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Phytochemical constituents and antioxidant activity of *Ricinus communis Linn* leaf and seeds extracts

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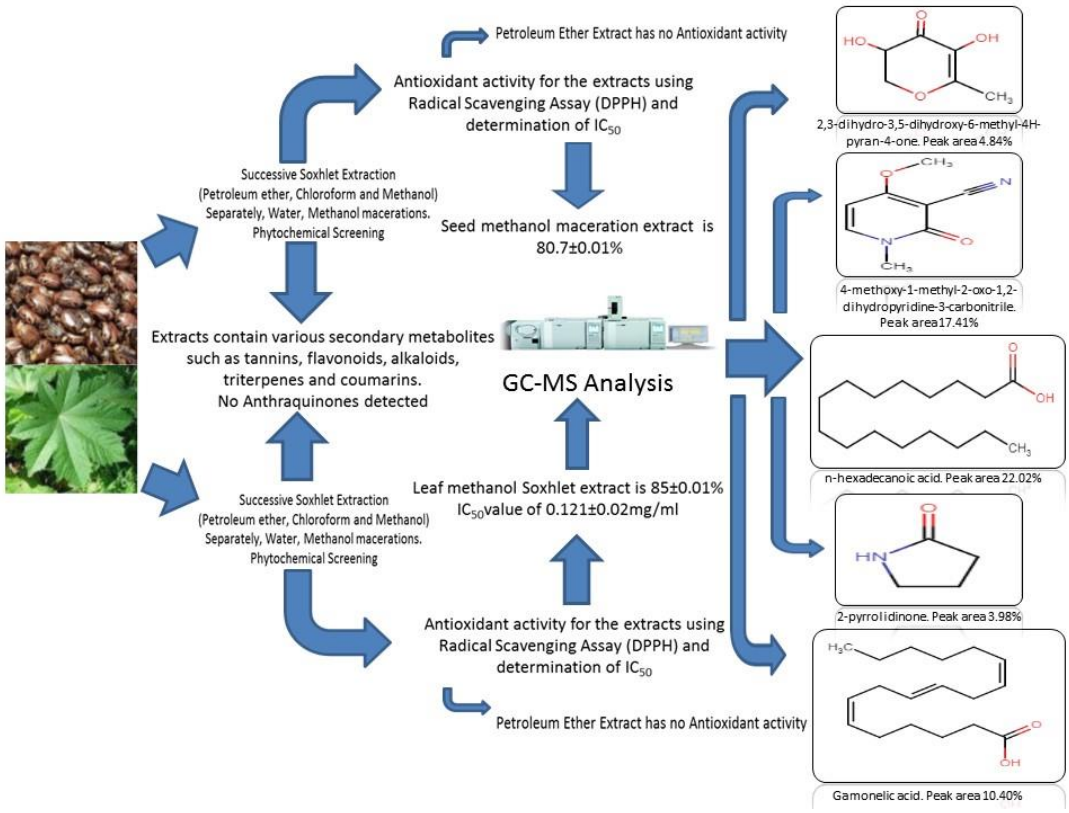

Abstract: The study investigates phytochemicals and antioxidant potential of leaf and seeds extracts of *Ricinus communis L.* *Ricinus communis L.* commonly known as castor plant belongs to the family *Euphorbiacea.* Phytochemical screening showed that both seeds and leaves of *Ricinus communis L.* have various secondary metabolites such as: tannins, flavonoids, alkaloids, triterpenes and coumarins,

while anthraquinones were not detected. Antioxidant potentials were assessed on the basis of radical scavenging effect of the stable 1,1-diphenyl-2-picrylhydrazil (DPPH). Leaf methanol Soxhlet extract showed the maximum antioxidant activity (85±0.01%) with IC_{50} value of (0.121±0.02mg/ml). Seed methanol maceration extract showed high antioxidant activity (80.7±0.01%) while petroleum ether extracts of both leaf and seeds showed no antioxidant activity. Spectroscopic analysis using GC-MS of the methanol maceration extract of leaf showed the presence of various compounds such as n-hexadecanoic acid with the highest peak area 22.02%, 4-methoxy-1-methyl-2-oxo-1,2-dihydropyridine-3-carbonitrile with peak area 17.41%, gamonelic acid with peak area 10.40%, 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one with peak area 4.84% and 2-pyrrolidinone with peak area 3.98%.

Keywords: *Ricinus communis L.*, antioxidant activity, phytochemical screening, IC_{50}, GC-MS

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Introduction

Natural plants are considered primary source of chemical compounds which have medicinal effect [1]. Among these medicinal plants, *Ricinus communis L.* (castor plant) which belong to spurge family *Euphorbiacea*and genus *Ricinus* [2]. The plant has wide traditional and medicinal uses for instance castor oil used as purgative, wound and scarf healer, expel worm and parasites from intestine, hair growth, expectorant, tonic, high nitrogen fertilizer, livestock feed, insecticidal, also it is used as laxative and to treat dermatitis, ringworm and wart [3]. The leaves have also been recommended in the form of decoction or poultice and as an application to breasts of women to increase the secretion of milk [4]. Phytochemical screening of the plant seeds and leaves showed that it has various important chemical constituents such as alkaloids, flavonoids, tannins, coumarin, terpenes, triterpenes, saponins, carbohydrates, fatty acids along with other useful compounds [5]. Aerial parts of this plant have been reported to have ricinine, N-demethylricinine, six flavonoid glycosides; kaempferol-3-O-β-Dxylopyranoside, Kaempferol-3-O-β-Dglucopyranoside,quercetine-3-O-xylopyranoside, quercetin-3-O-β-D-glucopyranoside, Kaempferol-3–O-β-rutinoside,quercetin-3-O-β-rutinoside [6]. The monoterpenoids (1,8-cineol camphor and α–pinene) and a sesquiterpenoid (β-caryophyllene), gallic acid, quercetin, gentisic acid, rutin, epicatechin and ellagic acid are the major phenolic compounds isolated from leaves [7]. Indole-3-acetic acid

has been extracted from the roots [8,9]. The seeds contain 45% of fixed oil lipases and a ricinine. The GLC analysis of castor oil exhibited the presence of esters from palmatic 1.2%, stearic 0.7%, arachidic 0.3%, hexadecenoic 0.2%, oleic 3.2%, linoleic 3.4%, ricinoleic 89.4% and dihydroxy stearic acid [6,10]. *Ricinus communis L*. is reported to have various pharmacological activities. The seeds have high antioxidant activity by DPPH at low concentrations due to presence of ricinoleic acid and methyl ricinolate which can be useful to treatment of oxidative diseases [11].

The different solvent extracts of roots of *Ricinus communis L.* (200mg\ ml) possess antimicrobial activity by using well diffusion method against pathogenic microorganism such as *Escherichia coli*, *straphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella*, *Typhimurium*, *Proteus vulgaris*, *Bacillus subtilis*, *Candida albicans* and *Aspergillusniger*. The hexane and methanol extracts showed maximum antimicrobial activity where the aqueous extract has no significant antimicrobial properties [12]. The ethanolic extract of *Ricinus communis L.* roots resulted antihistaminic activity at the dose 100,125, and 150 mg\kg intraperitoneally by using clonidine induced catalepsy in mice[13]. The *Ricinus communis l.* ethanol extract of roots possess antihistaminic activity due to presence of flavonoids or saponins[14]. A lactin isolated from *Ricinus communis*is ricin A, possesses antitumor activity that was more toxic to tumor cell than non-transformed cells [15]. The crude extract from root bark of *Ricinus communis* possesses central analgesic[16]. *Ricinus communis* seeds extract showed to have cytotoxic effect due to the presence of toxic material called ricin which mainly inhibits the synthesis of protein in cells and cause cell death; therapeutically it can be used to target cancer cells [17]. The leaves on the other hand have another range of cytotoxic phytochemicals, these compounds include three monoterpenoids, camphor, 1,8-cineole, α-pinene and sesquiterpenoid: β-caryophyllene [8]. Alkaloid ricinine (3-cyano-4-methoxy-N-methyl-2-pyridone) is one of the important phytochemicals which obtained from castor plant that belongs to the alkaloid piperidine group. Its present in all parts of the plant but especially in young plants and its only cyano-subsitiuted pyridine occurred naturally [18], Ricinine intake can cause vomiting and other toxic reaction such as liver and Kidney, damage, convulsions, and hypertension [19]. In order to understand the pharmacological significance and potential health risks, it is necessary that medicinal plants undergo phytochemical ingredients investigation because the scientific affirmation of bio-activity of the phytochemicals is a set up strategy for the discovery of new medication[20]. Thus searching for a new nontoxic, effective natural compounds with antioxidative activity has been intensified in recent years [21], in current literature review, phytochemical screening and antioxidant activities of *Ricinus communis L.* were assessed for cold macerated extracts

only and this may decrease the bioassay of the extracts in general due to antagonistic effect (opposite of synergistic effect), but in this study we carried out successive Soxhlet extraction for the same sample using different solvents in addition to macerated extracts. Previously we studied the antimicrobial assays and cytotoxic effect of *Ricinus communis L*. leaf and seeds extracts [22,23]. Continuously the purpose of this study is to Soxhlet extract and macerate *Ricinus communis L.* seeds and leaves using petroleum ether, chloroform, and methanol and compare the extracts in terms of phytochemicals and antioxidant activities, high antioxidant extract is subjected to GC-MS analysis to elucidate the chemical constituents responsible for high antioxidant properties.

MATERIALS AND METHODS

Materials

Plant material

Ricinus communis L. leaves and seeds samples were collected from Shambat Area identified and authenticated in the department of Botany– Alneelain University. Samples were placed in Alneelain University, College of Science and Technology.

Apparatus

Soxhlet extraction apparatus, multiplate reader spectrophotometer, GC-MS (GC/MS-QP2010-Ultra) from Japans'Simadzu Company., EZ-Fit Enzyme Kinematic program (Perrela Scientific Inc, U.S.A).

Chemicals and reagents

Acetic acid, Acetone, Benzene, Butanol, Chloroform, Ethanol, Ethyl acetate, Formic acid, Hexane, Methanol, Petroleum ether (60-80°C) and Toluene, Chemicals for antioxidant assays (Sephadex), reagents for phytochemical screening (Dragendroff's reagent, Hager's reagent, Mayer's reagent, Benedict's reagent, Millon's reagents, Kedde's reagent, Valser's reagent).

Methods

Collection of samples

The leaves and seeds of *Ricinus communis L.* were collected from the farms of agriculture college *SUST*, *Bahri*area. The taxonomy and identification of the plant carried out at the department of botany, college of science, *Alneelain University*. The leaves were washed with water from dusts and contaminants and dried under the room conditions, the seeds grinded with clean mortar and pestle.

Preparation of different extracts

The freshly collected leaves of this plant were cleared from dirt then, dried under shade for about 15days and then coarsely powdered in a mechanical grinder. The powder was macerated with methanol for 5 days, filtrate was collected and concentrated. The concentrated extract was dried using desiccator with anhydrous calcium chloride [24]. Soxhlet extraction was carried out using petroleum ether, chloroform and methanol.

Phytochemical screening of extracts

Phytochemical screening was carried out according to the method of Martinez [25] with many modifications.

Test for tannins

0.5 g of the extract was washed three times with petroleum ether, dissolved in 10 ml hot saline solution and divided in two tests tubes. To one tube 2-3 drops of ferric chloride added and to the other one 2-3 drops of gelatin salts reagent added. The occurrence of a blackish blue color in the first test tube and turbidity in the second one denotes the presence of tannins.

Test for sterols and triterpenes

0.5 g of the extract was washed three times with petroleum ether and dissolved in 10 ml of chloroform. To 5 ml of the solution, 0.5 ml acetic anhydride was added and then 3 drops of conc. H_2SO_4 at the bottom of the test tube. At the contact zone of the two liquids a gradual appearance of green, blue pink to purple color was taken as an evidence of the presence of sterols (green to blue) and or triterpenes (pink to purple) in the sample.

Test for alkaloids

0.5 g of the extract was heated with 5 ml of 2N HCl in water bath and stirred for about 10 minutes, cooled filtered and divided into two test tubes. To one test tube few drops of Mayer's reagent was added while to the other tube few drops of Valser's reagent was added. A slight turbidity or heavy precipitate in either of the tow test tubes was tanked as presumptive evidence for the presence of alkaloids.

Tests for flavonoids

0.5 g of the extract was dissolved in 30 ml of 80% ethanol. The filtrate was used for following tests:

A) to 3 ml of the filtrate in a test tube 1ml of 1% potassium hydroxide solution in methanol was added. Appearance of a yellow color indicates the presence of Flavonoids (Flavones and/or and flavonols).

B) to 2 ml of the filtrate 0.5 ml of 10 % lead acetate was added. Appearance of creamy turbidity was taken as an evidence of flavonoids.

Test for saponins

0.5 g of the extract was placed in a clean test tube. 10 ml of distilled water was added, the tube stoppered and vigorously shaken for about 30 seconds. The tube was then allowed to stand and observed for the formation of foam, which persisted for least an hour, was taken as an evidence for presence of saponins.

Test for coumarins

0.5 g of the extract was dissolved in 10 ml distilled water in test tube and filter paper attached to the test tube to be saturated with the vapor after a spot of 0.5N KOH put on it. Then the filter paper was inspected under UV light, the presence of coumarins was indicated if the spot has found to be adsorbed the UV light.

Test for anthraquinone glycoside

0.5 g of the extract was boiled with 5ml dil H_2SO_4 for two min, the filtrate was cooled and shacked with 5ml of chloroform or benzene, the chloroform (or benzene) layer was separated and 2 ml of ammonium solution was added. Arose of a pink color in the ammonia layer indicates the presence of Anthraquinone glycoside.

Test for carbohydrates

Molish test was carried out. 0.5 g of the extract was taken in test tube and solved with distilled water; 0.5ml of resorcinol was added. 1 ml of H_2SO_4 was poured slowly. Violet ring indicate the presence of carbohydrates.

Test of cardinoloides

0.5 g of the extract was treated with a small amount of Kedde reagent (Mix equal volumes of a 2% solution of 3, 5 dinitrobenzoic acid in menthol and a 7.5% aqueous solution of KOH). Development of a blue or violet color that faded out in l to 2 hrs. shows presence of cardinoloids.

Determination of antioxidant potential

DPPH radical scavenging assay method was used for determination of antioxidant activity of extracts according to Shimada *et al.* [26] with some modification. 0.5 mg of each

extract were weighed in an *Ependove* tube and dissolved in ethanolic DMSO (dimethylsulphoxide). In96-well plate, the test samples were allowed to react with 2.2-di (4tert-octylphenyl)-hydrazyl stable free radical (DPPH) for half an hour at 37 °C. The concentration of DPPH was kept as 300 mM. After the incubation the, decrease in absorbance was measured at 517 nm using multiplate reader spectrophotometer. Percentage radical scavenging activity was determined in comparison with a DMSO treated control group. All tests and analysis were run in triplicate.

Determination of IC_{50}

The IC_{50} (the concentration of test material which possess 50% inhibition of free radicals) of all the extracts and their fraction was determined by monitoring the effect of different concentrations ranging from 0.5 to 0.03 mg\l. The IC_{50} of the extracts and their fractions were calculated using EZ-Fit Enzyme Kinematic program (Perrela Scientific Inc, U.S.A) [26].

Gas chromatography- mass spectrometry analysis

The qualitative and quantitative analysis of the sample was carried out by using GM/MS technique model (GC/MS-QP2010-Ultra) from japans *'Simadzu* Company, with capillary column (Rtx-5ms-30m×0.25 mm×0.25µm).The sample was injected by using split mode, helium as the carrier gas passed with flow rate 1.61 ml/min, the temperature program was started from 60˚C with rate 10˚C /min to 300˚C as final temperature degree with 3 minutes hold time , the injection port temperature was 300˚C, the ion source temperature was 200˚C and the interface temperature was 250˚C.The sample was analyzed by using scan mode in the range of m/z 40-500 charges to ratio and the total run time was 26 minutes .Identification of components for the sample was achieved by comparing their retention times and mass fragmentation patents with those available in the library ,the National Institute of Standards and Technology (NIST).Results were recorded.

Results and discussion

Phytochemical screening

Phytochemical screening of *Ricinus communis L.* seeds and leaves extracts was carried out. The results presented in table 1 compare between the phytochemicals present in *Ricinus communis L.* seeds and leaves extracts when extracted with petroleum ether, chloroform, methanol and water, methanol maceration.

The comparison between petroleum ether extracts of *Ricinus communis L.* leaves and seeds clarify that more polar compounds (flavonoids and coumarin) are present in leaves, these compounds reported to have beneficiary effect on human body such as reducing risk of cancer, diabetes, cardiovascular and brain diseases [27].

Table 1. Phytochemical screening of *Ricinus communis L.* extracts.

Test	**Leaf**					**Seed**				
	P.E	**CH**	**ME**	**Water Mac**	**ME Mac**	**P.E**	**CH**	**ME**	**Water Mac**	**ME Mac**
Saponins	-	+	+	+	+	+	-	++	+	+
Coumarin	++	+++	+++	-	+	-	+	-	+	+
Alkaloids	-	-	-	++	-	-	-	-	+++	-
Flavonoids	+++	-	++	+++	+++	-	-	-	-	-
Tannins	-	-	++	+++	+	-	-	-	+	-
Sterols	+++	+++	+	-	++	++	+	+	-	+
Triterpenes	+++	++	++	+	+++	+++	+++	+++	++++	+
Anthraquinones	-	-	-	-	-	-	-	-	-	-
Carbohydrates	+++	++	++	+++	+++	+	+	+++	+++	+++
Cardinoloides	++	++	+	+++	+++	+++	+++	+++	++++	+++
Flavonols\-one	-	-	-	+	-	-	-	+	++	+

Keys: Leaf: *Ricinus communis L.* leaf extracts, Seeds: *Ricinus communis L.* seeds extracts, + Trace ++ Moderate +++ High - Negative. PE: Petroleum Ether, ME: Methanol, ME Mac: Methanol Maceration, CH: Chloroform, Water Mac: Water Maceration.

For the chloroform extracts; saponins, coumarins, and sterols are more prevalent in leaves, saponins reduce blood cholesterol levels, decrease blood lipids and lower cancer risk, while sterols act as membrane reinforcer and protecto [28,29].

Coumarins, flavonoids and tannins are prevalent in leaves methanol extract than the seeds methanol extract, while the seeds have flavonols and flavonones. Tannins accelerate blood clotting, reduce blood pressure and decrease serum lipid levels [30].

Seeds water maceration extract has wide range of compounds saponin, coumarin, alkaloid, tannin, triterpenes, carbohydrates, cardinoloides and flavonols\-one and that's is due to extraction of water to polar compounds present in seeds and seed shells and avoid of heating in maceration process kept the compounds intact, alkaloids protect the plant from predator and regulate its growth [31], also alkaloids known as anaesthetics, cardioprotective and anti-inflammatory agents [32]. Triterpenes act as antidiabetic and strong antioxidant agents[33]. Flavonoids and Anthraquinones are generally absent in seeds extracts and that's

is in accordance with Shahid *et al*. [34] phytochemical screening study which resulted in absence of flavonoids, anthraquinones and reducing sugars.

Meanwhile phytochemical screening of leaves presented in table 1 shows that methanol maceration extract has the wide range of compounds due to the extraction of almost all compounds in the leaves as the leaves consist mostly of polar compounds.

Kim *et al*. [35] reported that flavonoids are known to have antioxidant activity and that confirms the high antioxidant of methanol extract of *Ricinus communis L.* leaves and methanol maceration extract of leaves as table 2 shows.

Antioxidant activity

Extracts of *Ricinus communis L.* and one standard were tested for antioxidant activity using DPPH method, the metanol extract of leaf showed the maximum antioxidant activity with (85±0.01%) and IC_{50}values of (0.121±0.02 mg/ml). The metanol maceration extract of seed also showed antioxidant activity of (80.7±0.01%). However, petroleum ether extract showed lowest antioxidant activity (06±0.04%). The galic acid exhibited antioxidant of (92±0.01%) and IC_{50}values of (0.077m g/ml).

Table 2. Antioxidant activity of *Ricinus communis L.* extracts using DPPH and IC_{50} for methanol leaf extract.

Test	**Leaf**				**Seeds**				**Gallic acid**
	P.E	**CH**	**ME Sox**	**ME Mac**	**P.E**	**CH**	**ME Sox**	**ME Mac**	
%RSA±SD (DPPH)	06 ±0.04	06 ±0.06	85 ±0.01	49 ±0.05	09 ±0.01	37 ±0.01	53 ±0.01	80.7 ±0.01	92 ±0.01
IC_{50}±SDmg\l (DPPH)			0.121 ±0.02						0.077 ±0.01

Key: Leaf: *Ricinus communis L.* leaf extracts, Seeds: *Ricinus communis L.* seeds extracts, PE: Petroleum Ether, ME Sox: Methanol Soxhlet, ME Mac: Methanol Maceration, CH: Chloroform

Ricinus communis L. seeds extracts, petroleum ether has given low antioxidant potential (09%) followed by chloroform extract (37%) and methanol (53%) while the methanol maceration of seeds has exhibited the best antioxidant activity (80.7%), as the polarity of the solvent increases, the antioxidant activity of the extract increases [36].

Methanol extract of *Ricinus communis L.* leaves shows high antioxidant activity (85%) followed by *Ricinus communis L.* seeds methanol maceration (80.7%) while petroleum ether and chloroform extracts of both leaves and seeds exhibited low antioxidant activity.

The reason is that why methanol extract of leaves has exhibited higher antioxidant activity (85%) than the leaves methanol maceration extract (49%) might be due to antagonistic effect (inactive compounds block the action of active compounds), It has been suggested in the literature that antagonism among antioxidants can result from; regeneration of a less effective antioxidant by a more effective antioxidant, oxidation of a more effective antioxidant by the radicals of a less effective antioxidant [37,38], competitive formation of antioxidant adducts [39-42] or alteration of the microenvironment of one antioxidant by another antioxidant [43].

The results obtained is in accordance with other two previous studies, the first one is reported by Singh *et al.* [9] shows that methanol\water extract (8:2) of *R. communis L.* leaves showed strong DPPH radical-scavenging activity due to the presence of flavonoids in their extracts.

The second one is reported by Oleyede *et al.* [44] shows that the responsible chemical constituent of *Ricinus communis* which produce antioxidant activity are methyl ricinoleate, ricinoleic acid, 12-octadecadienoic acid and methyl ester. And this is confirmed by GC-MS results in table 3 which shows ricinoleic acid 22.2%, ricinoleate 1.82% and 12-octadecadienoic acid and methyl ester 2.36%.

GC-MS analysis of methanol extract of *Ricinus communis L.* leaf

Antioxidant activity of GC-MS analysis of methanol extract of *Ricinus communis L.* leaves has been carried out, Figure 1: GC-MS Chromatogram of *Ricinus communis L.* leaves methanol extract (see Supplementary Material) shows the name of separated compounds, retention time, peak area, molecular weight, molecula formula and fragmentation ions. GC-MS analysis of *Ricinus communis L.* leaves methanol extract shows that the leaves contain alkaloids, hydrocarbons, alcohols, esters, the most predominant compounds are n-hexadecanoic acid (palmitic acid) with the highest peak area 22.02%. 4-Methoxy-1-methyl-2-oxo-1,2-dihydopyridine-3-carbonitrile with peak area of 17.41% (this compound is expected to be N-methylricinine as GC-MS results shows) and gamonelic acid (gamma- linolenic acid) with 10.40%.

Compound 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (retention time 6.22, area percentage 4.84%, and molecular weight 144) has been identified as strong antioxidant using GC-MS and DPPH radical scavenging activity by Xiangying *et al*. [45] Compound 2-pyrrolidinone (retention time 5.32, area percentage 3.98%, and molecular weight 85) has been identified using GC-MS and HPLC in rich extract of *Brassica oleracea var. capitate*. The 2-pyrrolidinone rich extract exhibited high antioxidant activity against different cell lines (IC_{50}

for 2-pyrrolidinone was 2.5 µl/ml for Hela, 3 µl/ml for PC-3 cells at 24 h, and 1.5 µl/ml for Hela, 2 µl/ml for PC-3 cells at 48 h [46].

Conclusions and recommendation

Phytochemical screening of *Ricinus communis L.* leaves and sedes confirm that this plant is rich of different phytochemical compounds such as cardinoloids, triterpens, sterols, flavonoids, and saponin. These compounds are beneficial for the human body they function as cancer risk reducers, antidiabetics, anti-inflamatory agents, blood pressure and cholesterol reducers, membrane reinforcers and protectors.

Antioxidant activity of methanol extract of seeds and leaves are high meanwhile petroleum ether and chloroform extracts of both leaves and seeds are weak.

GC-MS analysis revealed that the methanol extract of leaf contain high amount of hexadecanoic acid (palimitic acid)., 4-methoxy-1-methyl-2-oxo-1,2-dihydopyridine-3-carbonitrile (N-methylricinine)., gamonelic acid., 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one., and 2-pyrrolidinone and these compounds are known to have high antioxidant activity.

Further chromatographic separation of methanol extract to isolate pure compounds is recommended to identify the compound (s) which responsable for high antioxidant activity of the metanol leaf extract.

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