

Research Article

Antidandruff activity of *Cassia auriculata* and *Cassia alata* through fatty acids mediated inhibition of *Malassezia furfur*

V. Prabhu*

PG Department of Chemistry, Nallamuthu Gounder Mahalingam College, Pollachi (Tamil Nadu), India

K. Poonkodi

PG Department of Chemistry, Nallamuthu Gounder Mahalingam College, Pollachi (Tamil Nadu), India

K. Pradeep

PG Department of Chemistry, Nallamuthu Gounder Mahalingam College, Pollachi (Tamil Nadu), India

S. Buvaneswari

PG Department of Chemistry, Nallamuthu Gounder Mahalingam College, Pollachi (Tamil Nadu), India

R. Mini

PG Department of Chemistry, Nallamuthu Gounder Mahalingam College, Pollachi (Tamil Nadu), India

K. Vimaladevi

PG Department of Chemistry, Nallamuthu Gounder Mahalingam College, Pollachi (Tamil Nadu), India

M. Anusuya

PG Department of Chemistry, Nallamuthu Gounder Mahalingam College, Pollachi (Tamil Nadu), India

Sibi G

Department of Biotechnology, Indian Academy Degree College-Autonomous, Bangalore (Karnataka), India

*Corresponding Author. Email: prabhusamruth@gmail.com

Article Info

[https://doi.org/10.31018/](https://doi.org/10.31018/jans.v12i4.2390)

[jans.v12i4.2390](https://doi.org/10.31018/jans.v12i4.2390)

Received: October 4, 2020

Revised: November 15, 2020

Accepted: November 20, 2020

How to Cite

Prabhu V. et al. (2020). Antidandruff activity of *Cassia auriculata* and *Cassia alata* through fatty acids mediated inhibition of *Malassezia furfur*. *Journal of Applied and Natural Science*, 12(4):532 - 540. <https://doi.org/10.31018/jans.v12i4.2390>

Abstract

Susceptibility of *Malassezia furfur* to certain medium chain fatty acids shed light onto novel strategies to control dandruff. This study explored antidandruff activity of the fatty acids and other bioactive compounds from flowers of *Cassia auriculata* and *Cassia alata*. The idea was supplementing the growth medium with fatty acids which are inhibitory to *Malassezia* so that plant-based antidandruff formulations could be developed based on the results. Chloroform and ethanolic flower extracts were tested there *in vitro* efficacy against *M. furfur* and the potential antidandruff compounds were identified by gas chromatography-mass spectrophotometry (GC-MS). Minimum inhibitory concentrations were determined for both the extracts and IC₅₀ values of 50 and 88 μM for chloroform extract of *C. auriculata* and *C. alata* were recorded. For ethanol extract, IC₅₀ values of 75 and 70 μM were exhibited by *C. auriculata* and *C. alata*, respectively. Inhibition of *M. furfur* through fatty acids from *Cassia* is the first report, and it is possible to include specific fatty acids in the growth media to inhibit the growth of *Malassezia* which could be later served as lead molecules in antidandruff formulations. Further, the presence of citronellol, pinitol, anthracenedione and chrysin in *Cassia* flower extracts and their antidandruff activity reported in this study needed further research on those compounds to formulate effective treatment of *Malassezia* associated diseases.

Keywords: Anti-dandruff, *Cassia alata*, *Cassia auriculata*, Fatty acids, *Malassezia furfur*

INTRODUCTION

Scalp has thick terminal hair, large numbers of sweat glands and sebaceous glands. Sebum secreted in stratum corneum provides a nutrient-enriched environment for microbial growth. *Malassezia* species

are found on mammalian skin (Findley *et al.*, 2013, Gemmer *et al.*, 2002; Sampaio *et al.*, 2011; Takemoto *et al.*, 2015) associated with several common skin disorders (Sugita *et al.*, 2010). *Malassezia* is a lipophilic yeast that uses sebum lipids as a nutrient

source and leaves unsaturated free acids to result in dryness of the stratum corneum that ultimately leads to dandruff (Ashbee and Evans, 2002). Dandruff is characterized by redness on the scalp with loosely attached oily flakes on the skin (Gupta *et al.*, 2004). Ketoconazole, zinc pyrithione, selenium sulfide and salicylic acid-containing preparations are commonly used in antidandruff shampoo formulations, but high resistance among *Malassezia* sp, recurrent dandruff rates and toxicity limits their usage on the human scalp.

Cassia belongs to the family Caesalpiniaceae and many studies have reported the use of *Cassia* due to its pharmacological potential. Various parts of *Cassia auriculata* and *Cassia alata* were studied for their antimicrobial activity (Murugan *et al.*, 2013; Timothy *et al.*, 2012). *Cassia* species is also reported in treating skin diseases (Singhal and Kansara, 2012; Khare 2007; Duke, 2002; Maity *et al.*, 2001; Atarzadeh *et al.*, 2017). Antifungal activity against *Malassezia furfur* by *C. auriculata* (Kumar *et al.*, 2008) and *C. alata* (Damodaran and Venkataraman, 1994) were reported earlier. However, the active ingredients present in the plants were not explored from the point of antidandruff activity. Further, *Malassezia* spp. are susceptible to certain medium chain fatty acids (Papavassilis *et al.*, 1999; Mayser, 2015; Bhattacharyya *et al.*, 2017). In light of this information, this study explored antidandruff activity of the fatty acids and their derivatives from flowers of *C. auriculata* and *C. alata*. The idea was supplementing the growth medium with fatty acids which are inhibitory to *Malassezia* so that plant based antidandruff formulations could be developed based on the results.

MATERIALS AND METHODS

Plant materials: Fresh flowers of *C. auriculata* and *C. alata* were collected from Pollachi (10.669823°N, 76.980639°E), Tamil Nadu, India. The flowers were identified and authenticated in the Department of Botany, Bangalore University. The flowers were washed and air-dried and powdered.

Preparation of flower extract: Flowers of *C. auriculata* and *C. alata* (50 g) was extracted successively with 500 ml of chloroform and 500 ml of ethanol by using a Soxhlet extractor for 4-6 h at a temperature not exceeding the boiling point. The chloroform and ethanol extracts were collected after filtration and evaporated to dryness under reduced pressure using a Rota vapour. The dried extracts were used for further studies.

Test organism: *Malassezia furfur* MTCC 1374 was obtained from Microbial Type Culture Collection, Chandigarh for this study.

Anti-malassezian activity: The antimicrobial activity of the test organisms to the *C. auriculata* and *C. alata* plant extracts was screened by using the agar well diffusion method. An inoculum suspension (1.2×10^3 CFU/ml) was swabbed uniformly to solidified 20 ml Sabouraud's Dextrose Agar (SDA) and was allowed to dry for 5 min. Holes of 6 mm in diameter were made in

the seeded agar using a sterile cork borer. Aliquot of 10 μ l from each plant extract (10, 20, 40, 80, 160 and 320 μ M) was added into each well on the seeded medium and allowed to stand on the bench for 1 h for proper diffusion and thereafter incubated at 37°C for 24 h. The resulting inhibition zones were measured in millimetres (mm). Ketoconazole was used as positive control and the studies were performed in triplicates.

Minimum inhibitory concentration assay: In vitro antidandruff activity was performed in 96 well microtitre plates using *M. furfur* MTCC 1374 in Sabouraud dextrose broth (Sibi *et al.*, 2014). Extracts in the concentrations of 200 μ M, 180 μ M, 160 μ M, 140 μ M, 120 μ M, 100 μ M, 50 μ M, 25 μ M, 10 μ M and 1 μ M were tested against *M. furfur*. Cell density at 1.2×10^3 CFU/ml was measured by following McFarland 0.5 standard solutions used. Optical density absorbance at 630 nm was read out in microtiter plate reader. Ketoconazole was used as a control in the study. The lowest concentration which inhibited the growth of the fungi was considered the minimum inhibitory concentration (MIC) of each extract. The inhibitory concentration (IC₅₀) value was calculated using the linear relation between the minimum inhibitory percentage and concentration of the extract.

GC-MS Profiling of bioactive compounds: To identify the compounds present in *C. auriculata* and *C. alata*, GC-MS analysis was carried out using an Agilent technologies GC systems with GC-7890A/MS-5975C model (Agilent Technologies, Santa Clara, CA, USA) equipped with HP-5MS column (30 m in length x 250 μ m in diameter x 0.25 μ m thickness of film). Spectroscopic detection by GC-MS involved an electron ionization system which utilized high energy electrons (70eV). Pure helium gas (99.995%) was used as the carrier gas with a flow rate of 1ml/min. The initial temperature was set at 45-150°C with the increasing rate of 8°C/min and holding time of about 4 min. Finally, the temperature was increased to 290°C for 4 min. One microliter of the prepared 1% of the extracts diluted with respective solvents was injected in a splitless mode. The relative quantity of the chemical compounds presents in each of the extracts of *C. auriculata* and *C. alata* was expressed as a percentage based on peak area produced in the chromatogram. Compounds were identified by comparing the mass spectral data with the NIST library (Prabhu *et al.*, 2020).

Statistical analysis: The experiments were done in triplicate and the data reported as the mean \pm SD. Analysis of variance was performed by one way ANOVA using SPSS statistical software, version 20. A probability value at P <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

An antidandruff activity using well diffusion assay revealed the inhibitory activity of both *C. auriculata* and *C. alata* against *M. furfur*. were prepared in chloroform

and ethanol. Quantitative results of the effect of chloroform and ethanol extracts of flowers at the concentrations of 20, 40, 60, 80 and 100 µg/ml on the size of the clear zones are represented in the table-1. The range of inhibition values observed between the extracts varied, and not all preparations exhibited the same anti-malassezian properties.

Anti-malassezian activity of ethanol extracts was higher than chloroform extract and the inhibitory was increased when the extract concentrations were higher. It was found that inhibitory concentration (IC₅₀) values of 50 and 88 µM were recorded for chloroform extract of *C. auriculata* and *C. alata* flowers, respectively. For ethanol extract, IC₅₀ values of 75 and 70 µM were exhibited by *C. auriculata* and *C. alata* respectively (Fig. 1).

The peaks are marked with retention time in the GC-MS chromatogram of the chloroform and ethanol extract of the flowers of *Cassia auriculata* and *Cassia alata*. Their retention time (RT) and the amount of their presence are indicated in Table 2, 3, 4, 5. A total of 59 and 37 compounds identified in chloroform and ethanol extract of *C. auriculata* and *C. alata* are shown in Fig. 2 and 3.

GC-MS analysis of chloroform extracts indicated the presence of fatty acids (i-Propyl 11,12-methyleneoctadecanoate, Octadecanoic acid, Hexadecanoic acid), fatty alcohols (Tetracosanol, Hexadecanol), sesquiterpenes and aromatic organic compounds in *C. auriculata* and *C. alata*. Of the total 59 compounds identified in the chloroform extracts, 30 were present in *C. auriculata*. The various compounds observed from chloroform extracts of *C. auriculata* and *C. alata* did not differ significantly from each other.

Fatty acids are carboxylic present in various plants as either saturated or unsaturated compounds with known antimicrobial activity (Rahuman *et al.*, 2000; Cerdeiras *et al.*, 2000; Dilika *et al.*, 2000; Lee *et al.*, 2002; McGraw *et al.*, 2002; Kumar *et al.*, 2010). Similarly, fatty acids esterified into polyhydric alcohols with antimicrobial effectiveness is reported (Mukherjee *et al.*, 2013; Togashi *et al.*, 2007; Kubo *et al.*, 1993). In our study, anti-malassezian activity of chloroform extracts of *Cassia* was due to the presence of fatty acids and fatty alcohols. This is the novel report that fatty

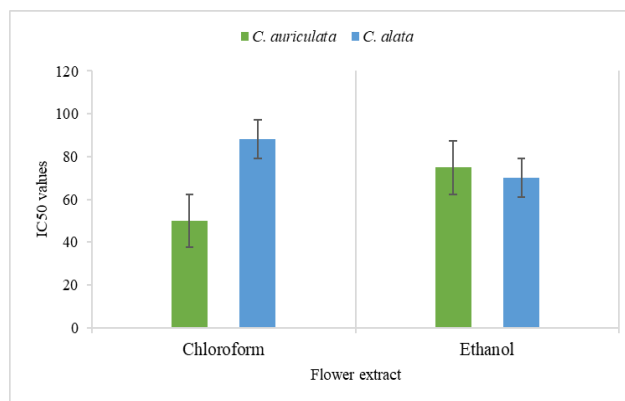


Fig. 1. IC₅₀ values of *C. auriculata* and *C. alata* flower extracts against *M. furfur* MTCC 1374.

acids from *Cassia* is inhibitory to *M. furfur* thus could help in the control of dandruff.

Anti-malassezian activity of medium-chain fatty acids is reported in earlier studies (Mayser, 2015; Bhattacharyya *et al.*, 2017), but the presence of fatty acids and fatty alcohols in *Cassia* species and their inhibitory activity against *Malassezia furfur* is reported for the first time through this study. From the results, it is possible to include specific fatty acids in the growth media to inhibit the growth of *Malassezia*, which could be later served as lead molecules in antidandruff formulations.

Various bioactive compounds were present in ethanol extract of *C. auriculata* and *C. alata* as revealed from GC-MS analysis. Some of the important phytoconstituents identified in this study were terpenoids (Citronellol), polyols (pinitol), glycosides (Anthracenedione, Mannopyranoside) flavonoids (Resorcinol), flavones (Chrysin) along with carbohydrates (Fig. 4 and 5; Table 3 and 4). Antimicrobial activities of the above identified phytochemical compounds from various sources are widely documented in earlier studies. For example, resorcinol is known for its keratolytic and anti-dermatophytic activities (Romagnoli *et al.*, 2016; Martindale, 1989). Ethanol extract of *C. auriculata* flowers showed the presence of resorcinol (0.66%) in this study which may be attributed to the antidandruff activity of plant. Inhibitory activity of citronellol against the fungi *Trichophyton*

Table 1. Anti-malassezian activity of *C. auriculata* and *C. alata* flowers against *M. furfur*. (Values are of triplicates).

Concentration (µg/ml)	Chloroform extract		Ethanol extract	
	Zone of Inhibition (mm)		Zone of Inhibition (mm)	
	<i>C. auriculata</i>	<i>C. alata</i>	<i>C. auriculata</i>	<i>C. alata</i>
20	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.00	0.0 ± 0.0
40	0.7 ± 0.47	0.3 ± 0.06	0.0 ± 0.06	0.1 ± 0.01
80	3.3 ± 0.11	2.6 ± 0.10	3.1 ± 0.20	4.3 ± 0.22
160	6.7 ± 0.20	4.7 ± 0.01	7.3 ± 0.01	7.7 ± 0.03
320	6.8 ± 0.25	5.2 ± 0.51	7.1 ± 0.33	7.8 ± 0.05
Ketoconazole (30 µg)	5.9 ± 0.74			

Table 2. Chemical constituents present in chloroform extract of *C. auriculata* flowers.

Name of the compound	Molecular formula	Retention time (min)	Total Percentage
Hexadecane	C ₁₆ H ₃₄	14.927	0.583
1-Octadecanol	C ₁₈ H ₃₈ O	15.619	3.2
Heptadecane	C ₁₇ H ₃₆	16.993	0.984
Pentadecane,2,6,10,14-tetramethyl-	C ₁₉ H ₄₀	17.121	1.103
Octadecane	C ₁₈ H ₃₈	18.968	1.910
Nonadecane	C ₁₉ H ₄₀	20.883	1.455
Eicosane	C ₂₀ H ₄₂	22.707	1.363
1-Eicosene	C ₂₀ H ₄₀	23.350	2.330
Heneicosane	C ₂₁ H ₄₄	24.468	0.959
Docosane	C ₂₂ H ₄₆	26.146	0.644
1-Docosene	C ₂₂ H ₄₄	26.778	2.054
Phenol,2,4-bis(1,1-dimethylethyl)-	C ₁₄ H ₂₂ O	27.263	3.860
Tricosane	C ₂₃ H ₄₈	27.775	0.996
Hentriacontane	C ₃₁ H ₆₄	29.328	0.414
Hexadecanoic acid,butyl ester	C ₂₀ H ₄₀ O ₂	29.512	0.626
n-Tetracosanol-1	C ₂₄ H ₅₀ O	29.949	1.792
Pentacosane	C ₂₅ H ₅₂	30.793	0.852
Phthalic acid, hept-4-yl isobutyl ester	C ₁₉ H ₂₈ O ₄	31.274	4.034
Phthalic acid, butyl-2-pentyl ester	C ₁₇ H ₂₄ O ₄	31.814	5.364
Heptacosane	C ₂₇ H ₅₆	31.971	1.348
Dibutyl phthalate	C ₁₆ H ₂₂ O ₄	32.164	17.63
1-Hexacosene	C ₂₆ H ₅₂	32.399	3.072
12-Oxotricyclo [5.3.1.1(2,6)]dodeca-3,8-diene,11-acetoxy-4,5,9-trichloro	C ₁₄ H ₁₃ Cl ₃ O ₃	33.697	0.793
Nonacosane	C ₂₉ H ₆₀	33.849	9.685
17-Pentatriacontene	C ₃₅ H ₇₀	34.196	1.247
l-(+)-Ascorbic acid 2,6-dihexadecanoate	C ₃₈ H ₆₈ O ₈	34.407	0.888
i-Propyl 11,12-methylene-octadecanoate	C ₂₂ H ₄₂ O ₂	35.145	1.768
Hexadecanoic acid	C ₁₈ H ₃₆ O ₂	36.103	0.632
Bis(2-ethylhexyl)phthalate	C ₂₄ H ₃₈ O ₄	37.089	26.104
Octadecane,-3-ethyl-5-(2-ethylbutyl)-	C ₂₆ H ₅₄	37.289	2.33

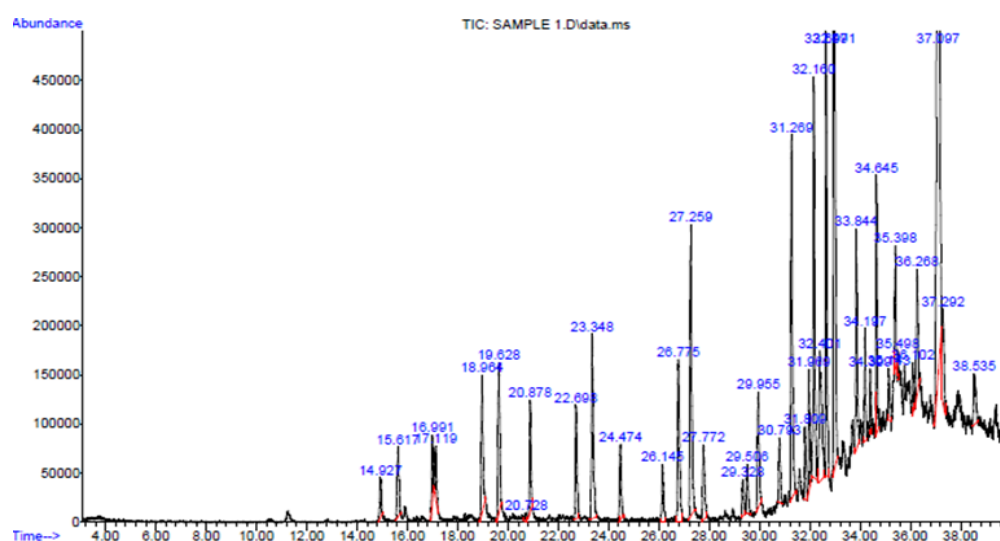
**Fig. 2.** Chromatogram of chloroform extract of *C. auriculata* flowers.

Table 3. Chemical constituents present in chloroform extract of *C. alata* flowers.

Name of the compound	Molecular Formula	Retention time (min)	Total Percentage
Hexadecane	C ₁₆ H ₃₄	15.012	0.556
1-Hexadecanol	C ₁₆ H ₃₄ O	15.704	0.975
Heptadecane	C ₁₇ H ₃₆	17.031	0.875
Hexadecane, 2,6,10-trimethyl-	C ₁₉ H ₄₀	17.134	1.015
Octadecane	C ₁₈ H ₃₈	18.977	1.656
1-Nonadecane	C ₁₉ H ₃₈	19.635	0.752
Benzyl alcohol	C ₇ H ₈ O	20.024	0.923
Nonadecane	C ₁₉ H ₄₀	20.865	2.178
Eicosane	C ₂₀ H ₄₂	22.673	1.757
1-Eicosene	C ₂₀ H ₄₀	23.308	1.006
Heneicosane	C ₁₂ H ₄₄	24.413	1.856
Docosane	C ₂₂ H ₄₆	26.112	1.683
Benzene,1,2,3-trimethoxy-5-(2-propenyl)-	C ₁₂ H ₁₆ O ₃	26.490	0.513
n-Tetracosanol-1	C ₂₄ H ₅₀ O	26.713	0.788
1,3-Benzodioxole, 4-methyl-6-(2-propyl)-	C ₁₁ H ₁₂ O ₃	26.879	0.265
Phenol, 2,4-bis(1,1-dimethylethyl)-	C ₁₄ H ₂₂ O	27.154	3.196
Tetracosane	C ₂₄ H ₅₀	27.726	6.097
Hexadecanoic acid, butyl ester	C ₂₀ H ₄₀ O ₂	29.437	0.279
Heptafluorobutyric acid, n-octadecyl ester	C ₂₂ H ₃₇ F ₇ O ₂	29.900	0.783
Pentacosane	C ₂₅ H ₅₂	30.787	6.624
Phthalic acid, butyl isohexyl ester	C ₁₈ H ₂₆ O ₄	31.182	4.17
Tetratriacontane	C ₃₄ H ₇₀	31.937	18.98
Phthalic acid, butyl hex-3-yl ester	C ₁₈ H ₂₆ O ₄	32.572	2.163
1-(+)-Ascorbic acid 2,6-dihexadecanoate	C ₃₈ H ₆₈ O ₈	34.323	6.188
Nonacosane	C ₂₉ H ₆₀	34.638	22.356
Octadecanoic acid	C ₁₈ H ₃₆ O ₂	36.000	1.890
Octadecane, 3-ethyl-5-(2-ethylbutyl)-	C ₂₆ H ₅₄	37.281	5.694
(2S,2'S)-2,2'-Bis[1,4,7,10,13-pentaoxacyclopentadecane]	C ₂₀ H ₃₈ O ₁₀	37.922	1.702
2-Hexadecanol	C ₁₆ H ₃₄ O	41.361	5.080

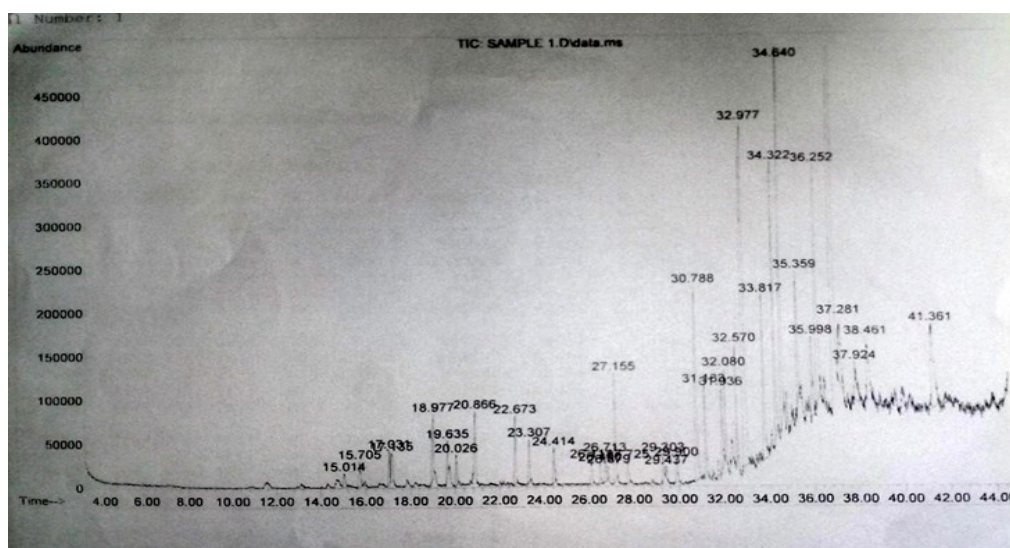
**Fig. 3.** Chromatogram of chloroform extract of *C. alata* flowers.

Table 4. Chemical constituents present in ethanol extract of *C. auriculata* flowers.

Name of the Compound	Molecular formula	Retention time	Total Percentage
Resorcinol	C ₆ H ₆ O ₂	14.4722	0.665
2-Oxiranemethanol, α-(1-methylethyl)-3-[1-(trimethylsilyloxy)pentyl]-	C ₁₄ H ₃₀ O ₃ Si	20.993	1.063
D-Pinitol, pentakis(trimethylsilyl) ether	C ₂₂ H ₅₄ O ₆ Si ₅	21.713	3.600
1,5-Anhydro-D-sorbitol, tetrakis(trimethylsilyl) ether	C ₁₈ H ₄₄ O ₅ Si ₄	22.003	5.520
Lactulose, octakis(trimethylsilyl) ether (isomer 1)	C ₃₆ H ₈₆ O ₁₁ Si ₈	22.324	5.002
Myo-Inositol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)-	C ₂₄ H ₆₀ O ₆ Si ₆	22.744	22.725
D-(-)- Erythofuranose, tris(trimethylsilyl) ether (isomer 2)	C ₁₃ H ₃₂ O ₄ Si ₃	22.95	12.160
D-(+)-Glucuronic acid γ-lactone, tris(trimethylsilyl) ether, methyloxime	C ₁₆ H ₃₅ NO ₆ Si ₃	23.463	9.551
Sedoheptulose, o-methyloxime, hexakis-O-(trimethylsilyl)-	C ₂₆ H ₆₅ NO ₇ Si ₆	23.61	5.028
d-Glucose, 2,3,4,5,6-pentakis-O-(trimethylsilyl)-, o-methyloxyme, (1Z)-	C ₂₂ H ₅₅ NO ₆ Si ₅	23.845	0.894
Inositol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)-, scyllo-	C ₂₄ H ₆₀ O ₆ Si ₆	24.331	5.093
D-(-)-Tagatose, pentakis(trimethylsilyl) ether	C ₂₁ H ₅₂ O ₆ Si ₅	25.983	6.350
Chrysin, bis(trimethylsilyl) ether	C ₂₁ H ₂₆ O ₄ Si ₂	30.594	2.304
Glucufuranoside, methyl 2,3,5,6-tetrakis-O-(trimethylsilyl)-, α-D-	C ₁₉ H ₄₆ O ₆ Si ₄	31.542	0.875
D-(+)-Turanoose, octakis(trimethylsilyl)ether	C ₃₆ H ₈₆ O ₁₁ Si ₈	31.747	7.487
9,10-Anthracenedione, 2-methyl-1,6-bis[(trimethylsilyloxy)-	C ₂₁ H ₂₆ O ₄ Si ₂	32.25	1.137
D-(-)-Ribofuranose, tetrakis(trimethylsilyl)ether(isomer 1)	C ₁₇ H ₄₂ O ₅ Si ₄	32.758	8.519
D-(-)-Ribofuranose, tetrakis(trimethylsilyl) ether (isomer 2)	C ₁₇ H ₄₂ O ₅ Si ₄	33.889	1.727
bis[2-Trimethylsiloxy]ethyl sulfone	C ₁₀ H ₂₆ O ₄ SSi ₂	35.507	0.301

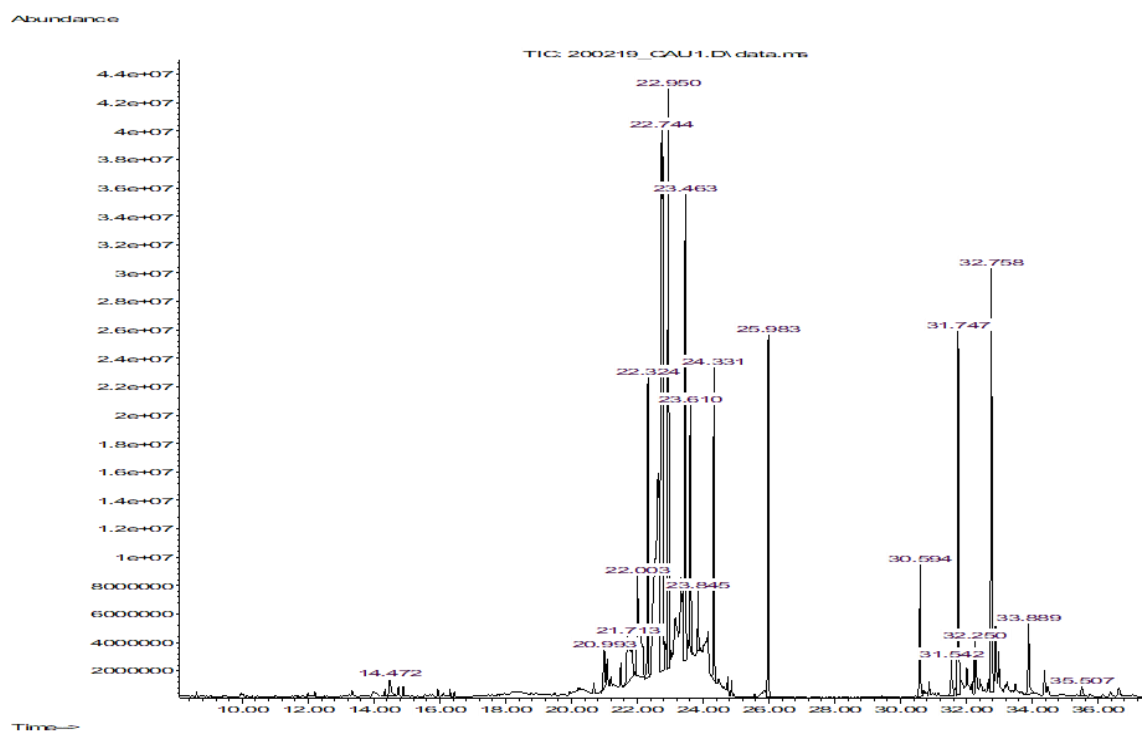
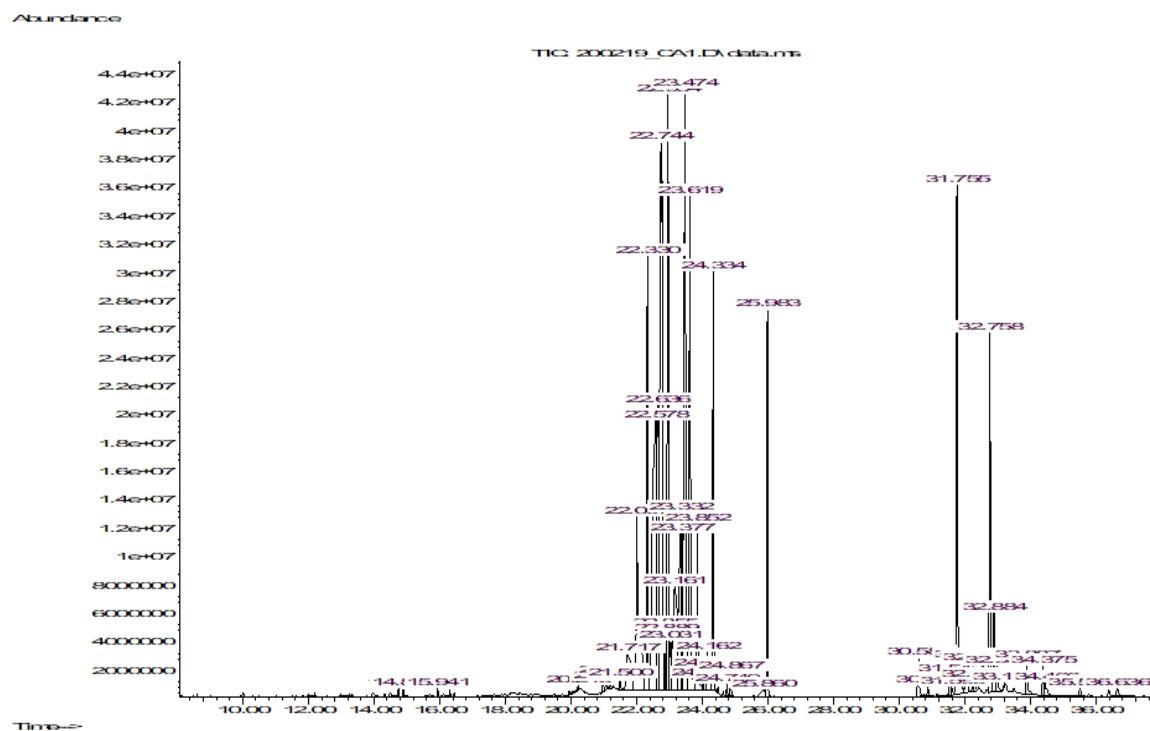
**Fig. 4.** Chromatogram of ethanol extract of *C. auriculata* flowers.

Table 5. Chemical constituents present in ethanol extract of *C. alata* flowers.

Name of the Compound	Molecular formula	Retention Time (Min)	Total Percentage
β -Citronellol, trifluoroacetate	C ₁₀ H ₂₀ O	14.745	0.112
1,4-Diethoxy-2-nitrobenzene	C ₁₀ H ₁₃ NO ₄	14.888	0.090
2(3H)-Furanone, dihydro-3,4-bis[(trimethylsilyl)oxy]-, trans-	C ₁₀ H ₂₂ O ₄ Si ₂	15.941	0.084
1,5-Anhydro-D-sorbitol, tetrakis(trimethylsilyl) ether	C ₁₈ H ₄₄ O ₅ Si ₄	22.007	6.379
D-(-)- Erythrofuranose, tris(trimethylsilyl) ether (isomer 2)	C ₁₃ H ₃₂ O ₄ Si ₃	22.33	6.238
D-Pinitol, pentakis(trimethylsilyl) ether	C ₂₂ H ₅₄ O ₆ Si ₅	22.744	23.094
β -D-Mannopyranoside, methyl 2,3,4,6-tetrakis-O-(trimethylsilyl)-	C ₁₉ H ₄₆ O ₆ Si ₄	22.954	9.539
D-(-)-Tagatose, pentakis(trimethylsilyl) ether, methyloxime (syn)	C ₂₂ H ₅₅ NO ₆ Si ₅	23.475	13.857
D-(+)-Talose, pentakis(trimethylsilyl) ether, methyloxime (syn)	C ₂₂ H ₅₅ NO ₆ Si ₅	23.619	9.062
Sedoheptulose, o-methyloxime, hexakis-O-(trimethylsilyl)-	C ₂₆ H ₆₅ NO ₇ Si ₆	23.852	2.508
Glucopyranose, 2,3-di-O-methyl-1,4,6-tris-O-(trimethylsilyl)-	C ₁₇ H ₄₀ O ₆ Si ₃	24.334	5.508
Myo-Inositol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)-	C ₂₄ H ₆₀ O ₆ Si ₆	25.983	5.046
Chrysin, bis(trimethylsilyl) ether	C ₂₁ H ₂₆ O ₄ Si ₂	30.589	0.734
D-(+)-Turannose, octakis(trimethylsilyl) ether	C ₃₆ H ₈₆ O ₁₁ Si ₈	31.755	9.725
D-(-)-Ribofuranose, tetrakis(trimethylsilyl) ether (isomer 1)	C ₁₇ H ₄₂ O ₅ Si ₄	32.758	5.286
3- α -Mannobiose, octakis(trimethylsilyl) ether (isomer 2)	C ₃₆ H ₈₆ O ₁₁ Si ₈	32.884	1.514
D-Ribofuranose, 1,2,3,5-tetrakis-O-(trimethylsilyl)-	C ₁₇ H ₄₂ O ₅ Si ₄	33.887	0.702
Silane, dimethyl(2-naphthoxy)heptadecyloxy-	C ₂₉ H ₄₈ O ₂ Si	34.375	0.495

**Fig. 5.** Chromatogram of ethanol extract of *C. alata* flowers.

rubrum was reported by Pereira *et al.* (2014) and Shin and Lim (2004) indicating its anti-dermatophytic activity. *Malassezia* is a dermatophytic yeast and the presence of citronellol from ethanolic extract of *C. alata* could have attributed the anti-malassezial activity in this study. Similar work done by Kavitha *et al.*, (2016) reported the susceptibility of *Malassezia japonica* to citronellol. Chrysin from beeswax is reported to protect the skin against pathogenic microorganisms (Gorecka *et al.*, 2020) and in this the study the presence of chrysin is noted in the ethanolic extract of *C. alata*. Furanones are naturally produced by plants and their antimicrobial activities are widely reported (Sharafutdinov *et al.*, 2019; Rathore *et al.*, 2015; He *et al.*, 2015). The anti-malassezial activity of ethanolic extract of *C. auriculata* and *C. alata* is due to one or other bioactive compounds identified through the GC-MS analysis. Further, studies are needed for the purification of the individual compounds and their activity against *Malassezia* to formulate drugs from *Cassia*. Species distribution in the etiology of dandruff is reported (Sharma and Sibi, 2017) and among the *Malassezia*, different species require different lipids for their growth (Mayser *et al.*, 1997). It is reported that medium-chain triglycerides and medium-chain free fatty acids are toxic for *Malassezia* species (Papavassilis *et al.*, 1999) and fatty acid monoesters could treat *Malassezia* associated diseases (Mayser, 2015). Identifying a bioactive compound from plants to control the growth of *Malassezia* is a way to develop plant-based drugs but different the susceptibility varies between *Malassezia* species (Sibi *et al.*, 2014).

Conclusion

Both chloroform and ethanol extracts from *C. auriculata* and *C. alata* flowers had the ability to inhibit *M. furfur* with MIC values of 50 μ M, 88 μ M, 75 μ M and 70 μ M thereby could serve as source of potential anti-dandruff compounds. The presence of fatty acids in chloroform extract and the antifungal compounds from ethanolic extract supported the potential application of *Cassia* species as potent natural sources for anti-dandruff activity. Thus, inhibition of *M. furfur* through fatty acids from *Cassia* flowers is a novel strategy to control dandruff and purification of antidandruff compounds and their mode of inhibition is needed to formulate effective treatment of *Malassezia* associated diseases.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

- Ashbee, H. R., Evans, E. G. V. (2002). Immunology of diseases associated with *Malassezia* species. *Clin Microbiol Rev.*, 15: 21-57. doi: 10.1128/CMR.15.1.21-57.2002
- Atarzadeh, F., Kamalinejad, M., Dastgheib, L., Amin, G., Jaladat, A.M. and Nimrouzi, M. (2017). *Cassia fistula*: A remedy from Traditional Persian Medicine for treatment of cutaneous lesions of *Pemphigus vulgaris*. *Avicenna J Phytomed.*, 7: 107–115. PMID: PMC5355816
- Bhattacharyya, A., Jain, N., Prasad, S., Jain, S., Yadav V., Ghosh S., Sengupta, S. (2017). Evaluation of therapeutic potential of VB-001, a leave-on formulation, for the treatment of moderate adherent dandruff. *BMC Dermatol.*, 17: 5. doi: 10.1186/s12895-017-0058-5
- Cerdeiras MP, Fernandez J, Soubes M, Vero S, Ferreira F, Moyna P, Olano I, Vazquez A (2000). A new antibacterial compound from *Ibicella lutea*. *J Ethnopharmacol.*, 73: 521-525. doi: 10.1016/S0378-8741(00)00339-1
- Damodaran, S., Venkataraman, S. (1994). A study on the therapeutic efficacy of *Cassia alata*, Linn. leaf extract against *Pityriasis versicolor*. *J Ethnopharmacol.*, 42: 19–23. doi: 10.1016/0378-8741(94)90018-3
- Dilika, F., Bremner, P.D. and Meyer, J.J.M. (2000). Antibacterial activity of linoleic and oleic acids isolated from *Helichrysum pedunculatum*: a plant used during circumcision rites. *Fitoterapia.*, 71: 450–452. doi: 10.1016/s0367-326x(00)00150-7
- Duke, J.A. *Handbook of Medicinal Herbs*. 2nd edition. Florida, USA: CRC Press; 2002.
- Findley, K., Oh, J., Yang, J., Conlan, S., Deming, C., Meyer, J. A., Schoenfeld, D., Nomicos, E., Park, M., Kong, H.H. and Segre, J.A. (2013). Topographic diversity of fungal and bacterial communities in human skin. *Nature.*, 498: 367-370. doi: 10.1038/nature12171
- Gemmer, C.M., Y.M. DeAngelis, Y.M., B. Theelen, B., T. Boekhout, T. and T.L. Dawson Jr, J.L. (2002). Fast, non-invasive method for molecular detection and differentiation of *Malassezia* yeast species on human skin and application of the method to dandruff microbiology. *J Clin Microbiol.*, 40: 3350-3357. doi: 10.1128/jcm.40.9.3350-3357.2002
- Gorecka, A.K., Gorecki, M., Rzepecka-Stojko, A., Balweez, R. and Stojko, J. (2020). Bee products in dermatology and skin care. *Molecules*. 25: 556. doi: 10.3390/molecules25030556
- Gupta, A.K., Batra, R., Bluhm, R., Boekhout, T. and Dawson, T.L. (2004). Skin diseases associated with *Malassezia* species. *J. Am. Acad. Dermatol.*, 51: 785–798. doi: 10.1016/j.jaad.2003.12.034
- He, X., Du, X., Zang, X., Dong, L., Gu, Z., Cao, L., Chen D., Keyhani NO., Yao, L., Qiu, J. and Guan, X. (2015). Extraction, identification and antimicrobial activity of a new furanone, grifolaone A, from *Grifola frondosa*. *Natural Product Research.*, 30: 941–947. doi: 10.1080/14786419.2015.1081197
- Kavitha, K., Sekar, P., Pirabhakaran, R. and Mukhopadhyay, T. (2016). Incidence of *M. japonica* in moderate dandruff condition in south India and its sensitivity against tea tree oil, geraniol, citronellol and ketoconazole. *International Journal of Recent Scientific Research.*, 7: 9383-9386.
- Khare, C.P. (2007). *Indian Medicinal Plants: An Illustrated Dictionary*. Berlin, Germany: Springer.
- Kubo, I., Muroi, H. and Kuo, A. (1993). Antibacterial activity of long-chain alcohols against *Streptococcus mutants*. *J. Agric. Food Chem.*, 41: 2447–2450. doi: 10.1021/jf00036a045
- Kumar, P.P., Kumaravel, S. and Lalitha, C. (2010). Screening of antioxidant activity, total phenolics and GC-

- MS study of *Vitex negundo*. *Afr. J. Biochem.*, 4: 191–195. doi: 10.5897/AJBR.9000213
17. Kumar, P.S., Sucheta, S., Deepa, V.S., P. Selvamani, Latha, S. (2008). Evaluation of antidandruff activity using polyherbal oil from six medicinal plants. *Journal of Biotechnology.*, 136: S408–S409. doi: 10.9790/264X-0501010106
18. Lee, J.Y., Kim, Y.S. and, Shin, D.H. (2002). Antimicrobial synergistic effect of linolenic acid and monoglyceride against *Bacillus cereus* and *Staphylococcus aureus*. *J Agric Food Chem.*, 50: 2193–2199. doi: 10.1021/jf011175a
19. Maity, T.K., Mandal, S.C, Bhakta, T., Pal, M. and, Saha, B.P. (2001). Metabolism of 1,8-dihydroxy 3-hydroxy methyl anthraquinone (aloe-emodin) isolated from the leaves of *Cassia tora* in albino rats. *Phytother Res.*, 15: 459-460. doi: 10.1002/ptr.975
20. Martindale, W. (1989). The Extra Pharmacopoeia. 28th ed. Pharmaceutical Press; London, UK: pp. 1637–1638.
21. Mayser, P. (2015). Medium chain fatty acid ethyl esters - activation of antimicrobial effects by *Malassezia* enzymes. *Mycoses.*, 58: 215–219. doi: 10.1111/myc.12300
22. Mayser, P., Haze, P., Papavassilis, C., Pickel, M., Gruender, K. and, Gueho, E. (1997). Differentiation of *Malassezia* species: selectivity of cremophor EL, castor oil and ricinoleic acid for *M. furfur*. *Br J Dermatol.*, 37: 208-213. doi: 10.1046/j.1365-2133.1997.18071890.x
23. McGraw, L.J., Jager, A.K. and, van Staden, J. (2002). Antibacterial effects of fatty acids and related compounds from plants. *South African Journal of Botany.*, 68: 417-423. doi: 10.1016/S0254-6299(15)30367-7
24. Mukherjee, K., Tribedi, P, Mukhopadhyay, B. and, Sil, A.K. (2013). Antibacterial activity of long-chain fatty alcohols against mycobacteria. *FEMS Microbiol. Lett.*, 338: 177-183. doi: 10.1111/1574-6968.12043
25. Murugan, T., Wins, J. A. and, Murugan, M. (2013). Antimicrobial activity and phytochemical constituents of leaf extracts of *Cassia auriculata*. *Ind J Pharm Sci*, 75: 122–125. doi: 10.4103/0250-474X.113546
26. Papavassilis, C., Mach, K.K. and, Mayser, P.A. (1999). Medium-chain triglycerides inhibit growth of *Malassezia*: implications for prevention of systemic infection. *Crit. Care Med.*, 27: 1781–1786. doi: 10.1097/00003246-199909000-00013
27. Pereira, F.O., Mendes, J.M., Lima, I.O., Mota, K.S., Oliveira, W.A., Oliveira, W.A. and, Lim, E.O. (2014). Antifungal activity of geraniol and citronellol, two monoterpenes alcohols, against *Trichophyton rubrum* involves inhibition of ergosterol biosynthesis. *Pharm Biol.*, 53: 228-234. doi: 10.3109/13880209.2014.913299
28. Prabhu, V., Manjula, V., Santhiya, K., Tamileela Kanali, G.P., Saravanakumar, P. and Sibi, G. (2020). Chemometric profile of *Calotropis gigantea* and its antioxidant activity through bioactive compounds from latex, leaves and flower extracts. *Asian Journal of Chemistry.*, 32: 2865-2872. doi:10.14233/ajchem.2020.22875
29. Rahuman, A.A., Gopalakrishnan, G., Ghouse, B.S., Arumugam, S. and Himalayan, B. (2000). Effect of *Feronia limonia* on mosquito larvae. *Fitoterapia.*, 71: 553–555. doi: 10.1016/s0367-326x(00)00164-7
30. Rathore, S.S., Isravel, M., Vellaisamy, S., Chellappan, DR., Raman, T. and Ramakrishnan, J. (2017). Exploration of antifungal and immunomodulatory potentials of a furanone derivative to rescue disseminated Cryptococcosis in mice. *Sci Rep.*, 7: 15400. doi: 10.1038/s41598-017-15500-8
31. Romagnoli, C., Baldisserotto, A., Vicentini, C.B., Mares, D., Andreotti, E., Vertuani, S. and Manfredini, S. (2016). Antidermatophytic action of resorcinol derivatives: ultrastructural evidence of the activity of phenylethyl resorcinol against *Microsporum gypsum*. *Molecules.*, 21: 1306. doi: 10.3390/molecules21101306
32. Sampaio, A.L.S.B., Mameri, A.C.A.C.A, Vargas, T.J.D.S., Ramos-eSilva M., Nunes, A.P and, Carneiro, S.C.S.D.S. (2011). Seborrheic dermatitis. *An. Bras. Dermatol.*, 86: 1061–1074. doi: 10.1590/s0365-05962011000600002
33. Sharafutdinov, I.S., Pavlova, A.S., Akhatova, F. S., Khabibrakhmanova, A.M., Rozhina, E.V., Romanova, Y.J., Fakhruullin, R., Lodochnikova, O.A., Kurbangalieva, A. R., Bogachev, M.I. and, Kayumov, A.R. (2019). Unraveling the molecular mechanism of selective antimicrobial activity of 2(5h)-furanone derivative against *Staphylococcus aureus*. *Int J Mol Sci.*, 20: 694. doi: 10.3390/ijms20030694
34. Sharma, S., and Sibi, G. (2017). Epidemiology and characterization of lipophilic yeast (*Malassezia*) from human dandruff samples using cultural and molecular methods. *International Research Journal of Biochemistry and Biotechnology.* 4: 68-74.
35. Shin S. and Lim, S. (2004). Antifungal effects of herbal essential oils alone and in combination with ketoconazole against *Trichophyton* spp. *J Appl Microbiol.*, 97: 1289-1296. doi: 10.1111/j.1365-2672.2004.02417.x
36. Sibi, G., Alam, M.A., Shah, J. and Razak, M. (2014). Susceptibility pattern of *Malassezia* species to selected plant extracts and antifungal agents. *International Journal of Green Pharmacy.*, 8: 226-230. doi: 10.4103/0973-8258.142675
37. Singhal, M., Kansara, N. (2012). *Cassia tora* Linn cream inhibits ultraviolet-B-induced psoriasis in rats. *ISRN dermatology.*, 346510. doi: 10.5402/2012/346510
38. Sugita, T., Boekhout, T., Velegraki, A., Guillot, J., Hadina, S. and Cabanes, F.J. (2010). Epidemiology of *Malassezia*-related skin diseases. T. Boekhout, E. Gueho, P. Mayser, A. Velegraki (Eds.), *Malassezia* and the skin, Springer Verlag. pp. 65-119. doi: 10.1007/978-3-642-03616-3_3
39. Takemoto, A., Cho, O., Morohoshi, Y., Sugita, T. and, Muto, M. (2015). Molecular characterization of the skin fungal microbiome in patients with psoriasis. *J Dermatol.*, 42: 166-170. doi: 10.1111/1346-8138.12739
40. Timothy, S.Y., Wazis, C.H., Adati, R.G. and Maspalma, I.D. (2012). Antifungal activity of aqueous and ethanolic leaf extracts of *Cassia alata* Linn. *Journal of Applied Pharmaceutical Science.*, 2: 182-185. doi: 10.7324/JAPS.2012.2728
41. Togashi, N., Shiraiishi, A., Nishizaka, M., Matsuoka, K., Endo, K., Hamashima, H and Inoue, Y. (2007). Antibacterial activity of long-chain fatty alcohols against *Staphylococcus aureus*. *Molecules.*, 12: 139-148. doi: 10.3390/12020139