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Antioxidant Screening of Various Solvent Extracts of *Cuminum cyminum* Cultivated in Bangladesh

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Abstract: *Cuminum cyminum* is a widely used spice and most valuable medicinal plant in South Asian countries to enhance vitality. This herb has been reported to be used as conventional remedy for many years. In this research, *C. cyminum* cultivated in Bangladesh was focused on exploring the scavenging ability using different solvents with green extraction technique. This study aims to screen the suitable solvent for the effective *C. cyminum* extraction followed by the phytochemical analysis and identification of its chemical constituents. The solvents, methanol and n-hexane, were used for the extraction using sonication extraction technique to screen the antioxidant activity of *C. cyminum.* Moreover, the different solvent ratios (0, 20, 40, 60, 80 and 100%) of ethanol and water were used to investigate the best combination of extracting solvents that can produce *C. cyminum* extracts with the most desirable and potent antioxidant activities. The metabolites were identified using gas chromatography-mass spectrometry. The methanol extract showed maximum yield (5.32±2.86%) and IC_{50} (3.02±1.97) value concerning n-hexane (3.02±0.78%, 5.08±0.95). However, the range of different solvent ratios (100% ethanol-0% ethanol) yield was 4.11±0.59-14.47±5.36%. On the other hand, the 60% ethanol exhibited the highest inhibitory potential (1.39±1.42µg/ml), and aqueous extract exhibited the lowest inhibitory potential (5.79±3.43µg/ml). Phytochemical investigation showed that tannin and glycosides were present in both extracts. Flavonoids showed a positive result in methanol extract, while anthraquinones and steroids showed a positive result in n-hexane extract. The identified metabolites using GCMS are D-carvone, 1, 3-benzodioxole, squalene, 2H, 6H-benzo [1, 2-b: 5, 4-b'] dipyran-2, 6-dione, D-limonene and apiol. *Cuminum cyminum* can reduce oxidative damage by potently inhibiting free radicals and can be utilized as natural compounds to scavenge free radical activity.

Keywords: Solvents, Extraction Yield, Antioxidant Activity, *Cuminum cyminum*, Bangladesh

1. Introduction

The medicinal plant contains a wide range of polar to nonpolar compounds that contribute to the antioxidant activity and act as free radicals scavengers that lead to many degenerative diseases. It is crucial to discover suitable extraction techniques in the innovation of nutraceutical based on plant products. Polar solvents are the ideal method for extraction of phenolics and other free radical scavenging activity containing phytochemicals from the natural product. This study focused on methanol, n-hexane, water, ethanol, and ethanol/water systems to identify appropriate extraction

solvent. Several studies have been reported on potent biological activities using appropriate solvent system with green extraction that can provide a potential to improve the release of secondary metabolites [1-4]. Before extraction, the solvent and extraction technique is essential to preserve material and enhance the shelf life by extracting potent chemical constituents. Studies on the effect of different solvent and extraction techniques on the phytochemicals and bioactivities of *C. cyminum* and other plant materials have been reported previously [5-7]. However, there is limited published data on the effects of solvents in antioxidant activities and metabolites constituents of *C. cyminum* cultivated in Bangladesh.

Cuminum cyminum, also known as 'jeera' (Apiaceae family), is a popular and the most useful spice in the world. It is widely used traditional medicine in the south asian countries especially this herbal plant is extensively found in tropical countries, such as India and Bangladesh. Cultivation of cumin (jeera) has also introduced different areas in Bangladesh. Cumin has a long history of cultivation in other countries all over the world. Many studies have been carried out to utilize *C. cyminum* plants for medical benefits. It is essential to know the suitable solvent for the effective cumin extraction followed by the phytochemical analysis and identification of its chemical constituents. Figure 1 shows the plant and seed of *C. cyminum*. It is reported to possess medicinal properties, like antimutagenic, antiviral, anticancer, antimicrobial, anti-inflammatory and inhibition potential [8]. The medicinal effects are probably due to anthraquinone, protein, alkaloid, flavonoid, coumarin, glycoside, resin, saponin, steroid and tannin compounds present in the plant. Extraction is a crucial step to ensure efficient extraction method to maximize the plant's benefit as pharmaceutical drug or food additives [9]. The variation in plant extracts' metabolites and pharmacological activity with the extraction solvent used is distinct [10]. Thus, a standardized approach is needed for assessing the bioactive compounds and their efficiencies.

Recently, botanical extracts with green extraction using binary solvent system have gained popularity to be used in healthcare products. Researchers are focused more on the optimization of extracting solvents with adequate levels of the active phytochemicals. The knowledge of the solvent on secondary metabolites may be beneficial on preserving natural products. So far, no study has been conducted on the effects of the solvent on *C. cyminum* chemical constituents, especially that of the Bangladeshi variety. Hence, the aim of this study is to investigate the best extracting solvents that can recover *C. cyminum* extracts with the most suitable and strong free radical scavenging activities. Samples were extracted using methanol and n-hexane for screening of bioactivity and ethanol-water binary solvent system (0, 20, 40, 60, 80 and 100%) for their food application. The bioactivities based on the tested solvents were also evaluated on the basis of the concentrations of their bioactive compounds. The results of this study might be recommended as the best extracting solvent to optimize *C. cyminum* extracts with good potential in selected pharmacological properties and high productivity.

Figure 1. *Cultivation of Cumin (Cuminum cyminum) in Bangladesh.*

2. Experimental

2.1. Chemicals

The ethanol, methanol, acetone, n-hexane, chloroform, hydrochloric acid, sulphuric acid and 2, 2-diphenyl-1 picrylhydrazyl (DPPH) were bought from Merck (Darmstadt, Germany). The standard catechin, pyridine, *N*-methyl-*N*-(trimethylsilyl) trifluoro acetamide purum (MSTFA) with 97.0% purity and methoxyamine hydrochloride (≥98%), ferric chloride, acetic anhydride was procured from Sigma-Aldrich (St. Louis, MO). The α-naphthol (Loba Chemicals, India), Dragendorff's reagent, Hager's reagents are used for the phytochemical analysis of cumin extract. Ultrapure water obtained from a Milli-Q system (Millipore, Milford, MA) was applied to make *C. cyminum* extracts and the reagents.

2.2. Plant Materials

The mature cumin seed was collected from the local area of Mohammodpur, Magura, Bangladesh. This plant was cultivated in December 2018. In 3 decimal lands, a total 120 kg seed was harvested. The voucher number of specimens was 376. The identification was done by Rajshahi University Herbarium, Department of Botany, University of Rajshahi, Bangladesh.

2.3. Sample Preparation

The collected seed was cleaned and dried at room temperature to avoid any debris leftover. After that, the samples were grounded to a fine powder by a grinding machine and stored in an air tight container in the refrigerator for further analysis.

2.4. Preparation of Plant Extracts

Two different types of extracts have been prepared. Firstly, the extractions were performed using methanol and n-hexane on a wet basis to screen antioxidant activity of the seed of *C. cyminum*. Based on activity, twenty-four extractions were done using another two solvents, ethanol and water at various ratios (0% ethanol, 20% ethanol, 40% ethanol, 60% ethanol, 80% ethanol and 100% ethanol) with four replicates [9]. For each solvent, about 6gm of the powdered material was soaked in 200ml of solvent. The sample was protected from light by covering with aluminium foil to prevent degradation. Samples were extracted using sonification method with the temperature of 25°C for 30 min. The extracts were centrifuged at 20°C for 15 min at 4000 rpm. Later the rotary evaporator was set at temperature 40°C to concentrate the crude and allowed for drying in air. The dried extract was kept in a refrigerator for further antioxidant activity, phytochemical and GCMS

analysis. Equation 1 was used to obtain the percentage of the extraction yield.

$$\text{Percentage of the extraction yield} = (\text{extract weight} / \text{sample weight}) \times 100 \quad (1)$$

Where the weight of extract = weight of beaker with extract-weight of the beaker

2.5. Antioxidant Activity

The free radical scavenging activity of various extracts was measured *in vitro* by DPPH assay according to the Shirwaikar and Kumar [11, 12] methods with some modifications. About 2ml of different extracts concentrations were added at an equal volume to a 0.1mM methanol solution of DPPH. After 30 min at room temperature, the absorbance was read at 517 nm. Radical scavenging activity was calculated using Equation 2. Then percentage radical scavenging activity was plotted against concentration, and from the graph, IC_{50} was calculated. Catechin was used as positive control.

$$\%\ \text{radical scavenging activity} = (\text{Acontrol-Asample} / \text{Acontrol}) \times 100 \quad (2)$$

Where Acontrol is the absorbance of the control, and Asample is the absorbance of the sample at 517 nm.

2.6. Phytochemical Analysis

Phytochemical tests were carried out qualitatively for the presence of different phytochemicals such as alkaloids, flavonoids, glycosides, anthraquinones, resins, saponins, steroids and tannins using the standard methods [13, 14].

2.6.1. Tests for Saponins

Frothing test: One drop of sodium bicarbonate solution was mixed to 5ml of the sample in a test tube and shaken vigorously. The tube was allowed to stand for 3 min, and the presence of saponins was confirmed with the formation of honey comb-like froth.

2.6.2. Tests for Tannins

A few drops of 5% w/v $FeCl_3$ solution were added to 1-2ml of the extract. The green colour indicated the presence of gallo-tannins, while brown colour indicates the presence of pseudo-tannins.

2.6.3. Tests for Flavonoids

Shinoda's test: A 2mg of extract was dissolved in 5ml of ethanol and to this ten drops of dilute hydrochloric acid was added and followed by adding a small piece of magnesium. Formation of pink colour indicated the presence of flavonoids.

2.6.4. Tests for Steroids

Salkowski reaction: A 2mg of dry extract was shaken with chloroform then sulphuric acid was added slowly to the chloroform layer by the sides of the test tube. Formation of red colour indicated the presence of steroids.

Liebermann-Burchard's test: A 2mg of sample extract was dissolved in acetic anhydride and heated to boiling. Once cooled down, about 1ml of concentrated sulphuric acid was added along the test tube's sides. The presence of steroids was confirmed through the formation of green colour.

2.6.5. Tests for Glycosides

Molisch's test: A 2mg of sample was mixed with 10ml of water, then the solution was filtrated and about 2-3 drops of Molisch's reagent was added. Later 2ml of concentrated sulfuric acid was introduced through the side of the test tube. The reddish violet ring appeared and indicated the presence of glycosides.

2.6.6. Tests for Alkaloid

Dragendorff's test: About 5ml of distilled water was added to 2mg of the extract, and then 2M hydrochloric acid was added. Then 1ml of Dragendorff's reagent was added, and the presence of alkaloids was confirmed through the formation of an orange-red precipitate

Hager's test: Few drops of Hager's reagent were added to 2mg of the extract in a test tube. Appearance of yellow precipitate confirmed the presence of alkaloids.

2.6.7. Test for Anthraquinones

About 5 g of seed powder was mixed with 20 ml of benzene and then filtered. Later, about 5ml of 10% ammonium hydroxide solution was added and shaken well. Presence of free anthraquinones was confirmed from the development of violet color.

2.7. GCMS Analysis

2.7.1. Derivatization Procedure

About 25 mg of samples were mixed with 50 µL of pyridine and sonicated for 10 minutes. Later the samples were centrifuged at 3000 rpm for 10 min in a microcentrifuge (TG16-WS table top high-speed centrifuge). The samples were dilute and vortexed with 100µL of methoxyamine HCl. Later it was incubated for 2 hrs at 60°C in an incubator shaker (DI-81220V, 300WAmb. +5 to 60°C, Korea). About 300 µL of MSTFA was mixed and incubated for 30 min at 60°C. After the samples were filtered, it was transferred into vials and let stand overnight at room temperature for the further GCMS analysis [15].

2.7.2. Analytical Method

The GCMS analysis was performed according to Canini [16] procedure with some modification. The GCMS analysis was performed on SHIMADZU GCMS QP-2020 equipped with auto-sampler (AOC-20s) and auto-injector (AOC-20i) using SH Rxi 5MS Sill column (30m×0.25mm; 0.25 µm). The carrier gas used was helium at 1.72 mL/min flow pressure. The oven temperature was programmed from 80.0°C with hold time 2.00 min. The temperature was raised at 5°C/min to a 150°C with holds time 5.00 min and final temperature of 280°C with holds time 8.00 min. The injector temperature was set to 230°C while

the ion source temperature was set to 280°C. The injection volume was set to 6.0 µL at 20:1 split less ratio mode. The ionization mass spectroscopic analysis was done with 70 eV. Mass spectra were recorded across the range of 45 m/z to 350 m/z for 55.0 min. Solvent cut time was 3.200 min and a total run time was 55.0 min. The components' identification was based on comparing their mass spectra with NIST08s, NIST08 and NIST14 libraries. The identification was confirmed when the similarity index attained in more than 80.

2.8. Statistical Analysis

One-way ANOVA with Tukey's comparison test with a significant level at $p < 0.05$ at 95% confidence level was performed using Minitab 16 (Minitab Inc., State College, Pa., U.S.A.).

Table 1. *Comparison of extraction yield and antioxidant activity of C. cyminum of methanol, and n-hexane.*

Solvent	Extraction yield (%)±SD	IC_{50} (µg/ml)±SD
Methanol	5.32±2.86[a]	3.02±1.97[b]
n-Hexane	3.02±0.78[b]	5.08±0.95[b]

Values represent the means ± standard deviation (SD), n = 3.
Values in each column with different superscript letters are significantly different (p<0.05).

3. Results and Discussion

3.1. Yield of Extraction and Antioxidant Activity

The % yield of extraction and antioxidant activity of *C. cyminum* extracts using different solvents has been shown in Table 1. The extracted yields from methanol and n-hexane solvents were found to be 5.32±2.86, and 3.02±0.78%, respectively. Methanol extract resulted in a higher yield of extraction compared to n-hexane. Hence, to achieve a higher yield of extraction, methanol extract will be a suitable solvent. This result agrees with Sultana and Sabina [3, 17] reported that a higher extraction yield was found when extracting the sample in aqueous methanol. This study showed that the methanol extract of cumin had a more scavenging effect on free radical. The antioxidant activity of *C. cyminum* decreased significantly with the solvent polarity. The polar solvents showed a more tremendous potential for extracting bioactive compounds responsible for oxidative cleavage associated with the variability in the extracted bioactive compounds and their concentrations at different solvent polarities. These findings also agree with Tunna [18] in which polar solvent methanol exhibited potent radical scavenging activity compared with that of a nonpolar solvent.

The percentage yields and inhibitory potential (IC_{50}) of different solvent ratio (0%-100% ethanol-water) has been shown in Table 2.

The percentage yields of the aqueous and ethanolic extracts were calculated using the equation (1). The percentage of extracted yield mainly depends on the solvent properties. Depending on the extraction solvent, the range of the extracts' yield was 4.11±0.59-14.47±5.36%, shown in Table 2.

Table 2. *Comparison of extraction yield and IC_{50} value of cumin in different ratios of solvent.*

Extraction solvent	Extraction yield (%)±SD	IC_{50} (µg/ml)±SD
Water	14.47±5.36[a]	5.79±3.43[a]
20%ethanol	11.82±1.17[d]	1.91±0.76[b]
40%ethanol	7.50±1.04[b]	2.44±0.82[b]
60%ethanol	7.77±1.41[e]	1.39±1.42[c]
80%ethanol	5.69±0.78[c]	1.44±0.74[b]
100% ethanol	4.11±0.59[de]	1.89±1.19[c]

Values represent the means ± standard deviation (SD), $n = 3$.
Values in each column with different superscript letters are significantly different (p<0.05).

The water extract exhibited the highest yield of extraction while ethanol extract exhibited the lowest yield of extraction that was 14.47±5.36% and 4.11±0.59%, respectively. The yield of different extract exhibited the following trend: 0%>20%ethanol>40%ethanol>60%ethanol>80%ethanol>100% ethanol which is in agreement with the previous studies by Sultana and Javadi [3, 9].

A lower IC_{50} value is desirable for higher inhibitory potential. The IC_{50} values of six different extracts include 100% ethanol, 80% ethanol, 60% ethanol, 40% ethanol, 20% ethanol, and 0% ethanol (100% water) used to determine inhibitory potential. In this study, the cumin extracts showed the IC_{50} ranged from 1.39±1.42 to 5.79±3.43µg/ml. The inhibitory potential of different samples was compared based on the resulting IC_{50} values.

The 60% ethanol exhibited the highest inhibitory potential (1.39±1.42µg/ml), and aqueous extract exhibited the lowest inhibitory potential (5.79±3.43µg/ml). A positive control (catechin) had an IC_{50} value of 1.01±0.70 µg/mL. The result of this study showed that the 60% ethanol had almost similar effect on free radical scavenging activity. The antioxidant activity showed the following trend: 60% ethanol>80% ethanol>100% ethanol>20% ethanol>40% ethanol>water (0% ethanol). Many recent studies investigated the inhibitory potential in aqueous solvent was found lower, which is comply with this for medicinal bio-plant [9, 19, 20].

This result also supported by other studies in which water to organic binary solvents system, such as methanol and ethanol, creates a more suitable medium because water dissolves polar compound and organic solvent dissolves less polar compound that facilitates the extraction of antioxidant activity containing compounds [21-23]. This study showed the binary solvent system (60%) had a more radical scavenging activity than with a single solvent. This observation was in agreement with the results reported by Lim et al., [24].

3.2. Identification of Compounds

3.2.1. Phytochemical Analysis

The result of the qualitative phytochemical investigation of methanol and n-hexane extracts of cumin seed are shown in Table 3. In this study, tannin and glycosides were present in both extracts. Saponins and alkaloids were absent in both extracts. Flavonoids showed a positive result in methanol extract whereas, anthraquinones and steroids showed a

positive result in n-hexane extract. Methanol extracts of cumin showed more antioxidant activity than n-hexane. It can be assumed that the methanol extract's better activity may be due to the presence of flavonoids, saponins, alkaloids, tannin and glycosides. This result is in agreement with the study reported by kulkula et al., (2013) [25]. Another study also reported that phenolic compounds, flavonoid groups, glycosides, tannin, alkaloid and terpenoid components are present in *C. cyminum* extracts responsible for bioactivities [26].

3.2.2. Identification of Compounds by GCMS

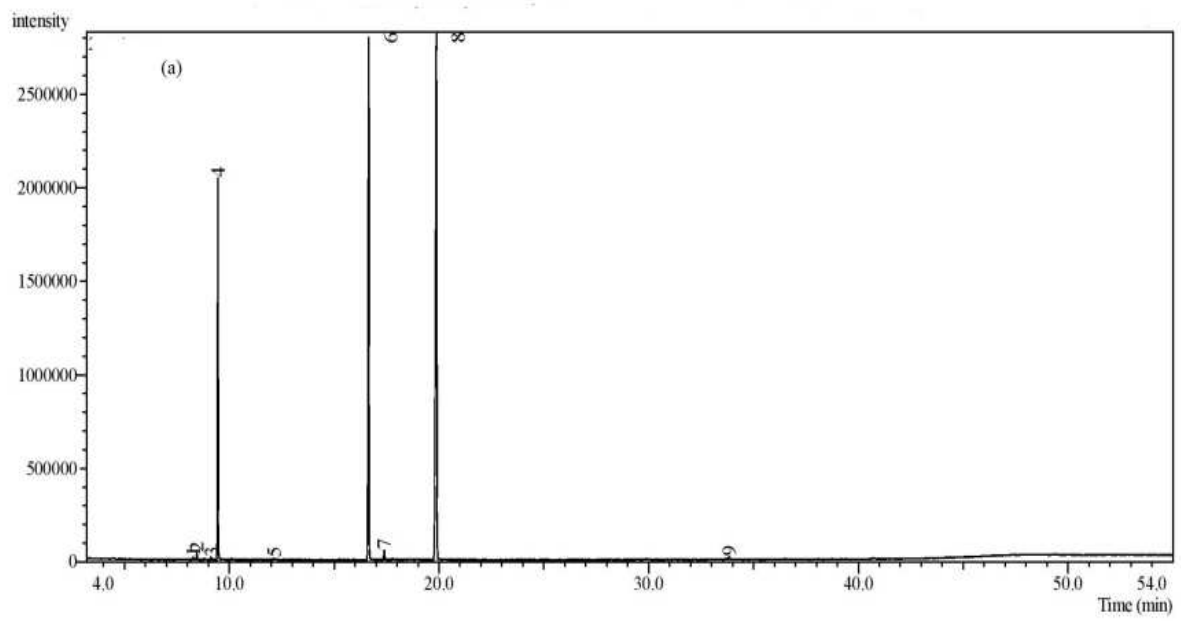
Antioxidant activity containing compounds in cumin has been identified by GCMS analysis. The analysis has been performed on three cumin extracts such as methanol, 60% ethanol and n-hexane. The spectral pattern of identified metabolites is shown in Figure 2 and confirmed by scrutinizing. The GCMS analysis has shown the compound mentioned in Tables 4, 5 and 6. Table 4 shows the methanol extract with nine significant compounds. Apiol, 1, 3-benzodixole and D-carvone were identified as significant compounds with the concentration of 26.56%, 29.10% and 43.22%, respectively.

In the 60% ethanol extract, a total of fifteen significant compounds have been detected by GCMS (Table 5). Of these, four compounds can be considered major bioactive constituents such as 9-octadecadienoic acid-methyl ester, 11-octadecanoic acid-methyl ester glycerin, and di-isooctyl phthalate with the concentration of 31.22%, 25.24%1, 3.22% and 10.43%, respectively. Besides, a total of six compounds were detected in n-hexane extract of cumin using GCMS. Among these six compounds, apiol 1, 3-benzodixole D-carvone, were detected as major phytochemicals with a concentration of 8.91, 36.71 and 53.71%, respectively which showed in Table 6. It can be concluded that a total of 24 different compounds has been detected in three extracts of cumin. Among these thirty compounds, D-carvone, 1, 3-benzodioxole and apiol are the major metabolites in methanol and n-hexane extract found in Bangladeshi cumin**.** Hashemian [27] reported that GCMS analyses in cumin oil, the major constituents of the essential oil were safranal (16.8%-29.0%), gamma-terpinene (14.1%-19.6%), gamma-terpinene-7-al (13.5%-25.5%), cuminaldehyde (17.5%-22.3%), beta-pinene (6.8%-10.4%), and p-cymene (4.1%-8.8%). Moreover, Mohammad [28] reported that GCMS analyses of *C. cyminum* L. from Alborz mountain contained α-pinene (29.2%), limonene (21.7%), 1, 8-cineole (18.1%), linalool (10.5%), and α-terpineole (3.17%) as the major compounds.

Table 3. *The qualitative phytochemical investigation of two different extracts of cumin seed.*

Sl. No.	Phytochemical	Name of test	Sensitivity in methanol	Sensitivity in n-hexane
1	Saponins	Frothing test:	_	_
2	Tanins		+	+
3	Flavonoids	Shinodas test	+	_
4	Steroids	Salkowskis test	_	+
		Liebermann-Burchard's test	_	+
5	Glycosides	Molischs test:	+	+
6	Alkaloids	Dragendroffs test:	_	_
		Hager's test:	_	_
7	Anthraquinones		_	+

The**"+"** indicates the presence of the relevant phytoconstituents, whereas **"–"**indicates the absence.



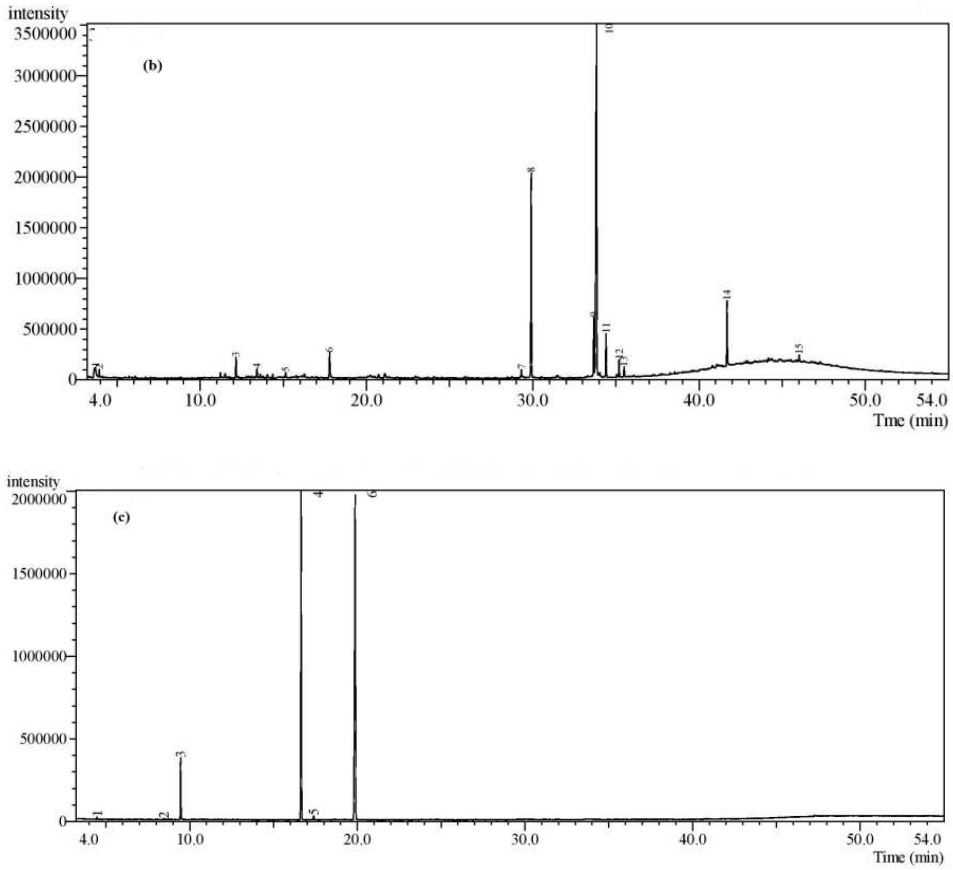


Figure 2. *GCMS Chromatogram of bioactive compounds in methanol (a), ethanol (b) and n-hexane (c).*

Table 4. *Metabolites identified in methanol extract of cumin using GCMS.*

No.	TentativeCompounds	RT	m/z	Concentration (%)
1	Cis-dihydrocarvone	8.27	67	0.10
2	Cyclohexanone	8.44	67	0.34
3	Cyclohexanol	9.13	93	0.06
4	D-carvone	9.45	82	26.56
5	1,2-Cyclohexanediol	12.12	71	0.05
6	1,3-Benzodioxole	16.64	192	29.10
7	Benzene	17.38	208	0.44
8	Apiol	19.87	222	43.22
9	6-Octadecenoic acid, methyl ester	33.83	55	0.11

RT is retention time, and m/z is mass to charge ratio

Table 5. *Bioactive compounds identified in 60% ethanol extract of cumin using GCMS.*

No.	Tentative Compounds	RT	m/z	Concentration (%)
1	Glycerin	3.68	61	13.22
2	Oxirane	3.92	57	1.90
3	1,2-Cyclohexanediol	12.15	71	1.25
4	Cis-p-mentha-1(7),8-dien-2-ol	13.41	71	0.27
5	Ascaridole epoxide	15.14	107	0.19
6	3',5'-dimethoxyacetophenone	17.77	180	3.77
7	Hexadecanoic acid, methyl ester	29.31	55	0.50
	9-octadecadienoic acid, methyl ester	29.90	74	31.22
9	9,10-octadecadienoic acid, methyl ester	33.67	67	3.78
10	11-Octadecanoic acid, methyl ester	33.84	59	25.24
11	Methyl stearate	34.40	74	5.37
12	Ethyle oleate	35.19	55	0.73
13	2H, 6H-Benzo[1,2-b:5,4-b']dipyran-2,6-dione	35.49	229	1.14
14	Diisooctyl phthalate	41.66	149	10.43
15	Squalene	46.02	69	0.92

RT is retention time, and m/z is mass to charge ratio

Table 6. *Bioactive compounds identified in n-hexane extract of cumin using GCMS.*

No.	Tentative Compounds	RT	m/z	Concentration (%)
1	D-Limonene	4.46	68	0.14
2	Cyclohexanone	8.45	67	0.09
3	D-carvone	9.45	82	8.91
4	1,3-Benzodioxole	16.64	192	36.71
5	Benzene	17.38	208	0.42
6	Apiol	19.86	222	53.71

RT is retention time, and m/z is mass to charge ratio

4. Conclusion

This research addressed possible radical scavenging activity of *C. cyminum* extract using different solvents based on antioxidant activity assay and the metabolites, responsible for bioactivity, identified through phytochemical and GCMS. This research showed that the combination of ethanol and water was the potent binary extraction system to get a higher inhibitory activity. The 60% ethanol-water ratio has a higher inhibition activity that could be used to decrease oxidative cleavage. Thus, this study's result gives scientific insight into the mechanism of *C. cyminum* in oxidative cleavage. This research can be preliminary data that could be useful to nutraceuticals and pharmaceuticals. Further researchers could reveal the potential pharmacological mechanism of antioxidant and related symptoms. All the tested extracts showed excellent activity due to the presence of promising bioactive compounds in *C. cyminum.* This preliminary investigation will give important evidence to excel in further animal trial and clinical studies to manage the cell organism's oxidative damage.

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