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Microscopical, phytochemical, and LC/MS analysis of *Ginkgo biloba* leaves

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Abstract

Every medicinal practitioner knows Ginkgo as the plant source of an extract that is good for memory improvement. *Ginkgo biloba* extract is classified as one of the medicines in the treatment of dementia and social exclusion brought by vascular or neurodegenerative disorders. In such disorders, the extract was reported to be successful in improving symptoms such as depression, attention, memory disturbances, vertigo, tinnitus, and anxiety. The aerial part of *Ginkgo bioloba* was obtained from China, Utilizing fresh leaves allows for a microscopic inspection, the concentrated extracts by soxhlet were screened by standard methods for the qualitative investigation of secondary metabolites present in the plant, and a small quantity of extract was analyzed by LC/MS instrument. Microscopical examination shows diacytic stomata, helical vessels, fiber, and unicellular unbranched trichomes. Qualitative analysis is positive for tannin, glycoside, flavonoid, terpene, and phenolic compounds detected while saponin, coumarin, and alkaloid gave a negative result. While LC/MS shows important compounds that have important biological activities such as phenolic acids, flavonoids, and flavonoid glycosides which are reported for their different pharmacological activity, the Ginkgo plant is a promising drug that can help in the treatment of different diseases and required further studies.

Keywords

Ginkgo bioloba; LC/MS; Metabolites; Qualitative analysis

Introduction

Every medicinal practitioner knows Ginkgo as the plant source of an extract that is good for memory improvement (1). Ginkgo is an ancient tree that belonged to millions of years ago and was able to survive for the present days in environmental conditions where close relatives had failed (2). Although Ginkgo prefers to grow in a warm, muggy, and depleted setting it can survive even in extreme cold weather (−3.3 °C) and under conditions of yearly precipitation. Moreover, Ginkgo was reported to possess high resistance to fire, ice, and air pollution, low salt levels, and heavy metals. In addition, the tree is susceptible to a few mold diseases (3). Nowadays, Ginkgo is grown in many world areas as a street tree in rural and urban landscapes or as a medicinal plant for the manufacturing of Ginkgo containing food supplements (4). The survival of the Ginkgo tree is thus one of the delightful gifts from nature to human beings.

Ginkgo biloba extract (EGb76) is classified as one of the medicines in the treatment of dementia and social exclusion brought by vascular or

neurodegenerative disorders. In such disorders, the extract was reported to succeed in improving symptoms such as depression, attention, memory disturbances, vertigo, tinnitus, and anxiety (5-7). Interplaying in the pathogenesis of neurodegenerative diseases are many factors. Abnormally metabolized apolipoprotein E, hyperphosphorylated Tau protein, and amyloid beta accumulation in cerebral parenchyma are the main pathogenic factors (8). In addition, inflammatory influences, oxidative stress, aberrant microglial function, and injury to cholinergic neurons are reported participants throughout the development of Alzheimer's illness (AD) (9-13). These effects of the extract are related to the presence of flavonoids (22.0-27.0%) and the platelet-activating factor antagonist terpenoids (5.0-7.0%). The important antioxidant flavonoids present in the extract are the flavonols isorhamnetin, kaempferol, myricetin, quercetin, and rutin. These portion of the mixture directly scavenge reactive oxygen species (ROS) and induce the expression of antioxidant enzymes such as superoxide dismutase (SOD) and glutathione reductase (GSH) which ultimately reduces the oxidative stress underlies the inflammatory scenario of AD (14, 15). The terpenoid fraction of the extract is composed of ginkgolides A, B, and C (.8-3.4%), bilobalide (2.6-3.2%), and less than 5 ppm of allergen ginkgolic acid) (16). Ginkgolide B antagonizes platelet-activating factor and hence, prevents the initiation of ROS, in addition to being a powerful ROS scavenger. Moreover, Ginkgolide B protects neurons against glutamate-induced excitotoxicity in cultured hippocampal neurons (17). Owing to these pharmacological actions and others, *Ginkgo biloba* extract helps in improving memory and learning ability, maintaining blood flow, reducing blood viscosity, and minimizing hypoxic damage in brain cells (18).

The medicine is tolerable at therapeutic doses; however, it causes gastric discomfort, allergy, and possible bleeding in patients under anticoagulant therapy or undergoing surgery (19).

Materials and Methods

Plants material

The aerial part of *Ginkgo bioloba* was obtained from China, the leaves were rinsed with distilled water after removing any remaining dirt or dust, then dried at 25 ºC for 14 days before being ground into a powder and weighed in preparation for additional research.

Microscopical examinations

Utilizing fresh leaves allows for a microscopic inspection, A Thin layer is scraped from the bottom of the leaves by a blade and placed on a slide, After adding and removing 2 drops of chloral hydrate 2-3 times to lighten the color and make the image more clear, the slide was covered, heated over a heater and then inspected under a microscope (20).

Preliminary phytochemical screening

The preliminary investigation was done after 20 g of shade-dried pulverized leaves of the plant were put inside a soxhlet apparatus thimble for extraction with 250 mL of absolute ethanol until the solvent became approximately colorless inside the soxhlet chamber and then the yield was filtered, concentrated by rotary evaporator at 45 °C, the concentrated extracts were screened by standard methods for the qualitative identification of active ingredients present in the plant (21).

Sample preparation for LC analysis

The sample was prepared by the addition of 2.0 mL of DMSO to a small quantity of extract, then brought up to 50 mL with acetonitrile. Each sample is then centrifuged for 3 min. about 4000 rpm. We then put 1.0 mL into an autosampler and injected about 3 uL.

Instrumentation LC/ MS parameters:

A Daltonik made by Bruker (Bremen, Germany) Compounds of interest were screened using an Impact II ESI-Q-TOF System outfitted with a Bruker Dalotonik Elute UPLC system (Bremen, Germany). We employed standards for m/z identification using Bruker TOF MS high resolution and precise analyst retention times after the separation process by chromatography.

A Bruker Solo 2.0_C-18 UHPLC column (100 mm x 2.1 mm x 2.0 µm) has been used for separation by chromatography, with a flow rate of 0.51 mL/min and a 4 °C temperature of the column. Eluent: acetonitrile and water containing 0.05% formic acid.

Gradient: 5% - 80% B linear gradient in 0–27 min; 95% B in 27–29 min; 5% B in 29.1 min; overall analysis time: 36 min at positive mode and 36 min at negative mode (3 uL) of injection.

The Ion Source Apollo II ion Funnel electrospray source has been used to power the MS apparatus. The nitrogen flow rate in dry gas was 8 L/min, the nebulizer gas pressure was 2.0 bar, the capillary voltage was 2500 V and the dry temperature was 200 ºC. The TOF repetition rate was up to 20 kHz22, the mass has a high resolution of about 50000 FSR (Full Sensitivity Resolution) and the accuracy of mass was^1 part/million (22).

Results and Discussion

Microscopical examination

The leaves of *Ginkgo bioloba* were diagnosed under the microscope by a diacytic stomata, helical vessel, fiber, and unicellular unbranched trichomes as clarified (Fig. 1).

Qualitative assessment results

Different tests for quality screening were made to determine the phytochemical ingredients of ginkgo leaves; these tests provide crucial details about the kinds of secondary metabolites found in plants that tannin, glycoside, flavonoid, terpene, and phenolic compounds are positively detected while saponin, coumarin, and alkaloid gave negative results and these results resemble many data reported in different previous studies (23-25)

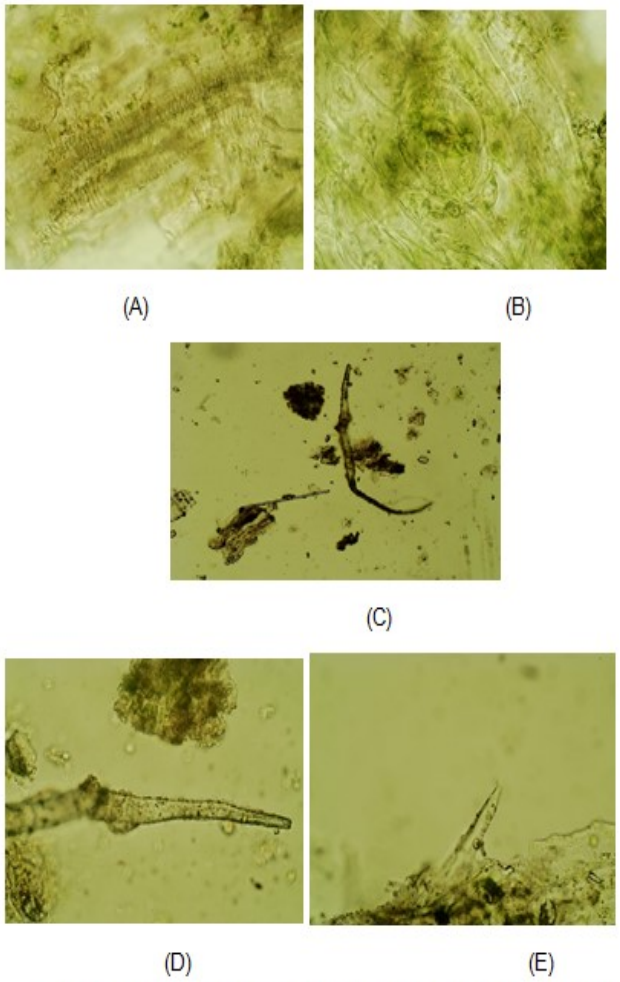

Fig. 1. A- helical vessel, B- diacytic stomata, C- fiber, and D, E-Unicellular unbranched trichomes.

(Table 1).

Identification of active compounds by LC/MS-MS

Since LC/MS-MS is a highly sensitive and accurate procedure used for active ingredients identification, it has been used for the identification of the ethanolic extract of plant leaves (26-28), LC/ MS-MS chromatogram is shown in Fig. 2 and the mass spectrum for compounds appeared in this part shown in (Fig. 3-6), while the list of compounds which are found in each part of the plant shown in (Table 2).

The results show important compounds that have important biological activities include phenolic acids (vanillic acid, caffeic acid, and p-Coumaric acid), flavonoids and flavonoid glycosides (Hispidulin, Apigetrin, Rutin, 3 -O-Neohesperidoside Kaempferol, 3-O-Neohesperidoside-7-Rha Kaempferol and 3-O-Neohesperidoside-7-Rha Quercetin which are reported for their antioxidant, anti-inflammatory, anticarcinogenic activity, anti-ischemia reperfusion, anti-thrombosis, anti-hypertension, anti-fibrosis, antimicrobial, antivirus, antihypertensive, antidiabetic, cytoprotective, vasoactive,

Table 1. Qualitative profile for phytochemicals found in *Ginkgo bioloba*

Phytochemical compound	presence
Saponin	Negative
Tannin	Positive
Glycoside	Positive
Coumarin	Negative
Flavonoid	Positive
Terpene	Positive
Alkaloid	Negative
Phenolic	Positive

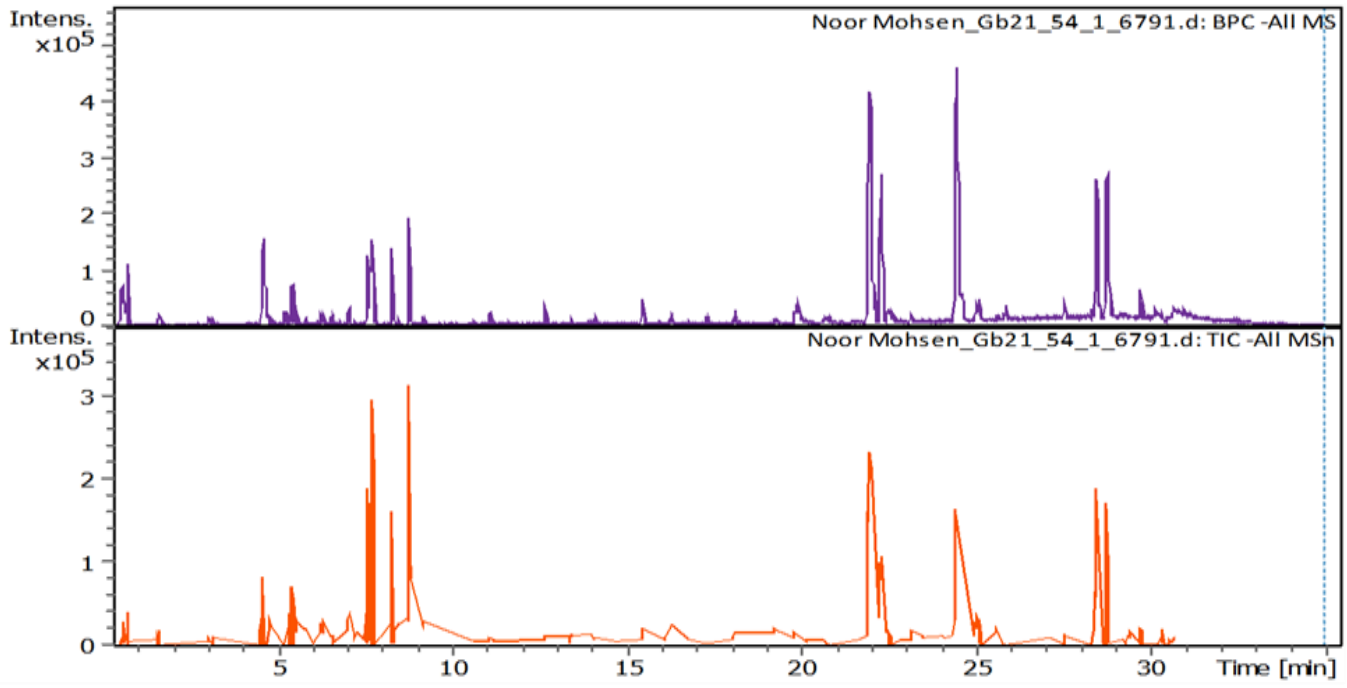

Fig. 2. LC/MS-MS chromatogram of leaves extract.

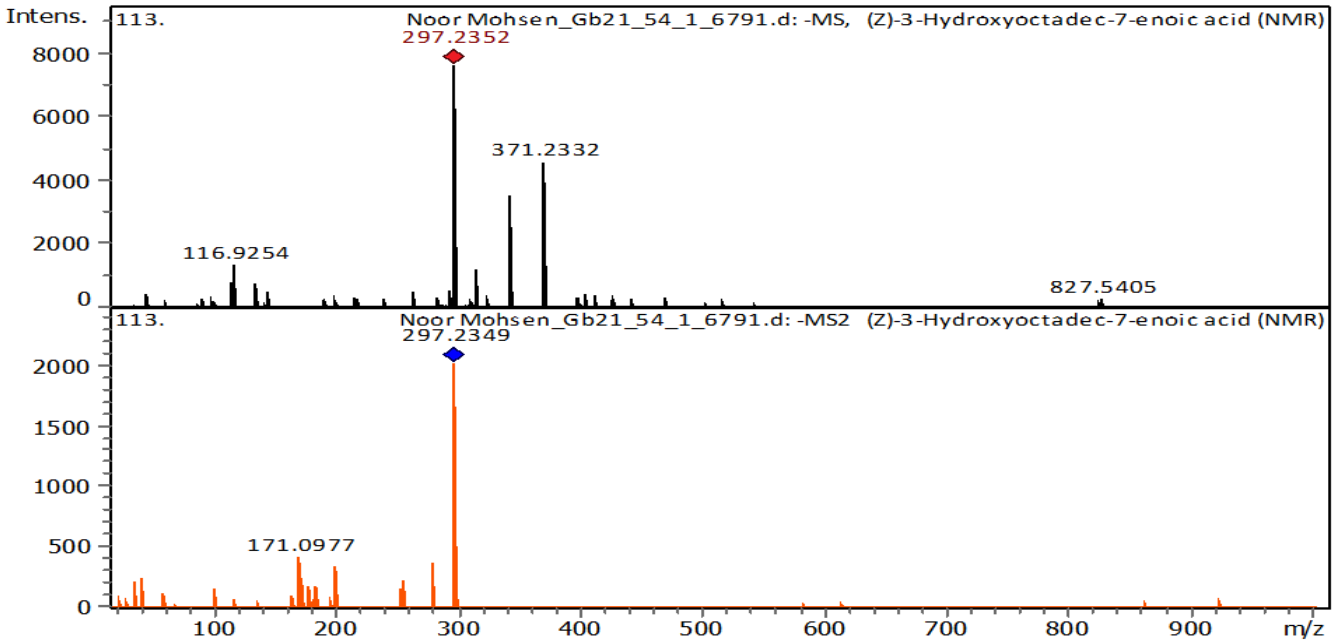

Fig. 3. The mass spectrum of (Z)-3-Hydroxyoctadec-7-enoic acid.

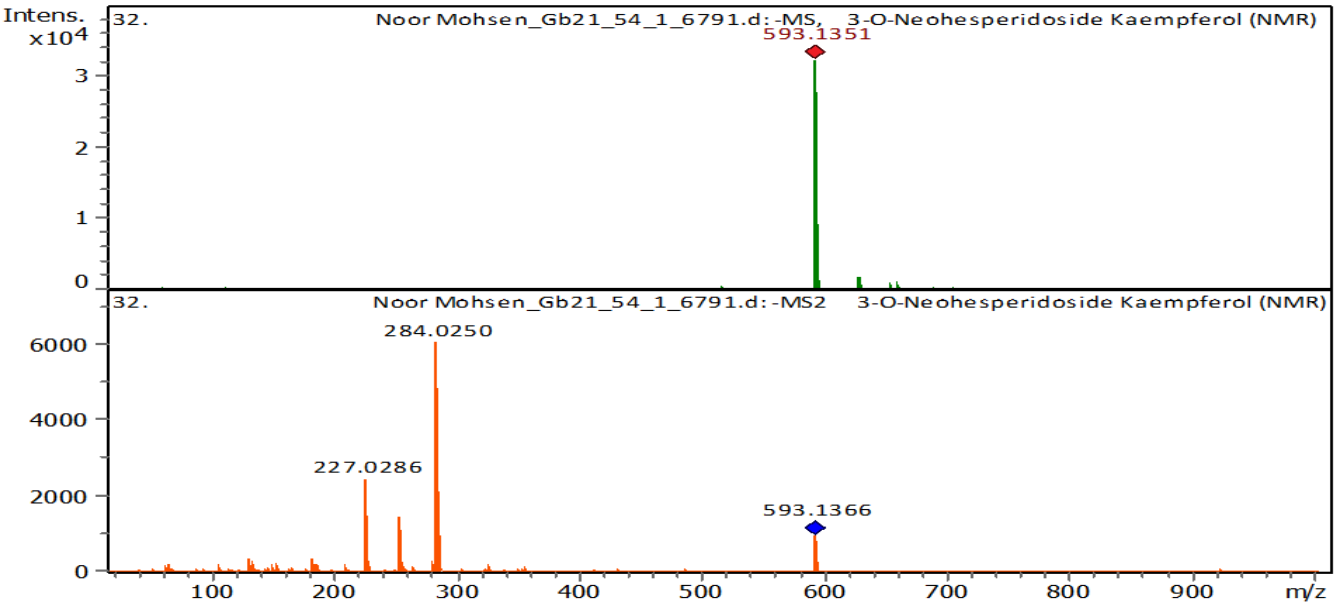

Fig. 4. The mass spectrum of 3-O-Neohesperidoside Kaempferol.

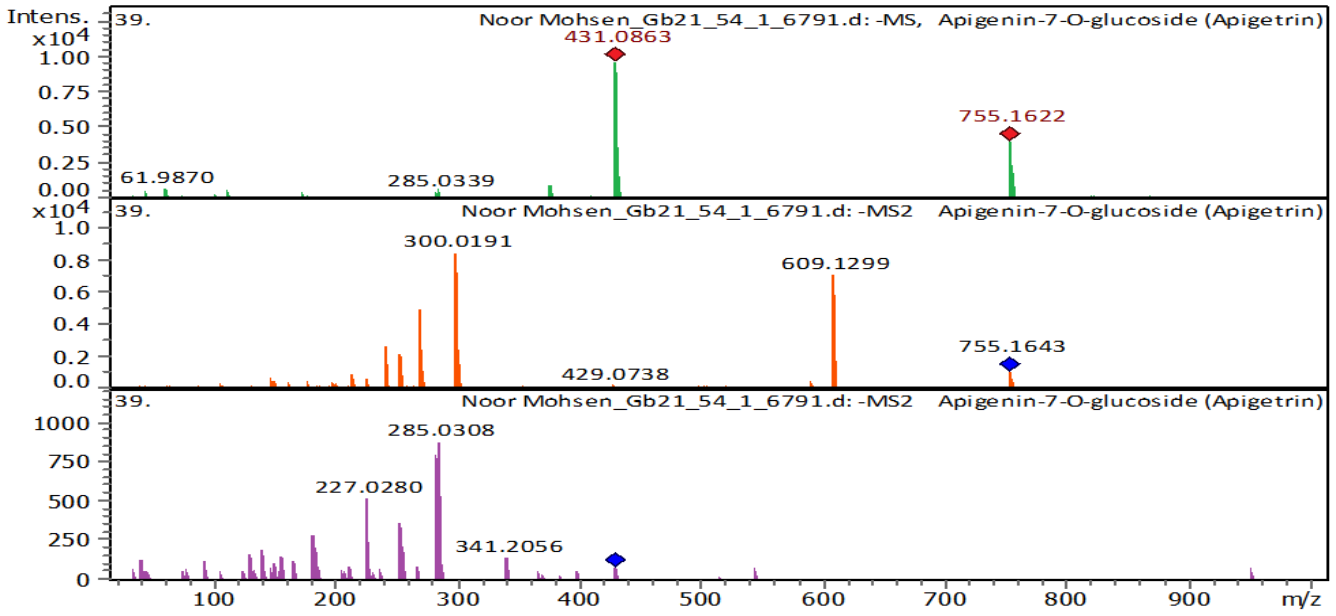

Fig. 5. The mass spectrum of Apigenin-7-O-glucoside (Apigetrin).

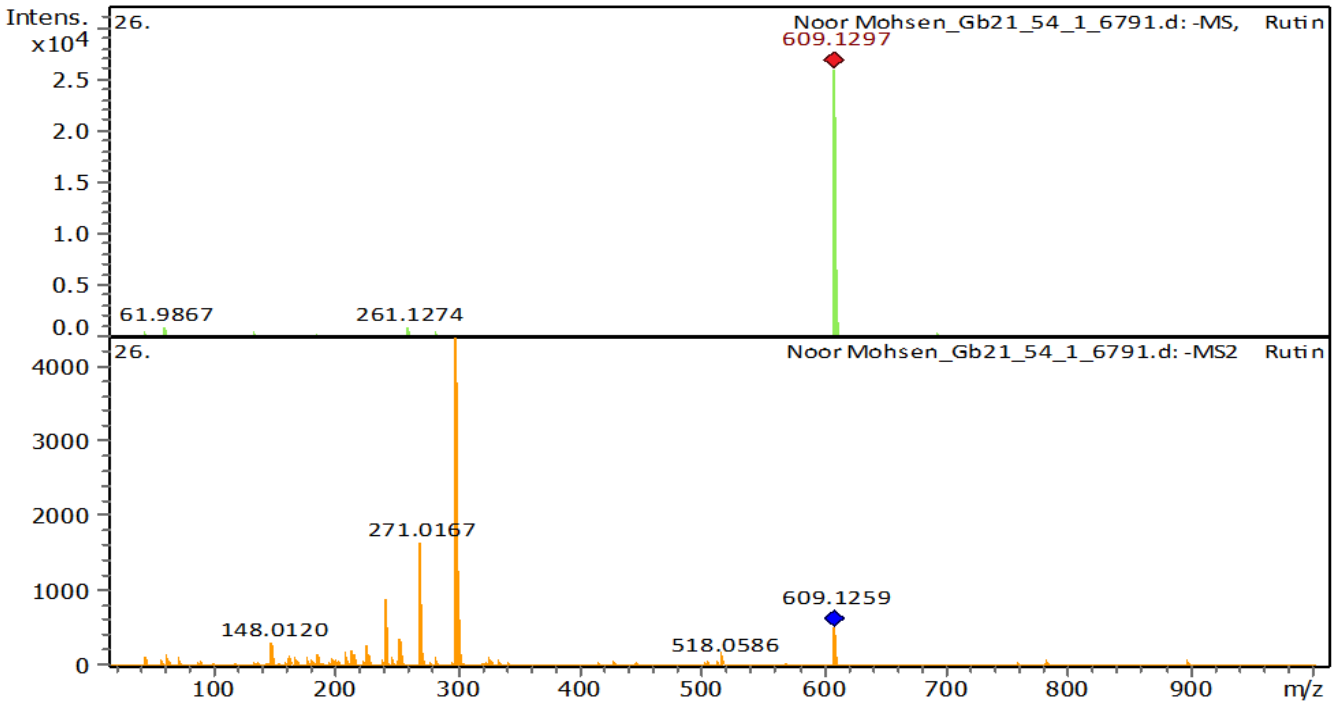

Fig. 6. The mass spectrum of rutin.

Table 2. Chemical compounds showed by LC-MS/MS in ethanolic leaves extract of *Ginkgo bioloba*

Compound	RT (min)	m/z meas.
Succinic acid	0.92	117.01998
(4 or 7) Hydroxy-Coumarin Plus Hydrate	6.97	161.02396
p-Coumaric acid	4.38	163.04063
Vanillic acid	4.27	167.03547
Caffeic Acid	3.18	179.03517
Caffeic Acid	3.76	179.03553
10E, 12Z-Linoleic acid	29.6	279.23314
Dihydrokaempferol	6.52	287.05578
(Z)-3-Hydroxyoctadec-7-enoic acid (NMR)	29.22	297.24333
Hispidulin	10.24	299.05516
Apigenin-7-O-glucoside (Apigetrin)	7.5	431.09699
3-O-Neohesperidoside Kaempferol (NMR)	7.07	593.15352
Rutin	5.59	609.14856
Rutin	6.28	609.14857
3-O-Neohesperidoside-7-Rha Kaempferol (NMR)	5.38	739.20517
3-O-Neohesperidoside-7-Rha Quercetin (NMR)	4.82	755.20035

hypolipidaemic, antiplatelet, antispasmodic and antitumor properties (29-37).

Conclusion

The Ginkgo plant is a promising drug that can aid in the healing of different illnesses and requires further studies.

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Authors' contributions

ER carried out the extraction and phytochemical studies, participated in the sequence alignment, and drafted the manuscript. HA carried out a microscopical examination. NM participated in the sequence alignment and LC/MS data analysis. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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