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Research Article

CHARACTERIZATION OF POLYPHENOLIC EXTRACT AND DNA RICH FRACTION FROM *OLDENLANDIA CORYMBOSA* (L)

***Seshadri, J. and Dr. Arunadevi, R.**

Department of Botany, Government Arts College, C.Mutlur, Chidambaram, Tamilnadu, India

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Abstract

Medicinal plants are often used as raw materials to extract active ingredients used in the synthesis of various medicines. The focus of this research is on the isolation and direct use of active medicinal ingredients or the development of semi-synthetic drugs or the active screening of natural products to obtain synthetic pharmacologically active compounds. The aqueous methanol extract (1 mL, 1 mg/mL) was mixed thoroughly with 1 mL of 50% Folin-Ciocalteu reagent. 1 mL of 2% Na_2CO_3, and centrifuged at 13400X g for 5 min. The polyphenolic rich extract was placed carefully in pre-coated aluminum silica gel 60 F, Merck F 254 using a micro capillary tube. Irradiating the samples with UV light, H_2O_2 was added to a mM concentration of 2.5mmol/L. The reaction volume was maintained in the lid of a polyethylene micro centrifuge tube placed directly on the surface of the transilluminator (8000 mW/cm) at 300 nm. Untreated pBR322 plasmid was included as a control for each gel electrophoresis. The extract development was viewed under UV240nm and 360 nm. The polyphenolic-rich fraction of the *Oldenlandia corymbosa L.* ensured the highest flavonoid content and exhibited the strongest antidiabetic activity.

Keywords: Spectrophotometer, Methanol, TLC, ABTS, Antidiabetic, Vitamin C.

INTRODUCTION

Polyphenols are normally happening compounds found to a great extent within natural products, vegetables, cereals, and refreshments. Polyphenols are auxiliary metabolites of plants and are by and large included in defense against bright radiation or animosity by pathogens. Towards the conclusion of the 20th century, epidemiological studies and related meta-analyses emphatically proposed that long-term utilization of diets rich in plant polyphenols advertised a few securities against the development of cancers, cardiovascular illnesses, diabetes, osteoporosis, and neurodegenerative infections. More than 8,000 polyphenolic compounds have been recognized in different plant species. All plant phenolic compounds emerge from a common halfway, phenylalanine, or a near antecedent, shikimic acid. Fundamentally they happen in conjugated shapes, with one or more sugar buildups connected to hydroxyl bunches, in spite of the fact that coordinate linkages of the sugar (polysaccharide or monosaccharide) to an fragrant carbon moreover exist. Affiliation with other compounds, like carboxylic and natural acids, amines, lipids and linkage with other phenol is additionally common (Kondratyuk and Pezzuto, 2004). Polyphenols may be classified into diverse bunches as a work of the number of phenol rings that they contain and on the premise of auxiliary components that tie these rings to one another. The most classes incorporate phenolic acids, flavonoids, stilbenes and lignans (Spencer *et al.,* 2008). The dissemination of phenolics in plants at tissue, cellular and subcellular levels isn't uniform. Insoluble phenolics are found within the cell wall, while dissolvable phenolics are found within the vacuoles of plant cells (Wink, 1997). Certain polyphenols, such as quercetin, are found in all plant items.

Corresponding Author:** ***Seshadri, J.
Department of Botany, Government Arts College, C.Mutlur, Chidambaram, Tamilnadu, India.

Flavanones and isoflavones are particular to certain nourishments, such as natural products, vegetables, grains, natural product juices, teas, wines, and decoctions. Most regularly, nourishments contain a complex blend of polyphenols. The external layer of plants contains higher sums of phenolics than the insides (Simon *et al.,* 1992). Various components impact the polyphenol substance of plants, counting development at gather, natural variables, handling and capacity. The polyphenol substance of nourishments is incredibly impacted by natural components as well as soil sort, daylight, and precipitation. Readiness essentially impacts the concentration and extent of different polyphenols (Manach *et al.,* 2004). For the most part, it has been watched that phenolic corrosive substance diminishes whereas anthocyanin concentration increments amid maturing. Numerous polyphenols, particularly phenolic acids, are specifically included in plant reactions to different sorts of stretch. Polyphenols contribute to mending by lignifying the harmed region, have antimicrobial properties, and their concentrations can increment after disease (Parr and Bolwell *et al.,* 2000).

MATERIALS AND METHODS

Total phenolic content

The total phenolic content (TPC) of aqueous methanol extract of *Oldenlandia corymbosa.L* was determined using the method by Gutfinger (1981). The aqueous methanol extract (1 mL, 1 mg/mL) was mixed thoroughly with 1 mL of 50% Folin-Ciocalteu reagent and 1 mL of 2% Na2CO3, and centrifuged at 13400X g for 5 min. The absorbance of the upper phase was measured at 750 nm using a spectrophotometer (ELICO (SL150) UV-Vis spectrophotometer) at 750 nm after incubation for 30 minutes at room temperature. Total phenolic content was expressed as catechol equivalents.

Estimation of flavanoid

A 1ml aliquot of each aqueous methanol extract of *Oldenlandia corymbosa.L* was mixed thoroughly with 1ml of 2% aluminium chloride and 0.5 ml 0f 33% acetic acid followed by the addition of 90% methanol and the content is thoroughly stirred and allowed to stand for 30 minutes (Delcour and de Varebeke, 1985). The *absorbance* was measured at 414 nm employing a UV-visible spectrophotometer. Quercetin was utilized as a standard.

Thin layer chromatography

Thin layer chromatography of polyphenolic rich extract of *Oldenlandia corymbosa.L* was performed using standard procedures (Harborne 1998).The polyphenolic rich extract wasplaced carefully in precoated aluminum silica gel 60 F, Merck F 254 using a microcapillary tube. The spots were allowed to dry for few minutes and the TLC plate was placed in the solvent mixture, Toluene, acetone and Formic acid (6:6:1). After drying, the TLC plates were observed under UV at 240nm and 360 nm in UV TLC viewer. The Rf value of the spots was calculated by using the standard formula,

$$\text{Retention factor Rf} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

In vitro* DNA cleavage protector activity of polypheonlic rich fraction from *Oldenlandia corymbosa.L

The experiments were performed in a volume of 20 ml containing 33mmol/L in bp (7.56 nmol/L) of pBR322 plasmid DNA in 5mmol/L phosphate buffer contained 10 mmol/L NaCl, pH 7.4, in the presence of different concentrations (200-400 mmol/L) of catechin, naringin, and rutin. Immediately prior to irradiating the samples with UV light, H_2O_2 was added to mM concentration of 2.5mmol/L. The reaction volumes were held in caps of polyethylene micro-centrifuge tubes, which were placed directly on the surface of a transilluminator (8000 mW/cm) at 300 nm. The samples were irradiated for 5 min at room temperature. After irradiation, 4.5 ml of a mixture containing 0.25% bromophenol blue, 0.25% xylene cyanol FF, and 30% glycerol was added to the irradiated solution. The samples were then analyzed by electrophoresis on a 1% agarose horizontal slab gel in Tris-borate buffer (45mmol/L Tris-borate, 1mmol/L reactive substances (TBARS) were determined as described by Stocks (Stocks and Dormandy, 1971) EDTA. Untreated pBR322 plasmid was included as a control in each run of gel electrophoresis, which was carried out at 1.5 V/cm for 15 h. Gel was stained in ethidium bromide (1 mg/ml; 30 min) and photographed on Polaroid-Type 667 positive land control.

RESULTS AND DISCUSSION

Phenolic content of stem extract of *Oldenlandia corymbosa.L*

In this context, the preliminary experiments revealed that 80% methanol was the best solvent for the extraction of phenolics from *Oldenlandia corymbosa.L* at 60 °C for 60 min since it afforded a maximum yield of phenolics. The yields dry plant of *Oldenlandia corymbosa. L*extracts ranged from 43% (w/w). Therefore, the total phenolic contents were reported as catechin equivalents (Table-1).

Table 1. Yield and phenolic content fleshy stem of *Oldenlandia corymbosa.L*

Sample	Yield of extract (g/100 g of defatted Content)	Total phenolic content (mg catechin equivalents per gram methanol extract)
Fruit phenolic extract of *Oldenlandia corymbosa.L*	53.1±1.4[a]	134.1±1.27[b]

[a]Data are expressed as mean ± standard deviation (n = 3) on a fresh weight basis.
[b]Means in each column sharing the same letter are not significantly (P = 0.05) different from other.

The partial characterization of phenolic extract of *Oldenlandia corymbosa.(L)* by TLC

The polyphenolic extract of *Oldenlandia corymbosa.(L)* loaded on Pre-coated TLC plates (60F 254 Merck) and developed with a solvent system of hexane, ethyl acetate and acetic acid in the ratio of 10:5:0.5. The developed plate was viewed under UV 240nm and 360nm. The Rf value of compounds were shown in Table-2 and Fig-1.

Table 2. Partial characterization of phenolic extract of *Oldenlandia corymbosa.L* by TLC

S.No.	Polyphenolicextract of *Oldenlandia corymbosa.L*		
	UV 240 nm Rf value	UV 360 nm Rf value	Visible Rf value
1.	0.64	0.64	-
2.	0.68	0.68	-
3	0.73	0.73	-
4	0.88	0.90	-
5	0.90	0.95	-

Analysis of polyphenolic rich fraction by GC–MS

The polyphenolic rich fraction of the *Oldenlandia corymbosa.L* ensured highest flavonoid content and exhibited the strongest antidiabetic activity. The GC-MS analysis is used to determine its chemical composition that may contribute to this activity. The GC-MS analysis showed a variety of phenolic compounds (Table-3and Fig-2). In addition, many cinnamic acid derivatives with the phenolic hydroxyl group were considered as antidiabetic and were supposed to have several health benefits due to their strong free radical scavenging properties.

Free radical-scavenging ability using ABTS assay of polyphenolic rich fraction of the *Oldenlandia corymbosa. L*

The radical scavenging ability was measured by ABTS assay as per given in table 3. The inhibition percentage of the ABTS radical activity was assessed on average and high free radical-scavenging values were found inpolyphenolic rich fraction of the *Oldenlandia corymbosa.L.* In ABTS assay, inhibition percentage was high in polyphenolic rich fraction of the *Oldenlandia corymbosa.L*89.6% with EC_{50} value 17.5µl/ml. The pure ascorbic acid was lower activity (Table-4 and Fig-3). Nevertheless, in present study, it is showed that these activities were mainlydue to phenolics and flavonoids. It is known that vitamin C (ascorbic acid) and carotenoids are chief source of discrepancy of antioxidant/ antiradical activities in plant materials.

Fig. 2. Partial characterization of phenolic extract of *Oldenlandia corymbosa*. L by TLC

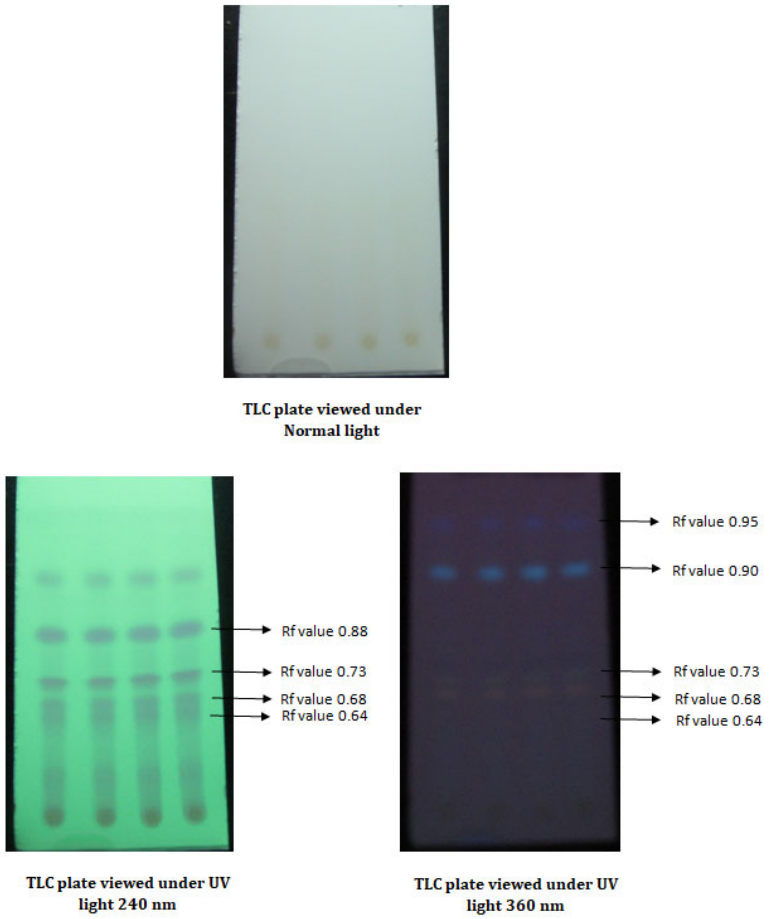
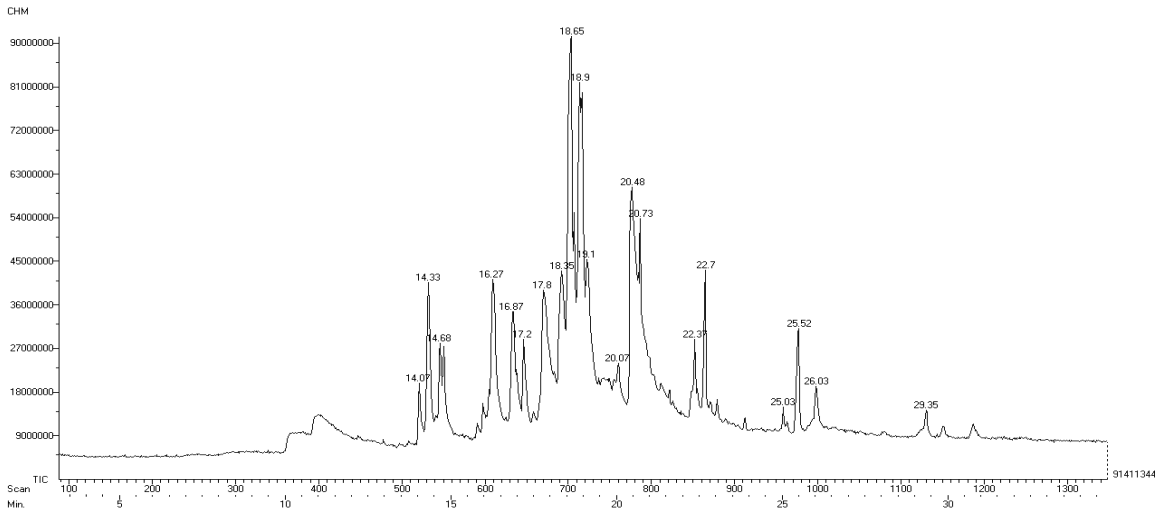
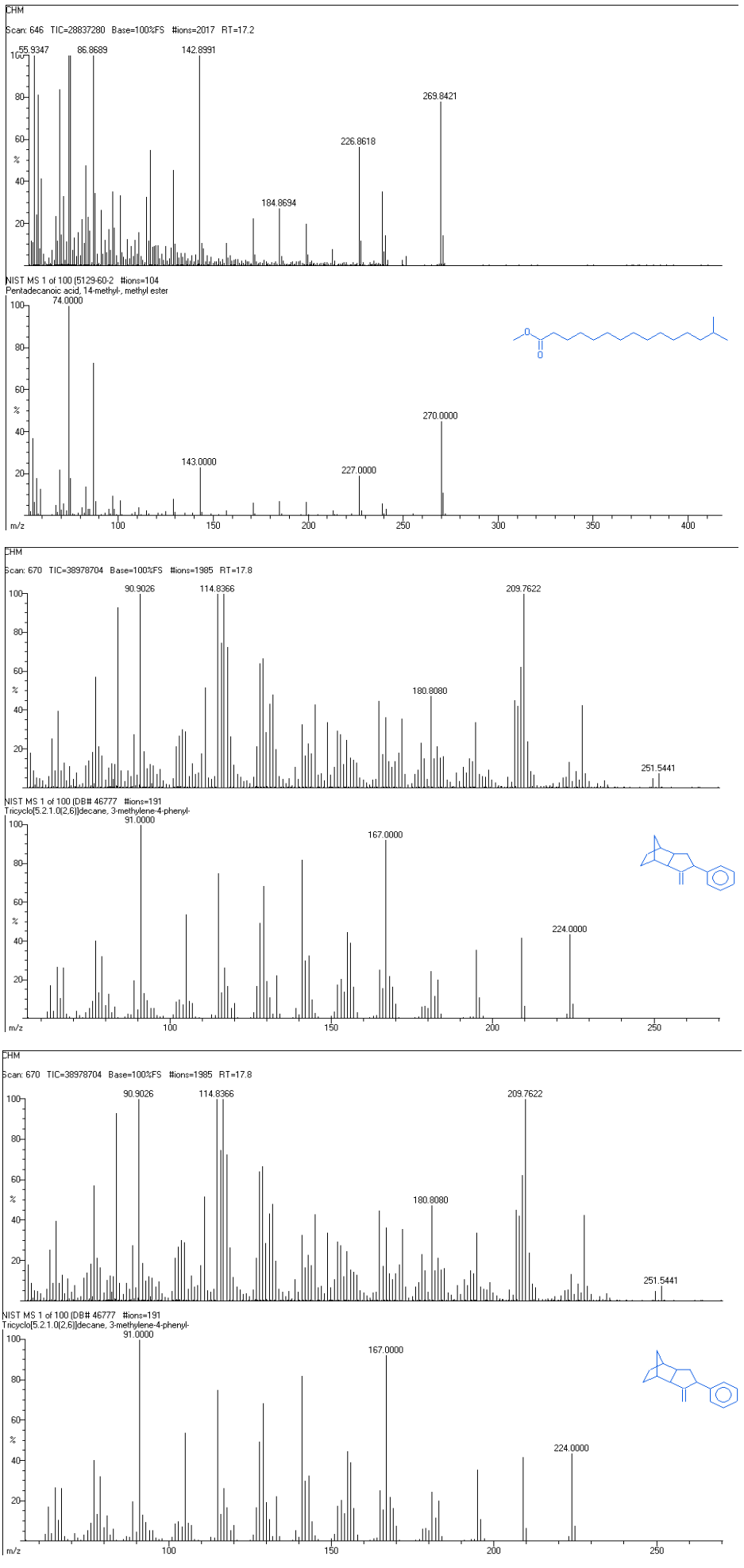


Table 3. Analysis of polyphenolic rich fraction of the *Oldenlandia corymbosa.L* leaves by GC–MS

S.No	Compound	Retention Time(min)	Molecular weight	Major peaks
1	Pentadecanoic acid, 14-methyl-, methyl ester	17.2	270	269, 226, 184
2	3-Methylene-4-phenyltricyclo	17.8	224	251, 209, 180
3	Tricyclo[7.2.2.0(3,8)]tridec-12-en-2-one, 5,6-epoxy-4-methyl	18.35	218	251, 227, 209
4	Tricyclododec carboxy ethoxy	19.65	273	259, 241, 222
5	*Benzamide, 2-amino-5-hydroxy*	18.9	228	259, 227, 209
6	Eicosatetraenoic acid	20.48	304	253, 238, 224
7	Hexadec-9-enoic acid	22.37	254	255, 218, 194
8	Tetratriacontane	22.67	478	308, 294, 266
9	8-Octadecenal	25.03	266	251, 218, 178
10	Heptacosane	25.53	380	379, 336, 280

Fig. 3. Analysis of polyphenolic rich fraction of the *Oldenlandiacorymbosa.L*by GC–MS

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143.0000
227.0000
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CHM
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90.9026
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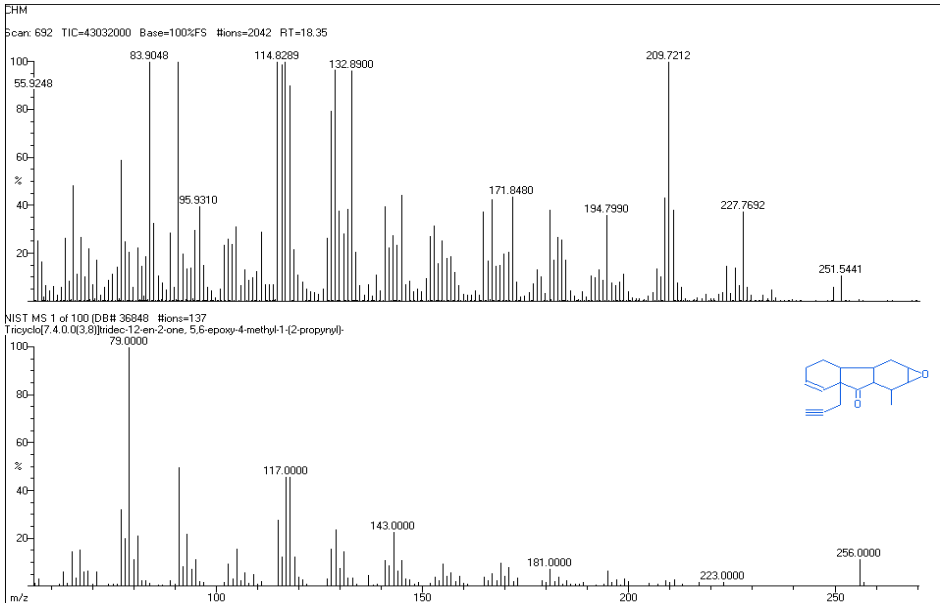
m/z
%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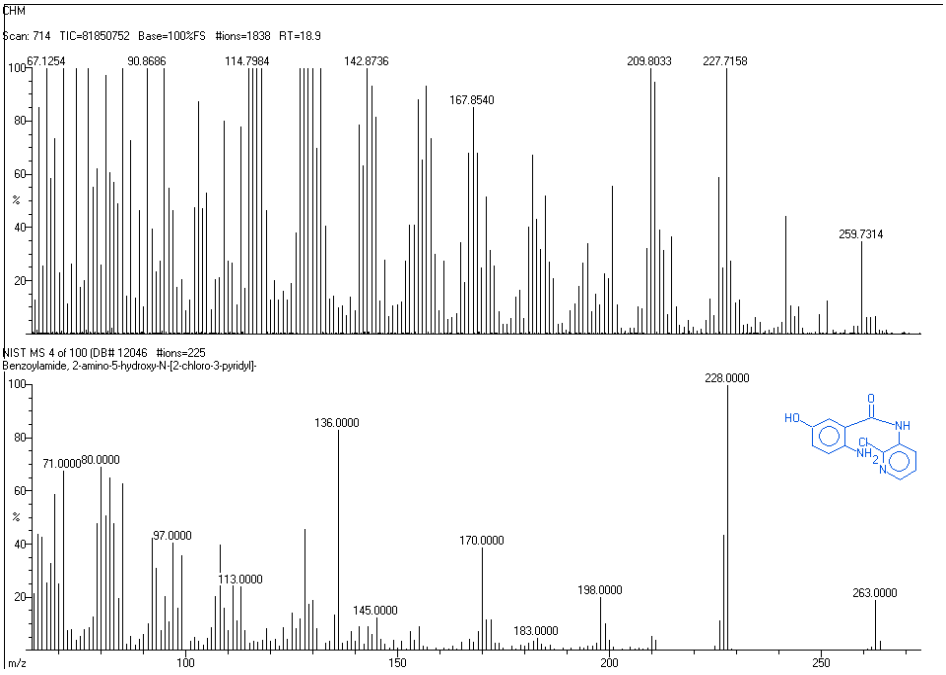
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259.7314
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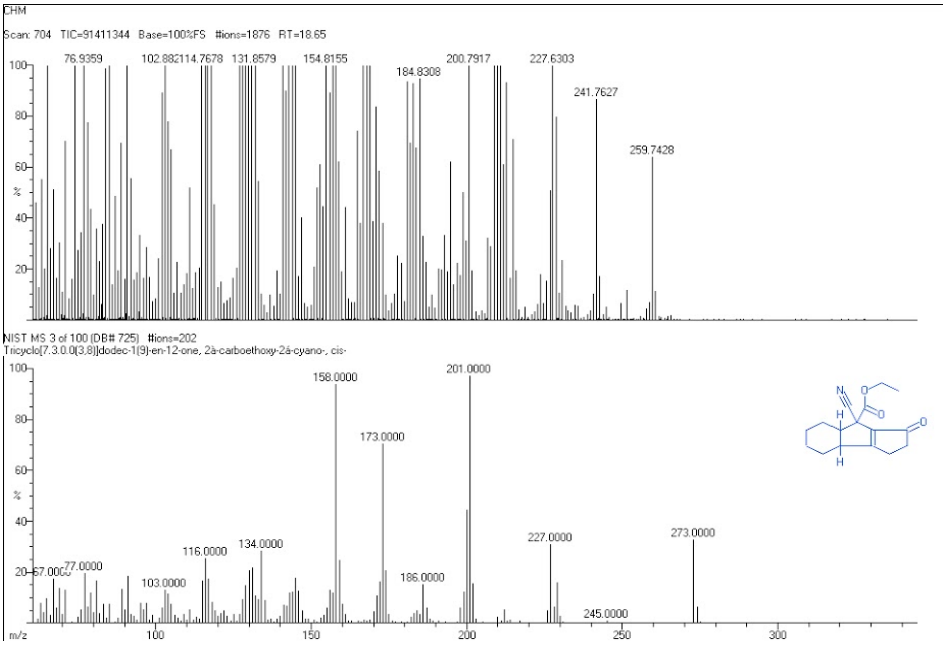
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116.0000
134.0000
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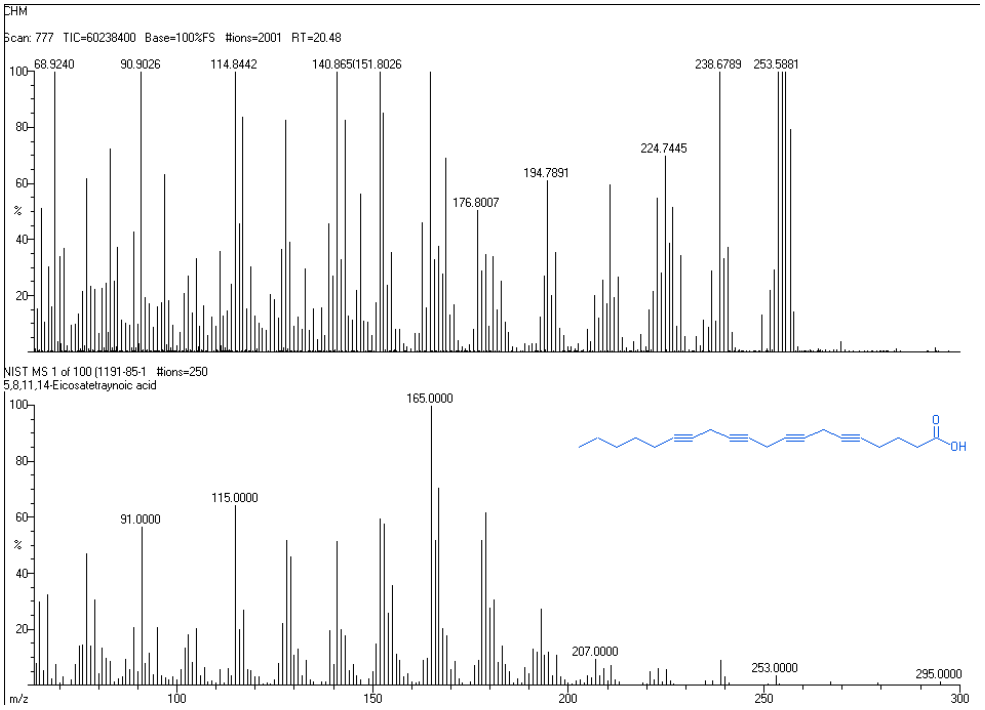
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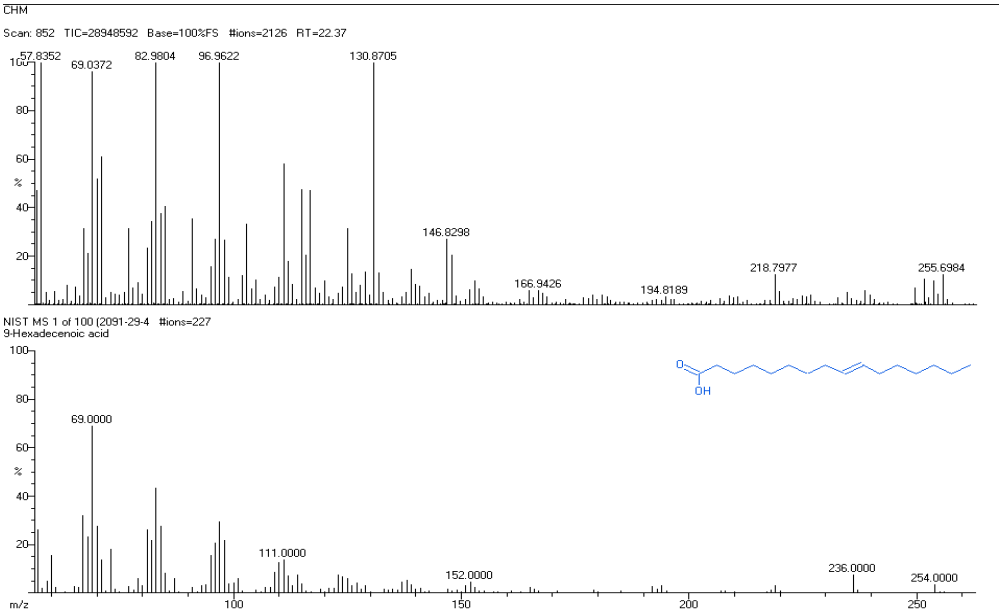
%
m/z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%
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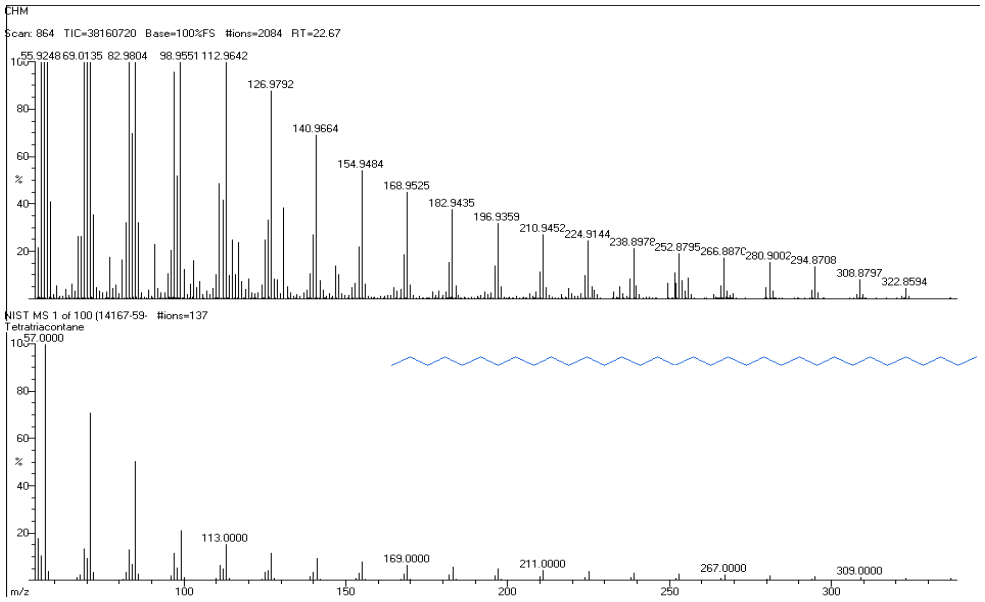
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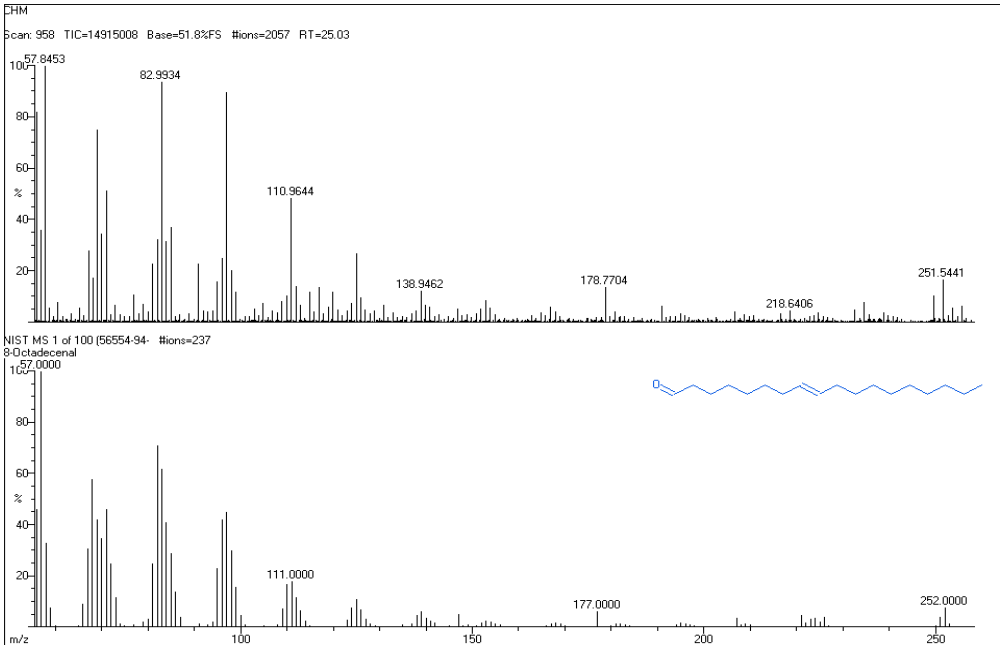
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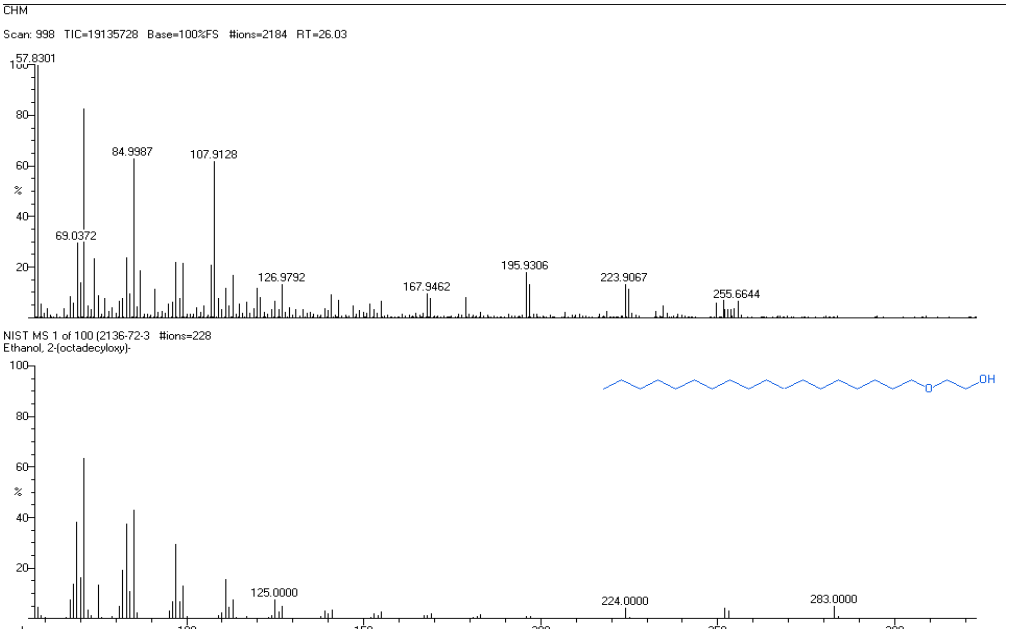
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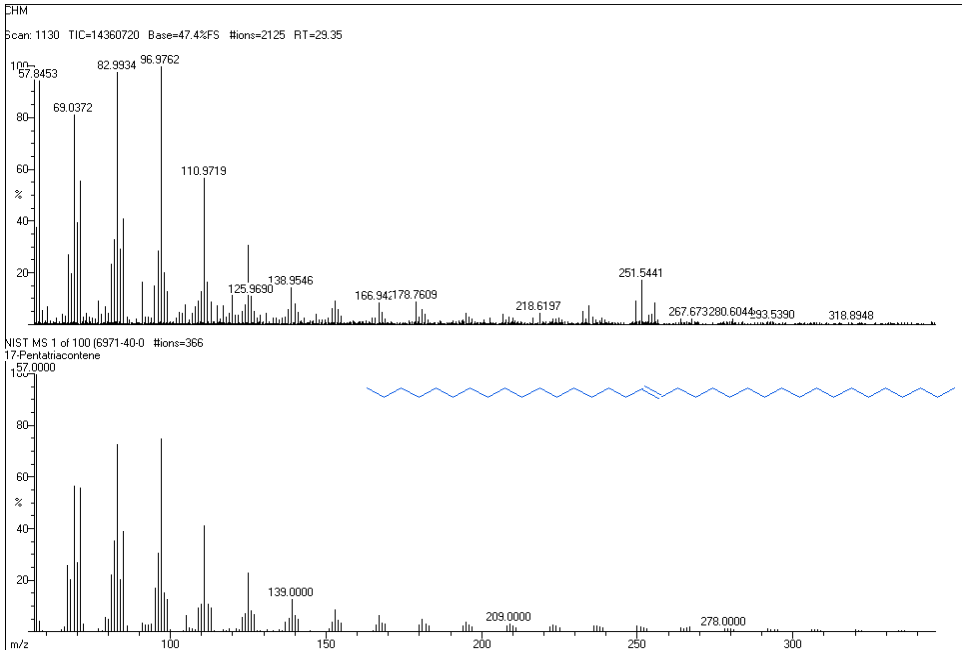
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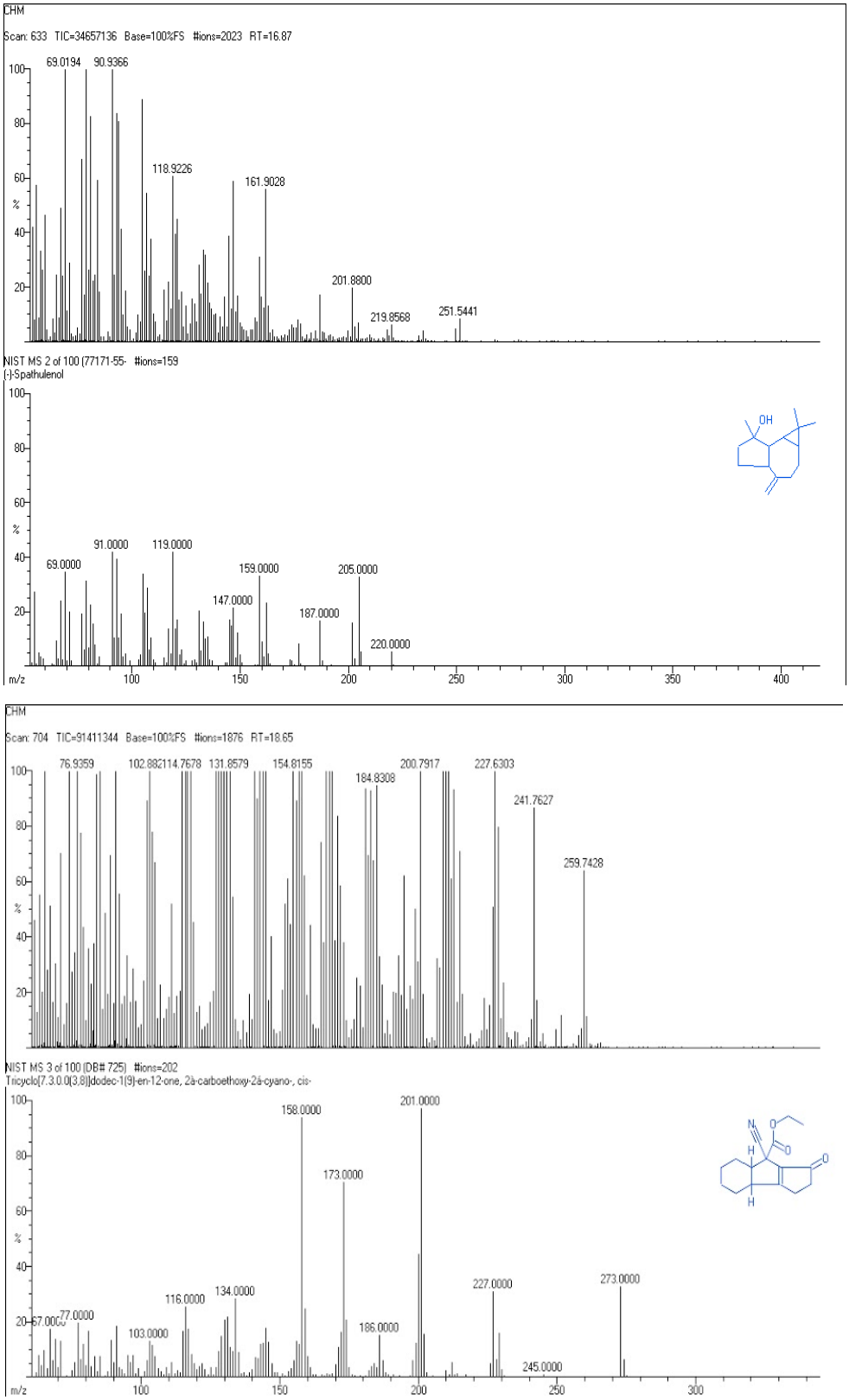

Table 4. Free radical-scavenging ability using ABTS assay of polyphenolic rich fraction of the *Oldenlandiacorymbosa.L*

Different concentration of extract	ABTS radical activity fruit of polyphenolic rich fraction of the *Oldenlandia corymbosa.L*	
	Polyphenolic rich fraction	ABTS radical activity Standard Vitamin-C
5 µl/ml	19.3±1.11	15.04±1.47
10 µl/ml	36.6±1.20	29.52±1.58
15 µl/ml	65.1±2.36	55.23±1.69
20 µl/ml	89.6±1.44	81.23±2.1
EC_{50} value	17.5	19.8

Results are expressed as percentage inhibit of ABTS ability with respect to control. Each value represents the mean+SD of three experiments

Fig. 4. Free radical-scavenging ability using ABTS assay of polyphenolic rich fraction of the *Oldenlandia corymbosa*.L

Polyphenolic rich fraction of the *Oldenlandiacorymbosa.L*

A- Control; B-5 µl/ml; C-10 µl/ml; D- 15µl/ml; E. 20 µl/ml of extract

Conclusion

Polyphenols have phenolic hydroxyl groups which are therefore valuable plant components for scavenging free radicals. These results obtained suggest that as the number of polyphenolic compounds increases, the antioxidant activity also increases. In the partial characterization of the polyphenolic extract of *Oldenlandia corymbosa.(L) by TLC, which showed 5 UV fluorescent compounds respectively.* The inhibition percentage of the ABTS radical activity was assessed on average and high free radical-scavenging values were found in polyphenolic extract of*Oldenlandia corymbosa.L* (89.6%).

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