.furfur*.

Corresponding author
R. Caroline Jeba
Department of Industrial Biotechnology
Dr M.G.R Educational & Research Institute University,Maduravoyal,
Chennai-95 , India
E-mail: janeshjeba@gmail.com

Please cite this article in press as R. Caroline Jeba et.al. Comparative study of anti dandruff activity of syzygium aromaticum and zingiber officinale. Indo American Journal of Pharm Research.2013:3(6).

Copy right © 2013 This is an Open Access article distributed under the terms of the Indo American journal of Pharmaceutical Research, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

INTRODUCTION

Medicinal plants have been used for centuries and have become part of complementary medicine worldwide because of their potential health benefits. In India, earliest references are available in Rigveda which is said to be written between 3500 – 1600 B.C. [1]. Plant metabolites are known to have direct positive effects in the treatment and management of infectious diseases and cancer. In addition, the indirect effects of plant metabolites through immunomodulation is well studied [2]. The medicinal plants are rich in secondary metabolites and essential oils of therapeutic importance [3]. Plants as a therapeutic option were achieving significance due to their safety profile besides being economical, effective and easily available. Plants play an essential role in the health care needs for the treatment of diseases and to improve the immunological response against much pathology [4].

Dandruff is a common scalp disorder affecting almost half of the pubertal population of any ethnicity and both genders [5]. It is a chronic scalp condition characterized by visible flakes induced by rapid turnover of scalp cells. In general, dandruff occurs after puberty and mainly affects males more than the females [6]. The pathogenesis of dandruff involves hyper proliferation, resulting in deregulation of keratinization. The corneocytes clump together, manifesting as large flakes of skin. The causative agents of dandruff belong to the group of scalp commensal lipophilic yeasts of the genus, *Malassezia*. *Malassezia* species are normal flora of skin and cause *Pityriasis versicolor* and foliculities under suitable conditions [7]. They are saprophytic lipophilic yeasts characterized morphologically by small cells exhibiting unilateral, enteroblastic and repetitive budding [8]. At present several species of *Malassezia* have been isolated which are *M. furfur, M. globosa, M. pachydermatis, M. restricta, M. obtusa, M. slooffiae , M. yamatoensis, M. dermatis, M. japonica, M. nana, M. caprae, M. equina, M. cuniculi* [9].

Several fungistatic compounds have been shown to improve dandruff condition. The main active ingredients include imidazole derivatives such as ketoconazole and other compounds such as selenium sulphide, zinc pyrithione (ZnPTO), piroctone olamine, cipropirox olamine, etc [5]. Herbal essential oils are promising sources for new natural antifungal drugs, which have good effects against pathogenic fungi compared with commercial synthetic antifungal drugs [8]. *Syzygium aromaticum* (cloves) contains a high percentage of eugenol, which has been identified as a compound exhibiting antifungal properties [10]. Previous studies have reported antifungal activity for clove oil and eugenol against yeasts and filamentous fungi, such as several food-borne fungal specie and human pathogenic fungi [11]. *Zingiber officinale* (Ginger), one of the most important spices in the world, is known for its medicinal and flavoring potentials. The medicinal properties are attributed to its spicy, pungent constituents, mainly gingerols, which stimulate the thermoregulatory receptors. This stimulation influences stomach and bile secretions by reflex action. [12].

In view of the above said facts the purpose of the current study is focused on evaluating the antidandruff activity of *Zingiber officinale* (Ginger) and *Syzygium aromaticum* (Cloves) against *Malassezia furfur.*

MATERIALS AND METHODS

General laboratory techniques recommended by was followed for the preparation of media, inoculation and maintenance of cultures.

Cleaning of glassware

The glassware were first soaked in chromic acid solution, (10% potassium dichromate in 25% sulphuric acid) for a few hours to remove tough residues, washed twice in tap water, then they were rinsed in metal distilled water and dried in an oven at 80ºC.

Sterilization

Sterilization of culture media and glassware were carried out in an autoclave at 121°C, 15 lbs for 20 minutes. Thermo labile substances are sterilized through millipore filter. All the experiments were conducted under laminar hood with strict aseptic conditions.

Chemicals

Analytical grade chemicals supplied by Loba, Hi-media, S.D.Fine chemicals, E-Merck, Qualigens and Sigma Chemicals (USA) were used.

MEDIA PREPARATION

Potato Dextrose Agar (g/L)

Potato	200.0
Dextrose	20.0
Agar	20.0
pH	6.5

Sabouraud's Dextrose Agar (g/L)

Dextrose	40.000
Mycological peptone	10.000
Agar	15.000
Final pH (at 25°C)	5.6±0.2

Nitrate Reduction Medium (g/L)

Beef (meat) extract	3.0
Gelatin peptone	5.0
Potassium nitrate (KNO_3)	1.0
pH	7.2±0.2

Nutrient Gelatin Medium (g/L)

Peptone	5.0
Beef extract	3.
Gelatin	120.0
pH	6.8±0.2

Urea Broth Base (g/L)

Peptone	1.0
Glucose	1.0
Sodium chloride	5.0
Disodium phosphate	1.2
KH_2PO_4	0.8
Phenol red	0.004
pH	6.8± 0.2

Plant material collection and preparation

The rhizome part of *Zingiber officinale* and the buds of *Syzygium aromaticum* were collected from Koyambedu market, Chennai. The samples were authenticated, and specimens were deposited in Arvind Remedies LTD.They were carefully washed with tap water, rinsed with distilled water, and air-dried for 1hr after which they were shade dried for 7days, powdered coarsely and stored at room temperature.

Direct extraction

Direct extraction with hexane, ethyl acetate and methanol was done following the method of [13]. In this method, finely ground plant material was extracted with hexane, ethyl acetate and methanol in the ratio of 1:10 in conical flask in shaking condition for overnight. The extract was filtered through the Whatmann No.1 filter paper in a separate container. The process was repeated 3times and the same plant material but using fresh solvent. The combined filtrate was subjected to condensation. The solvent was removed by placing the extracts

in distillation unit in the respective temperature. The extracted residues were weighed and re-dissolved in different solvents to yield 10mg/ml solutions ready for further analysis.

Isolation of dandruff causing agent

Samples were collected by scraping the lesions of patients and stored in sterile containers in refrigerator until use. Different media formulations (potato dextrose agar, Sabouraud's dextrose agar) were supplemented with olive oil and inoculated with the sample. The plates were incubated at 37°C for 3- 5days [6].

Morphological characterization of the isolate

Microscopic examination of the samples was performed after the treatment with KOH (20%) and 5% lactophenol cotton blue staining [14].

Biochemical tests

The organism was biochemically analysed by the following assays.

Catalase test

Using a sterile inoculating loop a small amount of organism from a 24hr colony was placed onto the microscopic slide. Using a dropper, 1drop of 3% H_2O_2 was placed on the microscopic slide and observed for immediate bubble formation [15].

Nitrate reduction test

The nitrate reduction medium was dispensed in test tubes. They were autoclaved for 15mins at 121°C, 15 psi and cooled before use [15].

REAGENTS

Sulfanilic acid solution (Reagent A)

8g of sulfanilic acid was dissolved in 1L of 5N acetic acid.

α-Naphthylamine solution (Reagent B)

6g of N, N-Dimethyl-1-naphthylamine was dissolved in 1L of 5N acetic acid. **Procedure**

The tubes were inoculated heavily with a fresh culture of the suspect organism. Another tube was kept as negative control without organism. The tubes were incubated at 35 to 37°C for 24 to 48hrs in an incubator. 5 drops of Sulfanilic acid solution and 5 drops of α-Naphthylamine solution were added into the tube containing culture to be tested and the negative control. The tubes were shaken well to mix reagents with medium and observed for a distinct red or pink color, which should develop within a few minutes, indicates presence of nitrate reduction.

Gelatin hydrolysis test

About 2 to 3ml of medium was dispensed into test tubes. The medium was autoclaved at 121°C (15 psi) for 15 minutes. The tubed medium was cooled in an upright position before use. A heavy inoculum of 24hrs old test culture was stab-inoculated into tubes containing nutrient gelatin. The inoculated tubes and an uninoculated control tube are incubated at 25°C, for up to 1 week, and checked every day for gelatin liquefaction. The tubes are immersed in an ice bath for 15 to 30 minutes. Afterwards, tubes were tilted to observe if gelatin has been hydrolyzed [16].

Urea hydrolysis test

The urea broth base was sterilized by autoclaving at 115°C for 20 minutes and was cooled to 55°C. To this 5ml of sterile 40% Urea Solution was added and mixed well. The tubes of Urea Broth were inoculated with 1ml of culture broth and incubated for 2 to 6hrs at 35°C and was observed for pink color [17].

Evaluation of antidandruff potential of the selected extracts

Various concentrations (250-1000µg/ml) of the extracts were prepared in DMSO from the resultant extract to determine its antidandruff activity. Control experiments were performed by using DMSO with identical concentration used to test the extract. Isolates from dandruff were inoculated by swabbing on the surface of gelled PDA plates. Wells of 8mm in diameter were performed in the PDA media, and each well was filled with 50µl of certain concentration of extract. The plates were kept in laminar air flow for 30 minutes for proper diffusion of the extract and thereafter incubated at 37ºC for 3-5days. The radius for the zone of inhibition was in millimeters and recorded against the corresponding concentration [6].

Broth dilution assay

Dilution assays are standard method used to compare the inhibition efficiency of the antimicrobial agents. 5ml of the potato dextrose broth, 0.1ml of the 24hrs growing culture (*M. furfur*) and the different concentration (100µg, 200µg….1000µg) of the crude extract dissolved in Dimethyl sulphoxide were taken in test tubes. The tubes were incubated at 37ºC for 24hrs. The optical densities were measured spectrometically at 600 nm . The percentage of viable cells was calculated using the following formula.

$$\%\ \text{of inhibition} = \frac{\text{Control O.D - Test O.D}}{\text{Control O.D}} \times 100$$

O.D = Optical density

Qualitative phytochemical screening

The methanol extracts were subjected to the following qualitative phytochemical analysis following standard techniques.

Detection of alkaloids

Solvent free extract (50mg) was stirred with few ml of dilute hydrochloric acid and filtered. The filtrate was tested carefully with various alkaloidal reagents as follows

Mayer's test

To a few ml of filtrate, a drop or two of Mayer's regent was added by the sides of the test tube. A white creamy precipitate indicated the test as positive.

Mayer's reagent

Mercuric chloride (1.358g) was dissolved in 60ml of water and potassium chloride (5.0g) was dissolved in 10 ml of water. The two solutions were mixed and made up to 100ml with water.

Detection of phenolic compound

Ferric chloride test

The extract (50mg) was dissolved in 5ml of distilled water. To this, few drops of neutral 5% ferric chloride solution were added. A dark green colour indicated the presence of phenolic compounds.

Detection of glycosides

50mg of extract was hydrolysed with concentrated hydrochloric acid for 2hrs on a water bath, filtered and the hydrolysate was subjected to the following test.

Borntrager's test

To 2ml of filtrate hydrolysate, 3mL of chloroform was added and shaken. Chloroform layer was separated and 10% ammonia solution was added to it. Pink color indicated the presence of glycosides.

Detection of flavonoids

A portion of the extract was heated with 10ml of ethyl acetate over a steam bath for 3 minutes. The mixture was filtered and 4ml of the filtrate was shaken with 1ml of dilute ammonia solution. A yellow coloration indicates the presence of flavonoids.

Detection of tannins

About 2ml of the aqueous extract was stirred with 2ml of distilled water and few drops of 0.1%FeCl3 solution were added. The formation of a green precipitate was an indication for the presence of tannins.

Detection of reducing sugars

The extract (100mg) was dissolved in 50ml of water and filtered. The filtrate was subjected to the following test.

Fehling's test

1ml of filtrate was boiled on water bath with 1ml each of Fehling's solution I and II. A red precipitate indicated the presence of sugar.

Fehling's solution

Fehling's solution I

Copper sulphate (34.66g) was dissolved in distilled water and made upto 500ml with distilled water.

Fehling's solution II

Potassium sodium tartarate (173g) and sodium hydroxide (50g) was dissolved in water and made up to 500ml.

Detection of saponins

Foam test

The extract (50mg) was diluted with distilled water and made up to 20ml. the suspension was shaken in a graduated cylinder for 15 minutes. A two cm layer of foam indicated the presence of saponins.

Detection of proteins

The extract (100mg) was dissolved in 10ml of distilled water and filtered through Whatmann No.1 filter paper and the filtrate was subjected to tests of proteins.

Biuret test

An aliquot of 2ml of filtrate was treated with one drop of2% copper sulphate solution. To this, 1ml of ethanol (95%) was added, followed by excess of potassium hydroxide pellets. Pink color in the ethanolic layer indicated the presence of proteins.

QUANTITATIVE ANALYSIS

Determination of Phenolic compound – Folin Ciocaltaeu's method

The total phenolic content of the plant extracts was determined by the Folin-Ciocalteau method [18]. Briefly, 200µl of diluted samples were added to 1ml of a 1:10 diluted Folin- Ciocalteau reagent. After 4min, 800µl of a saturated sodium carbonate solution (75g/L) was added. After 2hrs of incubation at room temperature, the absorbance at 760nm was evaluated using a spectrophotometer. The results were expressed as gram gallic acid equivalent (GAE)/100g DW of the plant material.

Determination of total flavonoids - Aluminium chloride test

The total flavonoid content was established in the reaction with aluminum chloride using a colorimetric method [18]. Briefly, 1ml of each extract was shaken for 1min and 0.1ml of 10% aluminum nitrate, 0.1ml 1M potassium acetate and 3.8ml of methanol was added. After 40min at room temperature, the absorbance was measured on the ultraviolet (UV)/Visible spectrophotometer at 415nm.

Determination of total tannins

The tannins content was determined using Folin denis reagent as described by [19]. In that method a standard calibration curve was prepared and the absorbance (A) against concentration of tannins at specific wavelength was estimated as follows:

Suitable aliquots of the tannin containing extract (1mg/ml) were pipetted in test tubes. The volume was made upto 1ml with distilled water. Then 2.5ml of sodium carbonate reagent was added. The tubes were shaken and the absorbance was recorded at 725nm after 40mins. The amount of total tannins was calculated as tannic acid equivalent form the standard curve.

Determination of carbohydrates

About 25mg of extract was weighed and was hydrolyzed by boiling it with 2.5N HCl for 2hrs and then cooled to room temperature. This mixture was then neutralized using solid sodium carbonate until the effervescence ceases. Make up the volume to 100ml with water. Centrifuge at 3500rpm for 10mins. Pipette out 0.5ml of supernatant with duplicates in two other test tubes. Make up the volume to 1ml with water in all test tubes. A tube with 1ml of water serves as a blank. 4ml of Anthrone reagent was added and heated for eight minutes in water bath and cooled. The green colour developed was read at 630nm. A standard graph of glucose was plotted, from which the carbohydrate content of the extract was determined [20].

Thin layer chromatography

The TLC was performed on pre-coated 5×1.5cm and 0.25mm thick plates. Methanol extract of Syzygium aromaticum was plotted on TLC plates .The plates were air dried and developed in suitable solvents for rapid screening methanol / chloroform in varying ratio 1:9, 0.5:9.5 and 0.25:9.75. The plates were run in the above solvent systems and dried at room temperature. Derivatisation of TLC plates was done by UV light at 254nm [21]. Different bands were observed and corresponding R_f values are determined. R_f value of each spot was calculated as:-

$$R_f = \text{Distance Travelled by the Solute} / \text{Distance Travelled by the Solvent}$$

Directbioautography

Bioautography was performed to determine the bioactive compound responsible for the antimicrobial activity. TLC was performed and the silica plates were placed in a sterile petri-plate and overlayed with PDB inoculated with 17hrs growing culture of *M. furfur* and the plate was closed and incubated for 24hrs. After incubation the inhibition bands were visualized [22].

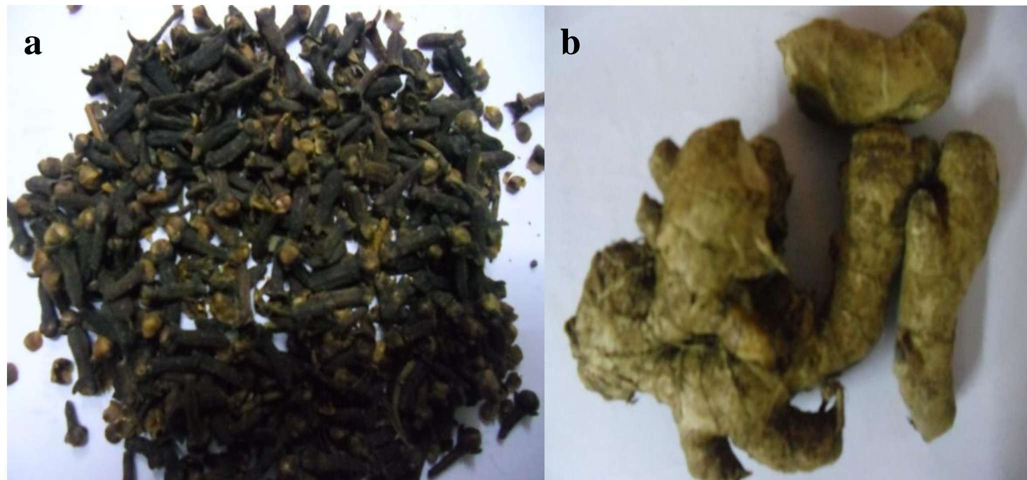
Compound inhibition by TLC

5ml of the potato dextrose broth, 0.1ml of the 24hrs growing culture (M. furfur) for control and another test tube treated with 850 µg/ml of the crude extract dissolved in Dimethyl sulphoxide were prepared. The tubes were incubated at 37ºC for 24hrs. The broths were centrifuged at 5000rpm for 15mins. Then the above mixture was transferred to the separating funnel. The solvent layer was collected and mixed with ethyl acetate and was allowed to dry. These samples were subjected to thin-layer chromatography (TLC) by loading on pre-coated silica gel 60 F254 plates (8cm×6cm;Merck). Ethyl acetate- hexane (1:9 v/v) mixture was used as the mobile phase. TLC chromatograms were scanned under UV light at 254nm. The fluorescent bands and the inhibited bands were marked [23].

RESULT

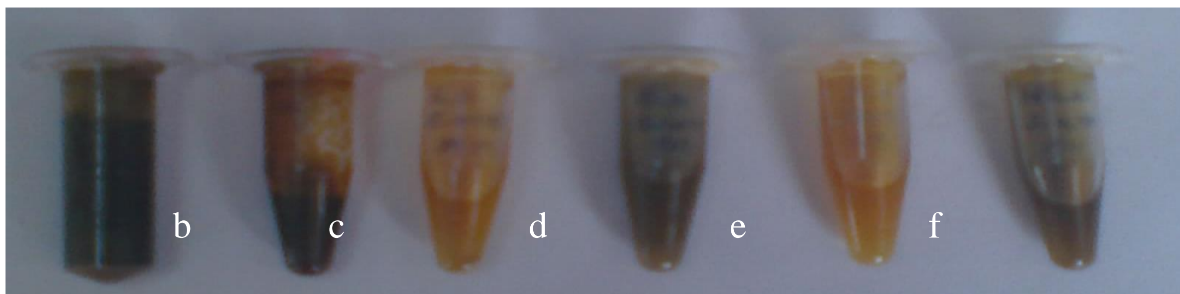
Extraction with different solvents

The extract of *Syzygium aromaticum* and *Zingiber officinale* were obtained by direct solvent extraction of Eloff's method (Fig. 2). In this method, different solvents were used namely hexane, ethyl acetate and methanol.



a-buds of *Syzygium aromaticum* **b-** rhizome of *Zingiber officinale*

Figure 1: *Syzygium aromaticum* and *Zingiberofficinale*



a- ***S.aromaticum*** **methanol extract** **b-*Z.officinale*** **methanol extract**
c- ***Z.officinale*** **ethyl acetate extract** **d-** ***S.aromaticum*** **ethyl acetate extract**
e- ***Z.officinale*** **hexane extract** **f-** ***S.aromaticum*** **hexane extract**

Figure 2: Crude extracts of *Syzygium aromaticum* and *zingiber officinale*

Isolation and sub-culture of dandruff causing organism

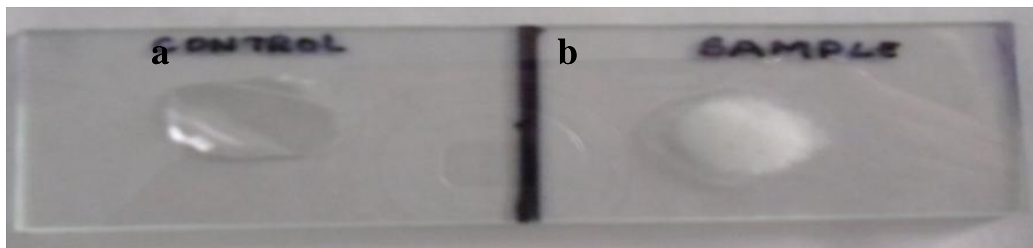
The flakes from different patients (Fig. 3a) were collected and cultured in Potato dextrose broth (PDB) and sub-cultured in Sabouraud's Dextrose Agar (SDA) and Potato Dextrose agar (PDA) by swabbing and streaking methods. Significant growth was observed in PDA (Fig. 3b).

a-Dandruff flakes **b-**Pure culture

Figure 3: Isolation and pure culture

Biochemical identification of the isolate
The various identification tests were performed to confirm the presence of *Malassezia furfur*.

Catalase test
The catalase enzyme catalyzes the decomposition of hydrogen peroxide during oxidation. The effervescence caused by liberation of free oxygen indicates a positive test (Fig. 4).



a- Control (without effervescence) **b-** Test (with effervescence)
Figure 4: Catalase test

Lactophenol cotton blue test
Grown isolates of *Malassezia furfur* smeared with lactophenol cotton blue were viewed in the microscope. The bottle shaped cells of *Malassezia furfur* were observed (Fig 5).

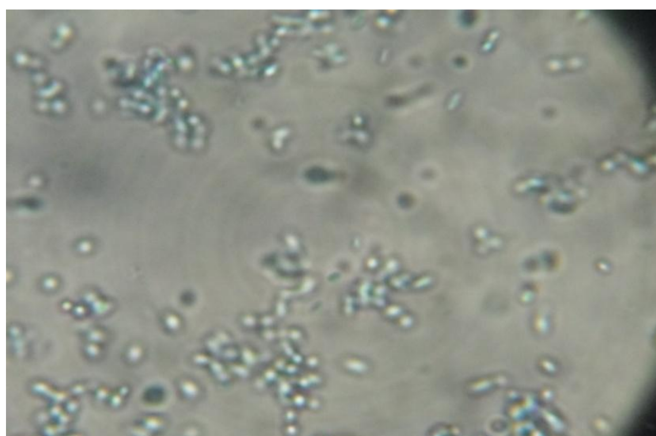


Figure 5: Microscopic view of *Malassezia furfur* showing bottle shaped cells

Nitrate reduction test
1ml of sample was inoculated in nitrate broth and 5 drops of Sulfanilic acid and α-Naphthalamine reagent were added. The solution gave a distinct light red colour which indicates a positive test (Fig. 6).

a- Control (Nitrate negative)

b- Test (Nitrate positive)

Figure 6: Nitrate reduction test

Urease test

Malassezia furfur culture was inoculated in Christensen's urea broth. Then, it was incubated for24hrs. Pink colour appeared which denotes the presence of urease positive organism (Fig. 7).

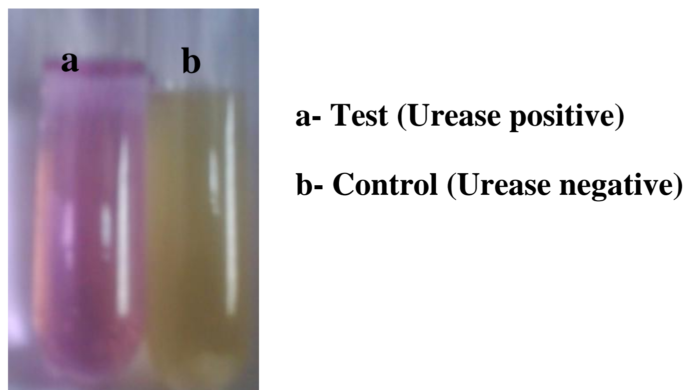


Figure 7: Urease test

Gelatin hydrolysis test

Pure culture of *Malassezia furfur* was inoculated into a sterile tube containing nutrient gelatin and incubated for 24hrs at 35-37ºC. The tube was placed on ice for few minutes and it was found that the media failed to solidify which indicates a positive test (Fig. 8).

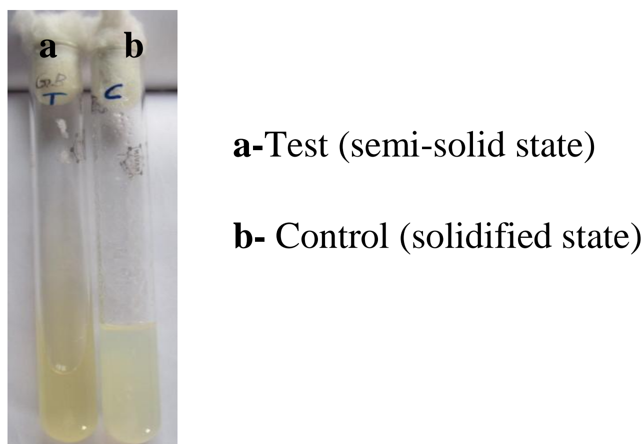
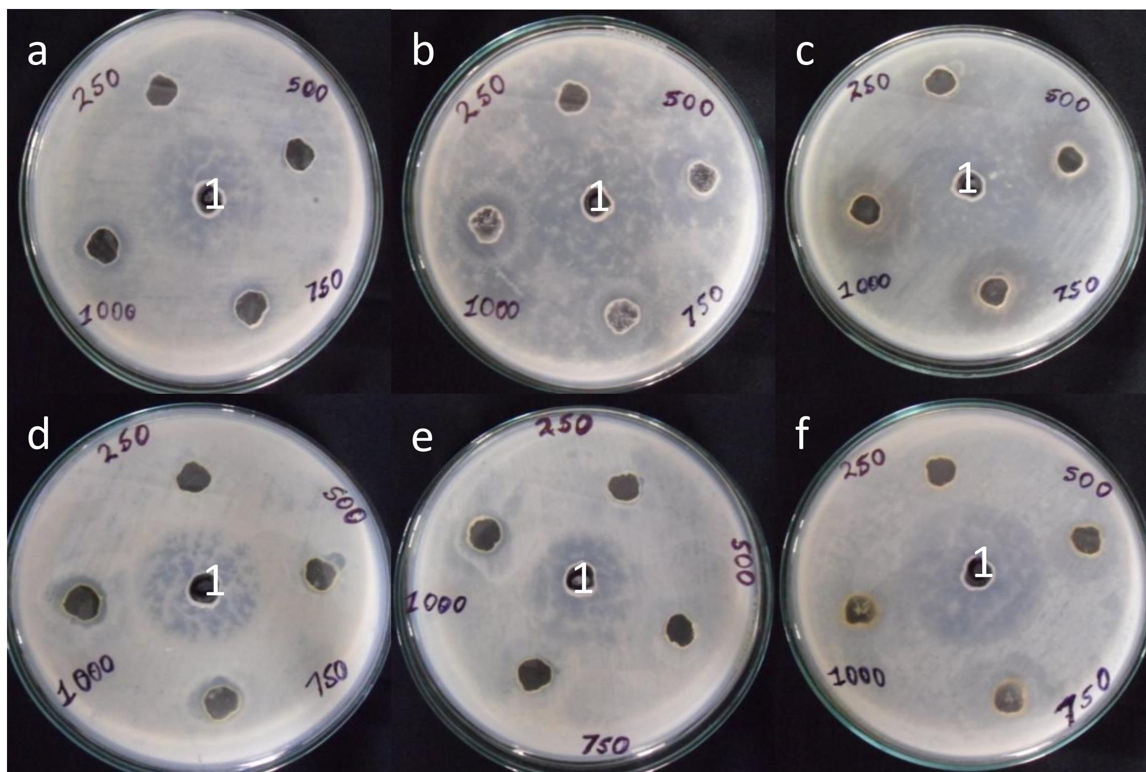


Figure 8: Gelatin hydrolysis test

Anti-dandruff assay

From the results it is clear that the methanol extracts of *S.aromaticum* and *Z.officinale* had significant inhibitory effect on *M. furfur* when compared with ethyl acetate and hexane extracts (Fig. 9). The methanol extract of *S. aromaticum* showed maximum inhibition with Zone of Inhibition 23mm. While, that for *Z. officinale* was observed to be 22mm. ZOI was observed for the methanol extract of *S. aromaticum* from 250µg/ml while it was 500µg/ml for the methanol extract of *Z. officinale* (Table1).The activity was found to be effective in the methanol extract of *Syzygium aromaticum* hence it was taken for further assay.



a- Syzygium aromaticum hexane b- *Syzygium aromaticum* ethyl acetate
c-Syzygium aromaticum methanol d- *Zingiber officinale* hexane
e-Zingiber officinale ethyl acetate f- *Zingiber officinale* methanol
1 – Standard (Ketaconazzole 100µg/ml)

Figure 9: Effect of extracts of *S.aromaticum* and *Z.officinale* on *M. furfur*

Table 1: Effect of plant extracts on *Malassezia furfur*

S. no	Concentration (µg/ml)	Zone of inhibition(mm)						
		Syzygium aromaticum			*Zingiber officinale*			Standard (100 µg/ml)
		Hexane	Ethyl acetate	Methanol	Hexane	Ethyl acetate	Methanol	
1	250	-	10.66±2.08	15±1	-	-	-	33.33±1.52
2	500	-	19±2.64	21.33±1.52	-	-	16.33±1.52	
3	750	11.33±1.52	20±1	22.33±1.52	10.66±1.52	-	19.33±2.51	
4	1000	13.33±1.52	21±2	22.66±1.52	12±1	11.66±1.2	22.33±2.51	

Values are expressed in mean±SEM

Broth dilution assay

Broth dilution assay was performed to know the minimum inhibitory concentration of *Syzygium aromaticum*. The MIC was 100µg/ml and IC_{50} value was found to be 850µg/ml (Table 2). The inhibitory effect was studied to be linear with the concentration of the sample (Fig. 10).

Table 2: Determination of IC_{50} of methanol extract of *S. aromaticum*

S.No	Concentration(µg/ml)	Inhibition (%)
1	100	0.68
2	200	2.18
3	300	2.73
4	400	5.32
5	500	15.98
6	600	21.72
7	700	27.32
8	800	32.92
9	900	60.24
10	1000	64.48

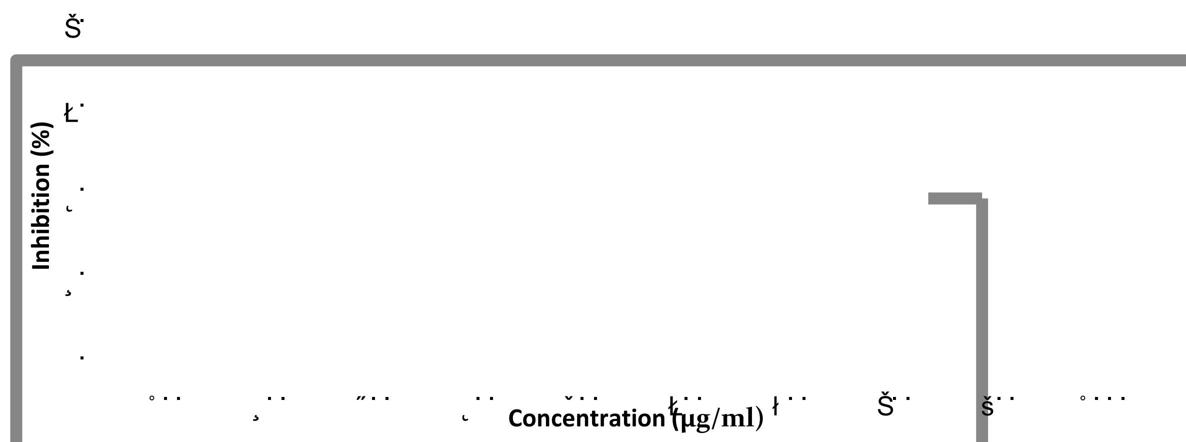
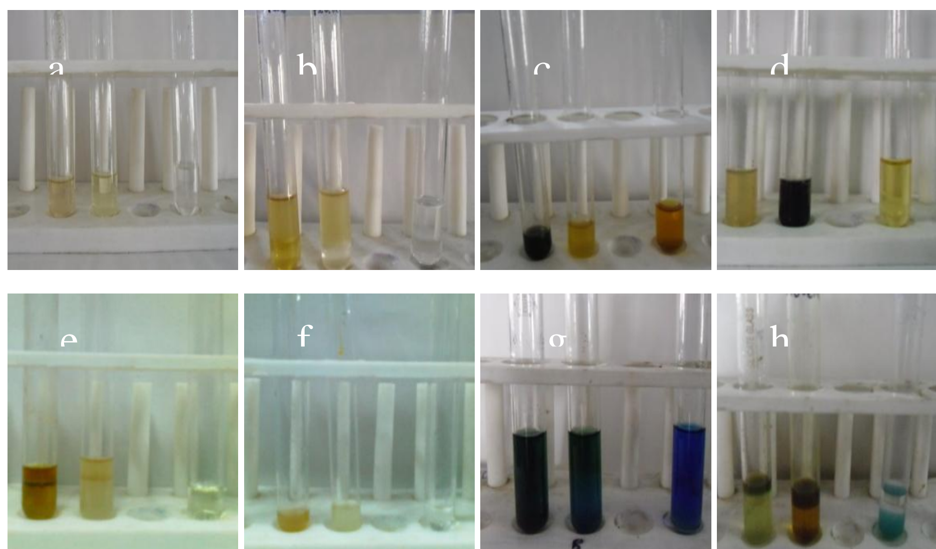


Figure 10: Determination of IC_{50} of methanol extract of *S. aromaticum*

Qualitative Phytochemical analysis



Qualitative phytochemical tests for alkaloids (a), flavonoids (b), phenols (c), tannins (d), glycosides (e), saponins (f), reducing sugars (g) and proteins (h)

Figure 11: Qualitative phytochemical analysis

In the qualitative analysis of *Syzygium aromaticum* the presence of flavonoids, tannins, glycosides, reducing sugars and phenolic compounds were determined. Qualitative analysis of *Zingiber officinale* showed the presence of flavonoids, glycosides, reducing sugars and proteins (Fig. 11).

Table 3: Qualitative phytochemical analysis

S.No	Phytochemical	*Syzygium aromaticum*	*Zingiber officinale*
1	Alkaloids	-	-
2	Flavonoids	+++	+
3	Phenols	+++	-
4	Tannins	+++	-
5	Glycosides	+	-
6	Saponins	-	-
7	Reducing sugars	+++	++
8	Proteins	-	+

- Not detectable using the assay followed + present in minor amount
++ present in moderate amount +++ present in higher amount

Quantitative estimation of phytochemicals

In the quantitative analysis of *Syzygium aromaticum* the total concentration of phenol was estimated to be 1052µg of GAE/g of extract, the total concentration of flavonoids was found to be 1299µg of QE/g of extract, the total concentration of tannins was found to be 1255µg of GAE/g of extract and the amount of reducing sugars was found to be 1052mg of Glucose/g sample.

Table 4: Quantitative estimation of phytochemicals in *S.aromaticum*

S .No	Phytochemical	Amount
1	Total phenolics	1052.66±1.52µg GAE/g sample
2	Total flavonoids	1299.33±2.08µg QE/g sample
3	Total tannins	1254.66±1.52µg GAE/g sample
4	Reducing sugars	1051.66±1.53mg Glucose/g sample

Values are expressed in mean±SEM

Thin layer chromatography

The ratio 0.25:9.75 showed prominent separation of compounds with 6 distinct bands of R_f value 0.102, 0.153, 0.282, 0.384, 0.538 and 0.897 (Fig. 12).

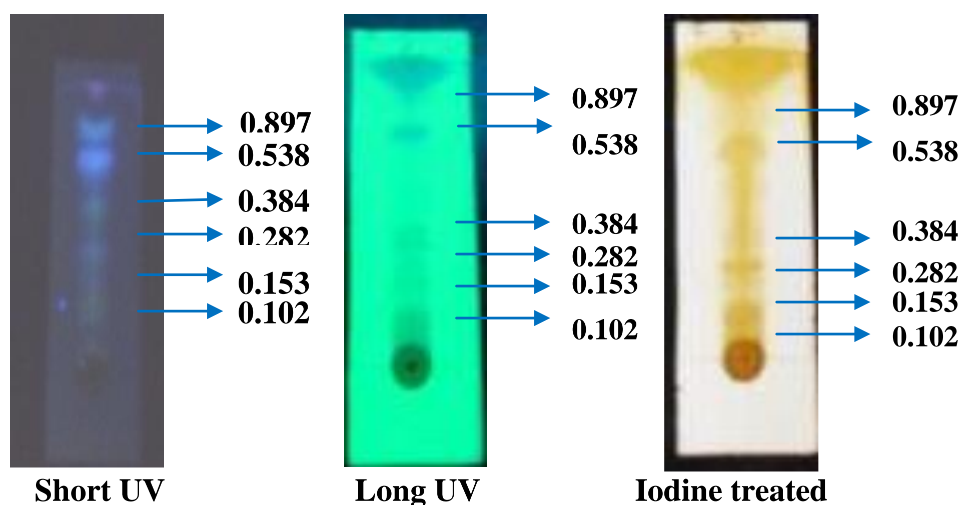


Figure 12: Thin Layer Chromatogram of methanol extract

Bio-autography

In the bio-autography, the zone of inhibition was found in the compound possessing the R_f value 0.153.

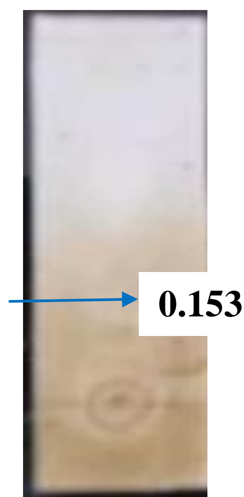


Figure 13: Bio-autography showing clear zone around compound with R_f value 0.153

Compound inhibition by TLC

In this method the band formed by the compounds in the *Malassezia furfur* was inhibited when treated with the extract of *Syzygium aromaticum.*

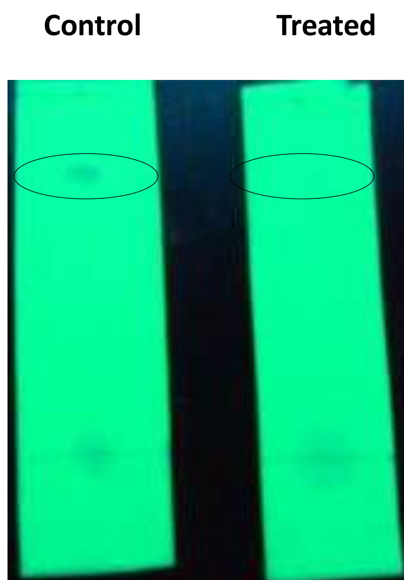


Figure 14: Compound inhibition by TLC

CONCLUSION

Nowadays, more individuals are susceptible to dandruff, which leads to both physiological and psychological problem. The treatment is essential to control the severity and harmful effects of dandruff. The currently available commercial agents such as Zinc pyrithione, Ketoconazole, Piroctone olamine, Cipropirox olamine, etc though are effective lead to certain side effects such as hair loss and irritation. Herbal products help in preventing dandruff without side effects.

Results of the present investigation reveal the antidandruff activity of the extracts of *Syzygium aromaticum* and *Zingiber officinale* against *Malassezia furfur*, the dandruff causing organism. Among the three solvents used the

methanol extract of *Syzygium aromaticum* shows higher antidandruff activity and can be used to formulate a potential therapeutic agent for dandruff.

The future research of the work could be to purify and isolate the compound that is responsible for the inhibition of the dandruff causing organism and develop it into a potential herbal product. This work will have importance in the field of cosmetics since it will be cost effective and there will be no side effects.

REFERENCES

1) Sirkar, N.N. (1989). Pharmacological basis of Ayurvedic therapeutics. In: Atal, C.K., Kapoor, B.M. (Eds.), Cultivation and utilization of medicinal plants. Published by PID CSIR.
2) Gomez-Flores. R., Verástegui-Rodríguez, L., Quintanilla-Licea, R., Tamez-Guerra1, R., Tamez-Guerra1, R., Rodríguez-Padilla, C. (2008). In vitro rat lymphocyte proliferation induced by *Ocinum basilicum*, *Persea americana*, *Plantago virginica*, and *Rosa spp*. Extracts. *Journal of Medicinal Plants Research*, 2(1), 005–010.
3) Prakash, P., Gupta, N. (2005). Therapeutic uses of *Ocimum sanctum Linn* (Tulsi) with a note on eugenol and its pharmacological actions: A short review. *Indian J Physiol Pharmocol*, 49(2), 125–131.
4) Borchers, A.T., Sakai, S., Henderson, G.L., Harkey, M.R., Keen, C.L., Stern, J.S., Terasawa, K., Gershwin, M.E. (2000). Shosaiko-to and other Kampo (Japanese herbal) medicines: a review of their immunomodulatory activities. *J Ethnopharmacol* 73, 1–13.
5) Prabhamanju, M., Shankar, SG.,Babu, k and Ranjith, MS,2009. Herbal vs. Chemical substances as antidandruff ingredients: which are more effective in the management of dandruff?-An overview. Egyptian Dermatology Online Journal 5(2):8.
6) Sibi, G., Gurmeetkaur, Devi, G., Dhananjaya, K., Ravikumar, KR and Mallesha, H, 2012. Anti-dandruff activity of *Ricinus communis* L.leaf extracts. International journal of current pharmaceutical research 4(3):74-76.
7) Berenji, F., Rakhshandeh, H and Ebrahimipour, H, 2010.*In vitro* study of the effects of henna extracts (*Lawsonia inermis*) on *Malassezia* species.Jundishapur J Microbiol 3(3): 125-128.
8) Naeini, A., Eidi, S and Shokri H, 2011.Fungitoxicity of *Zataria multiflora* essential oil against various *Malassezia* species isolated from cats and dogs with *Malassezia* dermatitis. African Journal of Microbiology Research 5(9):1057-1061.
9) Charles, W., Saunders, Scheynius, A and Heitman, J, 2012. *Malassezia* fungi are specialized to live on skin and associated with dandruff, Eczema, and Other Skin Diseases. PLoS Pathogens 8(6): e1002701.
10) Ranasinghe, L., Jayawardena, B and Abeywickrama, K, 2002. Fungicidal activity of essential oils of *Cinnamomum zeylanicum* (L.) and *Syzygium aromaticum* (L.) MerretL.M.Perry against crown rot and anthracnose pathogens isolated from banana. Letters in Applied Microbiology 35:208-211.
11) Pinto, E., Vale-Silva, L., Cavaleiro, C and Salgueiro, L, 2009. Antifungal activity of the clove essential oil from *Syzygium aromaticum* on *Candida, Aspergillus* and dermatophyte species. Journal of Medical Microbiology 58:1454-1462.
12) Onyenekwe, PC and Hashimoto, S, 1999. The composition of the essential oil of dried Nigerian ginger (*Zingiber officinale* Roscoe). Eur Food Res Technol 209:407-410.
13) Eloff, JN, 1998. *J. Ethnopharmacology* 60:1-8.
14) Gehan, S., El-Hadidy, Nahed, IM.,Gomaa, Abo bakr, RAE., Lobna, A and Metwally, 2007. Direct molecular identification of *Malassezia* species from skin scales of patients with Seborrheic dermatitis by Nested Terminal Fragment Length Polymorphism analysis. Egyptian Journal of Medical Microbiology 16(3):437-444.
15) Naveen, S., Karthika, S., Sentila, R., Mahenthiran, R and Michael, A, 2012. In-vitro evaluation of herbal and chemical agents in the management of dandruff. Journal of Microbiology and Biotechnology Research 2(6):916-921.
16) Vijayakumar, R., Muthukumar, C., Kumar, T and Saravanamuthu, R, 2006. Characterization of *Malassezia furfur* and its control by using plant extracts. Indian J dermatol 51(2):145-148.

17) Buick, SF, 1997. Development of an *In Vitro* diagnostic technique for *Malassezia furfur.*A thesis submitted in partial fulfillment of the requirement for the degree of Master of Science in Microbiology in the university of Canterbury.
18) Yang, C., Chang, F., Chang, H., Wang, S., Hsieh, M and Chuang, L, 2012.Investigation of the antioxidant activity of *Illicium verum* extracts. Journal of Medicinal Plants Research 6(2):314-324.
19) Sulieman, AME.,Issa, FM and Elkhalifa, EA, 2007. Quantitative determination of tannin content in some Sorghum cultivars and evaluation of its antimicrobial activity. Research Journal of Microbiology 2(3):284-288.
20) Yemm, EW and Willis, AJ, 1954. The estimation of carbohydrates in plant extracts by Anthrone 57:508-514.
21) Verma, A., Singh, N and Kumar A, 2013. Phytochemical investigation and thin layer chromatography of *Asparagus Racemosus* (*Asparagaceae*) methanolic leaves extract. International journal of Biology, Pharmacy and Allied sciences 2(1):187-191.
22) Choma, IM and Grzelak, EM, 2010. Bioautography detection in thin-layer chromatography. Journal of Chromatography A :1-8.
23) Pawar, NK and Arumugam, N, 2011. Leaf extract of *Centratherum Punctatum* exhibits antimicrobial, antioxidant and anti proliferative properties. Asian Journal of Pharmaceutical and Clinical Research 4(3):71-76.

***Submit your next manuscript to IAJPR and take advantage of*:**

- **Access Online first**
- **Double blind peer review policy**
- **No space constraints**
- **Rapid publication**
- **International recognition**

Submit your manuscript at: editorinchief@iajpr.com