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Antidermatophytic, the anticancer and antioxidant activity of *Cassia alata* ethanolic extract and its phytochemical analysis

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ABSTRACT

Cassia alata is used traditionally to cure various diseases like ulcers, stomach pain and fever. The potential of antidermatophytic, free radical scavenging and cytotoxic activity of ethanol leaves extracts of *C. alata* was studied. The preliminary phytochemical screening assay elucidates that presence of carbohydrates, tannin, phenol, saponin, flavonoid, steroid, terpenoids, glycosides, and alkaloids. Further phytochemical extraction was done by GC-MS, HPLC and FTIR. GC-MS analysis confirms that the presence of two major compounds namely Hexadecanoic acid and Oleic acid with four minor compounds. HPLC techniques revealed that the presence of two major compounds observed in two different retention time. FTIR spectrum revealed that the presence of carboxylic groups, alkanes, aldehydes and aliphatic amines in the ethanol extract of *C. alata* leaves. Cytotoxic effect of ethanol extract was assayed at different concentrations like 10, 25, 50 and 100 µg/ml against human epithelial carcinoma (A431). At100 µg/ml of ethanol concentration, 93.26±5.44 (%) and 82.45±2.44 (%) percentage of cancer cells were inhibited by ethanol extract and standard drug, respectively. Microscopic images of cancer cells treated with ethanol extract confirm the morphological changes and shrinkages of cells leads to cell death. These results were suggesting that Cassia alata exhibit high potential antioxidant, antidermatophytic and cytotoxic activity and also it could serve as alternative new resources for manufacturing new drugs against newly evaluating diseases.

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INTRODUCTION

Dermatophytes are mostly fungi that are causing infection in hair nail and skin by producing keratinases enzymes. These proteolytic enzymes are able to hydrolyse keratins which are main protein constituents of hair, nails and skin (Akcaglar *et al.,* 2011). Dermatophytes were colonizing on the host, and an ability to cause an infection leads to enzymatic degradation of keratin (Erbagci *et al.,* 2004; Simpanya, 2000; Abdel-Rahman, 2001; Macêdo *et al.,* 2005; Baeza *et al.,* 2007). Dermatophytes are cosmopolitan, and it also causes infection in several animal species by creating generally dry, smoothed and usually non-pruritic lesions, distributed on the skin (Pereira and Meireles, 2001). This infection is the most common and most probably caused skin disease in developing countries of tropical regions (Walsh and Groll 1999; Jain and Sharma 2003). These dermatophytes are otherwise known as superficial mycosis causes infections on millions of people and animals. Dermatophytes are a group of highly specialised filamentous fungi, the dermatophytes, which only infect keratinized structures (Burmester *et al.,* 2011). Fungal infections of the skin and nails are frequent

(Havlickova *et al.,* 2008). The large dermatophytes groups are mostly filamentous keratinophilic fungi genera are *Trichophyton, Microsporum* and *Epidermophyton* (Seebacher *et al.,* 2008). They are the members of the class Euascomycetes.

Natural products like plants have served as a research resource for most drugs inventions, providing a basis for chemical research and discovery of new drugs (Chin *et al.,* 2006). The search for plants with healing powers for various diseases dates back many years. Plants are having been used in a medicinal system for a long time. Plants are an important source of biologically active compounds, many of which are models for the synthesis of a large number of drugs (Yunes and Calixto, 2001; Simões *et al.,* 2003). Many antifungal agents are derived from plants resources like polyenes and griseofulvin, and they are having a broad spectrum of antifungal activity and not cause toxicity to the host (Carrillo-Muñoz *et al.,* 2006; Butler, 2005). Plants are highly utilized and great attention in the medical system due to the presence of a large number of secondary metabolic compounds found in plants.

The plant *Cassia alata* belongs to the family Fabaceae. It is the herbaceous plants grow up to 5 m, and it is grown in mostly a tropical climate. It is otherwise described as ringworm shrub, and it is more effective for ringworm diseases. This plant has highly active phytochemicals like alkaloids, anthraquinones responsible for antifungal, antimicrobial activity, abortifacient and analgesic. The single plant is used for curing skin diseases, stomach problems, and ringworm, asthma and aphthous ulcers. It has active components include the yellow chrysophanic acid. In this communication, we demonstrated that phytochemical screening of ethanol extract of *C. alata* leaves and to determine the ability of its antidermatophytic activity, free radical scavenging activity and cytotoxicity activity.

MATERIALS AND METHODS

Preparation of plant extract

Ethanolic extract of *C. alata* leaves was prepared by soxhlet extract method. In this method, the fresh leaves of *C. alata* were collected and washed with water and shade dried at room temperature for 3 – 4 days. Then the dried leaves were grounded into powder and through a fine mesh to get uniform sized powder. A 50 g of powdered leaf sample was packed with filter paper and inserted into soxhlet apparatus. A 200 ml of ethanol was taken in a bottom flask contains 200 ml of ethanol and the sample containing apparatus was boiled at 45°C for 24 hrs. Various phytoconstituents were extracted with ethanol and collected the solution. After boiling the filtrate was collected and evaporated with a rotatory evaporator to get the crude extract. This crude extract was further used to study the phytochemical characterization, DPPH scavenging activity, antidermatophytic activity and cytotoxicity assay.

Preliminary Phytochemical analysis

In this analysis, the extract was diluted at the concentration of 10mg/10 ml using double distilled water. The standard procedures were followed to analysis alkaloids, glycosides, carbohydrates, quinines, saponins, phenols, tannins, flavonoids, steroids, terpenoids, proteins and sugars compounds present in the ethanol extract of *C. alata* leaves described by Debiyi and Sofowora (1978), Trease and Evans (1989), Sofowora (1993) and Roopashree *et al.,* (2008).

Analytical techniques

Presence of phytoconstituents in ethanol crude extract with its molecular weight is determined by using Gas chromatography attached with Mass spectrometer. Molecular structure was performed using a Perkin-Elmer GC Clarus 500 system. HPLC system is used to a characterization of secondary metabolites present in the plant extract. The crude ethanol extract was injected into the HPLC column using the mobile phase containing 30:70 ml of acetonitrile and 0.1% of phosphoric acid. The bioactive functional groups present in the crude ethanol extract were characterized by FTIR spectrum at the wave number from 4000 to 500 cm^{-1}.

DPPH Free radical scavenging assay

The DPPH free radical scavenging activity of crude ethanol extract was analyzed at different concentrations. DPPH scavenging assay is a discoloration assay which is evaluated by addition of plant extract to a DPPH solution and measured in the absorbance at 517 nm (Halliwell 1994; Subhasree *et al.,* 2011) with slight modifications. The free radical DPPH was prepared in methanol at the concentration of 1 mM. A 500 µl of methanolic DPPH was taken in six test tubes. To this solution, 3 ml of different concentrations (5-50 µg/ml) of ethanol plant extracted in each test tube and incubated at 30 min at room temperature in dark conditions. After incubation, the ability to scavenging the stable DPPH free radical was recorded by measuring absorbance at 517 nm. The percentage of scavenging activity against the different concentrations of plant extract was plotted graph to determine the 50% inhibition concentration (IC_{50}) value. The percentage of DPPH inhibition by hexane solvent extracts of *C. alata* leaves was calculated by following the equation:

$$\% \text{ of Inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of test sample})}{\text{Absorbance of control}} \times 100$$

The inhibition concentration to scavenge 50% free radical (IC_{50}) is determined by plotting a graph of concentration (µg/ml) against percentage (%) of free radical inhibition.

An anti-dermatophytic activity of ethanol extracts of *C. alata* leaves

Collection of dermatophytes

Cultures of dermatophytes such as *Trichophyton mentagrophytes* (MTCC-7687), *Trichophyton rubrum* (MTCC-7859), *Epidermophyton floccosum* (MTCC-7880), *Microsporum audouinii* (MTCC-8197), and *Microsporum Canis* (MTCC– 3270) were collected from MTCC, Chandigarh.

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibition concentration of ethanol extract against dermatophytes was determined by performing agar well diffusion. The media Saboroud Dextrose Agar (SDA) was used to the assay of minimum inhibition concentration. The medium was prepared and sterilized in an autoclave and poured into Petri plates. Petri plates were allowed to solidify, and 0.1 ml of fungal inoculums spores was uniformly spread on media. 3 wells were punched with the diameter 6 mm using gel puncture and add different concentrations (25-75 µl/ml) of ethanol extract into each well. Minimum concentration required to inhibit the growth of fungi is known as minimum inhibition concentration (MIC). The all the plates were incubated at for 24 to 48 hrs and measure the zone of growth inhibition around the well. The difference between the activity of control and test sample ethanol extract is regarded as a percentage on inhibition. Terbinafine was used as a positive control against fungal dermatophytes, and its activity was considered as 100% of inhibition;

Percentage of Inhibition (%) = C-T/C × 100

C – Zone of inhibition activity of control

T – Zone of inhibition activity of ethanol extract

Determination of Minimum Fungicidal concentration

Invitro assay of the fungicidal effect of ethanol extract was determined at different concentrations (Espinel-Ingroff *et al.,* 2001 and 2002). 1 ml of fungal inoculums was inoculated into sterilized fresh Saboroud Dextrose broth. The ethanol extract was added into the broth at different concentrations from 10-100 µg/ml. The sample mixed broth was incubated at 37°C for 24 to 48 hrs. After the incubation, 0.1 ml of inoculums grown in different concentrations of ethanol extract containing broth were drawn and spread on Petri plate containing molten Sabouraud dextrose agar and incubated at 37°C for 72 h. The numbers of colonies were measured to calculate the minimum fungicidal concentration (MFC). MFC is defined as the lowest concentration that showed less than three colonies or visible growth on the plates is determined as 100% or 99% inhibition activity.

Cytotoxicity study

Cytotoxicity effect of ethanol extract was examined by MTT (3-(4,5 – dimethyl thiazol -2-yl)-5-diphenyltetrazolium bromide) assay (Francis and Rita 1986). The A431 cell lines were trypsinised and diluted using phosphate buffer. The 0.1 ml of diluted cell suspension was added into 96 well plates and incubated for 24 hrs in a CO_2 incubator. After the incubation, the cell culture was washed with MEM (minimum essential medium) medium containing 10% FBS (fetal bovine serum). Then add 100 µL of different concentrations of ethanol extract (10-100 µg/mL) were mixed to the cells in microtiter plates and the plates were then incubated at 37°C for 3 days in 5% CO_2 incubator. After 3 days, the sample drug was removed from wells and added 50 µL of MTT in PBS (phosphate buffered saline) into each well. The 96 well microtiter plate was incubated for 3 hours at 37° C in 5% CO_2 atmosphere. Finally, formazan crystals were formed which was dissolved by adding 100 µL of propanol and the plates were gently shaken. The synthetic drug 5-fluorouracil was used as positive control instead of plant extract. The absorbance intensity of solubilized formazan was measured using a microplate reader at the wavelength of 540 nm. Morphological analysis of test sample drug (ethanol extract) treated cells were examined under microscopic observations was noted every 24 hours of the time interval. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (IC_{50}) values is generated from the dose-response curves for each cell line.

$$\% \text{ of Inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of test sample})}{\text{Absorbance of control}} \times 100$$

RESULTS AND DISCUSSION

Phytochemical screening and antioxidant activity of *C. alata* leaves

Preliminary screening assay: The phytochemical analysis of ethanol leaf extract of *Cassia alata* was analysed for the compounds such as carbohydrates, tannin, phenol, saponin, flavonoid, steroid, terpenoids, glycosides, and alkaloids. All the active

phytoconstituents were present in the ethanol extract except quinines and proteins. Among the twelve phytochemicals, most of the phytochemicals (10/12) were present in the ethanol leaf extract (Table 1).

Table 1: Phytochemical screening of different solvents extract of C. alata leaves

S.No	Phytochemicals	Ethanol Extract
1	Alkaloids	+
2	Glycosides	+
3	Carbohydrates	+
4	Quinines	_
5	Saponins	+
6	Phenols	+
7	Tannins	+
8	Flavonoids	+
9	Steroids	+
10	Terpenoids	+
11	Proteins	_
12	Sugars	+

FTIR

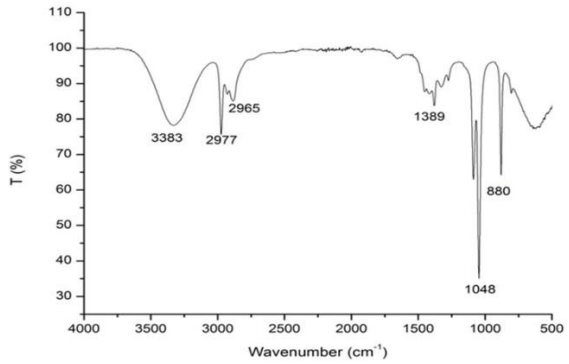

Figure 1: FTIR spectrum of ethanol extract of C. alata leaves

Table 2: FTIR spectrum shows various functional groups present in ethanol extract of C. alata leaves

S. No	Wavenumber (cm^{-1})	Functional groups
1	3383	N-H stretching amines and amides
2	2977	C-H stretch alkanes
3	2965	H-C=O stretching aldehydes
4	1389	N=O bend nitro groups
5	1048	C-N stretching aliphatic amines
6	880	N-H wag 1° and 2° amines

FTIR characterization technique was used to identify chemical bonds in compounds which are present in the ethanol extract of leaf of *C. alata*. Figure 1 shows the functional groups of ethanol extract of *C. alata* leave revealed a broad peak was observed at 3383cm^{-1} represents N-H stretching amines and amides. The absorption band at 2777 cm^{-1}corresponds to the presence of C-H stretch alkanes. The small peaks at 2965 and 1389 cm^{-1} revealed to H-C=O stretching aldehydes and N=O bend nitro groups respectively. Very sharp and narrow peaks were recorded at 1048 and 880 cm^{-1} characterized to C-N stretching aliphatic amines and N-H wag primary or secondary amines respectively (Table 3).

GC-MS analysis

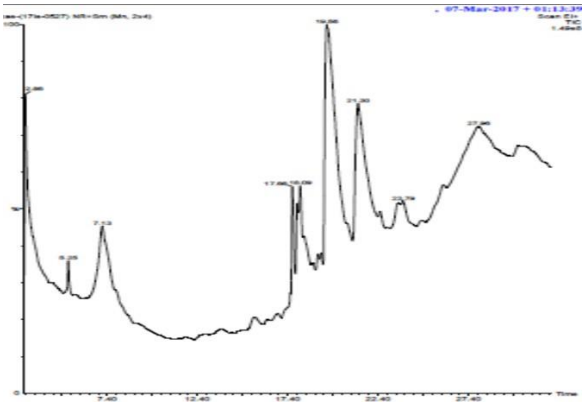

Figure 2: GC-MS chromatogram analysis of ethanol extract of C. alata leaves

The characterization technique of GC-MS spectrum of the ethanol extract of *C. alata* leaves showed 6 peaks indicate the presence of 6 different phytochemical components (Figure 2). The results of GC-MS chromatogram characteristics of ethanol extract revealed that presence of two major compounds namely Hexadecanoic acid and Oleic acid, the four minor compounds such as 2-Hydroxyethylhydrazine, Isopropyl-5-methylcyclohexyl 3-(1-(4-Chlorophenyl)-3-Oxobutyl)-C, Pentadecanoic acid, and cyclotrisiloxane, hexamethyl (Table 3).

HPLC analysis

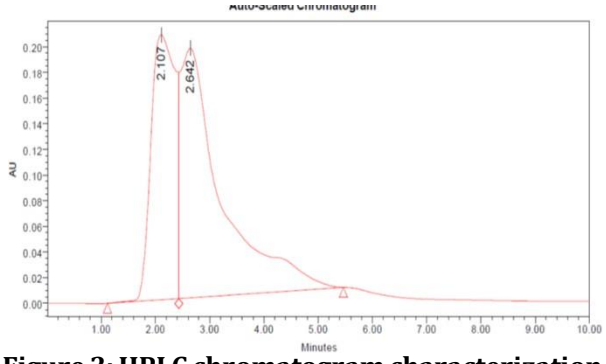

Figure 3: HPLC chromatogram characterization of ethanol extract of C. alata leaves

HPLC chromatograms showing two phytoconstituents of the ethanol extract of *C. alata* leaves shown in Figure 3. It appears to contain predominantly two compounds which are eluted at a retention time (Rt) of 2.107 min while the other peak was observed at an Rt of 2.642 min.

Table 3: GC-MS chromatogram analysis of ethanol extract of C. alata leaves shows phytoconstituents at retention time

Retention time	Name of the compound	Molecular weight	Molecular formula
7.13	2-Hydroxyethylhydrazine	76	$C_2H_8ON_2$
17.66	2-Hydroxyethylhydrazine	76	$C_2H_8ON_2$
18.09	Isopropyl-5-methylcyclohexyl 3-(1-(4-Chlorophenyl)-3-Oxobutyl)-C	524	$C_{30}H_{33}O_6Cl$
19.56	Hexadecanoic acid	256	$C_{16}H_{32}O_2$
21.30	Oleic acid	282	$C_{18}H_{34}O_2$
23.79	Pentadecanoic acid	242	$C_{15}H_{30}O_2$
27.96	cyclotrisiloxane, hexamethyl-	222	$C_6H_{18}O_3Si_3$

Table 4: Antioxidant activity of ethanol extract of C. alata leaves

Concentration of ethanol *C. alata* extract (µg/ml)	% Inhibition of DPPH free radical	
	C. alata ethanol extract	Standard
5	17.92±1.56	10.03±0.27
10	23.93±1.33	15.48±0.84
20	47.68±1.26	34.79±0.63
30	55.36±1.29	50.52±0.59
40	82.02±1.33	76.87±2.27
50	95.45±1.33	91.65±1.07

Antioxidant activity of chloroform extract of *C. alata*

The antioxidant activity of ethanol leaf extract of *C. alata* was determined by DPPH free radical scavenging activity. The activity of leaf extract was determined based on the measurement of the absorbance intensity in the Spectrophotometer at the shortest wavelength in 517 nm. The percentage of inhibition of DPPH free radical was increased with increasing the concentration of plant extract (Figure 4).

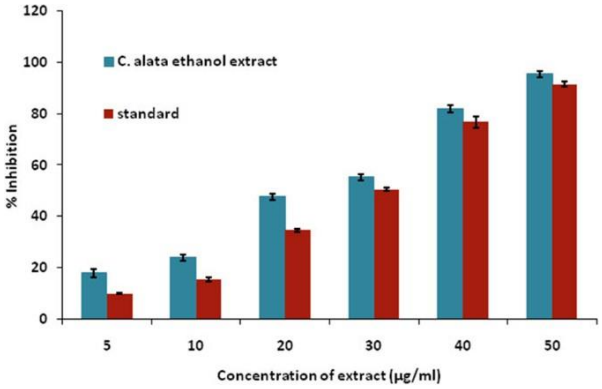

Figure 4: DPPH scavenging activity of Cassia alata ethanolic Extract

The IC50 value is defined as a sample that required to inhibiting the 50 % of DPPH concentration, was obtained from linear regression graph of mean percentage of the DPPH scavenging activity against the concentration of the ethanol leaf extract of *C. alata* (Figure 5). The results of linear regression analysis show the IC_{50} values of ethanol extract of *C. alata* leaves were found to be 24.56 µg/ml and the standard to be 29.92 µg/ml (Table 4). The total antioxidant activities of the plant extract may be due to the presence of flavonoids (Oyedemi et al 2010; Laloo and Sahu 2011; Kumar et al 2014), phenols (López-Vélez et al 2003; Lim et al 2007; Bourgou et al 2008; Jung et al 2008; Proestos *et al.,* 2013), alkaloids and tannins (Kumaran and Karunakaran 2007) in the leaves. These phytochemical constituents have the ability to reduce DPPH radicals by the pairing of an odd electron with hydrogen which gives the purple colour of DPPH turns into yellow. The reduced DPPH-H was obtained by the action of plant extract which serves as antioxidant agent.

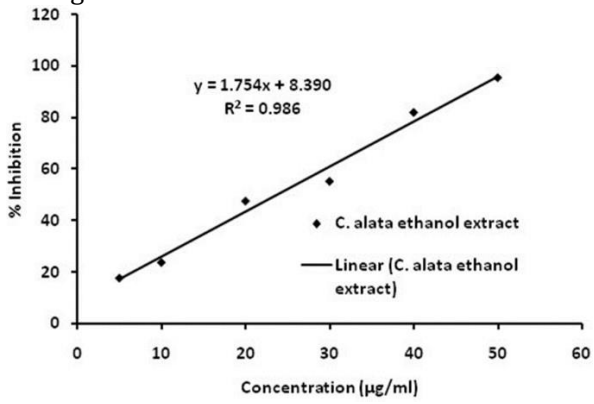

Figure 5: Linear regression analysis of DPPH free radical scavenging activity of ethanol extract of C. alata

The studied phytochemicals in leaves of C.alata ethanol extracts are pharmaceutically important. The phytochemical compounds are widespread in the plants are attractive as natural antioxidants (Sreeramulu and Raghunath 2010; Jaradat *et al.,* 2016). Many secondary metabolites like phenols, polyphenols, and flavonoids serve as important

Table 6: Percentage of the zone of inhibition, MIC and MFC

Dermatophytic fungi	*% Zone of Inhibition		MIC/MFC (µl/mL)	
	Control*	Ethanol extract	MFC	MIC
M. canis	19	12 (63.2%)	Nf<100	95
T.mentagrophytes	17	14 (82.4%)	75	63
T.rubrum	20	20 (100%)	25	25
E.floccosum	18	16 (88.9%)	Nf<100	95
M.audouinii	17	16 (94.2%)	50	65

*Control is considered as 100% inhibition. Percentage of inhibition was calculated by determining differences between control and extract; Nf<100 – No fungicidal activity below 100 µl/mL

sources of antioxidants to perform scavenging activity against free radical (Diplock 1997). Hence, this study presumed that the ethanol extract of *C.alata* might have a high amount of antioxidant properties which was comparable to that of the synthetic antioxidant standard used.

Screening of extracts for antidermatophytic activity

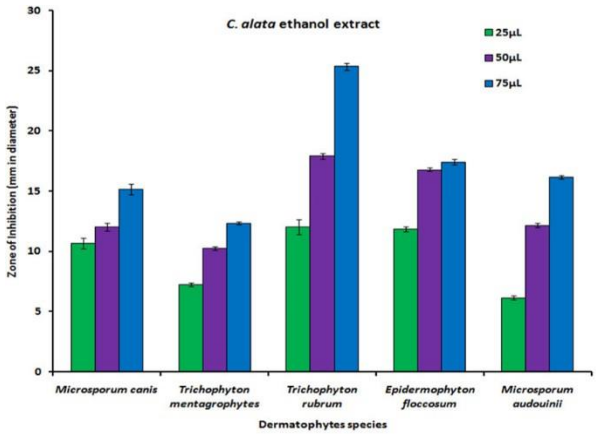

Figure 6: Anti-dermatophytic activity of ethanol extract of C. alata leaves at different concentrations

Table 5: Zone of inhibition of ethanol extract of C. alata leaves against dermatophytes

Dermatophytes	Zone of inhibition (mm in diameter)		
	25µL	50µL	75µL
Microsporum Canis	10.67± 0.44	12.03± 0.33	15.17± 0.44
Trichophyton mentagrophytes	07.27± 0.15	10.27± 0.13	12.37± 0.13
Trichophyton rubrum	12.03± 0.61	17.93± 0.23	25.37± 0.29
Epidermophyton floccosum	11.87± 0.19	16.80± 0.15	17.43± 0.23
Microsporum audouinii	06.17± 0.17	12.17± 0.17	16.17± 0.17

The results of antifungal sensitivity of chloroform extracts of *C. alata* leaves against the five different dermatophyte fungal strains are interpreted in table 5 and Figure 6. The ethanol extracts of *C. alata* leaves showed the most significant activity against *Trichophyton rubrum* with the zone of inhibition ranging from 12.03±0.61 to 25.37±0.29 mm. Minimum antifungal activity was examined against *Trichophyton mentagrophytes* with the inhibition zone 07.27±0.15 to 12.37±0.13 mm.

Percentage of the zone of inhibition: Terbinafine is used as positive control and its complete antifungal activity against pathogens is considered as 100% of inhibition. It was compared with extract of *C. alata* leaves. Chloroform extract shows 100 % of activity against *T.mentagrophytes* and minimum percentage of activity (44.4%) of hexane extract was noted against *E. floccosum*. Ethanol extract has the ability to complete control against all the pathogens above 60% of inhibition (Table 6).

Minimum Inhibitory Concentration and MFC determination

The profile of the medicinal plants used in this study is shown in Table 6. The results of antifungal activity of chloroform, ethanol and hexane crude extracts of C. alata leaves showed good activity on all the strains of *M. Canis*, *T.mentagrophytes*, *T.rubrum, E.floccosum* and *M.audouinii* tested at different concentrations, with ethanol extracts exerting slightly higher activity than water extracts as revealed by mean diameter of zone of inhibitions, minimum inhibitory concentration (MIC) and minimum fungicidal concentrations (MFC) (Table 6).

However, MIC and MFC values of 25.0 and 25.0 µl/ml were recorded for ethanol extract respectively against *T.rubrum* strain. MIC and MFC values of 50 and 46 µl/ml were recorded for chloroform extract respectively against *T.rubrum* strain. Likewise, hexane extract showed MIC, and MFC values of 25.0 and 20.0 µl/ml were recorded against *T.mentagrophytes*. from these results, ethanol extract shows more fungicidal activity as minimum concentration compared to other extracts of *C. alata* leaves.

Cytotoxicity assay

The result of discoloration MTT assay against A431 cancer cell lines revealed that decreased percent of cell viability was noted while treating with the drug ethanol extract of *C. alata* leaves. It induces

more cytotoxicity towards cancer cell lines human epithelial carcinoma (A431) and its effect also similar to that of the standard drug (5-fluorouracil). Microscopic images confirm that the ethanol extract induces cell death by causing morphological changes and shrinkage of cancerous cells (Figure 7). Cytotoxic effect of ethanol extract was assayed at different concentrations like 10, 25, 50 and 100 µg/ml. At100 µg/ml of ethanol concentration, 93.26±5.44 (%) and 82.45±2.44 (%) percentage of cancer cells were inhibited by ethanol extract and standard drug, respectively. This assay helps to confirm ethanol extract of *C. alata* leaves could be better, no toxic to noncancerous cells (Morshed *et al.,* 2011) and alternative source of anticancer drugs than the standard drug. The plant extract has shown remarkable dose-dependent activity against the selected cancer cell line (Figure 7 and Table 7). Among these four concentrations, 100 µg/mL of ethanol extract was the most effective in producing percentage growth inhibition.

Table 7: Determination of percentage (%) inhibition of human epithelial carcinoma by using ethanol plant extract

Concentration (µg/mL)	% of inhibition	
	Control	Plant extract
10 (µg/mL)	45.09±1.17	50.02±2.33
25 (µg/mL)	60.33±2.33	65.23±3.17
50 (µg/mL)	72.52±1.47	76.23±2.33
100 (µg/mL)	82.45±2.44	93.26±5.44

± Standard deviation

CONCLUSION

In the present investigation, the ethanol extract of leaves of *Cassia alata* exhibited effective DPPH scavenging ability, antidermatophytic activity against skin infecting dermatophytes and cytotoxic activity against human epithelial carcinoma cells. In addition, the ethanol extract was containing alkaloids, carbohydrates, flavonoids etc. examined preliminary phytochemical analysis based on chemical reaction. The presence of Hexadecanoic acid and Oleic acid was identified by GC-MS and HPLC. FTIR spectrum of ethanol extract revealed the presence of functional groups of bioactive compounds. Overall, the ethanol extract exhibited the highest antioxidant, antidermatophytic and cytotoxic activity while the standard drug showed better medicinal activities. Therefore, ethanol crude extract from leaves of *Cassia alata* could serve as promising sources of new drugs.

REFERENCES

Abdel-Rahman SM (2001) Polymorphic exocellular protease expression in clinical isolates of *Trichophyton tonsurans*. Mycopathologia. 150:117-120.

Akcaglar S, Ener B, Toker SC, Ediz B, Tunali S, Tore O (2011) A comparative study of dermatophyte infections in Bursa, Turkey. Med Mycol 49:602-607.

Baeza LC, Bailão AM, Borges CL, Pereira M, Soares CM, Mendes-Giannini MJ (2007) cDNA representational difference analysis used in the identification of genes expressed by *Trichophyton rubrum* during contact with keratin. Microbes Infect 9:1415-1421.

Bourgou S, Ksouri R, Bellila A, Skandrani I, Falleh H, Marzouk B (2008) Phenolic composition and biological activities of Tunisian Nigella sativa L. shoots and roots. C R Biol 331: 48-55.

Burmester A, Shelest E, Glöckner G, Heddergott C, Schindler S, Staib P *et al.,* (2011) Comparative and functional genomics provide insights into the pathogenicity of dermatophyte fungi. Genome Biol 12: R7.

Butler MS (2005) Natural products to drugs: natural product derived compounds in clinical trials. Nat Prod Rep 22: 162- 195.

Carrillo-Muñoz AJ, Giusiano G, Ezkurra PA, Quindós G (2006) Antifungal agents: mode of action in yeast cells. Rev Esp Quimioter 19:130-139.

Chin YW, Balunas MJ, Chai HB, Kinghorn AD (2006) Drug discovery from natural sources. AAPS Journal 8: E239-253.

Debiyi OO and Sofowora FA (1978) Phytochemical screening of medical plants, Iloyidia, 3: 234–246.

Diplock AT (1997) Will the good fairies, please prove to us that vitamin E lessens human degenerative disease? Free Rad Res, 27: 511-532

Erbagci Z (2004) Topical therapy for dermatophytoses: should corticosteroids be included? AmJ Clin Dermatol 5:375-384.

Espinel-Ingroff A (2001) In vitro fungicidal activities of voriconazole, itraconazole, and amphotericin B against opportunistic moniliaceous and dematiaceous fungi. J. Clin. Microbiol. 39:954-958

Espinel-Ingroff1 A, Fothergill A, Peter J, Rinaldi MG and Walsh TJ (2002) Testing Conditions for Determination of Minimum Fungicidal Concentrations of New and Established Antifungal Agents for *Aspergillus spp*.: NCCLS Collaborative Study, J. Clin. Microbiol, 40(9): 3204-3208

Francis D, Rita L (1986) Rapid colorimetric assay for cell growth and survival: modifications to the tetrazolium dye procedure giving improved sensitivity and reliability, J. Immunol. Methods, 89: 271-277

Halliwell B (1994) Free radicals, antioxidants, and human disease: curiosity, cause, or consequence? Lancet. 344: 721- 724.

Havlickova B, Czaika VA, Friedrich M (2008) Epidemiological trends in skin mycoses worldwide. Mycoses. 51 (Suppl. 4): 2-15.

Jain N and Sharma M (2003) Broad spectrum antimycotic drug for the treatment of ringworm infection in human beings. Current Sci (India); 85 suppl 1:30-34

Jaradat NA, Shawahna R, Hussein F, Al-Lahham S (2016) Analysis of the antioxidant potential in aerial parts of Trigonella arabica and Trigonella berythea grown widely in Palestine: a comparative study. Eur J Integr Med 8: 623–30.

Jung MJ, Heo SI, Wang MH (2008) Free radical scavenging and total phenolic contents from methanolic extracts of *Ulmus davidiana*. Food Chem. 108: 482–7.

Kumar S, Sandhir R and Ojha S (2014) Evaluation of antioxidant activity and total phenol in different varieties of *Lantana camara* leaves. BMC Research Notes 7:560.

Kumaran A, Karunakaran RJ (2007) In vitro antioxidant activities of methanol extracts of five Phyllanthus species from India. Leben Wissen Technol 40: 344-52.

Laloo D, Sahu AN (2011) Antioxidant activities of three Indian commercially available Nagakesar: An *in vitro* study. J Chem Pharm Res. 3: 277–83.

Lim YY, Lim TT, Tee JJ (2007) Antioxidant properties of several tropical fruits: A comparative study. Food Chem. 103:1003–8.

López-Vélez M, Martínez-Martínez F, Del Valle-Ribes C (2003) The study of phenolic compounds as natural antioxidants in wine. Crit Rev Food Sci Nutr. 43: 233–44.

Macêdo DP, Neves RP, Neves RP, Magalhães MC, Souza-Motta CM, Queiroz LA (2005) Pathogenic aspects of *Epidermophyton floccosum* Langeron et Milochevich as a possible a etiological agent of Tinea capitis. Braz J Microbiol 36:36-37.

Morshed MA, Uddin A, Rahman A, Hasan T, Roy S, Amin AA, *et al.,* (2011) In vitro antimicrobial and cytotoxicity screening of Terminalia arjuna ethanol extract. Int J Biosci 1:31–8

Oyedemi SO, Bradley G, Afolayan AJ (2010) *In-vitro* and *in-vivo* antioxidant activities of aqueous extract of Strychnos henningsii Gilg. Afr J Pharm Pharmacol. 4: 70–8.

Pereira DB, Meireles MCA (2001) Diseases caused by fungi and Oomycetes. Diseases of ruminants and horses 4:367-383.

Proestos C, Lytoudi K, Mavromelanidou OK, Zoumpoulakis P, Sinanoglou VJ (2013) Antioxidant Capacity of Selected Plant Extracts and Their Essential Oils. Antioxidants 2: 11-22.

Roopashree TS, Dang R, Rani RHS, and Narendra C, (2008) Antibacterial activity of antipsoriatic herbs: Cassia tora, *Momordica charantia* and *Calendula officinalis*, International Journal Applied Research in Natural Products, 1(3): 20–28

Seebacher C, Bouchara J, Mignon B (2008) Updates on the epidemiology of dermatophyte infections. Mycopathologia. 166: 335–352.

Simões CMO, Schenkel EP, Gosmann MG, Mello JCP, Ments LA, Petrovick PR (2003) Pharmacognosy: The medicinal plant - products of plant origin and development of drugs. 5a ed. p. 291-320.

Simpanya MF (2000) Dermatophytes: Their taxonomy, ecology and pathogenicity. Rev Iberoam Micol 17:1-12.

Sofowora A (1993) Phytochemical Screening of Medicinal Plants and Traditional Medicine in Africa, Spectrum Books Ltd, Ibadan, Nigeria.

Sreeramulu D, Raghunath M (2010) Antioxidant activity and phenolic content of roots, tubers and vegetables commonly consumed in India. Food Res Int 43:1017–20.

Subhasree B, Baskar R, Keerthana RL, Susan RL, Rajasekran P (2011) Evaluation of antioxidant potential in selected green leafy vegetables, Food Chem. 115: 1213-1220.

Trease GE and Evans WC (1989) "Phenols and phenolic glycosides," in Textbook of Pharmacognosy, 12: 343–383, Balliese, Tindall and Co Publishers, London, UK, 1989.

Walsh TJ and Groll AH (1999) Emerging fungal pathogens: evolving challenges to immunocompromised patients for the twenty-first century. Transplant Infectious Diseases, 1(4): 247-241.

Yunes RA, Calixto JB (2001) Plantas medicinais sob óptica da química medicinal moderna. 2001. Moderna – Chapecó: Argos.