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Research Article

Phytochemical Characterization of Extracts from *Fagraea Fragrans* and *Juglans Regia* by GC-MS Analysis

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Abstract: *Fagraea fragrans* and *Juglans regia* have several therapeutic applications. The present study aimed to analyze the phytochemical compositions of a methanolic crude extract from the stem bark of *F. fragrans* and an oily liquid from a chloroform extract of seeds of *J. regia*. Approximately, 220 g of methanolic crude extract (yield = *ca.* 5%) was obtained from the stem bark of *F. fragrans* from hot-solvent extraction, and approximately, 45 g of chloroform oily liquid (yield = *ca*. 1.5%) was obtained from the seeds of *J. regia* by maceration technique. The phytochemical compositions of these two extracts were analyzed separately by injecting an aliquot of 1.0 µL solution of each extract prepared in hexane to a GC-MS instrument fitted with a DB-5 column of the dimension 50 m × 0.25 mm, i.d., 0.25 µm film thickness. The presence of fourteen components, including fragrant aldehydes and bioactive sterols, was identified in the methanolic crude extract and the presence of eight components, including fatty acids, fatty esters, and γ-tocopherol, was identified in the chloroform oily liquid. For the first time, we report the presence of γ-tocopherol (3.46%), *n*-hexanoic acid (1.26%), ethyl hexadecanoate (4.70%), ethyl oleate (6.59%), and a high content of ethyl linoleate (29.93%) in this chloroform oily liquid. From this study, we concluded that *F. fragrans* and *J. regia* have bioactive compounds of pharmaceutical importance. Therefore, these compounds can be extracted from these plants for therapeutic applications.

***Keywords*:** *Fagraea fragrans*, Loganiaceae, *Juglans regia*, Juglandaceae, GC-MS analysis

1. Introduction

Fagraea fragrans belongs to the Loganiaceae family.[1,2] *F. fragrans* is indigenous to South and South East Asia, which includes India, Thailand, Burma, Vietnam, Cambodia, Laos, Malacca, Indonesia, and the Philippines.[3] *F. fragrans* is commonly found on dry forested slopes and in thickets at low altitudes.[3] *F. fragrans* is a tall tree with branches growing upwards from the main trunk. The leaves are elliptical and narrow towards the tip. The flowers appear as large bunches. The flowers begin creamy white and turn yellow on maturation.[1,2] They are very fragrant, especially in the late evening, and attract night-flying moths, which help with pollination.[1,2] The tree flowers towards the middle of the year and again at the end of the year.[1,2] The roundish fruits are small and are found in small bunches. They start as orange and turn to scarlet on maturity.[1,2] The thick and hard bark of *F. fragrans* contains an alkaloid, which is isomeric with

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strychnine and possesses similar properties.[3] Indigenous people have been used the bark as a febrifuge, especially to attack against *febris acuta*.[3] A traditional medicine in Cambodia uses the infusion of bark.[3] In Malacca, the bark has also been used to treat malarial fevers.[3] The presence of alkaloids in the leaves and fruits of *F. fragrans* have been reported.[4] The isolation of a pure alkaloid *viz*. gentianine has also been reported from the leaves and fruits of *F. fragrans*.[4] Stigmasterol and *β*-sitosterol have been isolated from the stem bark of this plant.[2] Our literature study showed that *F. fragrans* has not been extensively studied for its phytochemical compositions and pharmacological activities. Therefore, in this study, we aimed to analyse the phytochemical compositions of a methanolic extract obtained from the stem bark of *F. fragrans* collected in the Republic of Singapore by GC-MS. The presence of aromatic compounds, fragrant aldehydes, fatty acids, esters of fatty acids, and bioactive sterols was identified from this methanolic crude extract. Stigmasterol (24.565%), *β*-sitosterol (11.767%), and linoleic acid (4.595%) were found to be the major compositions in this methanolic crude extract. To the best of our knowledge, this is the first report of this kind especially the species gathered from the Republic of Singapore.

Juglans regia belongs to the Juglandaceae family.[2] *J. regia* is also known by other names such as walnut, common walnut, English walnut, and Persian walnut. *J. regia* grows up to 10-25 meters in height.[5] They have pinnately compound leaves with male and female flowers appearing on them separately. *J. regia* has hard stone Like fruits and a soft husk covers the nutmeat.[6] *J. regia* is found primarily in temperate areas.[6-8] *J. regia* can stand the cold weather up to -11 °C during winter dormancy.[6] The vegetative origin of the *J. regia* is the Eastern Balkans to the Himalayas and South-Western China.[9] However, the common walnuts have commercially been cultivated in many parts of the world, which include Southern and Eastern Europe, North and South America, Japan, China, Iran, and the foothills of the Himalayas.[7-11] *J. regia* finds many therapeutic applications in traditional medicine. The seeds are rich in many nutritious phytochemicals.[12] They are good sources of riboflavin, niacin, thiamine, pantothenic acid, vitamin E, vitamin B, melatonin, ellagic acid, carotenoids, and polyphenols.[12] These compounds have potential health effects against ageing, cancers, heart and brain stroke, inflammations, and neurological illnesses.[12-14] The fats in the seeds of *J. regia* have a beneficial effect on the formation of albumin in the body.[2] In traditional Chinese medicine, the seeds of *J. regia* have been used for relieving asthma.[2] The seeds of *J. regia* are also a good source of melatonin[15] and the increase in blood melatonin levels has a strong correlation with increased antioxidative capacity.[15] The roots of *J. regia* have been used to treat diabetes, the leaves have been used to treat fever, diabetes, rheumatic pains, and skin diseases, and the flowers have been used to treat rheumatic pain and malaria.[7,8,11,16] Additionally, various extracts from various parts of this plant have been exhibited significant bioactivities against experimental animals, which include antihypoglycaemic, anti-analgesic, antimicrobial, lipid Lowering activities, lipid peroxidation reduction, serum catalase levels elevation, antinociceptive and antiallodynic neuropathic pain.[4,17-20] Fatty acids such as oleic, linoleic, linolenic, palmitic, stearic, saturated, unsaturated, and cyclopropyl fatty acids have been reported from various extracts from the seeds of *J. regia*.[4,21-23] Additionally, various classes of secondary metabolites such as terpenoids, steroids, phenolics, quinones, flavonoids, flavonoid glycosides, carbohydrates, ascorbic acid, adenosine, and adenine have also previously been reported from various parts of *J. regia*.[24-32] The GC-MS phytochemical analysis of various solvent extracts *viz.* petroleum ether, hexane, ethyl acetate, chloroform-methanol (2:1, v/v) and chloroform-methanol-water (2:2:1.8, v/v/v) extracts prepared from the seeds of *J. regia* have previously been reported.[4,21-23] However, to the best of our knowledge, chloroform oily liquid extracted from the seeds of *J. regia* has not been reported previously. Therefore, in this study, we also aimed to analyze the phytochemical compositions of chloroform oily liquid obtained from the seeds of *J. regia* by GC-MS. The presence of eight components including fatty acids, fatty esters and *γ*-tocopherol were identified in this chloroform oily liquid. For the first time, we report the presence of *γ*-tocopherol (3.46%), *n*-hexanoic acid (1.26%), ethyl hexadecanoate (4.70%), ethyl oleate (6.59%), and a high content of ethyl linoleate (29.93%) in this chloroform oily liquid. The results are summarized in this article.

2. Materials and methods

2.1 *Plant materials*

A branch of *F. fragrans* weighing about 5 kg was cut from a tree inside the National University of Singapore (NUS) campus, Republic of Singapore. Associate Prof. Hugh Tan Tiang Wah, Department of Biological Sciences, NUS and

Mr. Chua Keng Soon, Senior Laboratory Officer (RMBR), Herbarium, NUS, identified the plant material. A voucher specimen (KM20041122) was deposited in the Herbarium, Department of Biological Sciences, NUS, Singapore. Approximately, 3 kg seeds of *J. regia* were purchased from a local market in Singapore and a voucher specimen (KManoSJ2003) was deposited in the Herbarium, Department of Biological Sciences, NUS, Singapore.

2.2 *Processing of plant materials*

In the case of *F. fragrans*, the leaves and twigs were removed from the branch using a chopper. The branch was then chopped into small pieces and allowed to air-dry at room temperature for three weeks. In the case of *J. regia*, the seeds were already purchased in dry condition and therefore, they were directly ground into a coarse powder using a pestle and mortar.

2.3 *Preparation of a crude extract from F. fragrans and an oily liquid from J. regia*

Approximately, 1.5 kg air-dried stem barks of *F. fragrans* and 2.5 L methanol were taken in a 5 L round-bottomed flask. The mixture was refluxed for 12 h in a fume-hood. The solution was filtered through a Whatman No.1 filter paper and the solvent was removed in *vacuo*. The methanolic crude extract thus obtained was kept separately in a china dish. The same plant material was again refluxed twice with methanol as previously and the crude extract was combined. A similar extraction procedure was repeated until all plant material was extracted with methanol. A combined methanol crude extract of *ca.* 220 g (yield = *ca.* 5%) was obtained and was stored at room temperature (29-31 °C) in a fume-hood before analysis.

Approximately, 1.5 kg seeds of *J. regia* and 2 L chloroform were taken in a 5 L round-bottomed flask. The mixture was macerated, i.e., extracted with chloroform at room temperature (29-31 °C) with occasional shaking in a fume-hood for 12 h. The solution was filtered through a Whatman No.1 filter paper and the solvent was removed in *vacuo* using a Buchi Rotavapor. The chloroform extract thus obtained was kept separately in a china dish. The same seed material was again macerated three times with chloroform as previously and the extracts were combined. The extraction procedure was repeated with the remaining 1.5 kg seeds of *J. regia* under the same extraction conditions as previously. The combined extract was left to stand for a few days in a fume-hood. A gum Like residue was settled at the bottom of the china dish and an oily liquid was settled on the top. Therefore, the oily liquid (*ca.* 45 g; yield = *ca*. 1.5%) was separated from the residue by simple decantation.

2.4 *Analysis of phytochemical compositions*

The GC-MS analysis of extracts from *F. fragrans* and *J. regia* was conducted on an Agilent GC-MS instrument. The injector was connected to a DB-5 column of the dimension 50 m × 0.25 mm, i.d., 0.25 μm film thickness and the system was fitted to a flame ionization detector (FID). The following temperature program was used. The oven temperature was fixed initially at 150 °C for 20 min and then increased by 10 °C/min until 200 °C, maintained for 5 minutes at this temperature and then increased by 5 °C/minutes up to 290 °C, which was maintained for a further 10 min. Helium was used as the carrier gas and was pumped through the column at a constant flow rate of 1 mL/minute. Approximately, 0.05 mg or 0.05 mL of each sample was dissolved in 5 mL of hexane. Each solution was filtered through a membrane filter (MF Millipore, pore size is 0.45 μm) to remove insoluble contaminants and other particulate matter. Aliquot of 1.0 μL of each solution was injected separately in the GC-MS column.

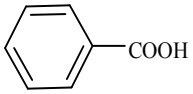
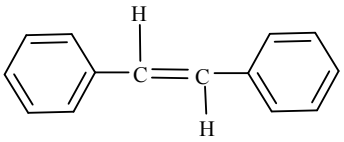
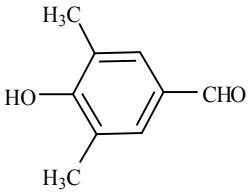
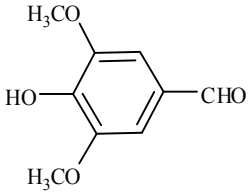
2.5 *Identification of phytochemical compositions*

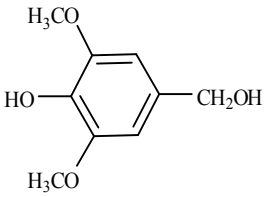
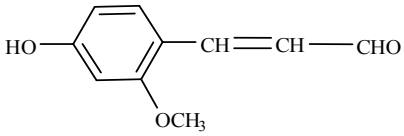
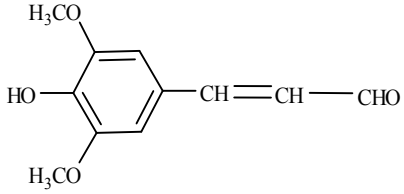
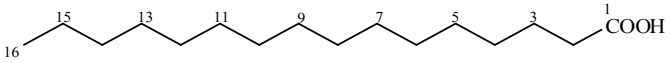
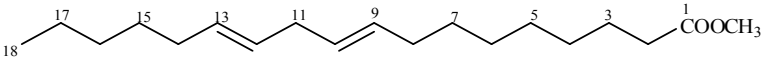
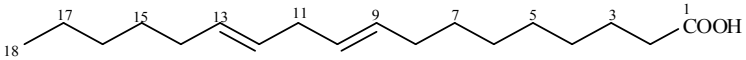
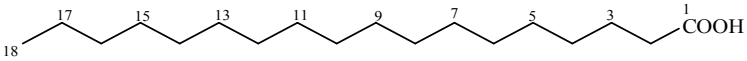
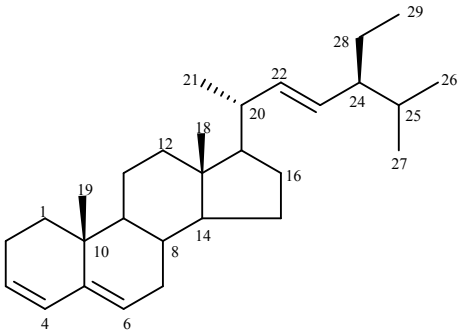
The phytochemical composition of each extract was identified by the direct comparison of their MS spectra with NIST library data. The relative quantities of individual compounds were calculated based on GC peak areas without using correction factors.

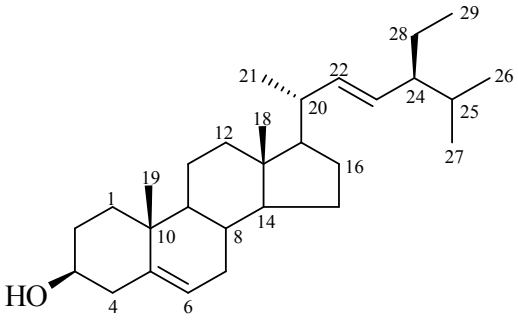
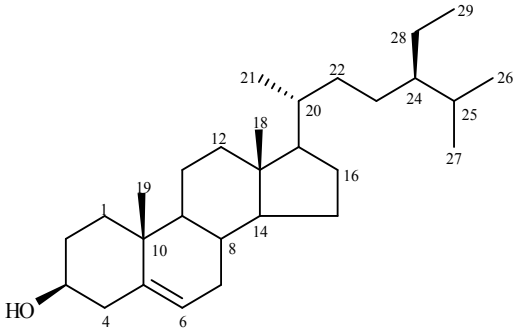
3. Results and discussion

GC-MS analysis of the methanolic crude extract from the *F. fragrans* showed the presence of fourteen compounds *viz.* benzoic acid, trans-stilbene, 4-hydroxy-3,5-dimethylbenzaldehyde, 4-hydroxy-3,5-dimethoxybenzaldehyde, 5-hydroxy-3,5-dimethoxybenzyl alcohol, 4-hydroxy-2-methoxycinnamaldehyde, 4-hydroxy-3,5-dimethoxycinnamaldehyde, *n*-hexadecanoic acid, methyl 9,12-octadecadienoate, 9,12 (Z, Z)-octadecadienoic acid, hexadecanoic acid, stigmasterol, stigmastan-3,5,22-triene and sitosterol. These fourteen compounds are listed in Table 1 in the order of their elution in the column. Our literature search showed that only a few reports on the phytochemical compositions and pharmacological activities of *F. fragrans* have been available. The presence of alkaloids in the leaves of *F. fragrans* has been reported.[33] At the beginning of the flowering season (May-June), the percentage of alkaloids content in the leaves has been found to be highest (0.32%) and lowest (0.005%) after fruiting had occurred (October-November). The presence of 0.10% alkaloids has been reported from the ripe fruits.[33] An alkaloid gentianine has been isolated from the leaves and fruits of *F. fragrans*.[33] However, this alkaloid isolated from other species is reported to have various pharmacological activities including the potential anti-inflammatory effect on experimental animals.[34] Stigmasterol and *β*-sitosterol have been isolated in a pure state from the stem bark of this plant.[2] Our study showed the presence of fragrant aldehydes and bioactive sterols in this methanolic crude extract (Table 1). Stigmasterol (24.565%) and *β*-sitosterol (11.767%) were identified as the main components (Table 1). Stigmasterol has a wide range of metabolic and therapeutic effects on animals and humans.[35] *β*-Sitosterol is another common bioactive phytosterol that also exhibited a wide range of biological and pharmacological activities.

Table 1. The GC-MS phytochemical analysis of a methanolic crude extract from stem-bark of *F. fragrans*

Peak No.	Rt (min.)	% of Area	Compounds identified	Structures of compounds
1	8.71	2.240	Benzoic acid	
2	13.72	0.793	*E*-Stilbene	
3	14.63	0.319	4-Hydroxy-3,5-dimethylbenz-aldehyde	
4	14.87	0.221	4-Hydroxy-3,5-dimethoxy-benzaldehyde	

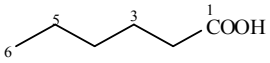
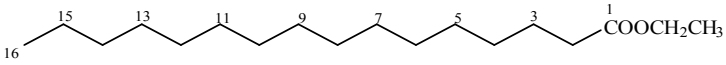
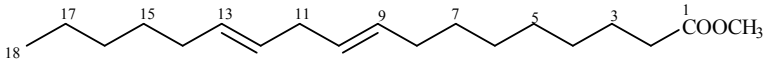
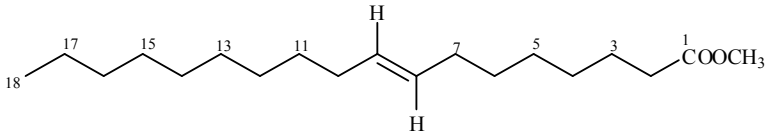
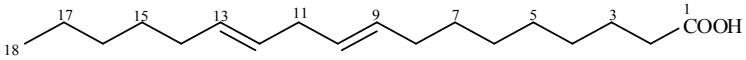
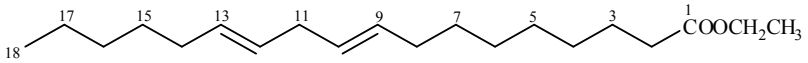
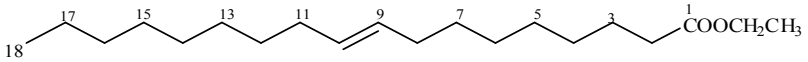
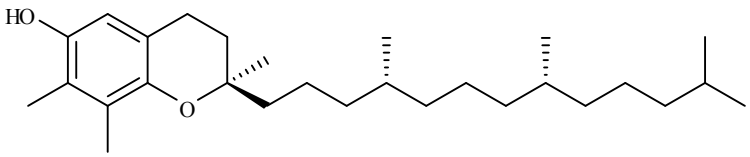
5	15.21	0.457	4-Hydroxy-3,5-dimethoxybenzyl alcohol	
6	15.48	0.458	4-Hydroxy-2-methoxycinnamaldehyde	
7	18.10	0.543	4-Hydroxy-3,5-dimethoxycinnamaldehyde	
8	18.46	1.407	*n*-Hexadecanoic acid (Palmitic acid)	
9	20.42	0.310	Methyl 9,12-octadecadienoate	
10	21.03	4.595	9,12 (*Z*,*Z*)-Octadecadienoic acid (Linoleic acid)	
11	21.54	0.784	Octadecanoic acid (Stearic acid)	
12	34.02	0.435	Stigmastan-3,5,22-triene	

13	35.92	24.565	Stigmasterol	
14	36.20	11.767	*β*-Sitosterol	

Rt = Retention time in minutes

GC-MS analysis of the chloroform oily liquid obtained from the seeds of *J. regia* showed the presence of eight compounds *viz. n*-hexanoic acid, ethyl hexadecanoate, methyl 19, 12-octadecadienoate, methyl 8(*E*)-octadecenoate, 9,12 (*Z*, *Z*)-octadecadienoic acid, ethyl linoleate, ethyl oleate, and *γ*-tocopherol. These eight compounds are listed in Table 2 in the order of their elution in the column. Our study showed the presence of fatty acids, fatty esters, and *γ*-tocopherol in the oily liquid (Table 2). Fatty acids and their esters have exhibited many biological and pharmacological activities, which include antioxidant and antifungal effects.[36] Fatty acids such as linoleic, linolenic, oleic, palmitic and stearic acids have been reported as active constituents for such effects.[37,38] In the present study, in addition to linoleic acid (9.30%), the presence of esters of fatty acid such as ethyl hexadecanoate (4.70%), methyl 9,12-octadecadienoate (2.77%), methyl 8(*E*)-octadecenoate (16.55%), ethyl linoleate (29.93%) and ethyl oleate (6.59%) were identified. Among the fatty esters, ethyl linoleate (29.93%) was found to be a major component followed by methyl 8(*E*)-octadecenoate (16.55%). Ethyl linoleate has previously been reported in other studies. This ethyl linoleate has exhibited many biological and pharmacological activities, which include antibacterial, anti-inflammatory[39] and anti-acne agent.[40] Due to these potential activities, ethyl linoleate has been used in the cosmetic industry.[40] Additionally, ethyl linoleate showed a decrease in melanin production and exhibited tyrosinase activity in α-melanocyte stimulating hormone (α-MSH) B15F10 cancer cell lines.[40] Furthermore, ethyl linoleate has been used as a biomarker for identifying Foetal Alcohol Syndrome (FAS).[41,42] The second major component identified in this oily liquid was methyl 8-octadecenoate. This methyl 8-octadecenoate has previously been reported in other studies and it exhibited antioxidant, nematicide, pesticide, insectifuge, cancer preventive, hypochloesterolemic, antianhydrogenic, hemolytic, lubricant, and 5α-reductase inhibitor activities.[43] The other major component identified in this oily liquid was linoleic acid. This linoleic acid has been previously reported in other studies and it has been used to reduce acute inflammatory pain, chronic blood-glucose levels, serum insulin elevation, and normalize glycated hemoglobin levels.[4]

Table 2. The GC-MS phytochemical analysis of an oily liquid from seeds of *J. regia*

Peak No.	Rt (min.)	% of Area	Compounds identified	Structures of compounds
1	8.29	1.26	*n*-Hexanoic acid	
2	8.75	4.70	Ethyl hexadecanoate	
3	10.30	2.77	Methyl 9,12-octadecadienoate	
4	10.47	16.55	Methyl 8(*E*)-octadecenoate	
5	11.29	9.30	9,12 (*Z*,*Z*)-Octadecadienoic acid (Linoleic acid)	
6	11.75	29.93	Ethyl linoleate	
7	11.96	6.59	Ethyl oleate	
8	26.88	3.46	*γ*-Tocopherol	

Rt = Retention time in minutes

The GC-MS phytochemical analysis of various solvent extracts such as petroleum ether, hexane, ethyl acetate, chloroform-methanol (2:1, v/v) and chloroform-methanol-water (2:2:1.8, v/v/v) extracts prepared from the seeds of *J. regia* have been previously reported and are summarized in Table 3.[4,21-23] The chemical compositions and percentage of oil contents in the seeds *of J. regia* depend on several factors, which include collection from different geographic locations, cultivars, and irrigation rate.[21] The percentage of oil content can vary from 50-70%.[21]

Table 3. Phytochemical compositions of various solvent extracts obtained from the seeds of *J. regia* were collected at various locations[4,21-23]

S. No.	Locations of collections of seeds of *J. regia*	Solvent used	Compounds identified by GC-MS	Percentage	Reference
1	Western region of Romania	Petroleum ether	Fatty acids:		21
			Palmitic acid	9.70	
			Stearic acid	3.48	
			Oleic acid	13.62	
			9,12-Octadecadienoic acid (Linoleic acid)	56.57	
			Linolenic acid	12.09	
2	From a herbalist in Beirut, Lebanon	Hexane	Fatty acids:		20
			Palmitic acid	8.30	
			Palmitoleic acid	0.80	
			Stearic acid	1.90	
			Oleic acid	10.70	
			9,12-Octadecadienoic acid (Linoleic acid)	55.00	
			Linolenic acid	16.70	
			Volatile oils:		
			Heptadecane	14.90	
			Tetradecanes	13.30	
			Hexadecane	13.20	
			Octadecane	12.80	
			Nonadecane	11.00	
			Myristicin	10.60	
			Eicosane	7.70	
			Heneicosane	6.80	
			Pentadecanes	4.10	
			Others	traces	
			Sterols:		
			Cholesterol	0.009	
			Campesterol	0.063	
			Clerosterol	0.021	
			β-sitosterol	0.110	
			δ-5-avenasterol	0.027	

3	From a herbalist in Beirut, Lebanon	Ethyl acetate	Fatty acids:		20
			Palmitic acid	5.60	
			Palmitoleic acid	0.10	
			Stearic acid	2.80	
			Oleic acid	14.20	
			9,12-Octadecadienoic acid (Linoleic acid)	45.00	
			Linolenic acid	8.10	
			Arachidic acid	0.10	
			Volatile oils:		
			Heptadecane	10.20	
			Tetradecanes	0.60	
			Hexadecane	5.20	
			Nonadecane	7.30	
			Myristicin	11.80	
			Heneicosane	6.80	
			E-Pinocarveol	3.30	
			Myretenal	6.30	
			E-*β*-caryophyllene	8.30	
			Caryophyllene oxide	6.10	
			Heneicosane	2.00	
			Others	traces	
			Sterols:		
			Cholesterol	0.001	
			Campesterol	0.010	
			Stigmasterol	0.012	
			Clerosterol	0.220	
			β-sitosterol	0.110	
			δ-5-avenasterol	0.100	
			Others	traces	
4	Purchased from a local market in Jerusalem, Israel	Chloroform-methanol (2:1, v/v)	Fatty acids and fatty esters:		23
			Palmitic acid	4.81	
			Stearic acid	3.62	

			Oleic acid	21.34	
			9,12-Octadecadienoic acid (Linoleic acid)	65.40	
			9,12,15-(*Z,Z,Z*)-Octa decadienoic acid	6.11	
			Methyl-9,10-methylene octadecanoate	0.16	
5	Collected in Evia island, Greece	Chloroform-methanol-water (2:2:1.8, v/v/v) (Bligh-Dyer method)	Neutral lipids (fatty acids compositions as methyl esters):		22
			9,12-Octadecadienoic acid (Linoleic acid)	74.00	
			Hexadecanoic acid	10.40	
			9,12,15-Octadecatrienoic acid	10.00	
			Others	trace	
			Sphingolipids (fatty acids compositions as methyl esters):		
			9,12-Octadecadienoic acid (Linoleic acid)	55.60	
			9,12,15-Octadecatrienoic acid	19.40	
			Hexadecanoic acid	16.20	
			Octadecanoic acid	4.50	
			9-Octadecenoic acid	2.50	
			Others	traces	
			Phospholipids (fatty acids compositions as methyl esters):		
			9,12-Octadecadienoic acid (Linoleic acid)	50.90	
			Hexadecanoic acid	22.60	
			9,12,15-Octadecatrienoic acid	17.20	
			Octadecanoic acid	4.8	
			Octadecenoic acid	2.3	
			Others	traces	
			Sterols:		
			β-sitosterol	84.60	
			δ-5-avenasterol	7.30	
			Campesterol	4.60	
			Cholesterol	1.10	
6	Purchased from a local market in Singapore	Chloroform	Fatty acids and fatty esters		Present study
			n-Hexanoic acid	1.26	

Ethyl hexadecanoate	4.70
Methyl-9,12-octadecadienoate	2.77
Methyl-8(*E*)-octadecenoate	16.55
9,12-Octadecadienoic acid (Linoleic acid)	9.30
Ethyl linoleate	29.93
Ethyl oleate	6.59
γ-Tocopherol	3.46

Fatty acids such as oleic, linoleic, linolenic, palmitic, and stearic acids have been reported in a petroleum ether seed oil extract from *J. regia*.[21] The major component is linoleic acid (56.57%) (Table 3). However, in this study, linoleic acid was identified as the only component common to this chloroform seed oil extract and it was found to be only (9.30%). The major component identified in this chloroform extract was ethyl linoleate (29.93%) followed by methyl-8(*E*)-octadecenoate (16.55%). Hexane and ethyl acetate extracts from the seeds of *J. regia* revealed the presence of volatile components, several sterols, and fatty acids.[4] The major component identified in these hexane and ethyl acetate extracts was again linoleic acid with 55.00 and 45.00%, respectively (Table 3). Additionally, the hexane seed oil extract exhibited a more lipid peroxidation reduction, while the ethyl acetate seed oil extract exhibited a more acute blood-glucose level reduction and serum catalase levels elevation in experimental male Albino white mice.[4] At higher doses, both of these two oil extracts showed profound biological activities such as antinociceptive potentials and anti-allodynic neurapathic pain.[4] Linoleic acid (9.30%) was also identified in our chloroform seed oil extract, but we did not find the presence of sterols. The seed oil extract from the *J. regia* extracted by a solvent mixture of chloroform and methanol (2:1 v/v) showed the presence of several saturated and unsaturated fatty acids and a cyclopropyl fatty ester.[23] The presence of linoleic acid (65.40%) is the major component in this oil followed by oleic acid (21.34%) (Table 3). However, in our study, the presence of linoleic acid (9.30%) was identified but we did not identify the presence of cyclopropyl fatty ester. The extraction of total lipids has been reported from the seeds of *J. regia* by using Bligh-Dyer method (chloroform-methanol-water, 2:2:1.8, v/v/v,).[22] The resulting extract has been subjected to further fractionation by various chromatographic techniques.[22] The GC-MS analysis of these fractions showed the presence of sterols and various classes of lipids such as neutral lipids, sphingolipids and phospholipids (Table 3).[22] The presence of linoleic acid is the major component (50.90-74.00%) in all these fractions. As stated previously that linoleic acid (9.30%) was also identified in our chloroform seed oil extract but we did not find the presence of sterols. Additionally, the major components identified in our chloroform oily liquid were ethyl linoleate (29.93%) followed by methyl 8(*E*)-octadecenoate (16.55%). The comparative study (refer to Table 3) showed that linoleic acid has been found to be common to all studies but with various proportions. However, for the first time, we report the presence of *γ*-tocopherol (3.46%), *n*-hexanoic acid (1.26%), ethyl hexadecanoate (4.70%), ethyl oleate (6.59%), and a high content of ethyl linoleate (29.93%) in the chloroform oily liquid.

Several other secondary metabolites from various parts of *J. regia* have previously been reported.[24] For example, sesquiterpens, oxygentated sesquiterpens, *β*-caryophyllene, caryophyllene oxide, *β*-pinene, germacrene and alcohols have been identified from the leaves of *J. regia*.[25] Juglone (5-hydroxy-1,4-naphthalenedione), syringaldehyde, phenolic acids, chlorogenic acid, ellagic acid, caffeic acid, *p*-coumaric acid, syringic acid, sinapic acid and ferulic acid have been reported from ripe fruits.[26] 2-Methyl-1,4-naphthoquinone, 1,4-naphthoquinone, and plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone) have been reported from the husks of walnuts.[27,28] The presence of sterols[29], carbohydrates[29], and ascorbic acid[30] have been reported from various parts of the walnut tree. Flavonoid glycosides, hydrolyzable tannins together with adenosine and adenine have also been reported from this plant.[31,32]

The selection of solvent for extraction of plant materials depends mainly on the plant parts chosen for research as well as the objective of the research. In general, seeds are rich in non-polar (volatile hydrocarbons) and low polar (fatty acids and esters of fatty acids) lipid compounds. Additionally, seeds can also have medium and more polar lipids

(sphingolipids and phospholipids), sterols, and other compounds. Non-polar solvents such as hexane and petroleum ether have been used for extracting non-polar and low polar lipid compounds. Medium polar solvents such as chloroform, ethyl acetate, chloroform-methanol, and chloroform-methanol-water have been used for extracting medium polar compounds. These medium polar solvents also have the tendency to extract non-polar and low polar lipid compounds. Additionally, a mixture of solvents increases the extracting power of compounds present in the plant materials. Aqueous-based solvents such as water, ethanol-water, acetone-water, and methanol-water at various proportions and ethanol (100%) and methanol (100%) have been used for the extraction of more polar compounds. In the previous studies, extracts obtained from the seeds of *J. regia* using various solvents such as petroleum ether, hexane, ethyl acetate chloroform-methanol, and chloroform-methanol-water have been analyzed for their phytochemical compositions.[4,21-23] However, chloroform oily extract obtained from the seeds of *J. regia* has not been reported previously. Therefore, in the present study, chloroform was chosen as a solvent to extract oily liquid from the seeds of *J. regia*.

Plant materials such as stem barks, roots, and leaves are rich in various classes of secondary metabolites. Additionally, these parts of plant materials may also have a combination of any or all types of non-polar, low polar, medium polar, and more polar lipid compounds. Therefore, the selection of solvents for extraction from these parts of materials is based on the research requirement. Methanol (100%) has been regarded as a versatile solvent and it is one of the highest polar solvents among the common solvents. Furthermore, methanol, as a single solvent, could extract various classes of secondary metabolites and various types of non-polar, low polar, medium polar, and more polar compounds. Therefore, in this study, methanol was chosen as a solvent to extract as many as compounds possible from the stem bark of *F. fragrans*.

4. Conclusion

Both *F. fragrans* and *J. regia* find therapeutic applications in traditional medicine. For example, the stem bark of *F. fragrans* has been used to treat malarial fevers and *J. regia* has been used to treat asthma, diabetes, and malaria. In the present study, a methanolic extract obtained from the stem bark of *F. fragrans* was analyzed for phytochemical compositions by GC-MS. The presence of fourteen components including aromatic compounds, fragrant aldehydes, fatty acids, esters of fatty acids, and bioactive sterols were identified from this methanolic crude extract. Stigmasterol (24.565%), *β*-sitosterol (11.767%), and linoleic acid (4.595%) were found to be the major compositions in this methanolic crude extract. These identified compounds have been exhibited a variety of biological and pharmacological activities. In the case of *J. regia*, an oily liquid obtained from a chloroform extract of seeds of *J. regia* was analyzed for phytochemical compositions by GC-MS. The presence of eight components including fatty acids, fatty esters, and *γ*-tocopherol were identified from this oily liquid. For the first time, we report the presence of *γ*-tocopherol (3.46%), *n*-hexanoic acid (1.26%), ethyl hexadecanoate (4.70%), ethyl oleate (6.59%), and a high content of ethyl linoleate (29.93%) in this chloroform oily liquid. From this study, we concluded that both *F. fragrans* and *J. regia* showed the presence of bioactive compounds. Therefore, these compounds can be extracted from these plants for therapeutic applications.

Conflict of interest

The authors have no conflicts of interest to declare.

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