Ultrasonics Sonochemistry 88 (2022) 106048

Contents lists available at ScienceDirect

Ultrasonics Sonochemistry

journal homepage: www.elsevier.com/locate/ultson

Bioactive characterization of ultrasonicated ginger (*Zingiber officinale*) and licorice (*Glycyrrhiza Glabra*) freeze dried extracts

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ARTICLE INFO

Keywords:
Ginger
Licorice
Phytochemicals
HPLC
FESEM
FTIR

ABSTRACT

Ginger (*Zingiber officinale*) and Licorice (*Glycyrrhiza glabra L.*) are one of the most popular spices having a wide range of bioactive compounds that have varied biological and pharmacological properties. The study was aimed to extract polyphenols from Himalayan medicinal herbs ginger and licorice in different solvents using ultrasonication technique. The extraction efficiency (EE) was determined, and the extracts were characterized for physical properties (particle size, colour values), total phenolics, flavonoids, antioxidant properties, and structural and morphological features. Ultra-sonicated ginger in aqueous phase had the highest EE of polyphenols (15.27%) as compared to other solvents. Similar trend was observed in licorice with EE of 30.52 % in aqueous phase followed by ethanol: water (50: 50), and methanol: water (50:50) with 28.52% and 26.39%, respectively. The preliminary screening showed the presence of tannins, phenolics, flavonoids, saponins and carbohydrates, steroids and alkaloids in all the extracts. The phenolic and flavonoid content of dried ginger was found higher in ethanolic extracts compared to fresh ones as revealed by HPLC. Similarly, for licorice, the ethanolic fractions had the highest polyphenolic content. The representative samples of ginger (ethanol: water 75:25 and ethylacetate: water 75:25) and licorice (ethanol: water 70:30 and methanol: water 50:50) were studied for FESEM and particle size. The results showed the agglomerated extract micro-particles with a diameter of 0.5–10 μm and increased particle size (ginger: 547 and 766 nm), and (licorice: 450 and 566 nm). The findings could be beneficial for the advancement of ginger and licorice processing, for the comprehension of these herbs as a source of natural antioxidants in different food formulations.

1. Introduction

Natural products with promising antibacterial activity are the focus of numerous studies in modern medicine due to the overall trend toward searching for novel natural bioactive compounds and the rising resistance of infections to synthetic antibiotics. Additionally, the food sector is increasingly relying on natural ingredients. Ginger (Zing*iber officinale*) contains abundant phytochemicals and is primarily used in foods as antioxidant rich spice. The underground rhizome of the *zingiber officinale* plant is known as ginger or ginger root. Depending on the kind, the flesh ginger rhizome can be red, yellow, or white in colour. It has a brownish skin that can be thick or thin, and is eaten as a delicacy, medicine, or spice. Ginger consists of 40–70% starch, 1.5–3% essential oil, 6–20% protein, 2–11% fixed oil, 9–12% water and 8–10% ash, along with pungent principles and other saccharides [1] in addition it is a rich source of antioxidant and antimicrobial polyphenols and flavonoids [2]. Besides its food uses, ginger root has been discovered to aid in cholesterol reduction, arthritis pain treatment, digestive issues, expectorant properties, and gesture-intestinal stimulation [3]. According to Nicoll & Henein [4], the diseases viz. cardiovascular, stroke, diabetes, common cold, rheumatism, asthma, catarrh, gingivitis, toothache, and constipation, have all been treated with ginger in medicinal and therapeutic preparations for its pharmacological effects like anti-platelet, immune-modulatory, anti-tumour, anti-apoptotic, anti-inflammatory, antiviral,

Abbreviations: EE, Extraction efficiency; RP-HPLC, Reverse Phase High performance liquid chromatography; DPPH, 1 1-diphenyl-2-picrylhydrazyl; AOA, Antioxidant activity; QE, Quercetin equivalent; AAE, Ascorbic acid equivalent; GAE, Gallic acid equivalent; TCA, Trichloroacetic acid; TBA, Thiobarbituric acid; APS, Average particle size; DLS, dynamic light scattering; FESEM, Field emission scanning electron microscopy; EDS, energy-dispersive X-ray spectroscopy; FTIR, Fourier transform infrared spectroscopy; ATR, Attenuated total reflectance.

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https://doi.org/10.1016/j.ultsonch.2022.106048
Received 15 April 2022; Received in revised form 20 May 2022; Accepted 23 May 2022
Available online 26 May 2022

anti-microbial, analgesic, antioxidant, and anti-hyperglycaemic properties. The main active constituents in ginger are gingerol, zingerone, paradol and shogaol [5] which are responsible for ginger's odour and flavour [6]. Gingerols have the potent antioxidant and anticancer properties which can help in the prevention and treatment of diseases [7].

Glycyrrhiza glabra L. commonly known as licorice belongs to the family Leguminosae or Fabaceae. It is an ethno medicinal sweetener and calming herb that is still frequently used today. It is increasingly being utilized as a healthful component in a several commercial products, including pharmaceuticals, foods, beverages, and as a flavouring agent for confectionery and beverages as well as in cosmetic products [8]. Licorice also known as Mulethi is well recognized for antioxidants and antimicrobial activities [9]. The two main classes of bioactive compounds are saponins and flavonoids, which contain glycyrrhizin and glabridin or glabrene, respectively, as the major constituents. It contains various nutrients like proteins, amino acids, simple sugars, polysaccharides, carbohydrates, minerals (manganese, calcium), vitamins (E, B1, B2, B3, B5, and C), tannins, coumarins, phytosterols (stigmasterol and sitosterol), and glycosides [10]. Roots have been reported to show antacid, anti-inflammatory, demulcent, diuretic, anti-ulcer, tonic, expectorant, sedative and laxative properties [11]. They also possess antimicrobial, antipyretic, anxiolytic and antiherpes properties [12]. Therefore, licorice derivatives can be used in food as encapsulated polyphenolic compounds due to their antioxidant and antimicrobial properties. However, some of the functional properties, such as the antioxidant activity of dried ginger may be affected by prolonged heating [13]. Hence, freeze-drying the extracted active components of ginger and licorice is critical for the production of high-quality dried ginger and licorice products. Despite higher initial equipment expenditures, it could result in increased colour, nutritional, and flavour stability, as well as indistinguishable modifications to the natural raw product [14]. The study aimed at the extraction of bioactive compounds from ginger and licorice rhizomes using green extraction technique i.e. ultra-sonication. The extracts were freeze-dried and evaluated for nutraceutical, phytochemical, physio-morphological and structural properties.

2. Materials and methodology

2.1. Materials

Fresh ginger rhizomes (*Zingiber officinale*) and dried roots of licorice (*Glycyrrhiza glabra* L.) were procured from the local vegetable marketplace of Hazratbal, Srinagar, J & K, India. All the reagents and chemicals were purchased from Hi-media Pvt. Ltd, India. The standards were bought from Sigma-Aldrich (USA).

2.2. Ultrasound-assisted extraction

2.2.1. Preparation of ginger extracts

The method described by Arawande et al. [15] was used with some modifications. The foreskin of fresh ginger rhizomes was manually removed, dried, and processed into a fine powder in a laboratory mixer. 10 g (dry weight basis; db) of fresh and dried samples were mixed with 100 mL of different solvents (ethanol, ethyl acetate, acetone, and water), magnetically stirred for 6 h and left as such overnight. The mixture was sonicated using ultrasonicator (Probe sonicator) device (Athena Technology) for 15 min at a frequency setting of 60 Hz and a pulse mode function of 5 s on and 3 s off. The extraction temperature was kept below 35 °C by submerging the flask in ice throughout the procedure. The samples were centrifuged at 4000 × g for 15 min and the filtrate was concentrated at 40 °C in a vacuum rotary evaporator (Equitron, Roteva, Germany). The subsequent extracts sample were lyophilized (Lyovapor L-200, Butchi, Germany) at – 55°C and the yield obtained was a crude extract of ginger polyphenols. The obtained extract was weighed and the extraction value of each solvent was calculated using the following formula:

$$\%\ Extractive\ value\ of\ solvent = \frac{Weight\ of\ extract\ (g)}{Weight\ of\ sample\ (g)} \times 100$$

2.2.2. Preparation of licorice extracts

The method described by Luo, et al. [16] was used for extraction of licorice. The dried rhizomes of licorice were crushed into a fine powder using laboratory grinder. A sample of 10 g (db) was placed in a flask (amber coloured) and 100 mL of different solvents viz. ethanol: water (50: 50 and 70:30), methanol: water (50:50 and 70:30), and water (100%) was added. Each mixture was magnetically stirred for 6 h and left as such overnight. The mixture was extracted by ultra-sonication (Athena Technology) for 15 min and the extraction temperature was kept below 35 °C by submerging the flask in ice throughout the procedure. The frequency was set at 60 Hz, and the pulse function (Pulse mode) was tuned at 5 s on and 3 s off. This was followed by centrifugation at 4000 × g for 15 min and then filtration to remove particles and obtain clear extracts. The clear extracts obtained were concentrated by rotary vacuum evaporator (Equitron, Roteva, Germany) under reduced pressure at 40 °C to eliminate any volatile alcohol. The extracts were then freeze-dried in a lyophilizer (Lyovapor L-200, Butchi, Germany) at –55°C. The freeze dried powder samples were stored in laminated pouches. The extraction efficiency was determined by using the formula given below:

$$\%\ Extractive\ value\ of\ solvent = \frac{Weight\ of\ extract\ (g)}{Weight\ of\ sample\ (g)} \times 100$$

2.3. Moisture content

The moisture content of fresh and dried ginger samples was estimated using gravimetric method [17]. Each sample (5 g) was taken in previously dried and weighed petri-dish and placed in the oven (NSW-143, Oven Universal) maintained at 105 ± 1 °C until the constant weight was obtained. The per cent moisture content was calculated as:

$$Moisturecontent(\%) = \frac{(W_2 - W)}{(W_1 - W)} \times 100$$

Where, W is Weight of empty petri-dish; W_1 is Weight of petri-dish with sample before drying; W_2 is Weight of petri-dish with sample after drying to constant weight.

2.4. Qualitative and quantitative phytochemical screening

The phytochemical screening of ginger and licorice extracts for the presence of phenolics, tannins, saponins, carbohydrates, alkaloids, glycosides, flavonoids, steroids, and protein (amino acid), was carried out according to the method reported by Sofowora [18].

2.4.1. Test for tannin

Sample extracts (0.5 g) were taken in test tubes, added with 2 mL of water and then heated in a water bath at boiling temperature for 7–8 min. The mixture was filtered and 1 mL of 10% $FeCl_3$ solution was added to it. The appearance of blue-black colour indicated the presence of tannin.

2.4.2. Test for flavonoid

Sample extract (0.2 g) in a test tube was added with 5 mL of distilled water followed by the addition of 1 mL of 1% $AlCl_3$ solution and shaken rigorously. The existence of flavonoids was indicated by a light-yellow precipitate.

2.4.3. Test for phenol

Samples extract (0.5 g) was mixed with 1 mL of 10% $FeCl_3$ solution in

Table 1
Moisture content and powder yield of dried ginger rhizomes.

Ginger	Weight
Whole rhizome	3 kg
Peeled rhizomes	2 kg & 370 g
Peels	630 g
Yield of powder	
3000 g	305.31 g
1000 g	101.77 g
100 g	10.17%
Moisture content (%)	
Fresh (ginger)	$86.73^{a} \pm 1.02$
Dried (ginger)	$8.716^{b} \pm 0.34$

Mean values in the same row which is not followed by the same letter are significantly different ($p \leq 0.05$). Values represent mean ± standard deviation ($n = 3$).

Table 2
Phytochemical screening analysis of extracts.

Constituents	Ginger	Liquorice
Alkaloids	+	+
Saponins	-	+
Glycosides	+	+
Phytosterols/Steroids	–	+
Triterpenoids	–	+
Tannins	+	+
Carbohydrates	+	+
Phenols	+	+
Flavonoid	+	+
Proteins	+	–
Amino acids	+	–

a test tube. A rich bluish-green colour revealed the presence of phenol.

2.4.4. Test for saponins

About 0.2 g of sample extracts were shaken with 4 mL of distilled water in a test tube and heated on a water bath till boil. The formation of a creamy mass of small bubbles (frothing) indicated the presence of saponins.

2.4.5. Test for carbohydrate (Molish test):

Sample extract 2 mL (1 mg/mL) was added with two drops of alcoholic alpha naphthol solution. After thoroughly shaking the mixture, 2–3 drops of strong sulphuric acid was carefully added around the walls of the test tube. Appearance of the violet ring at the junction showed the presence of carbohydrates [19].

2.4.6. Test for glycoside

A sample extract (0.2 g) taken in a test tube was added with 2.5 mL of

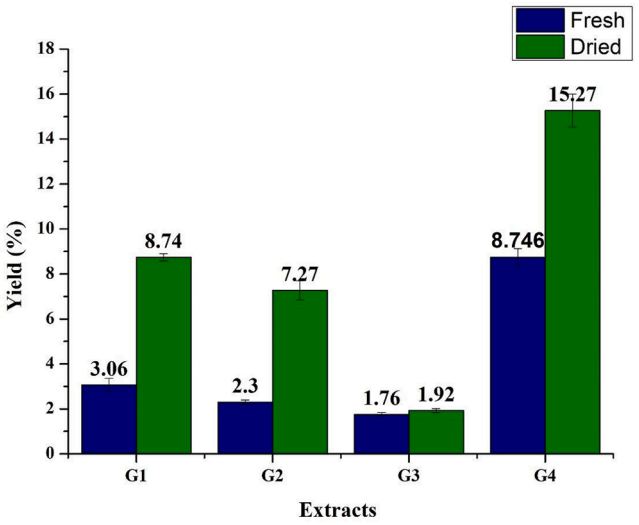


Fig. 1a. % Yield of extracts obtained from ginger (fresh and dried) using different solvents G1: ginger (fresh & dried) in ethanol: water (75:25); G2: ginger (fresh & dried) in ethyl acetate: water (75:25); G3: ginger (fresh & dried) in acetone: water (75:25); G4: ginger (dried) in ethanol: water (50:50) and water (100%).

Table 3
Calibration data for phenolics and flavonoid compounds present in ginger.

Peak	Phenolics Compound	R_t	Regression line	LOD	LOQ	R^2
1	Gallic acid	1.527	Y = 36474.29X + 0.072	0.0011	0.003	0.996
2	Vanillic acid	5.715	Y = 42317.47X + 8.36	0.0008	0.002	0.993
3	Ferulic acid	1.922	Y = 38974.62X-14.99	0.0010	0.003	0.988
4	Chlorogenic acid	2.966	Y = 15.284X-20.865	0.0003	0.0009	0.988
Flavonoids						
1	Kaempferol	13.256	Y = 35356X + 4.004	0.0002	0.0007	0.99
2	Quercetin	7.624	Y = 3269.4198X + 0.336	0.0006	0.0020	0.998
3	Isorhamnetin	8.767	Y = 119756.62X + 11.034	0.0002	0.0007	0.999
4	Rutin	10.574	Y = 10552.48X-76.870	0.0003	0.0008	0.98
5	Catechin	5.190	Y = 85.4583X-11.95	0.0005	0.0005	0.99
6	Caffeic acid	9.565	60.727X + 112.865	0.0003	0.0009	0.978

Table 4
Calibration data for phenolics and flavonoid compounds present in liquorice.

Peak	Phenolic compounds Compound	R_t	Regression line	LOD	LOQ	R^2
1	Vanillic acid	5.280	Y = 42317.47X + 8.36	0.0008	0.002	0.993
2	Ferulic acid	1.813	Y = 38974.62X-14.99	0.0010	0.003	0.988
3	Chlorogenic acid	2.729	Y = 15.284X-20.865	0.0003	0.0009	0.988
Flavonoids						
1	Kaempferol	13.085	Y = 35356X + 4.004	0.0002	0.0007	0.99
2	Quercetin	8.083	Y = 3269.4198X + 0.336	0.0006	0.0020	0.998
3	Rutin	10.574	Y = 10552.48X-76.870	0.0003	0.0008	0.98
4	Catechin	5.190	Y = 85.4583X-11.95	0.0005	0.0005	0.99
5	Caffeic acid	3.746	60.727X + 112.865	0.0003	0.0009	0.978

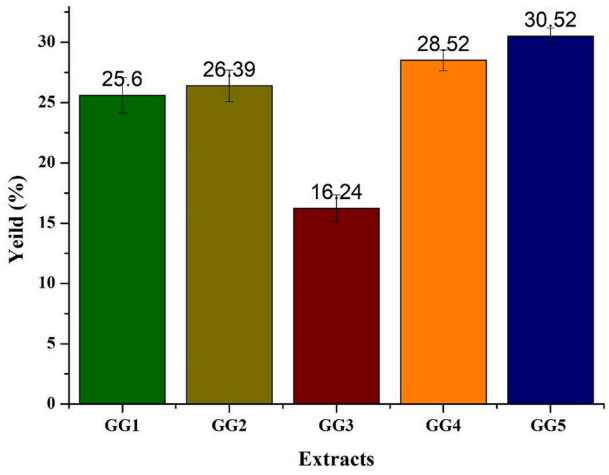


Fig. 1b. % Yield of extracts obtained from G. Glabra (Licorice) using different solvents GG1: licorice in methanol: water (70:30); GG2: licorice in methanol: water (50:50); GG3: licorice in ethanol: water (70:30); GG4: licorice in ethanol: water (50: 50); GG5: licorice in water (100 %).

dilute sulphuric acid and heated for 15 min. The samples were cooled and neutralized with 5 mL of Fehling solution A and B, respectively. The presence of glycoside was indicated by the appearance of brick red precipitate.

2.4.7. Test for protein

Sample extracts (0.2 g) were mixed with 5 mL of distilled water and left for 3 h. Later, the mixture was filtered. 0.1 mL Millon's reagent was applied to 2 mL of filtrate. The appearance of yellow precipitate showed the presence of protein.

2.4.8. Test for steroids (Salkowski test)

Take 0.2 g of extract combined with 2 mL of chloroform in a test tube and shake well. A layer was formed to which 2 mL of concentrated sulphuric acid (99% purity) was added. The occurence of steroids was shown by the formation of a violet/blue/green/reddish-brown ring at the contact. The yellowish colour of the lower layer signified the presence of triterpenoids.

2.4.9. Test for alkaloids

Take 2 mL of sample extract (1 mg/mL) in a test tube; add 2–3 drops of Wagner's reagent to it. The presence of alkaloids was revealed by the appearance of reddish-brown colour [20].

2.5. Quantitative estimation of phytoconstituents

Harbone's method [21] was used to determine the quantitative concentrations of alkaloids. Tannins were estimated using Allen and co-workers' method [22] Obadoni and Ochuko's [23] approach was used to quantify saponins.

2.6. Quantification of polyphenols by high performance liquid chromatography (HPLC)

The phenolic compounds such as vanillic acid, gallic acid, chlorogenic acid, and ferulic acid were separated, identified, and quantified in ginger and licorice extracts by using HPLC. An Agilent HPLC 1260 infinite series equipped with quaternary pumps, a diode array detector (DAD), a manual injection port supported by Agilent Chemstation 4.03 software was used for analysis. The separation was performed on an SB-C18 analytical HPLC column (4.5 × 150 mm) kept at 25 °C. The mobile phase used was methanol and water in a 3:2 ratio with a flow rate of 0.8 mL/min under isocratic conditions, for 15 min. The solvents were of HPLC grade and were degassed for 10 min with sonication after passing through a 0.22 μm filter. The detector was set at a wavelength of 280 nm.

The modified method of Landim et al. [24] was used for the separation and quantification of flavonoids such as rutin, kaempferol, quercetin, caffeic acid, catechin, and isorhamnetin. The mobile phase consisting of 100% methanol (C), methanol, water, and acetic acid (D) in the ratio of 100:150:5 were used. The mobile phase was run under gradient elution as solvent C 0% from 0 to 4 min and D 100%, solvent C from 4 to 30 min was linearly decreased to 0% and solvent D increased to 100% from 4 to 30 min. The flow rate of mobile phase was kept as 1.3 mL/min. The retention time and UV spectrum (360 nm) were used for

Table 6
Phenolic constituents (mg/g) of liquorice extracted using different solvent concentrations.

Compound (mg/g) Phenolics	G. Glabra Ethanol (70%)	Methanol (70%)	Ethanol (50%)	Methanol (50%)	Water (100%)
Gallic acid	ND	ND	ND	ND	ND
Vanillic acid	2.34	2.05	1.98	3.02	1.98
Ferulic acid	6.53	6.21	5.89	6.81	5.03
Chlorogenic acid	0.91	0.87	0.81	0.98	0.51
Flavonoids					
Quercetin	12.51	12.02	11.86	12.86	10.21
Kaempferol	18.03	17.01	17.01	18.51	16.85
Isorhamnetin	ND	ND	ND	ND	ND
Caffeic acid	1.13	1.09	1.01	1.59	0.95

G. Glabra: glycyyrhiza glabra, ND: not detected.

Table 5
Phenolic constituents (mg/kg) of ginger (fresh and dried) rhizomes extracted in different solvent concentrations.

Compound (mg/kg)	G. fresh					G. Dried				
Phenolics	Acetone (75%)	Ethanol 50 %	75 %	EA (75%)	Water (100%)	Acetone (75%)	Ethanol 50 %	75 %	EA (75%)	Water (100%)
Gallic acid	30.47	31.24	35.41	32.4	21.41	35.43	39.34	41.30	48.31	24.10
Vanillic acid	74.31	86.43	89.41	89.31	71.33	89.00	93.10	97.00	94.30	86.43
Ferulic acid	121.31	103.41	201.42	181.31	81.31	160.30	189.4	218.4	190.43	94.50
Flavonoids										
Quercetin	56.41	58.65	87.76	78.76	31.76	64.65	67.76	101.7	89.76	40.65
Kaempferol	13.085	18.87	29.76	24.65	13.87	21.98	25.76	37.76	31.78	19.78
Isorhamnetin	141.89	134.3	201.56	197.56	102.45	161.56	167.7	247.8	217.34	134.67
Rutin	16.65	21.65	28.65	23.65	9.65	18.87	25.76	31.67	28.65	11.54
Catechin	69.54	119.45	161.67	143.34	57.67	98.32	132.4	175.1	157.08	87.57
Caffeic acid	15.44	18.65	23.45	21.45	13.76	19.56	21.45	38.23	29.43	18.76

G. fresh: fresh ginger extract, G. Dried: dried ginger extract.

Table 7
TPC (mg/g), TFC (μg/ml), DPPH (%), RP (AAE/g), Lipid peroxidation (% ILP) of ginger extracts.

Solvents	Fresh DPPH	TPC	TFC	RP	ILP	Dried DPPH	TPC	TFC	RP	ILP
Acetone (75%)	$46.78^{c} \pm 2.27$	$34.50^{c} \pm 2.35$	$4.875^{b} \pm 0.87$	$0.50^{c} \pm 0.001$	$62.4^{c} \pm 0.51$	$87.74^{b} \pm 2.00$	$57.25^{e} \pm 2.07$	$7.25^{b} \pm 4.00$	$1.10^{a} \pm 0.01$	$62.8^{d} \pm 0.32$
Ethanol (75%)	$73.13^{b} \pm 1.65$	$54.01^{a} \pm 2.81$	$6.25^{c} \pm 0.43$	$0.65^{b} \pm 0.001$	$72.8^{b} \pm 0.44$	$77.51^{d} \pm 3.83$	$114.60^{b} \pm 3.38$	$18.125^{c} \pm 0.11$	$0.96^{a} \pm 0.006$	$73.1^{c} \pm 0.47$
Ethyl acetate (75%)	$79.13^{a} \pm 2.26$	$45.58^{b} \pm 0.74$	$6.375^{a} \pm 2.87$	$1.006^{a} \pm 0.002$	$74.8^{a} \pm 0.25$	$91.65^{a} \pm 0.52$	$101^{d} \pm 5.00$	$10.5^{d} \pm 2.41$	$1.17^{a} \pm 0.006$	$75^{b} \pm 0.20$
Ethanol (50%) - - - - -						$82.72^{c} \pm 1.47$	$155.19^{a} \pm 2.81$	$6.125^{e} \pm 0.375$	$0.69^{c} \pm 0.01$	$76.1^{a} \pm 0.30$
Water (100%) - - - - -						$60.06^{e} \pm 1.24$	$104.21^{c} \pm 1.41$	$5.68^{a} \pm 4.93$	$0.81^{b} \pm 0.001$	$76.2^{a} \pm 0.50$

Mean values in the same column which is not followed by the same letter are significantly different ($p \leq 0.05$). Values represent mean ± standard deviation ($n = 3$). TPC: total phenolic content; TFC: total flavonoid content; RP: reducing power; ILP: inhibition of lipid peroxidation.

Table 8
TPC (mg/g), TFC (μg/ml), DPPH (%), RP (AAE/g), Lipid peroxidation (% ILP) of Liquorice extracts.

Solvents	DPPH	TPC	TFC	RP	ILP
Ethanol (70%)	$68.87^{b} \pm 2.00$	$188.62^{a} \pm 1.17$	$58.50^{b} \pm 0.87$	$0.73^{b} \pm 0.001$	$70.8^{a} \pm 0.51$
Ethanol (50%)	$45.83^{d} \pm 0.79$	$54.01^{e} \pm 2.81$	$52^{d} \pm 0.43$	$0.52^{d} \pm 0.001$	$61.9^{d} \pm 0.44$
Methanol (70%)	$50.17^{c} \pm 0.83$	$108.32^{c} \pm 1.05$	$55.25^{c} \pm 2.87$	$0.60^{c} \pm 0.002$	$62.4^{c} \pm 0.25$
Methanol (50%)	$76.43^{a} \pm 0.37$	$168.91^{b} \pm 4.70$	$96.25^{a} \pm 0.52$	$0.83^{a} \pm 0.05$	$69^{b} \pm 0.33$
Water (100 %)	$21.48^{e} \pm 0.17$	$79.35^{d} \pm 0.91$	$96.75^{a} \pm 0.43$	$0.47^{e} \pm 0.01$	$60.6^{e} \pm 0.11$

Mean values in the same column which is not followed by the same letter are significantly different ($p \leq 0.05$). Values represent mean ± standard deviation ($n = 3$). TPC: total phenolic content; TFC: total flavonoid content; RP: reducing power; ILP: inhibition of lipid peroxidation.

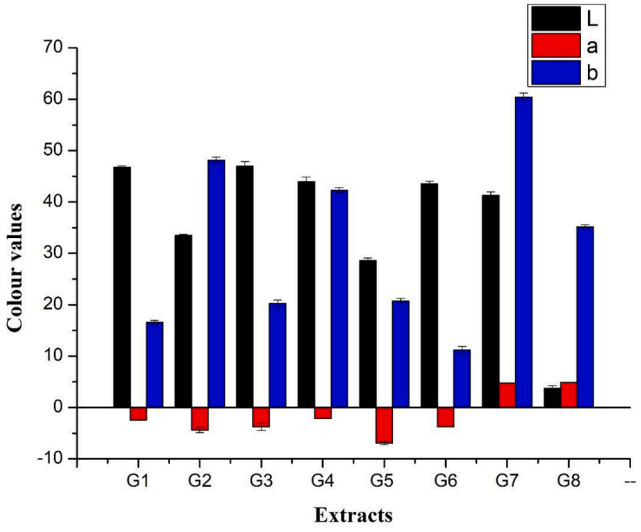


Fig. 2a. Colour (L, a, b) values of extracts obtained from ginger using different solvents G1: ginger (fresh) in acetone: water (75:25); G2: ginger (dried) in acetone: water (75:25); G3: ginger (fresh) in ethanol: water (75:25); G4: ginger (dried) in ethanol: water (75:25); G5: ginger (fresh) in ethyl acetate: water (75:25); G6: ginger (dried) in ethyl acetate: water (75:25); G7: ginger (dried) in ethanol: water (50:50); G8: ginger (dried) in water (100%).

the detection by comparison between the standards and the sample peaks.

The sample was prepared by using the procedure described by Caballero-Ortega et al. [25] with some changes. In a 50 mL centrifuge tube, dried ginger, and licorice powder extracts (10 mg) were suspended in 1 mL of methanol: water (1:1) and homogenized. The tubes were placed in an incubator shaker for 48 h at 4 °C in dark. The samples were then centrifuged at 10,000 × g for 30 min and filtered using a 0.2 μm syringe filter. The tubes were wrapped in aluminum foil to prevent photodegradation and kept at 4 °C until further analysis.

Standard stock solutions of 1 mg/mL of each chlorogenic acid, catechin, vanillic acid, ferulic acid, gallic acid, quercetin, caffeic acid, rutin, and kaempferol standards were prepared in methanol and water (1:1) and stored at 4 ℃. However, in the case of isorhamnetin, methanol, water, and DMSO (1:1:1) were used for the preparation of the standard stock solution. Working serial dilutions of 7, 6, 5, 4, 3, 2, and 1 μg/mL were prepared for calibration and filtered through a 0.2 μm syringe filter before injection. The unknown compounds in the sample were quantified using the linear regression equations (Table 3 and 4) of each standard.

2.7. Total phenolic content (TPC)

The TPC was determined by the Folin– Ciocalteau's method of Cindric et al. [26] with slight modifications. 2 g of freeze dried ginger and licorice samples were re-dissolved in 50 mL of their respective extraction solvents and refluxed for 2 h. All the samples were filtered and residue was collected, and refluxed again for 1 h, and then filtered. The filtrate was collected (0.2 mL) in an eppendorf tube containing 0.8 mL of distilled water and 5 mL of Folin–Ciocalteau's reagent was added to each tube. Then after 5 min 4 mL of 20 % sodium carbonate was added and incubated in the dark for 90 min. The absorbance was measured at 760 nm with a UV–visible spectrophotometer (U-2900, Hitachi, Tokyo, Japan). The phenolic content was determined using the standard curve of gallic acid and expressed as mg of gallic acid equivalents per gram (mg GAE/g) of the sample.

2.8. Total flavonoid content (TFC)

The TFC of the extracts was determined using the method of Krishnaiah et al. [27] with slight modifications. The freeze dried samples (100 mg) were re-dissolved in 15 mL of methanol: water (50% v/v) were

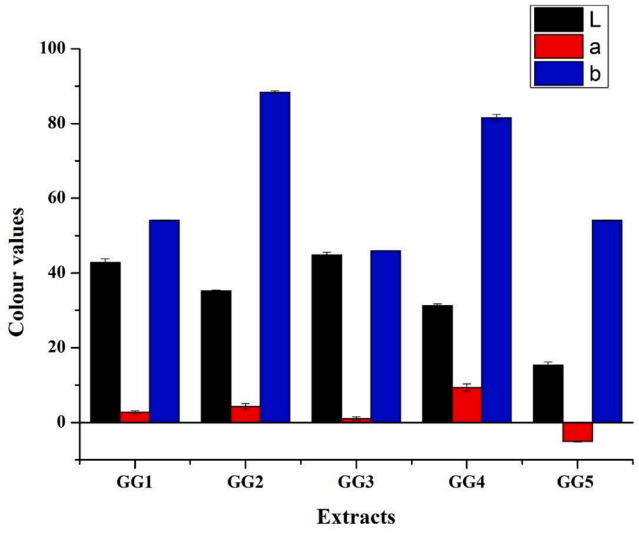


Fig. 2b. Colour (L, a, b) values of extracts obtained from *G. Glabra* (licorice) using different solvents. GG1: licorice in methanol: water (70:30); GG2: licorice in methanol: water (50: 50); GG3: Licorice in ethanol: water (70: 30); GG4: licorice in ethanol: water (50: 50); GG5: licorice in water (100 %).

combined and centrifuged at 2000 × g for 10 min. The supernatant (5 mL) was added with an equal volume of aluminium trichloride solution (2% w/v in methanol). After 10 min., the absorbance at 415 nm was measured against a blank using a UV–vis spectrophotometer (U-2900, Hitachi, Tokyo, Japan). The standard curve of quercetin (0–100 μg/mL) was prepared and TFC was calculated in terms of mg quercetin equivalents per gram (mg QE/g).

2.9. Antioxidant properties:

2.9.1. DPPH radical-scavenging activity

The DPPH radical scavenging activity of the samples was measured by using a modified method of Brand-Williams et al. [28]. A 100 mg sample (db) was extracted by using 10 mL of methanol for 2 h and centrifuged at 3000 × g for 10 min. The upper layer (100 μL) was collected and treated with 3.9 mL of a DPPH solution (6x10^{5}mol/L). The absorbance (A) of the samples was measured using UV–Vis spectrophotometer (Model, UV-2450, Shimadzu, Japan) at 515 nm after 0 and 30 min against a methanol as a blank. Antioxidant activity was calculated using the equation given below:

$$DPPH\ \ radical\ \ scavenging\ \ activity\ \ (\%) = \left\{1 - \left[\frac{A\ \ of\ \ sample\ \ at\ \ t = 30}{A\ \ of\ \ sample\ \ at\ \ t = 0}\right]\right\} \times 100$$

2.9.2. Reducing power

The reducing power of the extract samples was calculated by following the procedure of Liu et al. [29]. Briefly, the freeze dried samples were re-dissolved in their respective extraction solvents (15 mg/mL) and 2.5 mL of the sample solutions were added with 2.5 mL of phosphate buffer (0.2 M; pH 6.6), and 2.5 mL of potassium ferricyanide (10 mg/mL) were mixed and placed in a water bath at 50 °C for 20 min. The samples were cooled to room temperature and 2.5 mL of 10% TCA was added followed by centrifuged at 3,000 × g for 10 min. The supernatant was collected, mixed with 0.5 mL of ferric chloride solution (1 mg/mL), and the absorbance at 700 nm was measured. The results were expressed in ascorbic acid equivalents per gram (AAE/g of extract) by preparing the standard curve of ascorbic acid.

2.9.3. Inhibition of lipid peroxidation (ILP)

The ILP values of the extracts were measured using the modified method of Wright et al. [30]. The freeze dried samples (200 mg) were re-dissolved in their respective solvents. The supernatant layer (250 μL) was added with 1 mL of linoleic acid (0.1 % in ethanol), 0.2 mL of ascorbic acid (200 mM), 0.2 mL of ferric nitrate (20 mM), and 0.2 mL of hydrogen peroxide (30 mM). The mixture was incubated in a water bath at 37 °C for 1 h and reaction was stopped by adding the 1 mL of TCA (10% w/v), followed by TBA (1 mL, 1% w/v). After boiling for 20 min in a water bath, the reaction mixture was centrifuged at 5000 × g for 1 min and the absorbance was measured at 35 nm against a blank using a UV spectrophotometer (U-2900, Hitachi, Toky, ofn). The following formula was used to determine the percentage inhibition of lipid peroxidation:

$$\%inhibition = [1 - (Absorbance\ \ of\ \ Smp/Absorbance\ \ of\ \ control)] \times 100$$

2.10. Colour

The Colour Flex Spectrocolorimeter (Hunter Lab Colorimeter D-25, Hunter Associates Laboratory, Ruston, USA) was used to assess the colour values L* (lightness), a* (redness), and b* (yellowness) of the freeze dried powdered samples.

2.11. Fourier transform infra-red (FTIR) spectroscopy

FTIR (Cary 630 FTIR, Agilent Technologies, Virginia, USA) was used for structural characterization of the freeze dried powder samples. The analysis was carried out using the Resolution Pro software version 2.5.5 (Agilent Technologies, USA).

2.12. Field emission scanning electron microscopy (FESEM)

The shape, size, and elemental analysis of the extracts were examined using a FESEM (Hitachi-PU) in conjunction with energy-dispersive X-ray spectroscopy (EDS; BRUKER XFLASH 6130). The extracts were dried at 37 °C and the pictures were acquired at accelerating voltage of 15 kV.

2.13. Particle size analysis of dried extracts

The sample (0.01 percent, w/v) was suspended in distilled water and sonicated for 30 min at 40 kHz in a sonicator bath to fully disperse the powder. A particle size analyser (Litesizer, 500, Anton Paar, Austria) was used to determine the average particle size of dried particles.

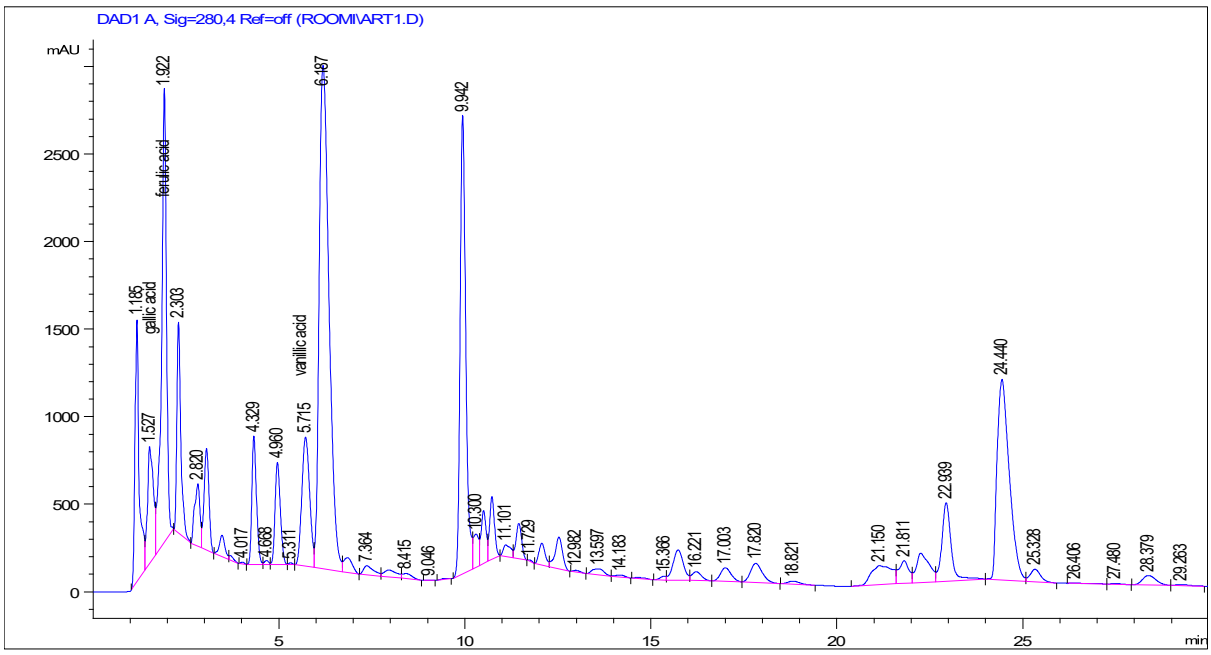
2.14. Statistical analysis

All investigations were carried out in triplicate. The research data were presented as a mean value with a standard deviation. At a 95 percent confidence level (p 0.05), statistical analysis was performed using Duncan's Multiple Range Test.

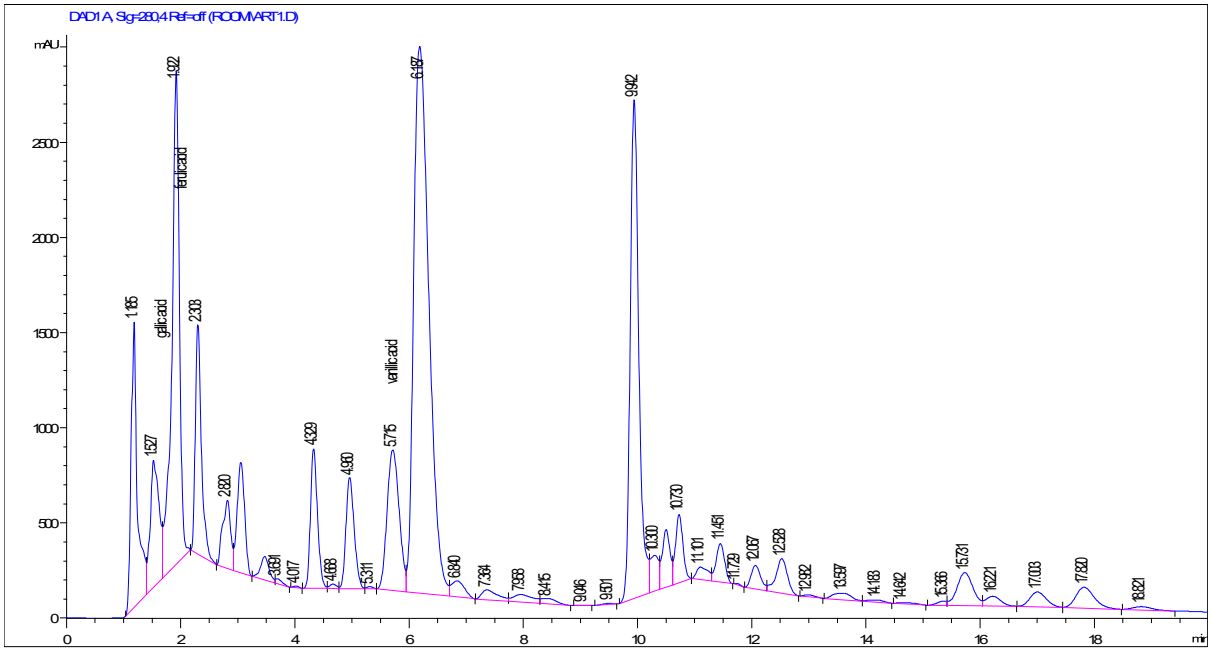
3. Results and discussion

3.1. Powder yield, moisture content and extraction value for ginger

The average powder yield obtained was 305.31 g per 3000 g of fresh ginger which is about 10.77% (Table 1). The moisture content of fresh ginger was found as 86.73 ± 1.02% and after drying it was observed as 8.71 ± 0.34%. On comparing the extraction efficiency of different solvents for both fresh and dried ginger samples, it was found that extraction of crude polyphenols from dried ginger was higher as compared to fresh samples. This might be due to the fact that more surface area was available for maximum solute–solvent interactions and mass transfer in dry ginger powder resulting in greater extraction values. The increase in yield differed amongst solvents with aqueous extracts showing significantly the highest extraction yield as compared to other solvents (Fig. 1a). The highest value for extraction yield of 15.27% was



(a)



(b)

Fig. 3. (a) & (b). HPLC chromatogram of ginger phenolics (a) fresh and (b) dried extracts.

obtained in aqueous extracts and the lowest yield was found in ethyl acetate extract of fresh samples (1.76%). The yield of dried ginger in ethanol: water (75:25) and ethanol: water (50:50) exhibited almost similar extraction values of 8.740 and 8.746%. Therefore, sonicated extraction in an aqueous medium showed maximum extraction yield followed by ethanol: water (50:50), ethanol: water (75:25) and acetone: water (75:25). Arawande et al. [31] also observed the highest extraction values of ginger in water (16.62%), followed by ethanol (13.88%) and the least value for acetone extract (10.14%). Different solvent-extracts of the same plant contain different bioactive components which may have varying medicinal and therapeutic benefits.

3.2. *Extraction value for licorice*

The extraction value of any solvent is a measure of the ability of the solvent to obtain a bioactive material from a given sample [31]. The extraction yield of licorice in different solvents is presented in Fig. 1b. It is clear from the figure (Fig. 1b) that extraction value of licorice (30.52%) in water was the highest and this was followed by ethanol: water (50:50) with a value of 28.52% and the least value of 16.24% was

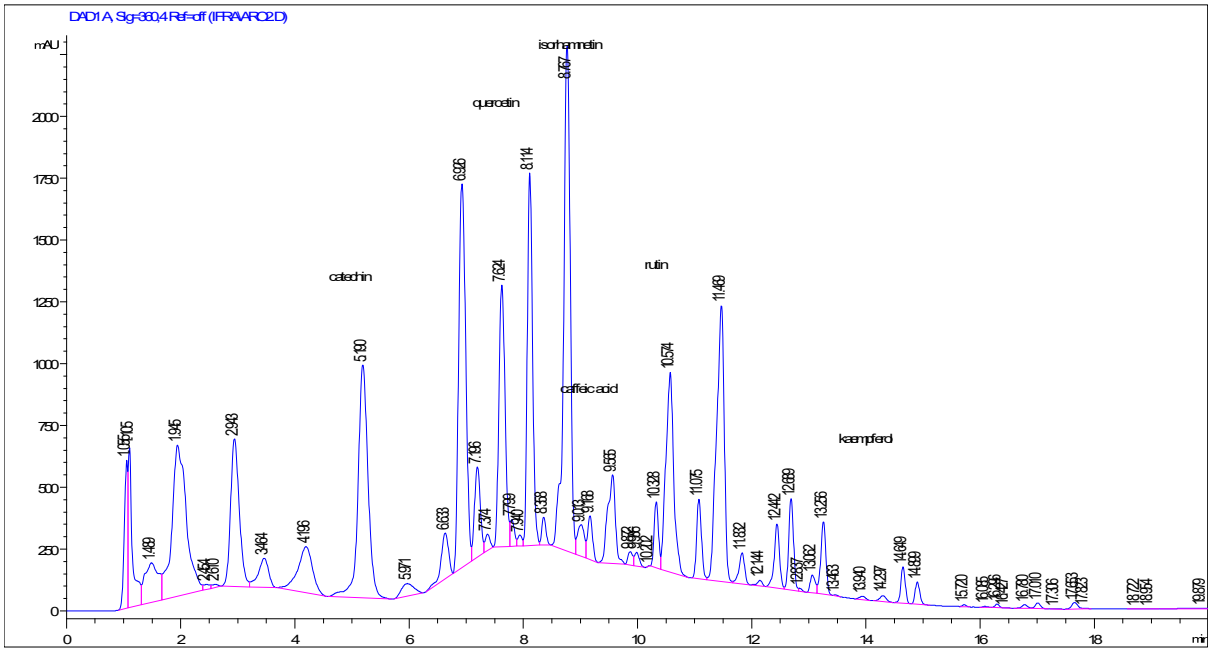


Fig. 4. (a) & (b). HPLC chromatogram of ginger flavonoids.

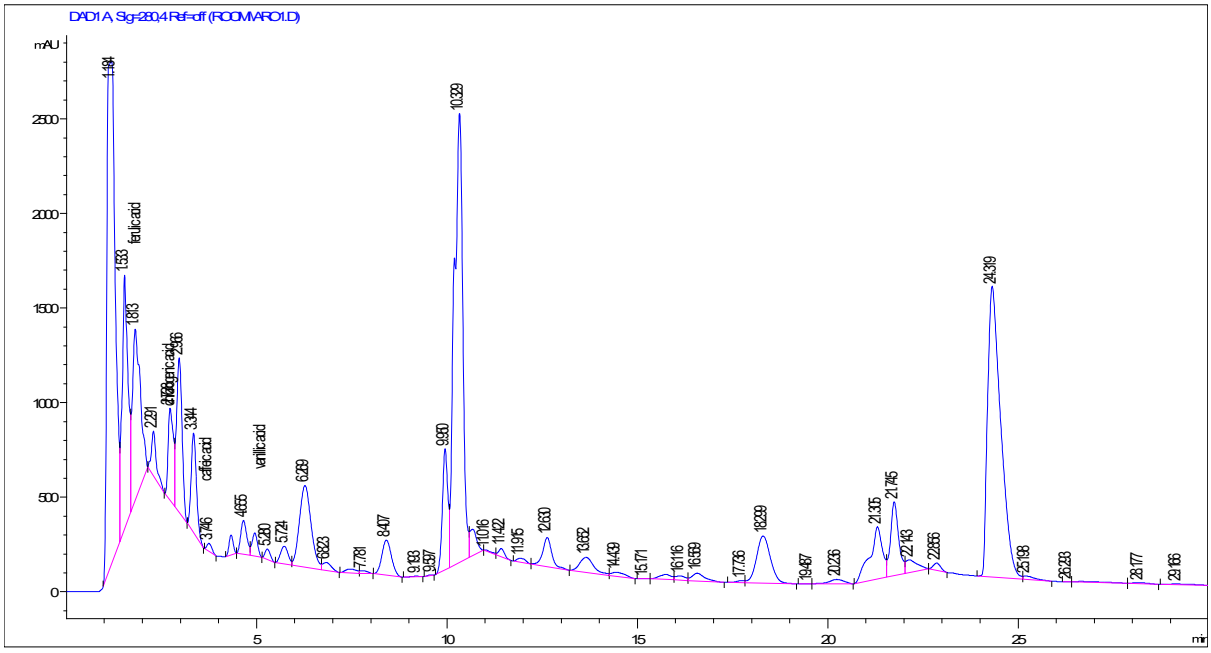
observed in ethanol: water (70:30) extract. Therefore, the extraction of licorice samples varied significantly in all the solvents. It has been reported that increasing the solvent concentration enhances the cell membrane degradability of the raw materials resulting in higher extraction recovery from licorice root [32]. Thakur et al. [33], evaluated the phytochemical, and antioxidant properties of licorice root extracts. It was reported that solvents with high concentration are expected to be efficient in extracting bioactive ingredients and as most of the active components are polar in nature; polar solvents are expected to have high extraction values.

3.3. Qualitative and quantitative screening of extracts

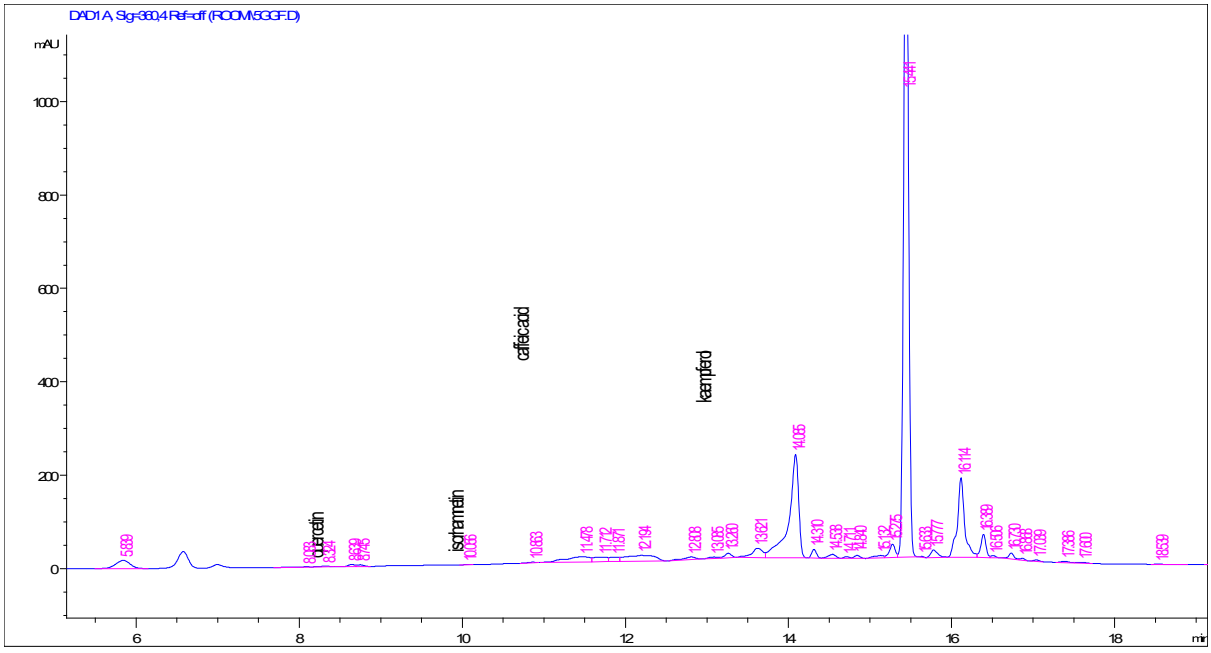
The investigated phytochemical constituents of the ginger and licorice extracts are given in Table 2. The preliminary screening indicated the presence of phytochemicals like flavonoids, phenolics, tannins, carbohydrates, steroids, glycosides, and alkaloids in licorice and ginger extracts except saponins which were present in licorice only. In addition, the protein & amino acids were not detected in licorice but were present in ginger. Among the screened constituents, the amount of major quantified metabolites such as alkaloids and tannins in ginger extract were observed as 10.35 ± 0.80 g/100 g and 13.06 ± 0.50 g/100 g, respectively, The observed results are in accordance with the findings of Adekunle et al. [34] who reported the alkaloids (12.33%), tannins (10.80%) and saponins (nil), respectively, in ginger extracts. In case of the licorice the amount of saponins and tannins were observed as 22.20 ± 0.90 g/100 g and 38.64 ± 0.11 mg/g, respectively. The preliminary qualitative analysis carried out by Archana, and Vijayalakshmi [35], for licorice extract showed the presence of phenols, flavonoids, tannins, and saponins. The results showed the phenols as 211, 281.66, 263 mg/g; 152.66, 187.33, 183.33 mg/g of flavonoids, 21.33, 41, 34.3 mg/g of tannins in aqueous, ethanol and hydro-alcoholic extracts of licorice.

3.4. Total phenolic content (TPC) and total flavonoid content (TFC) of extracts

The phenolics and flavonoid content of plants has been attributed to the majority of their antimicrobial and antioxidant capabilities. Polyphenols are antioxidants that exist naturally in food sources. Although their antioxidant capacity explains their pharmacological activity, it is not impossible that their biological effects extend far beyond the decrease of oxidative stress [36]. The total phenolic content of ginger (fresh and dried) extracts determined using different solvents measured in milligrams of GAE per gram of gallic acid are represented in Table 7. The data obtained revealed that the TPC in ginger extracts obtained from fresh and dried samples varied significantly ($p \leq 0.05$) among the solvents. Highest TPC content was observed in hydroalcoholic extracts [Ethanol: water (50:50 and 75:25)], with the value of 155.19 and 114.60 mg/g GAE followed by aqueous extract (104.211 mg/g GAE). Falleh et al. [37] reported that the composition and amount of phenolic compounds varies greatly based on many internal and extrinsic factors. This results in the variation of antioxidant activity as well as total phenolic and flavonoid concentrations from cultivar-to-cultivar. Extraction also has a considerable impact on the final extract in terms of composition, content, and characteristics [38]. The kind of solvent and the concentration of phenolic compounds have a significant impact on the recovery of these compounds. Ethanol: water (50:50) and aqueous (100%) extracts revealed the lowest tfc values (6.125 and 5.68 μg/mLQE) while the higher tfc of 18.125 and 10.50 μg/mLQE was found in ethanol: water (75:25) and ethylacetate: water (75:25) extracts, respectively (table 7). The values observed for tpc and tfc of ginger extracts was found to be close to that found by Yousfi et al. [39] For licorice, highest tpc of 188.62 ± 1.17 mg/g GAE was found in ethanol: water (70:30) followed by methanol: water (50:50) with tpc value of 168.91 ± 4.70 mg/g GAE) while the later showed maximum tfc of 96.25 ± 0.52 μg/mLQE than former (58.50 ± 0.87 μg/mLQE) when compared to the other extraction solvents (table 8). According to Archana and Vijayalakshmi [35], the aqueous, ethanol, and hydro-alcohol extracts showed the total phenols and flavonoids at estimation values of 211, 281.66, 263GAE/g and 152.66, 187.33, 183.33QE/g of the licorice extracts. Phytochemicals and antioxidant activity of extracts from licorice root have been studied by Tohma et al. [40] using a variety of solvents. Ethanol was found to be a better solvent than water in tests. Increases in TPC were seen by increasing the concentration of ethanol in this investigation, which is consistent with the research findings of Tohma et al. [40].



(a)

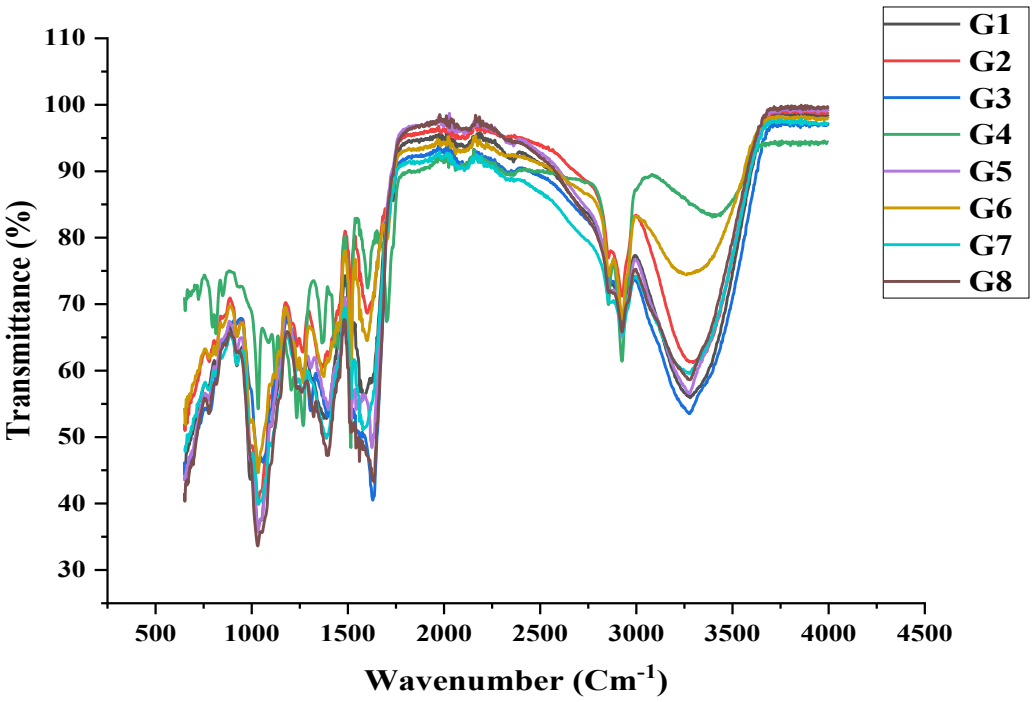


(b)

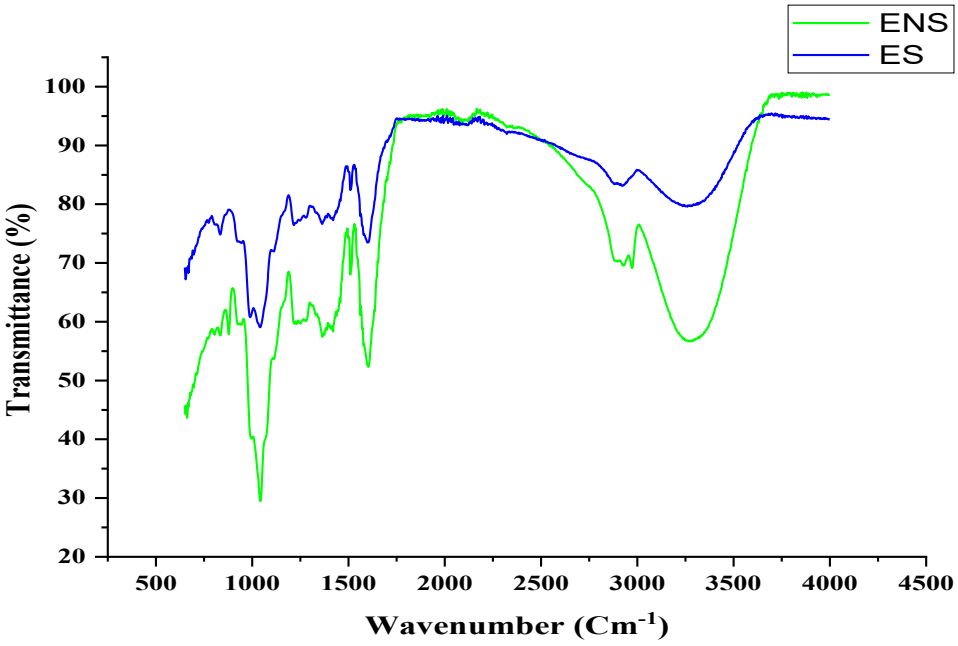
Fig. 5. (a) & (b). HPLC chromatogram of phenolics and flavonoids from liquorice extracts.

3.5. DPPH free radical scavenging activity, reducing power and inhibition of lipid peroxidation of extracts

Polyphenols have been shown to play a major function in the antioxidant capacity of medicinal plants in earlier studies [41]. A diamagnetic molecule is formed when DPPH, a stable free radical, acquires an electron or hydrogen radical and is generally used substrate for evaluating the antioxidant properties of antioxidants. Antioxidants were tested for their ability to neutralize DPPH radicals, which are widely used as a test substrate. Tables 7 and 8 show the antioxidant effects of ginger and licorice on DPPH radical scavenging. The antioxidant and free radical scavenging properties of ginger rhizome extracts in acetone, ethyl acetate, ethanol, and aqueous form were studied. Based on these results, significantly ($p \leq 0.05$) highest antioxidant activity of 91.65 ± 0.52 % was observed in ethyl acetate: water (75:25) extracts of dried ginger powder followed by 87.74 ± 2.00% for acetone: water (75:25). While this percentage remains limited to 60.06 ± 1.24% for the aqueous extracts. Yousfi et al.[39] observed the excellent antioxidant activity of 92.13% and 95.53% for ethanol extracts of ginger, which are in good confirmation with the antioxidant results of present study. Ginger extracts may have free radical scavenging properties due to the worldwide engagement of active ingredients like polyphenols and flavonoids [42].



(a)



(b)

Fig. 6. (a) & (b). FTIR spectroscopic measurements of sonicated-freeze dried (a) ginger (fresh & dried) (b) liquorice extracts. G1, G2: ginger (fresh & dried) in acetone: water (75: 25); G3, G4: ginger (fresh & dried) in ethanol: water (75:25); G5, G6: ginger (fresh & dried) in ethyl acetate: water (75: 25); G7, G8: ginger (dried) in ethanol: water (50: 50) and water (100%). ENS: extract not sonicated; ES: extract sonicated.

In licorice, the highest AoA (76.43 ± 0.37%) and RP (0.83 ± 0.05AAE/g) were perceived by methanol: water (50:50) extracts followed by ethanol: water (70:30) having 68.8 ± 2.00% of AoA and 0.73 ± 0.001AAE/g of RP), respectively. When polyphenols have a high reducing power, this indicates that they have a high antioxidant capacity, which is determined by their ability to convert the Fe^{3+}/ferricyanide complex to the ferrous form [29]. In contrary, ethanol: water (70:30) exhibited comparably the highest ILP of 70.8 ± 0.51% than

Table 9
Particle size distribution, average hydrodynamic particle size and polydispersity index of sonicated-freeze dried extracts.

Parameter	Z_1	Z_2	GG_1	GG_2
Average hydrodynamic particle size (nm)	$547^{b} \pm 17.52$	$766^{a} \pm 16.02$	$450^{b} \pm 4.11$	$566^{a} \pm 6.73$
Particle size distribution (nm)				
Dv(10)	$76^{a} \pm 0.50$	$722^{b} \pm$	$252^{b} \pm 2.04$	$555^{a} \pm 16.08$
Dv(50)	$954^{b} \pm 15.32$	$3210^{a} \pm 21.67$	$304^{b} \pm 3.10$	$1620^{a} \pm 21.50$
Dv(90)	$3414^{a} \pm 25.50$	$1344^{b} \pm 15.01$	$357^{b} \pm 1.20$	$4036^{a} \pm 25.71$
Polydispersity index	$35^{a} \pm 0.40$	$27^{b} \pm 0.11$	$40^{a} \pm 0.82$	$37^{b} \pm 0.10$

Mean values in the same row which is not followed by the same letter are significantly different ($p \leq 0.05$). Values represent mean ± standard deviation ($n = 3$). Z_1 and Z_2, represents ginger extract in ethanol and ginger extract in ethylacetate, while as GG_1 and GG_2 represents liquorice extract in ethanol and liquorice extract in methanol, respectively. Dv (10), Dv (50) and Dv (90) signifies the points in the size distribution, up to and including which, 10 %, 50 % and 90 % of the total volume of material in the sample is contained.

methanol: water (50:50) extract with 69 ± 0.33% ILP. Archana and Vijayalakshmi[35] reported the maximum scavenging property of licorice rhizomes using methanol: water (50:50) extracts. The larger the percentage of DPPH radicals scavenged, the stronger the antioxidant activity [43]. Natural antioxidants, such as ginger, have been linked to a reduced risk of tumour and cardiovascular and degenerative diseases, according to a number of studies [44,45].

3.6. Colour

The colour characteristics could be exploited for rapid assessment of some antioxidant properties of the ginger extracts of the samples [46]. Colour properties (L*a*b* measurements) of ginger and licorice extracts are shown in Fig. 2 (a, b). The colour of the extracts differed significantly ($p \leq 0.05$) among the solvents. The L* value of ginger extracts varied from 3.70 to 46.97, a* varied from −6.935 to 4.93 and b* value from 11.17 to 60.41, respectively. It was observed that the lightness of dried ginger extracts were less as compared to fresh ones. The aqueous extract of dried ginger sample was the darkest among all extracts. Similar findings were reported by Singha and Muthukumarappan [47] on colour attributes of ginger extract. Licorice powder extracted by using different solvents followed the order as; ethanol: water (70:30) > methanol: water (70:30) > methanol: water (50:50) > ethanol: water (50:50) > aqueous (100%) with L* values of 44.83, 42.87, 35.28, 31.29, and 8.78. The a* value was found to decrease with highest values shown by ethanol: water (50:50) as 9.35 and lowest a*value of −5.1 in aqueous extracts revealed the greenish colour of extract. Similarly, the highest b* value of 88.43 was observed in methanol: water (50:50) and lowest b* value of 40.4 for aqueous extracts.

3.7. Quantification of polyphenols in ginger and licorice by RP-HPLC

The phenolic compounds and flavonoids in ginger rhizomes and licorice extracts were quantitatively estimated by RP-HPLC method. The representative chromatograms of the extracts obtained by using different solvents are shown in Figs. 3 & 4, respectively. The compounds were detected based on retention time and using UV–visible spectrum as previously described by Caballero-Ortega et al. [25]. The retention time of the phenolic acids such as gallic acid, vanillic acid, and ferulic acid extracted from ginger was observed as 1.52 min, 5.71 min and 1.92 min, respectively. The amount of gallic acid, vanillic acid, ferulic acid was calculated from the regression equation (Table 3) and the highest value was found in ethanol: water (75:25) (41.30, 97.00, 218.4 mg/kg) and ethylacetate: water (75:25) (48.31, 94.30, 190.43 mg/kg) extracts, while the lowest was observed in aqueous extracts (24.10, 86.43, 94.50 mg/kg), respectively (Table 5). Likewise, the amount of flavonoids (quercetin, kaempferol, isorhamnetin, rutin, catechin and caffeic acid were found highest in ethanol: water (75:25) (101.7, 37.76, 247.8, 31.67, 175.1 and 38.23 mg/kg) extracts. Whereas the lowest flavonoid content was shown by aqueous extraction (40.65, 19.78, 134.67, 11.54, 87.57 and 18.76 mg/kg). The results showed higher amount of both phenolics/flavonoids in dried ginger samples as compared to fresh extracts with isorhamnetin, ferulic acid, catechin and quercetin as the predominant compounds. These findings are in line with the results reported by Ghafoor et al. [48] and Tohma et al. [40] who reported the improved antioxidant properties of dried samples as compared to fresh ones. This might be due to lower moisture level and higher dry matter content, along with the probable deactivation of the polyphenol-oxidase enzymes during drying [48]. In the case of licorice, the retention time of the phenolic acids such as ferulic acid, vanillic acid, and chlorogenic acid was observed 5.28 min, 1.81 min and 2.72 min, respectively. The amount of vanillic acid, ferulic acid and chlorogenic acid was calculated from the regression equation (Table 3). The highest value was seen in methanol: water (50:50) (3.02, 6.81 and 0.98 mg/kg) and lowest in aqueous (1.98, 5.03, 0.51 mg/g) extracts, respectively (Table 6). Similarly, the amount of quercetin, kaempferol and caffeic acid was found highest in methanol: water (50:50) followed by ethanol: water (70:30), methanol: water (70:30) and water (100%) of licorice extracts, respectively (Fig 5 a,b). While the gallic acid and isorhamnetin were not found in either of these solvents comparable to the observations made by Memariani et al. [49] According to Trabelsi et al. [50] pure methanol-based leaf extracts contained the greatest concentration of polyphenols, indicating the wide range of phenolic content and antioxidant activity among the various solvents. Numerous bioactive compounds have been derived from licorice primarily triterpene saponins and flavonoids having broad biological activity [51]..

3.8. Fourier transform infra-red (FTIR) spectroscopy

The molecular structure and chemical bonding of biological materials can be determined by using FTIR. As shown in the Fig. 6 (a) that the spectrum in general revealed a wide band in the range of 3000 to 3500 cm^{-1} which may be due to hydrogen bonds and stretching of the O–H bond of carbohydrates or carboxylic acids. The residual water peak between 2850 and 3000 cm^{-1} may be ascribed to SP_3 C–H stretching. The peaks at wavelengths 1730–1750 cm^{-1} may be attributed to the C = O stretching of carboxylic acids and esters. While the peaks related to C-O bond of organic acids and sugars were observed between 1060 and 1150 cm^{-1}. The spectral peaks at 1017 cm^{-1}, 1105 and 1142 cm^{-1} could be described as C-O stretch of C-O-C bonds and C-O–H bonding. At 3200–3300 cm^{-1}, a broad band was attributed to the O–H stretching of (intermolecular) hydrogen-bonded and asymmetric CH_2 vibrations (O–H stretching). Kumar et al. [52] reported the peaks at 1603, 1190, 813, 850, and 723 cm^{-1} were strong indicators of flavonoids and alkaloids of ginger extract. Furthermore, multiple prominent peaks at wavenumbers 680, 940, 1170, 1470, and 1770 cm^{-1} were identified in relation to the spectra of 6-gingerol, showing that it is the main constituent of the extract [53]. The results are consistent with the annotations of Varakumar et al. [54] for FTIR spectroscopy of extracted oleoresin from ginger rhizome powder. In case of licorice, prominent spectral bands were observed in the region at 2800–2900, 1708, 1601, 1464, 1376, 1008, 880, 716 and 654 cm^{-1} (Fig. 6b) mainly ascribed to C–H, C–O, C = O,—C–O–C and C = C stretching modes most of which might be the characteristic of flavonoids and terpenoids [55].

3.9. Particle size and morphological characteristics of extracts

Dynamic light scattering (DLS) was used to determine the average particle size (APS) of 547 ± 17.52 and 766 ± 16.02 nm in ginger extracts while in licorice extracts the APS was observed as 450 ± 4.11, and

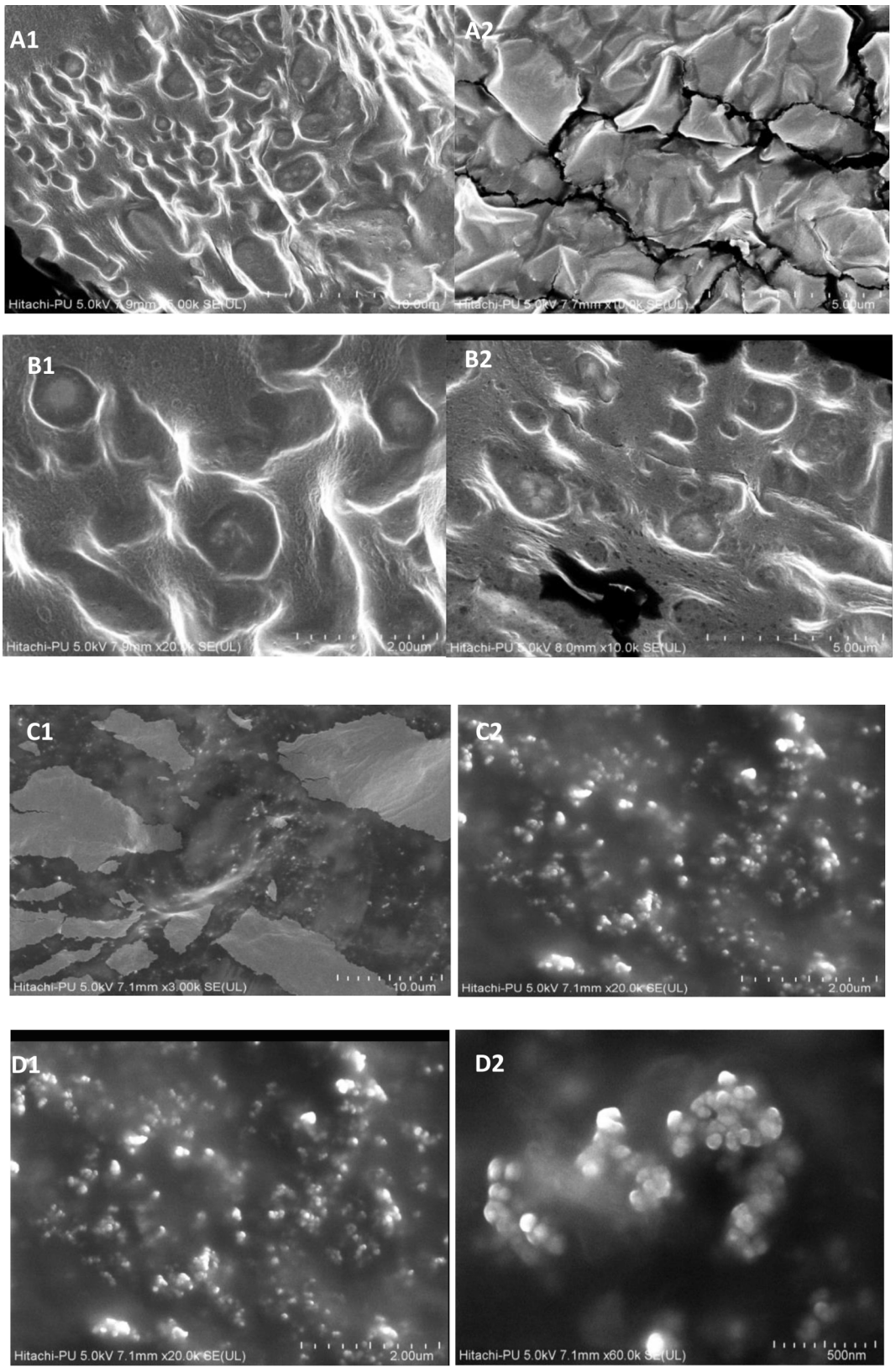


Fig. 7. Field emission scanning electron micrographs of ginger: A_1, A_2 & B_1, B_2 represent the ethanol: water extract (75:25); C_1, C_2 & D_1, D_2 represent the ethyl-acetate: water extract (75:25), respectively.

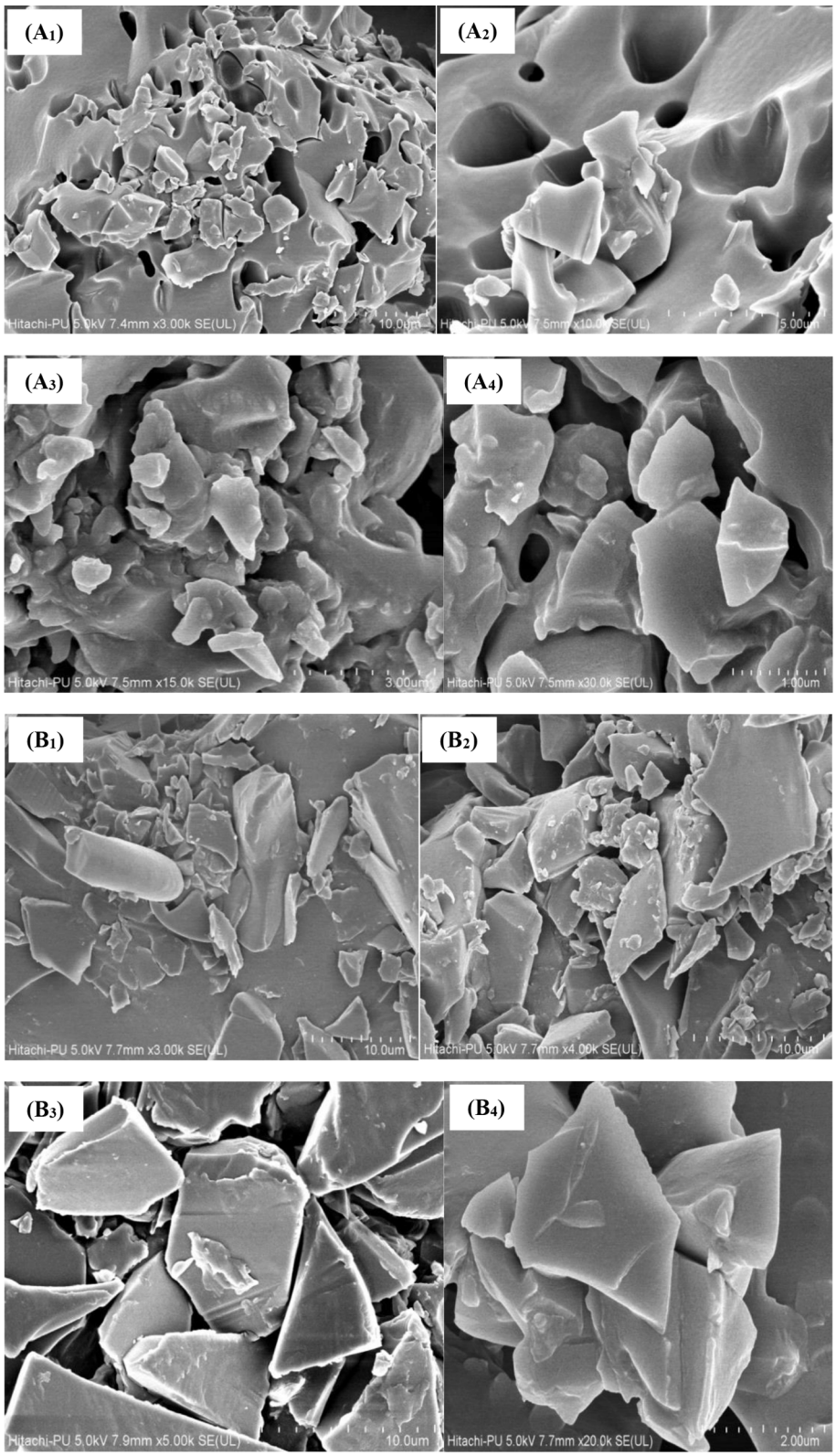


Fig. 8. Field emission scanning electron micrographs of liquorice: A_1, A_2, A_3 & A_4 represent the ethanol: water extracts (70:30); B_1, B_2, B_3 & B_4 represent the methanol: water extract (50:50), respectively.

566 ± 6.73 nm, respectively as described in Table 9. The particle size analysis showed freeze drying enhanced the adhesive-agglomerative property of extracts (ginger and licorice) thus increased particle sizes, producing many agglomerated micro-particles with a diameter around 0.5–10 μm (Figs. 7 & 8) at variable magnifications and more porous granules with a bimodal intra-granular distribution [56] as displayed by FESEM micrographs (Figs. 7 & 8). It was discovered that the surface of licorice extract seems to be more perforated, with granules that have been broken and compressed, resulting in greater yields of a target compound being released from the matrix.

4. Conclusions

It can be concluded from the study that the ultrasound treatment significantly increased the yield of bioactive compounds. The maximum extraction efficiency of polyphenols in dried ginger and licorice was found in aqueous extracts. The different solvents showed different extraction yield in ginger and followed the order aqueous > ethanol > acetone > ethylacetate. However, in case of licorice the extraction in different solvents showed the order aqueous > ethanol > methanol. In terms of antioxidant properties, phenolics and flavonoids ethylacetate and ethanol significantly proved to be better solvents as compared to other extraction solvents for ginger. Similarly, for licorice the better antioxidant properties and phenolics/flavonoids were observed in solvents like ethanol and methanol. The results emphasized that ginger and licorice extracts dried by freeze-drying had significantly increased flavonoid and total phenolic content as shown by HPLC analysis, thus displaying a good correlation with higher antioxidant activity. Thus, the nutraceutical potential of ginger and licorice, as well as their distinct flavour, make them promising ingredients for functional food formulations for value-added products of commercial importance.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

The authors gratefully acknowledge the Indian Council of Medical Research (ICMR), Government of India for financial assistance by awarding the Research Associate Fellowship to Dr. Romee Jan (3/1/2/193/2020-Nut).

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