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Original Article

SPECTROPHOTOMETRIC, HPTLC AND GC-MS STUDIES ON SELECTED SPICE EXTRACTS

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ABSTRACT

Objectives: The present work was carried out with a view to analyze the major phytochemical compounds of Indian spices, cumin (*Cuminum cyminum* L.), fenugreek (*Trigonella foenum-graecum* L.) and fennel (*Foeniculum vulgare* Mill.).

Methods: Hydro-alcoholic (30% ethanol in water, v/v) extracts were prepared from selected spices and analyzed for extract yield, loss on drying (LOD), pH and phytochemical compounds such as total alkaloids, phenols, flavonoids and tannins were quantified by spectrometric methods. High performance thin layer chromatography (HPTLC) and gas chromatography coupled with mass spectrometer (GC-MS) techniques were employed to reveal the phytochemicals of selected spice extracts.

Results: Hydro-alcoholic extracts from selected spices revealed the pH to be 5.36-5.62, loss on drying (12.54–15.41%) and extract yield (8.62–29.19%). Among the investigated samples, higher levels of alkaloids (2.032%) and tannins (0.809%) were found in fenugreek while fennel exhibited highest content of total phenolics (3.39%) and flavanoids (2.621%). The hydro-alcoholic extracts were subjected to HPTLC analysis and the results suggested the presence of three different major phytochemical compounds in cumin and fenugreek, whereas fennel extract displayed only one major peak. Cumin, fenugreek and fennel extracts showed relatively similar spots with Rf values of 0.51, 0.62, 0.90 and 0.97, which indicates the presence of four similar type of flavonoids in each extract. Presence of some volatile compounds in extracts was identified by GC-MS analysis.

Conclusion: Due to the presence of various phytochemical constituents and favorable extract yield, LOD and pH, the presently investigated spice extracts could be used in drug formulations.

Key words: Cumin, fennel, fenugreek, Phytochemicals, HPTLC profile, GC-MS analysis.

INTRODUCTION

Spices are used all over the world to improve the taste and flavour of food products. In addition, they have medicinal properties, and can be beneficial in the prevention of different human diseases. Epidemiological and *in vitro* studies strongly suggest that phytochemical of spices have potential protective effects against many diseases. Therefore, they could be used as anti-mutagenic, antibacterial, antiviral and anti-inflammatory agents [1]. There is increasing evidence that consumption of phytochemical compounds present in spices may lower the risk of serious health disorders [2, 3]. In India, the spices such as cumin (*Cuminum cyminum* L.), fennel (*Foeniculum vulgare* Mill.) and Fenugreek (*Trigonella foenum-graecum* L.) are important ingredients used in the food.

Cumin (*Cuminum cyminum* L.) is an aromatic plant belonging to the Apiaceae family and is used to flavor foods, to impart fragrances, and used in medicinal preparations [4]. In Asian countries, it is recommended in digestive disorders [5]. In traditional medicine, cumin is considered as a stimulant, carminative, and astringent, and is often prescribed in gastrointestinal, gynecological, and respiratory disorders. It is also used for treating tooth-ache, diarrhea, and epilepsy [6]. Anti-oxidant and anti-microbial [7, 8], hepato-protective [9], anti-diabetic [10] and anti-cancer [11] properties of cumin seeds were already reported. Hypocholesterolemic effect of cumin was investigated using various models [12–15]. Chemical composition and nutraceutical potential of cumin seeds are reviewed by Sowbhagya [16]. Cuminaldehyde provides the characteristic aroma to cumin seeds [17].

Foeniculum vulgare Mill. (fennel), a perennial herb with a characteristic aniseed flavor, belongs to the Apiaceae family and is cultivated worldwide. For centuries, fennel seeds have been used as a traditional herbal medicine in Europe and mainland China [18]. A number of beneficial properties such as anti-inflammatory, analgesic, antibacterial, and antioxidant [19, 20], dyspeptic disorders [21], hepato-protective activity [22], anti-depressant effect [23] have been attributed to fennel seeds. Hypolipidemic effect of volatile oil of fennel was evaluated [24–26]. Fennel seeds are reported to contain phytochemicals such as polyacetylenes (falcarinol, falcarindiol, falcarindiol-3-acetate) and polyphenols (caffeic acid, gallic acid, apigenin-7-o-glucoside, ferulic acid, syringic acid, isovitexin, phloridzin) [27].

Fenugreek (*Trigonella foenum-graecum* L.) is an annual herb belonging to the family Fabaceae widely grown in India [28]. Fenugreek seeds were used as tonic and lactagogue [29] as well as for the treatment of weakness and edema in legs [30]. The anti-dyslipidemic effect of fenugreek seed extracts and phytochemicals were evaluated [31, 32]. Protective effect of fenugreek seed extract against gastric ulcer was studied in animal model [33]. Anti-cancer effect of fenugreek extract containing diosgenin was evaluated in lung cancer cell line [34]. Anti-inflammatory activity of herbal ointments formulated with ethanolic extract of fenugreek was analyzed by Jyothi *et al.* [35]. Several studies indicated the hypoglycemic and hypolipidemic properties of fenugreek seeds [36]. The seeds of fenugreek contain chemical constituents such as saponins, coumarin, fenugreekine, sapogenins, phytic acid, scopoletin and trigonelline and diosgenin [37].

Even though few reports are available on the phytochemical composition of selected spices (cumin, fennel and fenugreek), the use of hydro-alcoholic solvent system (30% ethanol in water V/V) to extract the phytochemicals are not yet studied and evaluated for chemical composition of hydro-alcoholic extracts of cumin, fennel and fenugreek. Hence, the present work was carried out to evaluate the physico-chemical properties and phytochemical compounds with the help of Spectrophotometry, HPTLC and GC-MS techniques.

MATERIALS AND METHODS

Plant materials

Spices namely fenugreek, fennel, and cumin were purchased from local market, Thanjavur, Tamil Nadu, India. The spices were authenticated in the Center for Advanced Research in Indian System of Medicine (CARISM), SASTRA University, Thanjavur.

Chemicals

Gallic acid and quercetin were procured from Natural Remedies, Bangalore. All other chemicals used were of AR grade.

Preparation of extract

The materials of cumin, fenugreek and fennel were powdered in domestic mixer. About 500 g of each powdered sample was macerated with 1000 ml of solvent (30% ethanol in water, v/v) and kept for 3 d with occasional shaking. The extracts were filtered using Whatman No. 1 paper and the filtrate were evaporated to dryness over a water bath at 90ºC. The extract yield calculated was found to be 8.62%, 29.19%, and 18.10% w/w for cumin, fenugreek and fennel, respectively.

Determination of loss on drying

All the three extracts (500 mg each) were taken in a pre-weighed petri-dish with lid and kept in a hot-air-oven at 105ºC for three hours. Then, the samples were cooled to room temperature in a desiccator and weighed in an electronic balance. Based on the differences in the weight, the loss on drying (LOD) was calculated and expressed on the percentage basis.

Measurement of pH

For the measurement of pH, the extracts (250 mg) were taken in a beaker and dissolved in 25 ml of distilled water. The pH of the solution was measured using pH meter (Model: LI120, Make: Elico).

Estimation of total alkaloids

The total alkaloid content of spice extracts was estimated according to the method described in Indian Pharmacopoeia [38]. The extracts were weighed (5 g each) separately and 100 ml of alcoholic ether mixture (4:1 ratio, v/v) was added with 2 ml of dilute ammonia solution, shaken well and allowed to stand for 1 h. Then the solution was filtered with Whatman No. 41 paper and filtrate were collected in a separating funnel and 30 ml of 1 N sulfuric acid was added and shaken well. The acid layer was collected in another separating funnel. Then 25 ml of 0.5 N alcoholic sulfuric acid (3:1) was added, extracted for 3 min and the acid layer was collected in the separating funnel. The extraction was repeated until the solution becomes colorless. The collected acid layer was extracted with chloroform in order to remove the extraneous matter and the pH was adjusted to 10 with dilute ammonia solution until alkaloids get precipitated. The chloroform layer was collected into a pre-weighed beaker through a funnel containing sodium sulphate and evaporated to dryness over a water bath. The weight of the residue was measured and the results are expressed in percentage basis.

Estimation of total phenolics

The total phenolic content of the extracts was determined using Folin-Ciocalteu reagent [39]. Different concentrations of the standard and samples were prepared and mixed with 1.5 ml of Folin Ciocalteu reagent, and after 5 min 4 ml of 20% Na_2CO_3 solution was added and made up to volume with distilled water. Then the absorbance was recorded at 765 nm. Gallic acid was used as standard and the results were expressed in percentage basis.

Estimation of total flavonoids

Total flavonoid content was determined using aluminium chloride ($AlCl_3$) according to the method of Zhishen *et al.* [40] using quercetin as a standard. The extract (0.1 ml) was added to 0.3 ml distilled water followed by 5% $NaNO_2$ (0.03 ml). After 5 min at 25 °C, $AlCl_3$ (0.03 ml, 10%) was added. After further 5 min, the reaction mixture was treated with 0.2 ml of 1 mM NaOH. Finally, the reaction mixture was diluted to 1 ml with water and the absorbance was measured at 510 nm. The results were expressed in percentage basis.

Estimation of tannins

The extract (1 g) was digested with 50 ml of water and heated on a water bath for 30 min with frequent stirring [41]. The supernatant was collected into a volumetric flask and the extraction was repeated until the solution becomes colorless. The solution was cooled and made up to a volume of 100 ml with distilled water, from which 25 ml was taken with 750 ml of water and 25 ml of indigo sulphonic acid solution. The contents were titrated against 0.1 M potassium permanganate solution with constant stirring until golden yellow colour appears. A blank was also performed without the sample. Each ml of 0.1 M potassium permanganate solution is equivalent to 0.004157 g of tannins. Based on the titration value, the total tannin content was calculated.

HPTLC analysis for flavonoids

HPTLC analysis was performed to obtain the characteristic finger printing profile of spice extracts. The extract (500 mg) was dissolved in water-alcohol (7:3, ratio, v/v) and 20 µl was applied on a pre-coated silica gel plates (60 F254, 0.2 mm thickness, 10 x 10 cm size, Merck, Germany) by using an automizer of HPTLC (CAMAG Linomat-5, Muttenz, Switzerland). The plate was developed in the solvent system to a distance of 8 cm using the mobile phase (Toluene: Ethyl acetate: Formic acid, 2:1:1 ratio). After development, the plate was dried in a hot-air-oven and visualized at 254 and 366 nm. The plate was scanned densitometrically and the Rf values and colour of the resolved bands were recorded and the profile pattern was presented in table 3.

GC-MS analysis

The extracts were analyzed using Gas Chromatographic system coupled with Mass Spectrometry (Perkin Elmer, Model: Clarus-500). Silica capillary column (30 m x 0.25 mm, 0.25 µm film thickness, Elite-5 MS non-polar fused) was used. Oven temperature was programmed with an increase of 8 °C/min to 280 °C; injector temperature was 280 °C; carrier gas was helium with the flow rate of 1 ml/min. Sample (2 µl) was injected with split ratio of 1:10. Ionization energy 70 ev was used in the electron ionization mode; ion source temperature was set at 150 °C, mass was scanned in the range of 40-450 amu. The resulted mass spectrum was compared with inbuilt NIST library database and fragments of various compounds present in the extracts were identified and presented in the fig. 2.

RESULTS AND DISCUSSION

Extract yield

Among the presently investigated spices, the hydro-alcoholic extract of fenugreek exhibited the highest yield (29.19%), which is followed by fennel (18.10%) and cumin (8.62%) (table 1). The extract yield of fenugreek of the present study was higher than that of an earlier study on ethanolic extract of fenugreek (25.32%) [42]. Hydro-alcoholic extract yield of fennel of our present study (18.10%) is comparable to that of water extract yield of fennel (16.31–20.87%) and higher than that of alcoholic extractives (5.59–7.00%) of fennel [43]. In general, all the presently investigated spices exhibited good extract yield compared to the previous study and hence suitable for herbal drug formulation with different therapeutic action.

Table 1: Extract yield, loss on drying and pH of hydro-alcoholic extracts of spices[1].

S. No.	Parameters	Cumin	Fenugreek	Fennel
1	Extract yield (%)	8.62±0.23	29.19±0.18	18.10±0.12
2	pH value	5.58±0.02	5.62±0.01	5.36±0.02
3	LOD (%)	13.60±0.11	15.42±0.02	12.55±0.02

[1]Values are reported as mean±SD of three separate determinations.

Extract pH

The hydro-alcoholic extracts of selected spices exhibited slightly acidic pH (5.36–5.62) (table 1). The pH of fennel extract was found to be slightly lower to that of the previous report on fennel (5.89–6.00) [43]. The pH of the presently studied spice extracts are fall in a similar range and hence suitable for further drug development.

Loss of drying

The LOD result indicates that the extracts are slightly hydroscopic in nature. The loss on drying was high in fenugreek (15.42%) when compared to cumin and fennel extracts. The LOD of fennel of the present study (12.55%) was found to be higher than the earlier

report on fennel (9.63–10.10%) [43]. According to Indian Pharmacopoeia [38], the LOD of herbal drugs should not exceed 35% (w/w) and the presently analyzed extracts revealed LOD of 12.55–15.42%. The spice extracts of the present investigation indicate that they have the additional advantage of being easier to store as they are less liable to microbial or hydrolytic spoilage.

Phytochemical compounds

The total alkaloid concentration of spice extracts was found to be 0.91–2.03% (table 2). The alkaloid content of fenugreek of the present study (2.03%) was found to be higher when compared to previous reports on alkaloid level of Nigerian spices such as *Piper guineenses* (1.54%), *Xylopia aethiopica* (1.44%), *Monodora myristica* (1.32%), *Tetrapleura tetrapetra* (1.46%) and *Allium sativum* (1.22%) [44]. Alkaloids are natural product that contains heterocyclic nitrogen atoms and are basic in nature. They possess many pharmacological activities including anti-hypertensive effects (many indole alkaloids), anti-arrhythmic effect (quinidine, spareien), anti-malarial activity (quinine), and anti-cancer actions (dimeric indoles, vincristine, vinblastine). Some alkaloids have stimulant property such as caffeine and nicotine, morphine are used as analgesic and quinine used as the anti-malarial drug [45].

Table 2: Phytochemical compounds of hydro-alcoholic extracts of spices[1]

S. No.	Phytochemical compounds	Cumin	Fenugreek	Fennel
1	Total alkaloids (%)	0.91±0.16	2.03±0.08	1.48±0.15
2	Total flavonoids (%)	1.92±0.24	2.02±0.11	2.62±0.14
3	Total phenols (%)	3.04±0.06	3.19±0.13	3.39±0.08
4	Total tannins (%)	0.65±0.13	0.81±0.05	0.49±0.12

[1]Values are reported as mean±SD of three separate determinations.

The flavonoid concentration of presently analyzed spice extract falls in the range of 1.92–2.62% (table 2). The flavonoid content of presently studied spices was higher when compared to an earlier report on fenugreek (0.65%) [42] and fennel (0.53%) [46], which might be due to the use of specific hydro-alcoholic solvent system. Flavonoids are phenolic substances found in vascular plants with over 8000 individual known compounds. Apart from their physiological roles in the plants, flavonoids are important components in the human diet, although they are generally considered as non-nutrients. Indeed, the level of intake of flavonoids from diet is considerably high as compared to those of vitamin C (70 mg/day), vitamin E (7-10 mg/day), and carotenoids (beta-carotene, 2-3 mg/day) [47]. Many studies have suggested that flavonoids exhibit biological activities, including antiallergenic, antiviral, anti-inflammatory, and vasodilating actions. However, interest has been devoted to the antioxidant potential of flavonoids, which is due to their ability to reduce free radical formation and to scavenge free radicals.

Presently studied spice extracts revealed 3.04–3.39% of total phenolic concentration (table 2). The total phenolic content of fenugreek was found to be higher when compared to a previous report on fenugreek (0.68%) [42], cumin (1.8%) [48] and fennel (0.87%) [46]. Phenolic compounds are the largest category of phytochemicals and are most widely distributed in the plant kingdom. They are plant secondary metabolites, and have an important role as defense compounds. phenolics exhibit several properties beneficial to humans and its antioxidant properties are important in determining their role as protecting agents against free radical-mediated disease processes. Phenolics possess diverse biological activities, for instance, antiulcer, anti-inflammatory, antioxidant, antitumor, antispasmodic and antidepressant activities [49]. The tannin concentration of spice extracts was observed to be 0.49–0.81% (table 2). The tannin content of fenugreek of the present study (0.81%) was found to be higher when compared to previous reports on tannin level of Nigerian spices such as *Xylopia aethiopica* (0.24%), *Monodora myristica* (0.18%), *Tetrapleura tetrapetra* (0.22%) and *Allium sativum* (0.06%) [44].

Tannins are a heterogeneous group of high molecular weight polyphenolic compounds with the capacity to form reversible and irreversible complexes with proteins [50]. Several health benefits have been attributed for the intake of tannins and some epidemiological associations with the decreased frequency of chronic diseases have been established [51]. The tannin-containing plant extracts are used as astringents, against diarrhoea, as diuretics against stomach and duodenal tumors [52], and also used as anti-inflammatory, antiseptic, antioxidant and haemostatic pharmaceuticals [53].

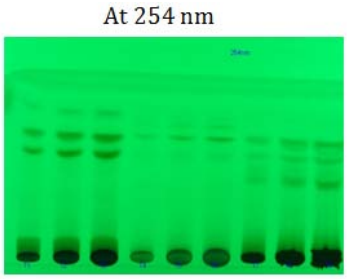
Phytochemical composition of presently investigated hydro-alcoholic extracts of spices showed that fenugreek extract contained higher levels of both total alkaloids (2.032%) and tannins (0.8098%) (table 2). The total phenolics (3.39%) and flavonoids (2.62%) concentrations were found to be high in fennel extracts when compared to cumin and fenugreek. Hence, presence of phytochemicals such as alkaloids, phenols, tannins and flavonoids in the presently investigated spice extracts might be responsible for the medicinal properties of the spices selected in the present work.

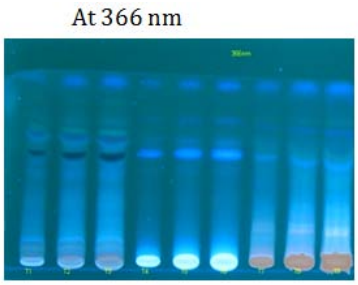
HPTLC profile

Among the modern analytical tools HPTLC is a powerful analytical method equally suitable for qualitative and quantitative analytical tasks. HPTLC is playing an important role in today's analytical world, not in competition to HPLC but as a complementary method. HPTLC method deals with qualitative and quantitative analytical applications such as herbal and dietary supplements, nutraceuticals, and various types of medicines. It is used in quality control and in purity checks, in the detection and identification of pharmaceutical raw materials, drugs and their metabolites in biological media. HPTLC method is also a very powerful tool for identification of the presence of adulterants in herbal products based on the characteristic image produced and much useful for determining the presence and the quantification of both inadvertent substitution as well as intentional adulteration of prescription drugs [54]. Hydro-alcoholic extracts of the selected spices were subjected to HPTLC analysis and the results revealed the presence of similar type of phytochemical compounds in cumin and fenugreek, whereas fennel extract displayed only one major peak. Cumin, fenugreek and fennel extracts showed nearly similar spots with Rf values of 0.51, 0.61, 0.90 and 0.96. Based on mobile phase composition used in this study, these spots could be flavonoids [39]. In an earlier study, presence of diosgenin and quercetin in fenugreek seeds was reported using HPTLC technique [55]. Similarly, flavonoid compounds were identified through HPTLC in *Polygonatum odoratum* [56], *Alstonia macrophylla* [57], *Urena lobata* [58] and *Cyamopsis tetragonoloba* [59]. In the present investigation, similar type of flavonoids with relatively near Rf value must exist in all the three spice extracts. So, this characteristic HPTLC profile could be used as a finger printing of the selected spice extracts to identify and authenticate the presence of these extracts in herbal drugs.

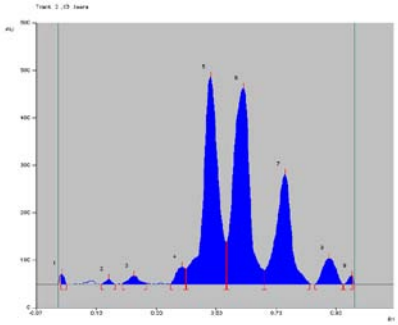
GC-MS analysis

Gas chromatography (GC) has been the choice of analysis of volatile compounds in plant extracts. The phytochemical constituents are identified using combination of GC with mass spectrometry (MS). GC-MS is a sophisticated technique used to identify the volatile phytochemical compounds in plant/drug extracts with the help of mass spectral libraries. The GC-MS analysis revealed the presence of six major phytochemical constituents in cumin extract like O-Cymene, 4-H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, alpha-D-Glucopyrsnoside, Cumic acid, 1,4,4,7a-Tetramethyl-2,4,5,6,7,7a-hexahydro-1H-indene-1, 7-diol and Benzene, 1-(1,3-dimethyl-3-butenyl)-4-methoxy. Similarly Rebey *et al.* [48] reported the presence of alpha-Thujene and p-Cymene in hexane extract of cumin. Further, 3-Pyridinecarboxylic acid, 2(5H)-Furanone, 3-hydroxy-4,5-dimethyl (Satalone), Ethyl á-d-riboside, Pyranoside, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl were found to be major compounds in fenugreek extract. Our results are in agreement with that of previous work reported by Arti [60] in fenugreek. Fennel extract revealed the presence of 2-Furancarboxaldehyde, 5-(hydroxymethyl)-, 2-Propanone, 1-(4-methoxyphenyl)-, Oxazolidine, 2-ethyl-2-methyl-were found to be major constituents.



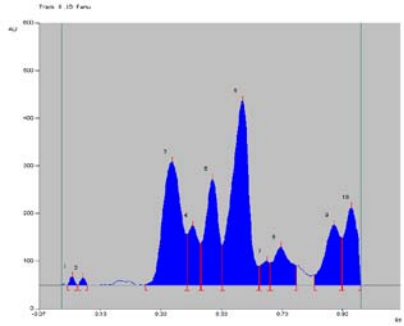


Peak display observed in Cumin extract



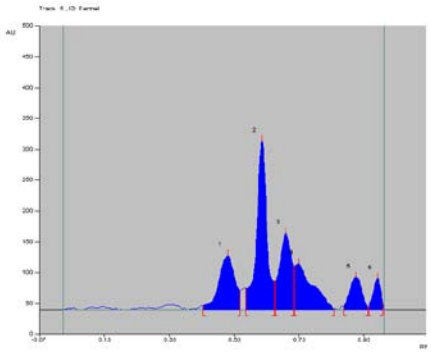
Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %	Assigned substance
1	0.01	19.7	0.01	21.6	1.74	0.03	0.3	188.0	0.41	unknown *
2	0.14	0.3	0.17	10.1	0.82	0.19	0.1	153.3	0.33	unknown *
3	0.21	1.9	0.25	17.4	1.41	0.29	2.0	475.1	1.03	unknown *
4	0.37	0.1	0.41	35.8	2.89	0.43	32.0	837.9	1.82	unknown *
5	0.43	32.3	0.51	435.5	35.21	0.56	88.3	16476.5	35.69	unknown *
6	0.56	88.3	0.62	413.3	33.42	0.69	28.5	17120.9	37.09	unknown *
7	0.69	28.6	0.76	231.5	18.71	0.84	2.1	8913.8	19.31	unknown *
8	0.86	0.0	0.90	54.1	4.37	0.95	0.1	1799.9	3.90	unknown *
9	0.95	0.2	0.98	17.5	1.42	0.98	15.4	194.9	0.42	unknown *

Peak display observed in Fenugreek extract



Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %	Assigned substance
1	0.02	0.4	0.03	18.0	1.25	0.05	0.7	198.6	0.37	unknown *
2	0.06	0.6	0.07	14.5	1.00	0.09	0.5	173.1	0.32	unknown *
3	0.28	1.5	0.36	258.2	17.92	0.41	106.0	12010.4	22.40	unknown *
4	0.41	106.3	0.43	124.2	8.62	0.46	86.6	3269.7	6.10	unknown *
5	0.46	87.1	0.50	222.4	15.43	0.53	81.7	7201.7	13.43	unknown *
6	0.53	82.5	0.60	386.5	26.82	0.65	39.0	16366.3	30.53	unknown *
7	0.65	39.5	0.68	49.6	3.44	0.69	45.9	1100.1	2.05	unknown *
8	0.69	46.1	0.72	79.3	5.50	0.78	40.2	3447.0	6.43	unknown *
9	0.83	21.2	0.90	125.7	8.72	0.93	98.3	4911.9	9.16	unknown *
10	0.93	98.4	0.96	163.0	11.31	0.99	0.0	4931.8	9.20	unknown *

Peak display observed in Fennel extract



Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %	Assigned substance
1	0.43	6.6	0.51	87.7	13.23	0.54	31.5	3383.6	17.43	unknown *
2	0.56	34.7	0.61	273.4	41.25	0.65	46.3	6822.7	35.14	unknown *
3	0.65	46.8	0.69	124.1	18.72	0.71	70.6	3525.1	18.16	unknown *
4	0.71	70.8	0.73	74.5	11.23	0.83	1.4	3221.7	16.59	unknown *
5	0.86	5.1	0.90	52.4	7.91	0.94	4.8	1536.9	7.92	unknown *
6	0.94	5.0	0.97	50.7	7.65	0.99	11.0	925.2	4.77	unknown *

Fig. 1: HPTLC profile of spice extracts (Spots T1-T3: Cumin extract, Spots T4-T6: Fennel extract, Spots T7-T9: Fenugreek extract)

Table 3: HPTLC profile pattern of hydro-alcoholic extracts of spices

S. No.	Name of the sample	No. of spots	R_f value	Colour of the spot (AT 366 nm)
1	Cumin extract	4	0.51	Black
			0.62	Blue
			0.90	Green
			0.96	Blue
2	Fenugreek extract	6	0.50	Blue
			0.60	Blue
			0.68	Blue
			0.72	Green
			0.90	Blue
			0.96	Blue

3	Fennel extract	6	0.51	Blue
			0.61	Blue
			0.69	Blue
			0.73	Blue
			0.90	Blue
			0.97	Blue

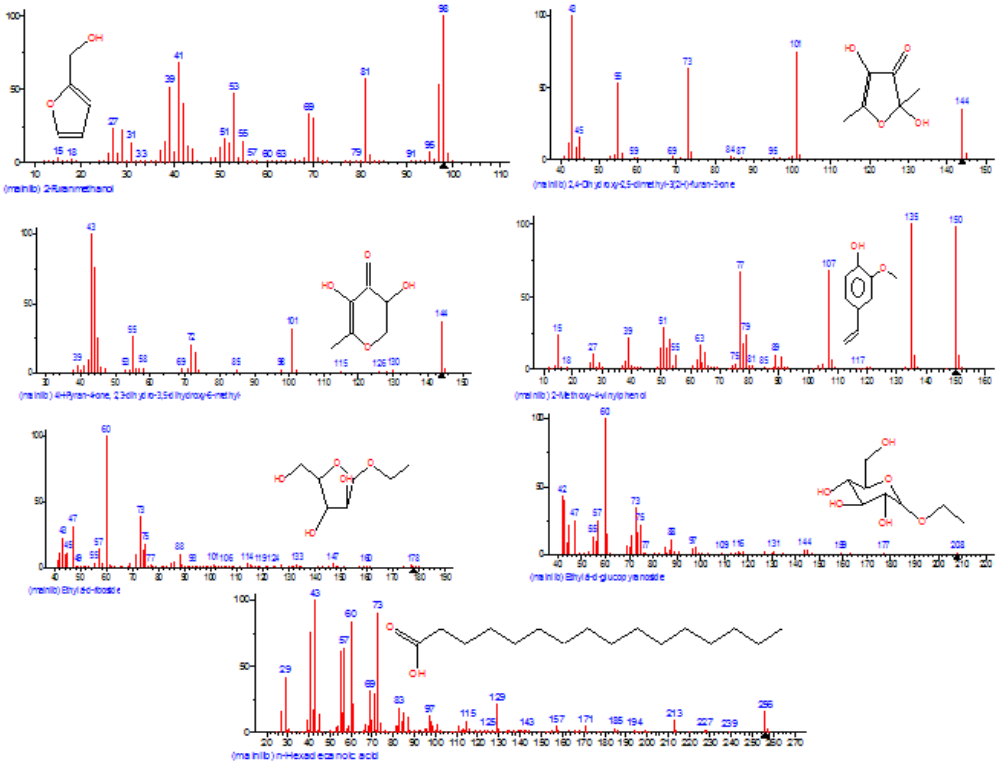


Fig. 2: Mass fragmentation pattern and structures of volatile compounds in selected spice extracts

Table 4: Data on similar GC-MS fragmentation patterns in the selected spice extracts

S. No.	Name of the compound	Peak area (%)		
		Cumin	Fenugreek	Fennel
1.	Name: 2-Furanmethanol Formula: C5H6O2 MW: 98	2.4349	0.3356	2.6382
2.	Name: 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one Formula: C6H8O4 MW: 144	0.5249	0.5091	0.9402
3.	Name: 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- Formula: C6H8O4 MW: 144	9.2617	4.9510	16.7284
4.	Name: 2-Methoxy-4-vinylphenol Formula: C9H10O2 MW: 150	1.3155	0.1159	1.6124
5.	Name: Ethyl á-d-riboside Formula: C7H14O5 MW: 178	1.0236	9.0651	0.6597
6.	Name: Ethyl à-d-glucopyranoside Formula: C8H16O6 MW: 208	3.2412	4.8457	0.8380
7.	Name: n-Hexadecanoic acid Formula: C16H32O2 MW: 256	4.1666	1.5368	3.9477

In GC-MS study, seven common phytochemical constituents (2-Furanmethanol, 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, 2-Methoxy-4-vinylphenol, Ethyl á-d-riboside, Ethyl à-d-glucopyranoside, n-Hexadecanoic acid) have been identified in all the three spice extracts. All these phytochemicals were reported to possess antimicrobial, anti-inflammatory, antioxidant, anti-cancer and hypocholesterolemic activities [61, 62]. Presence of these volatile phytochemicals might be responsible for the various therapeutic properties exhibited by the selected spice extracts.

CONCLUSION

The present study conducted on selected spice extracts revealed the extract yield, loss on drying, pH, phytochemicals content, HPTLC finger printing and GC-MS profile of cumin, fennel and fenugreek. The applied solvent system (30% ethanol in water, V/V) brought out the presence of notable levels of phytochemicals like total phenols, tannins, flavonoids and alkaloids from the spices with remarkable extract yield. Hence, such solvent system could be considered to recover these bioactive compounds for pharmaceutical applications. Further, the HPTLC profile of selected spice extracts offer a finger printing pattern for the rapid identification of the constituents of spice extracts in drug formulation and also indicated the presence of four similar type of flavonoids in each of the spice extract. The GC-MS analysis revealed the presence of various volatile compounds and also seven similar compounds in the presently investigated spice extracts. Presence of phytochemical compounds in hydro-alcoholic extracts might be responsible for the health claims and therapeutic potential of the investigated spices.

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CONFLICT OF INTERESTS

Authors declared no conflict of interest

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