



A COMPARATIVE STUDY ON ANTIDANDRUFF ACTIVITY OF PLANT EXTRACTS OF *CASSIA AURICULATA* AND *LEUCAS ASPERA*

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Abstract: The plant samples of *Cassia auriculata* and *Leucas aspera* were collected from the nearby areas of Kancheepuram and Thiruvallur Districts, Tamil Nadu, India. Aqueous and ethanol extracts of leaves and flowers of both plants were prepared. The presence of flavonoids, steroid, saponins, alkaloids and tannins are associated with the antifungal activity of these extracts. The dandruff collected from people with visible flakes was inoculated on sterile Sabouraud dextrose agar (SDA) incubated at 32 °C to 37 °C for 3 to 5 days. Identified fungal species of *Malassezia furfur* was isolated by pure culture in Sabouraud dextrose agar medium with added Chloramphenicol as antibiotic. The antifungal drug Ketoconazole was used as a control. The aqueous and ethanol leaf and flower extract of *L. aspera* showed higher antifungal activities than the same extract of *C. auriculata* were tested by Disc Diffusion Method.

Index Terms: *Leucas aspera*, *Cassia auriculata*, Dandruff, SDA, *Malassezia furfur*

1. INTRODUCTION

In developing countries it has been seen that the increase in research using medicinal plants in the treatment of microbial infections (Ahmed and Beg, 2001; Portillo *et al.*, 2001; Rios and Recio, 2005; Webster *et al.*, 2008; Hofling *et al.*, 2010 and Svetaz *et al.*, 2010). The report of Duraipandiyan *et al.*, 2011 and Spampinato and Leonardi, 2013 stated that the plant extracts which has antidandruff properties can be used efficiently as a substitute to chemical drugs due to insufficient number of drugs, high toxicity and prolonged treatment.

1.1. *Cassia Auriculata* L.

Cassia auriculata L. belonging to family fabaceae and is cultivated in many parts of India. Its local names are matura tea tree, ranawara or avaram. It is an evergreen shrub with closely placed, alternate, stipulate, paripinnate compound leaves. The flowers are bright yellow, bisexual and irregular. It has been

reported that all parts of the plants such as the flower, leaves, stem, root, and unripe fruit are used as Ayurvedic medicine due to the presence of antipyretic, hepatoprotective (Vedavathy and Rao, (1993), antidiabetic, antiperoxidative and antihyperglycemic and microbial activity (Kumar *et al.*, (2002).

1.2. *Leucas Aspera* (Wild) Link

Leucas aspera belonging to the family Lamiaceae and is commonly called as Thumbai. It is distributed throughout India and is found in dry, open, sandy soil. *L. aspera* is an annual herb and the leaves are opposite with short petioles. Flowers are white, small, and it is directly attached to the base without a peduncle or stalk and are arranged in auxiliary whorls or dense terminals (Nirmala, 2018). It is used to treat scorpion bites in traditional medicine of the Philippines and also an antipyretic, antioxidant, antimicrobial, and cytotoxic activities (Das *et al.*, 2011; Sarathambal *et al.*, 2001; Babu *et al.*, 2016 and Nair and Abdulla, 2017).

1.3. Dandruff

Dandruff is nothing but separation of small white flakes of dead skin cells of the scalp. Dandruff is a common problem faced by many people of all age groups. Approximately 5% of the population is affected by dandruff and it commonly occurs after puberty, between 20 - 30 years and the dandruff affects males more than females (Czop and Wcisło, 2013).

Lipophilic basidiomyceteous fungus *Malassezia* species is the causative organism for dandruff which grows healthy in sebum (Borelli, 2000; Ro and Dawson, 2005 and DeAngelis *et al.*, 2005). Eradication of dandruff causing fungus would be an effective treatment of dandruff. According to Dawson (2007) there might also be a number of genetic and environmental factors playing a role. The condition may get worse in the winter and it is not always due to poor hygiene (Ranganathan and Mukhopadhyay, 2010). The commercially available anti dandruff medicines are amphotericin B clotrimazole, imidazole derivatives, ketoconazole, salicylic acid, selenium sulphide, tar derivatives and zinc pyrithione etc.

1.4. *Malassezia* Species

Malassezia is a fungi, previously called as *Pityrosporum* and belongs to the family *Malasseziaceae* (Saunte *et al.*, 2020) and it causes hair and skin infections Seborrheic dermatitis and dandruff etc. (Ranganathan *et al.*, 2001). *Malassezia* species like *M. furfur*, *M. globosa* and *M. restricta* are causative organism of dandruff and is medically termed as *Pityriasiscapitis*. It is a disorder of scalp and also causes major cosmetic problem of hair fall and it needs effective remedy (Nematian *et al.*, 2006).

1.5. Objectives

The present study was focused on the antidandruff activity of the leaf and flower extracts obtained from the plants of *Cassia auriculata* and *Leucas aspera* against dandruff causing fungi, *Malassezia furfur*. This is the first report of using these two plants as antidandruff efficacy.

2. MATERIALS AND METHODS

2.1. Collection of Plants

The plant samples of *Leucas aspera* and *Cassia auriculata* were collected from the nearby areas of Kancheepuram and Thiruvallur Districts, Tamil Nadu, India (Plate 1 and 2). The species of the plants were identified by Dr. J. Amalorpavam, Assistant Professor, Department of Botany, Queen Mary's College,

Chennai - 600 004. The fresh leaves and flowers of both the plants were collected and washed with tap water and twice with distilled water to remove the dust particles and other adhering impurities. The leaves and flowers were allowed to dry at room temperature for about one week.

Plate 1 *Cassia auriculata*



Plate 2 *Leucas aspera*



2.1.1. Aqueous extract

The aqueous extract of leaves and flowers of plants, *Cassia auriculata* and *Leucas aspera* were prepared by soaking 100 gms of powdered materials of leaves and flowers of both plants in 500 ml of distilled water for 24 hours. Using the muslin cloth, the extract was filtered and this process was repeated for three times to prepare clear aqueous leaf and flower extract of 100%. These were stored in an air tight container as a stock extracts at 4° C in the refrigerator until the examination. Further, it was diluted to make different concentrations of the extract such as 25%, 50%, 75% (on the basis of volume) (Al-Manhel and Niamah, 2015).

2.1.2. Ethanol Extract

The ethanol extracts of leaves and flowers of both plants like *C. auriculata* and *L. aspera* were prepared in accordance with Zamin, 2014.

2.2. Phytochemical Screening of *L. aspera* and *C. auriculata*

The different concentrations of aqueous and ethanolic leaf and flower extracts of *L. aspera* and *C. auriculata* were analyzed for the presence of phytochemicals, such as alkaloids, reducing sugars, glycosides, proteins, amino acids, saponins, steroids, tannins, flavonoids and anthraquinones according to the method described by Harborne, 1973; Baker and Thormasberg, 1983; Trease and Evans, 1989; Sofowora, 1993; Sahm and Washington, 1990 and Brindha *et al.*, 1991.

2.2.1. Detection of Alkaloids (Wagner's Test)

Three ml of aqueous and ethonolic leaf and flower extract of each plants was diluted with 1 ml of hydrochloric acid. To this, added few drop of Wagner's reagent (solution of Iodine in Potassium Iodide). Formation of reddish brown precipitate indicates the presence of alkaloids.

2.2.2. Detection of Reducing Sugar (Fehling's Test)

To one ml of Fehling solution A, and 1 ml of Fehling solution B is added and mixed well and then heated in a water bath for 1 minute. To this, equal volume of aqueous and ethanol extract was added and

then heated for 5 to 10 minutes. Appearance of yellow to brick red colour indicates the presence of reducing sugar.

2.2.3. Detection of Glycosides, (Borntrager's Test)

One ml of dilute sulphuric acid was added in a test tube containing equal volume of aqueous and ethanolic leaf and flower extract boiled for 5 minutes and cool the filtrate and shake with equal volume of chloroform, then separate the lower layer of chloroform and added half volume of dilute ammonia, shake it well. Formation of rose pink to red colour in the ammonical (lower phase) layer indicates the presence of glycoside.

2.2.4. Detection of Protein (Biuret's Test)

To 3 ml of aqueous and ethanolic leaf and flower extract, 1 ml of 4% sodium hydroxide and 1 ml of 1% copper sulphates were added. Formation of violet or pink colour indicates the presence of proteins.

2.2.5. Detection of Amino Acid (Ninhydrin Test)

To 1 ml of aqueous and ethanolic leaf and flower extract, 2 drops of 5% Ninhydrin (in butanol) solution was added. The mixture was heated over a water bath for 10 minutes and allowed to cool. The presence of amino acids was indicated by the formation of purple or blue colour.

2.2.6. Detection of Saponins (Foam Test)

To a small quantity of aqueous and ethanolic leaf and flower extract, 2 ml of water was added and shaken well. Persistence of foam produced for 10 minutes indicates the presence of saponins.

2.2.7. Detection of Steroids (Salkowski Test)

To 1 ml aqueous and ethanolic leaf and flower extract, 10 ml of chloroform was dissolved and then added equal volume of concentrated sulphuric acid from the walls of the test tube and then shaken well. Formation of reddish blue colour in the upper layer and green fluorescence in the acid layer indicates the presence of steroids.

2.2.8. Detection of Tannins (Ferric chloride reagent test)

Few drops of 5% ferric chloride was added to small quantity of aqueous and ethanolic leaf and flower extract. The presence of tannin is indicated by the appearance of dark green colour or deep blue colour.

2.2.9. Detection of Flavonoids (Ferric chloride reagent test)

Two ml of extracts were treated with few drops of sodium hydroxide solution separately in a test tube. Formation of intense yellow colour, which becomes colourless on addition of few drops of dilute acid, indicates the presence of flavonoids.

2.2.10. Detection of Anthraquinone (Borntrager's reaction)

Take one ml of the aqueous and ethanolic leaf and flower extract in a test tube, 20 ml of chloroform was added. This was heated in steam bath for 5 minutes and the extract was filtered and allowed to cool. To the filtrate, an equal volume of 10% ammonia solution was added and shaken well. Appearance of bright pink colour indicates the presence of anthraquinones.

2.4. Sample Collection

Scrapings of dandruff were collected from people with visible flakes. Sampling was done by scraping the head using sterile scalpel and it was observed under compound microscopy with 10 % KOH and was stained with methylene blue (Plate 3).

2.5. Isolation of Fungi in Pure culture

The dandruff collected was inoculated on sterile Sabouraud dextrose agar plate bought from Indiamart, Chennai, Tamil Nadu. It was incubated at 32°C to 37 °C for 3-5 days. Characteristic white growths around the flakes were indicative of an organism causing dandruff (Plate 3).

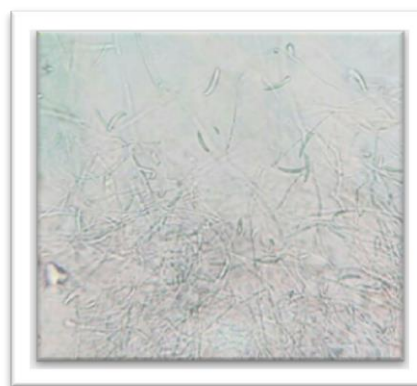
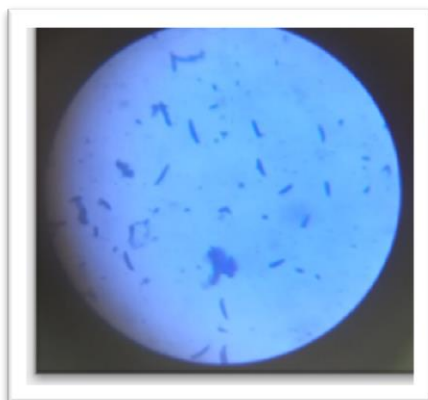
2.6. Growth and Identification

The organism was identified based on cultural, microscopic and biochemical methods. The colonies were identified as *Malassezia furfur* in accordance with Dr. P. Palani, Assistant Professor, Center for Advanced Study in Botany (CAS), University of Madras, Guindy Campus, Chennai – 600025 (Plate 3). Identified fungal species of *Malassezia furfur* was isolated by pure culture in Sabouraud dextrose agar medium with added chloramphenicol. The agar provides a selective media for the growth of medically significant fungi while the antibiotic chloramphenicol serves to inhibit the growth of unwanted bacterial floras (Sabouraud, 1892).

2.7. Inoculum Preparation

The pure culture so obtained was inoculated using buds onto Sabouraud Dextrose agar by spread plate method and was incubated at 37 °C for two days. Discs of varying concentration (100%, 75%, 50% and 25%) of both aqueous and ethonolic plant extracts were prepared from stock solutions of leaves and flowers of both plants *Cassia auriculata* and *Leucas aspera* in order to assess the minimum inhibitory concentration (MIH) of each extract. Disc was prepared with 2% Ketoconazole as control. The antifungal activity of different concentrations of aqueous and ethanolic plant extracts were tested by Disc Diffusion Method.

Plate 3 Culture and Growth Pattern of *Malassezia furfur* on Sabouraud's Dextrose Agar Media and Microscopic Observation of *Malassezia furfur*



The plates with discs were stored in the inoculation chamber for 48 hours. After that the plates were taken out and the zone of inhibition for each concentration of the extract was measured in millimeters. Experiment was done with three replicates per extract concentration.

2.8. Statistical Analysis

The triplicate results of antifungal activities were analyzed using mean, standard deviation and standard error

3. RESULTS AND DISCUSSION

The analysis of phytochemical components of aqueous and ethanolic leaf and flower extracts of *Cassia auriculata* and *Leucas aspera* and its antidandruff activities were shown in table 1,2 and 3.

3.1. Phytochemical Constituents of Aqueous and Ethanolic Leaf and Flower Extracts of *Cassia auriculata*

Table 1 showed that the qualitative analysis on phytochemical of aqueous leaf extracts of *C. auriculata* and revealed the presence of alkaloids, reducing sugars, glycosides, proteins, saponins, steroids, tannins, and flavonoids whereas, the amino acids and anthraquinones were not observed. While the ethanol extract of leaf reported the same except reducing sugars and steroids. The aqueous extract of flower of *C. auriculata* indicated the presence of all the phytochemicals, except reducing sugars, amino acids, and anthraquinones while the ethanolic extracts of flower indicated that except steroids all the phytochemical components were present.

Table 1 Phytochemical Screening of Aqueous and Ethanolic Leaf and Flower Extracts of *Cassia auriculata*

Sl. No.	Phytochemical Test	Aqueous Extract		Ethanolic Extract	
		Leaf	Flower	Leaf	Flower
1	Alkaloid	+	+	+	+
2	Reducing Sugar	+	-	-	-
3	Glycosides	+	+	+	+
4	Protein	+	+	-	+
5	Amino acid	-	-	+	-
6	Saponin	+	+	+	+
7	Steroid	+	+	-	-
8	Tannin	+	+	+	-
9	Flavonoids	+	+	+	+
10	Anthroquinone	-	-	-	-

Table 2 Phytochemical Screening of Aqueous and Ethonolic Leaf and Flower Extracts of *Leucas aspera*

Sl. No.	Phytochemical Test	Aqueous Extract		Ethanolic Extract	
		Leaf	Flower	Leaf	Flower
1	Alkaloid	+	+	+	+
2	Reducing Sugar	+	+	-	-
3	Glycosides	+	+	-	-
4	Protein	+	-	+	-
5	Amino acid	-	-	+	-
6	Saponin	+	+	+	+
7	Steroid	+	+	+	-
8	Tannin	+	+	+	-
9	Flavonoids	+	+	+	+
10	Anthroquinone	+	+	-	-

3.2. Phytochemical constituents of Aqueous and Ethonolic Leaf and Flower Extracts of *Leucas aspera*

The phytochemicals study of aqueous leaf extracts of *Leucas aspera* reported that absence of amino acid and presence of all other tested components. At the same time as the ethonolic extract of leaves showed the same as above except reducing sugars, glycosides and anthraquinones (Table 2). Whereas, the aqueous flowers extract of *L. aspera* indicated that except the proteins and amino acids the presence of all the phytochemicals, whereas, the ethanol extracts of flower denoted the presence of alkaloids, saponins and flavonoids only.

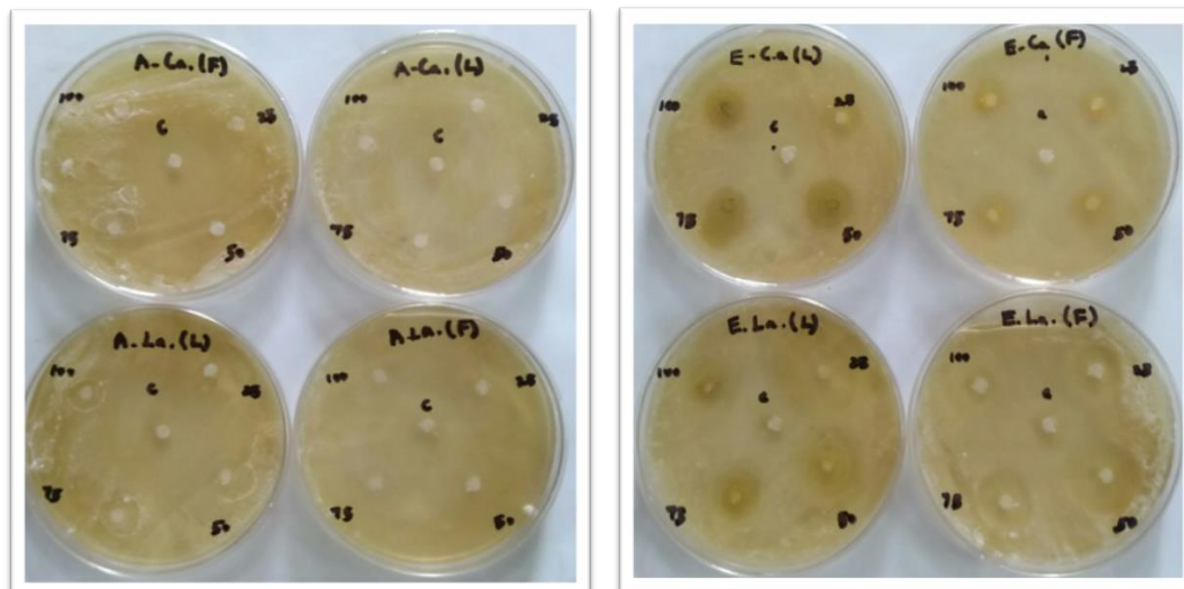
The presence of flavonoids, saponins and alkaloids supports the significant bioactivity exhibited by the crude and the different concentration of extracts against the *Malassezia furfur*. These results are positively agreed with the reporting of Bukola *et al.*, (2008) who stated that the antifungal activity of these plant extracts is probably associated with the presence of flavonoids, steroid, saponins and tannins. Similarly, sterols (Kavita *et al.*, 2014), flavonoids (Cushnie *et al.*, 2005), tannins (Scalbert, 1991) saponins, (Akinpelu *et al.*, 2014) glycosides and (Kouam *et al.*, 2011) have been stated to have significant inhibitory properties against different diseases causing pathogens.

3.3. Antidandruff Activity of Aqueous and Ethanol Extracts of Leaves and Flower of *Cassia auriculata* and *Leucas aspera* against *Malassezia furfur*

Generally Sabouraud's dextrose agar medium is used for the culturing of dermatophytes. The present work observed that the organism grown well at $32^{\circ}\text{C} \pm 5^{\circ}\text{C}$ and is more significant with the report of Vijaykumar *et al.*, (2006); Meena and Pavithra (2015) and Nirmalkar and Randhir (2018).

The antifungal activities of different concentrations of aqueous and ethanol extracts of leaves and flowers of *C auriculata* and *L aspera* against dandruff causing fungi, such as, *Malassezia furfur* was evaluated by the presence of or absence of zone and measured the individual zone diameters (mm). The values are mean of triplicates and were analyzed statistically by means of standard deviation and standard error.

Plate 4 Plates showing Zones of Inhibition for Aqueous and Ethanol Leaf and Flower Extracts of *C. auriculata* and *L. aspera* against *Malassezia furfur*



It was observed that the antifungal effect of aqueous extracts of leaves and flowers of both plants varied from each other and also in different concentrations within the same plant. From the results it was observed that the aqueous leaf extract of *L. aspera* have greater antifungal activities (0.67 mm to 1.1 mm) than the same extract of *C. auriculata* (0.23 mm to 0.53 mm) Whereas, the aqueous leaf extracts of *L. aspera* has the highest antifungal activity (1.1 mm) in 25% followed by 50% (0.83 mm), 100% (0.7 mm) and 75% (0.67 mm). But the activity of *C. auriculata* was higher (0.53 mm) in 100% concentration followed by 75%, 50% and 25%. Whereas, the inhibition zone of control, Ketoconazole was ranging from 1.87 mm to 2.13 mm (Table 3 and Plate 4).

Table 3 Mean Radius of Zones of Inhibition of Aqueous Leaf and Flower Extracts of *C. auriculata* and *L. aspera* against *Malassezia furfur*

Zone of inhibition in mm (Aqueous)					
Extract	100%	75%	50%	25%	ketoconazole 2% (W/V)
<i>C. auriculata</i> (Leaf extract)	(0.53 ± 0.09)	(0.47 ± 0.12)	(0.23 ± 0.03)	(0.33 ± 0.03)	(1.87 ± 0.09)
	(0.152)	(0.208)	(0.057)	(0.057)	(0.153)
<i>C. auriculata</i> (Flower extract)	(0.23 ± 0.03)	(0.4 ± 0.06)	(0.23 ± 0.03)	(0.5 ± 0.06)	(2.0 ± 0.06)
	(0.058)	(0.1)	(0.058)	(0.1)	(0.1)
<i>L. aspera</i> (Leaf extract)	(0.7 ± 0.06)	(0.67 ± 0.09)	(0.83 ± 0.09)	(1.1 ± 0.15)	(2.13 ± 0.19)
	(0.1)	(0.153)	(0.153)	(0.265)	(0.322)
<i>L. aspera</i> (Flower extract)	(0.6 ± 0.06)	(0.9 ± 0.58)	(0.5 ± 0.06)	(0.7 ± 0.15)	(2.0 ± 0.12)
	(0.1)	(0.1)	(0.1)	(0.265)	(0.2)

Values are Mean of Triplicates ± Standard Error; Values in Parentheses indicates Standard Deviation.

The highest measurement of zone of inhibition of aqueous flower extract of *L. aspera* was 0.9 mm in 75% concentration followed by 25% (0.7 mm), 100% (0.6 mm) and 50% (0.5 mm) while the inhibition zone of aqueous flower extract of *C. auriculata* was ranging from 0.23 mm – 0.5 mm).

3.4. Antifungal Activities of Various Concentration of Ethanol Extracts of Leaves and Flowers of the Plants *Cassia auriculata* and *Leucas aspera*

The results of inhibitory zone for different percentages of concentrations of leaf and flower extracts of both plants showed that the ethanol extract of leaves of *L. aspera* was the highest value (1.5 mm) in 25% concentrations followed by 50% (1.1 mm), 75% (0.97mm) and 100% (0.83 mm) whereas, the *C. auriculata* was 1.03 mm, 0.9 mm, 0.73 mm and 0.6 mm for 50%, 100%, 25% and 75% respectively. Likewise, the ethanol flower extract of *L. aspera* was having more anti dandruff activity than that of the *C. auriculata* (Table 4 and Plate 4).

Table 4 Mean Radius of Zones of Inhibition of Ethanolic Leaf and Flower Extracts of *C.auriculata* and *L. aspera* against *Malassezia furfur*

Zone of inhibition in mm (Ethanol)					
Extract	100%	75%	50%	25%	Ketoconazole 2% (W/V)
<i>C. auriculata</i> (Leaf extract)	(0.9 ± 0.06)	(0.6 ± 0.06)	(1.03 ± 0.12)	(0.73 ± 0.03)	(1.8 ± 0.15)
	(0. 1)	(0. 1)	(0. 208)	(0. 057)	(0. 265)
<i>C. auriculata</i> (Flower extract)	(1. 03 ± 0.09)	(0.83 ± 0.09)	(0.83 ± 0.09)	(0.73 ± 0.03)	(1.9 ± 0.06)
	(0. 153)	(0. 153)	(0. 153)	(0. 058)	(0. 1)
<i>L. aspera</i> (Leaf extract)	(0.83 ± 0.08)	(0.97 ± 0.03)	(1.1 ± 0.57)	(1.5 ± 0.57)	(1.8 ± 0.15)
	(0. 153)	(0. 057)	(0. 1)	(0. 1)	(0. 265)
<i>L. aspera</i> (Flower extract)	(0.6 ± 0.57)	(1.03 ± 0.08)	(1.23 ± 0.08)	(0.9 ± 0.06)	(1.73 ± 0.15)
	(0. 1)	(0. 153)	(0. 153)	(0. 1)	(0. 252)

Values are Mean of Triplicates ± Standard Error; Values in Parentheses indicates Standard Deviation

Finally it was found that compared to aqueous extract, the ethanol extract has more significant antifungal activity. This is because of solvent dependent activity or the presence of selective kind of phytochemical products present in the plant extracts.

Arunachalam *et al.*, (2019) reported that the solvents might have great potentials as disinfectants. Alteration of the extraction process and the use of supplementary solvents in extraction may produce extracts with more effective antimicrobial activity. These findings significantly agreed with the results of present investigations that compared to aqueous extract the ethanol extract has more significant antidandruff activity against *Malassezia furfur* this is because of solvent dependent activity.

The present findings are inspiring in the sense that the plant extracts were able to inhibit some experimental isolates which had been revealed to be resistant to generally recommended antibiotics. The aqueous and ethanolic leaf and flower extracts of both *C. auriculata* and *L. aspera* inhibited *Malassezia furfur*, the organisms involved in dandruff and dermatophytic related infections.

It is concluded that the result of the present study indicated the presence of different components of Phytochemicals in the leaves and flowers of *Cassia auriculata* and *Lucas aspera* which are actively involved in controlling the dandruff causing fungi.

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