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Research Article

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Studies on the antioxidant activity of ethanol extract of *Cassis alata* using FT-IR, HPLC and GC-MS analysis

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Abstract

The aim of the present investigation is to screening of phytochemical constituents for its free radical scavenging activity of ethanol extract of *Cassia alata* leaves. The preliminary screening was carried out by standard chemical method. It evidenced that the presence of phytochemicals such as alkaloids, carbohydrate, phenols, tannins, falvanoids, steroids, protein and sugar in the ethanol extract. Various functional groups like alcohols, phenols, carboxylic acid and nitro groups were associated with the extract were characterized using Fourier transform infrared spectroscopy (FTIR). A few common compounds available in the extract were identified by Gas chromatography – Mass spectrum (GC-MS) analysis. The DPPH free radical scavenging activity and 50 % inhibitory concentration (IC_{50}) was calculated and compared with the standard synthetic drug BHT. The IC_{50} value of leaf extract was found to be 24.56 µg/ml respectively. This study revealed that *C. alata* ethanol extract of leaves has alternative drugs for free radical it could be acted as a novel source of free radical scavengers.

Keywords: *Cassia alata*, ethanol extract, antioxidant, HPLC, GC-MS

Introduction

Medicinal plants are important to the global economy as approximately 85% of traditional medicine preparation involve the use of plants or plant extracts [1]. *Cassia alata L* is a beautiful flowering shrub that grows about 1 to 2 m in height. It produces pretty yellow flowers in a column that resemble yellow candlesticks-earning its common name candlestick or candle bush. It is belongs to the family Fabaceae. It is native to the Amazon Rain forest and can be found in Peru, Brazil, French Guiana, Guyana, Suriname, Venezuela and Colombia [2]. Found in many habitats, preferring disturbed, rather open vegetation such as roadsides, river banks, rain forest edges, lake shores, pond and ditch margins, open forest, orchards and around villages. The plant is highly valued in many areas of the tropics for its medicinal virtues. It is commonly gathered from the wild, mainly for medicinal use but also as a food and source of materials [3]. It is often cultivated for medicinal purposes, and also as an ornamental plant in tropical to warm temperate areas. *C.alata* used as a traditional medicine, particularly valued for its laxative effect and

its effective treatment of several skin conditions, including ringworm and scabies [4, 5]. Crude leaf extracts have shown antibacterial activity against a range of bacteria and also antitumor activity [6]. The bark is used to treat skin diseases, diarrhoea, worms, parasitic skin diseases, scabies and eczema [7].

Free radicals are unstable and as chemical molecules independently having one or more unpaired electrons and they play role in metabolic activity. While exceeding the amount of free radicals in the body causes cell damage and tissues. This imbalance between free radical and antioxidant systems leads to cause of cardiovascular, diseases, cancer, aging etc [8]. Some of the common free radicals are nitric oxide, hydrogen peroxide, hydroxyl radical, superoxide anion radical etc. These are otherwise known as reactive oxygen species [9].

An antioxidant is defined as a molecule that preventing the oxidation of other molecule. Currently synthetic antioxidants like BHT and BHA are available to slowing or inhibit the free radicals formation and low effectiveness. To overcome this problem plants are utilized in therapeutic applications. Plants are having natural antioxidants like phytochemicals such as phenols, flavanoids etc capable to scavenging harmful free radicals [10]. In this study ethanol extract of *Cassia alata* leaves was screened and characterized by FTIR, GC7-MS and HPLC for DPPH free radical scavenging activity.

Materials and Methods

Preparation of ethanol extract

The leaves were washed with tap water and distilled water. Washed leaves were air dried at room temperature for 7 days. The dried leaves were pulverized into fine powder. The ethanol extract of the *C. alata* leaf was prepared by soaking 10 g of fine powder in 100 ml of chloroform solvent for 24 hours. Then, the extract was filtered using what man No.1 filter paper and collects the filtrate. The collected filtrate was packed in airtight container and stored in dark conditions. The extract was concentrated by vacuum rotary evaporator for the study of phytochemical screening and antioxidant studies.

Phytochemical screening of ethanol extracts

Presence of bioactive phytochemicals like alkaloids, carbohydrate, phenols, tannins, falvanoids, steroids, protein, sugar, glycosides, quinines, saponins and terpenoids in ethanol extract was carried out by following standard methodologies as described by Harborne [11,12], Kokate, [13], Trease and Evans [14] and Edeoga *et al* [15].

Finally the plant extracts were analysed using FTIR analysis, GC-MS analysis and HPLC.

Antioxidant activity of ethanol extract of *C. alata* leaves

Antioxidant activity of ethanol extract of leaf was analysed by Spectrophotometric method on the basis of determination of scavenging activity of DPPH free radical. BHT was prepared at different concentrations (5, 10, 20, 30, 40, and 50 µg/ml) and considered as standard. Stock of test sample was prepared by dissolving 10 mg in 10 ml chloroform at concentration of 1 mg/ml. From this stock solution, different concentration of 5, 10, 20, 30, 40, and 50 µg/ml was prepared. DPPH free radical was prepared by dissolving 1 mM DPPH in 3 ml methanol and kept in dark conditions to protect from sunlight by covering aluminum foil. A 3 ml of different concentrations of leaf extract and standard were mixed with 0.5 ml of DPPH solution and incubated in dark conditions for 30 min. After incubation, the absorbance at 517 nm is determined using UV-vis Spectrophotometer. Methanol was used as blank. The percentage of free radical scavenging of leaf extract was calculated by following equation

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

The inhibition concentration to scavenge 50% free radical (IC50) is determined by plotting a graph of concentration (µg/ml) against % of free radical inhibition.

Results and Discussion

Phytochemical analysis

Medicinally valuable plants are having large number of pharmaceutically important compounds which are considered to able to be study for investigation of new herbal drugs for many harmful life threatening diseases like cancer, ulcer and tumors. Bioactive molecules from medicinal plants proved which inhibit microbial growth and radical scavenging activity. In this study preliminary phytochemical screening of ethanol extract of *C. alata* leaves was analysed for antioxidant activity. The result of phytochemical

screening of ethanol extract of *C. alata* leaves are shown in Table 1. It concluded that the presence of phytochemicals such as alkaloids, carbohydrate, phenols, tannins, falvanoids, saponins, steroids, protein and sugar. Other phytochemicals such as glycosides, quinines, and terpenoids steroids were not present in chloroform leaf extract of *C.alata*. Alkaloids, saponins and flavanoids are attributed to the medicinal properties of plants.

Table 1: Phytoconstituents screening of ethanol extract of *C. alata* leaves

Phytochemicals	Presence/Absence
Alkaloids	+
Glycosides	-
Carbohydrates	+
Quinines	-
Saponins	+
Phenols	+
Tannins	+
Flavanoids	+
Steroids	+
Terpenoids	-
Proteins	+
Sugars	+
Alkaloids	+

FTIR

FTIR characterization studies are used to identify the functional molecules of the phytochemicals present in the plant extract or other materials. Figure 1 shows six different absorption peaks at wave numbers which are corresponds to functional molecules of the ethanol extract of *C. alatas* leaves Table 2. The strong and broad band was observed at 3383 cm^{-1} corresponds to N-H stretching amines and amides. The weak band at 2977 cm^{-1} indicates the presence of **C-H** stretch alkanes. A very weak band was observed at 2965 cm^{-1} corresponds to H-C=O stretching aldehydes. The bands 1389 and 1048 cm^{-1} are assigned to N=O bend nitro groups and C-N stretching aliphatic amines, respectively. The narrow bands shown at absorption peak 880 are designated to N-H wag 1° and 2° amines respectively. Hence this result concluded that the ethanol extract of *C. alata* has active functional groups like, alkanes, amine, aldehydes and nitro groups etc. These functional groups are associated with the bioactive phytochemicals in the leaf extract.

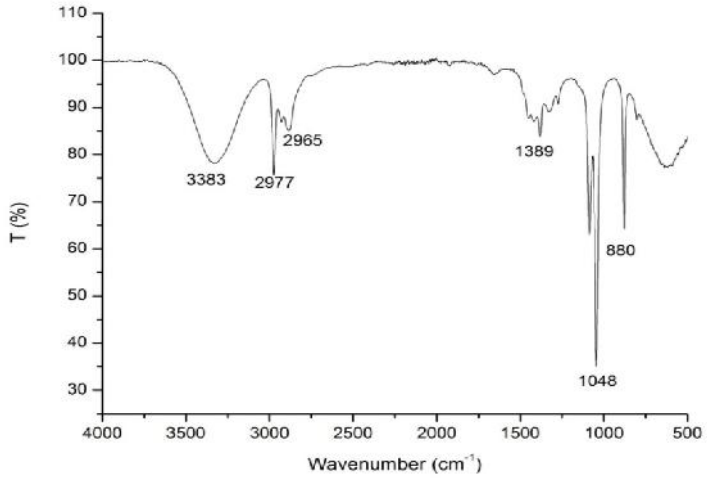

Figure 1: FTIR characterization shows the functional groups present in ethanol extract of *Cassia alata* leaves

Table 2: Functional groups of ethanol extract of *Cassia alata* leaves analysed by FTIR

S.No	Wave number (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

GC-MS

GC-MS analysis of leaves of *C. alata* ethanol extract showed the presence of 9 components at the different retention time (Figure 2). Propylene glycol, 4-Pentadecanol, 2-Hydroxyethylhydrazine, Isopropyl-5-methylcyclohexyl 3-(1-(4-Chlorophenyl)-3-Oxobutyl)-C, Hexadecanoic acid, Eicosanoic acid, Oleic acid, Pentadecanoic acid, cyclotrisiloxane, hexamethyl. The molecular weight and formula of 9 main components is presented in Table 3.

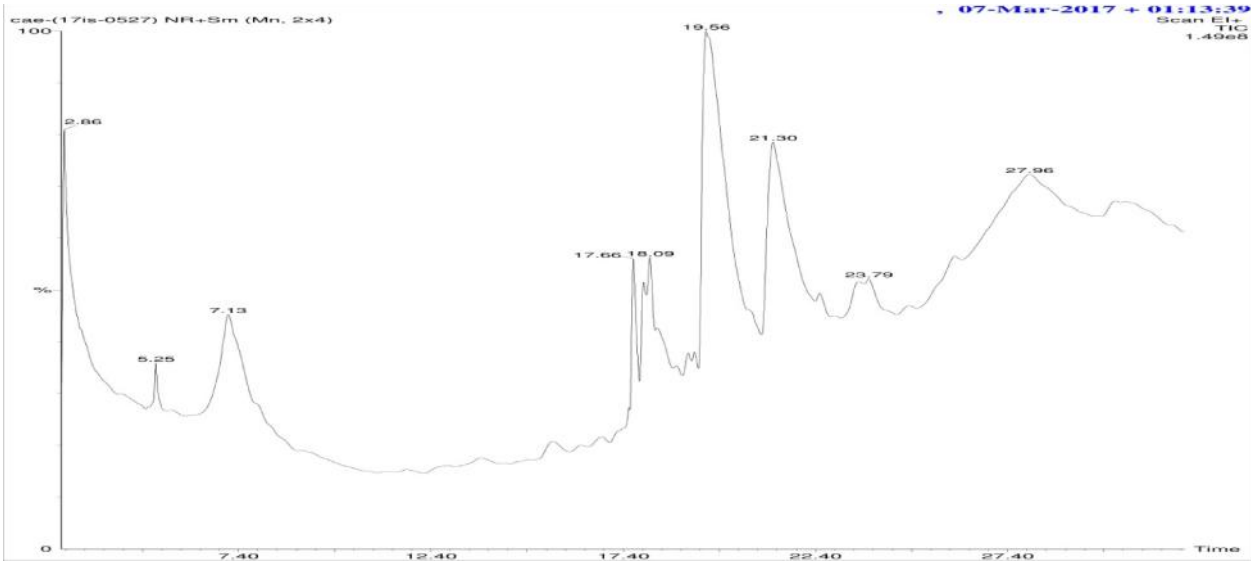

Figure 2: GC-MS analysis of ethanol extract of *Cassia alata* leaves

Table 3: GC-MS analysis of ethanol extract of *Cassia alata* leaves

Retention time	Name of the compound	Molecular weight	Molecular formula
2.86	Propylene glycol	78	$C_3H_8O_2$
5.25	4-Pentadecanol	228	$C_{15}H_{30}O_2$
7.13	2-Hydroxyethylhydrazine	76	$C_2H_8ON_2$
17.66	Isopropyl-5-methylcyclohexyl 3-(1-(4-Chlorophenyl)-3-Oxobutyl)-C	524	$C_{30}H_{33}O_6Cl$
18.09	Hexadecanoic acid	256	$C_{16}H_{32}O_2$
19.56	Eicosanoic acid	312	$C_{20}H_{40}O_2$
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	$C_6H_{18}O_3Si_3$

HPLC

HPLC technique was used to identification of phyto-chemical components of the herbal plants. Each phyto-constituents of the plant extract exhibit a characteristic peak under certain retention times. Figure 3 shows two peaks for two compounds present in the ethanol extract of *Cassia alata* leaves.

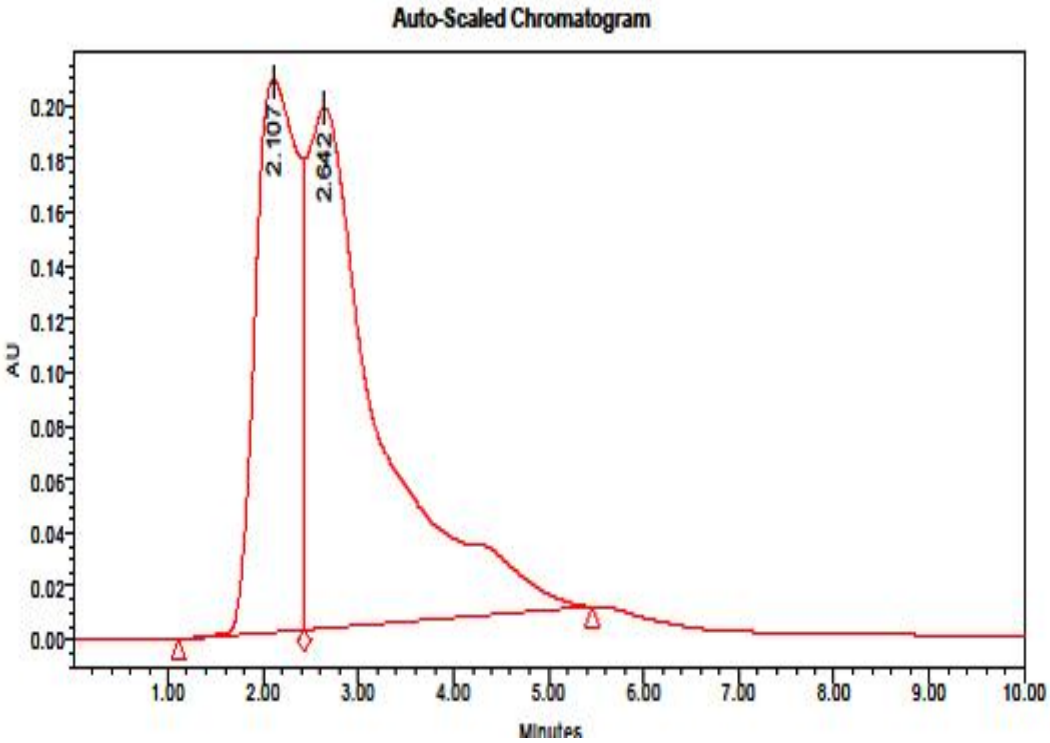

Figure 3: HPLC analysis of ethanol extract of *Cassia alata* leaves

Antioxidant activity of ethanol extract of leaves of *C. alata*

Ethanol leaf extract of *C. alata* exhibited greater antioxidant activity compared to standard BHT at different concentration (5, 10, 20, 30, 40 and 50 µg /ml). In a dose dependant manner, percentage of the antioxidant activity of leaf extract was increased as increasing the concentration (Figure 4). The leaf extract at a concentration of 5µg/ml showed a percentage inhibition was found to be 17.92±1.56 and for 50 µg/ml it was 95.45±1.33. The BHT at a concentration of 5µg/ml exhibited a percentage inhibition was found to be 10.03±0.27and for 50 µg/ml it was noted as 91.65±1.07 (Table 4). The 50% inhibition concentration (IC_{50}) value of leaf extract was found to be 24.56 µg/ml respectively. Regression analysis shows the good linear relation in plant extract towards concentration and inhibition activity (Figure 5).

Antioxidant activity is determined on the basis of the stable DPPH free radical accepting an electron from molecules. It is visually identified by changing color from purple to yellow. The antioxidant activities of the plant may be due to the presence of flavonoids in the leaves. Tannins have astringent properties and biological activities like antioxidant, anti-oxidant, antimicrobial and anti-inflammatory properties [16].

Our report revealed that the ethanol extract of *C. alata* shows excellent antioxidant activity due to the presence of phytochemicals like phytol, esters and fatty acids. Phytol has antioxidant activity was proved [17]. Similarly, isolated phytol from *Lagasea mollis* and reported that it act as antimycobacterial agent [18]. Ara *et al.*, [19] reported that the compounds octadecanal has the antimicrobial effect against pathogenic microorganisms.

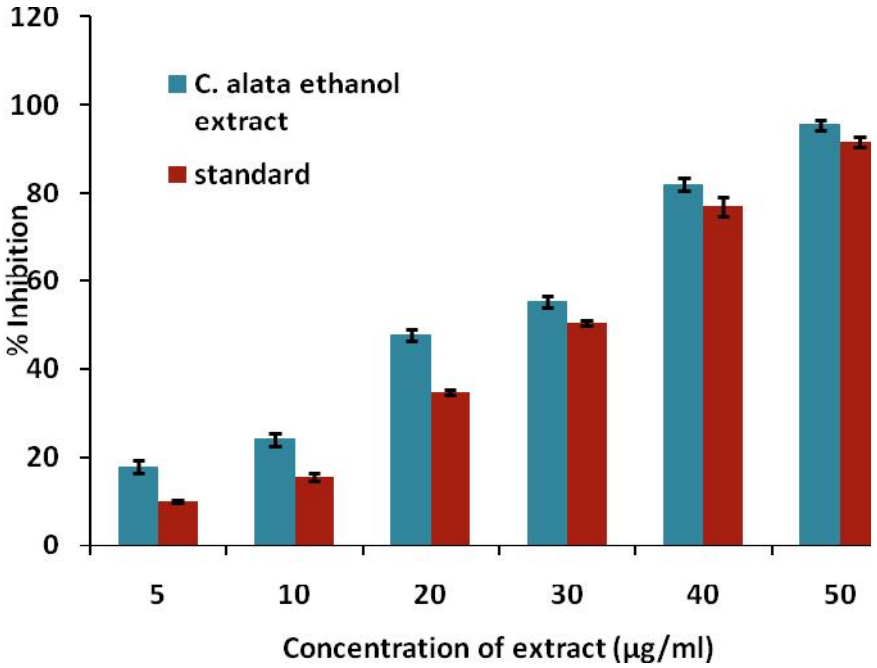

Figure 4: DPPH free radical scavenging activity of ethanol extract of *Cassia alata* leaves

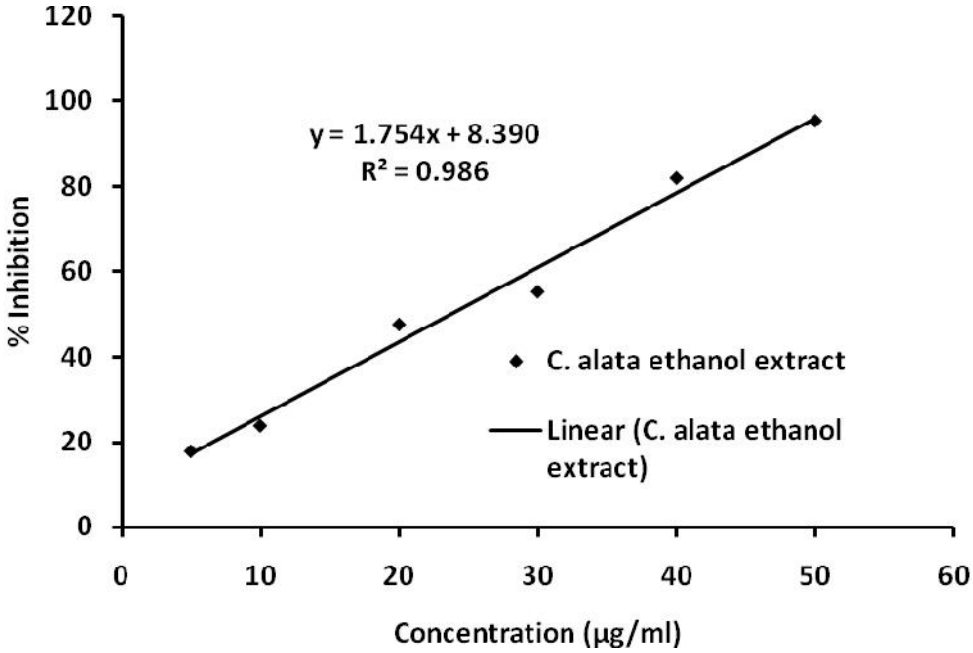

Figure 5: Regression graph of DPPH free radical scavenging activity of ethanol extract of *C. alata*

Table 4: DPPH free radical scavenging activity of ethanol extract of *Cassia 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

± Standard deviation

Conclusion

The qualitative preliminary screening shows the presence of alkaloids, carbohydrate, phenols, tannins, falvanoids, saponins, steroids, protein and sugar were established. FTIR shows the available functional bioactive molecules in the ethanol extract of C. *alata* leaves. The 9 major components present in *Cassia alata* ethanol extract of leaves were identified by GC-MS. HPLC demonstrated the two potential compounds present in the extract at different retention time (Rt). The potential antioxidant activity of *Cassia alata* ethanol extract of leaves was established by measuring DPPH radical scavenging at different concentrations. The activity was compared with synthetic drug shows greater percentage of inhibition of free radical.

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