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PHYTOCHEMICAL EXTRACTION AND SCREENING OF BIOACTIVE COMPOUNDS FROM CUMINUM CYMINUM L. SEEDS EXTRACT

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Abstract:

The medicinal plants gifted by nature have been explored by humans to find out their respective values in the medical field. Cuminum cyminum L. has been utilized for ages as a traditional medication. The aim of this study is to evaluate the phytochemical screening of methanolic extract of C. cyminum L. seeds. To compare the phytochemical analyses of C. cyminum L. methanol extract and its constituents (tannins, flavonoids, alkaloids, saponins, and phenols), an experiment was carried out. We have analyzed phytochemicals such as alkaloids, phenols, tannins, saponins, and flavonoids in Cumin. The results of the study showed that C. cyminum L. is a potential source of bioactive phytochemicals, which can be used as a plant-based antioxidant, antibacterial and antidiabetic agent. This is valuable information to support further studies to exploit and apply it in functional food, pharmaceutical, and nutraceutical applications.

Keywords: *Cuminum Cyminum L., Antioxidant, Nutraceutical, Pharmaceutical.*

Introduction:

Spices are not really a food, rather its ingredient to impart aroma, color and taste. They are unique and indispensable constituents for any cooking materials in our day-to-day life. Spices are specifically defined as plant items that can be used in a variety of forms, including broken, powdered, dried, ripe, or fresh. [1,2] Essentially, they are low-volume high-value crops playing pivotal role in our national economy and providing a strong footing in the international market. Coloring, flavoring, aromatic and pungent properties of spices are due to the presence of essential oils and oleoresins [3]. Since time immemorial spices are also regarded to be therapeutically useful in the management of stomachache, leprosy, cough, loss of appetite, rheumatoid pain, convulsion and inflammation [4] packed with some precious biochemical compounds including protein, tannin, saponin, flavonoids, tannic acids, anthocyanin, cumarin and so on, which have a positive effect on our health [5,7]. These phytochemicals improve memory, brain function, and many other bodily illnesses in addition to curing mental, neurological, and cognitive issues.

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(A) (B) (C)

(D) (E) (F)

Figure: (A) Natural habitat, (B) Inflorescence, (C) A stem,
(D) Leaves of Cumin, (E) A flower and (F) Fruiting of Cumin

Phytochemicals (from Greek Phyto, meaning ‘‘plant’’) are biologically active, naturally occurring chemical compounds essentially classified as primary or secondary constituents, depending on their role in plant metabolism [8]. Common sugars, proteins, amino acids, pyrimidines and purines found in nucleic acids, chlorophylls, etc. are examples of primary ingredients. Secondary constituents are the remaining phytochemicals, viz., alkaloids, flavonoids, saponins, phenolics, flavonoids and glycosides [9,10]. They have biological activity in the plant host and play a role in plant growth or defense against competitors, pathogens or predators. The plant chemicals that protect plant cells from environmental hazards like pollution, stress, drought, UV exposure and pathogenic attack are called phytochemicals [11,12], uniquely protecting humans against diseases too [11,12].

Current project deals with qualitative analysis of alkaloids, cardiac glycosides, flavonoids, tannins, saponins, resins and phenols.

Materials and Methods:

Collection of samples:

The different spice samples of *C. cyminum* were purchased from the local market, Kandivali, Mumbai.

Preparation of extracts:

The collected spice samples were dried and grinded into a fine powder which can be used for extraction.

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Figure: *C. cyminum* seeds with powder

Pharmacognostical evaluation:

Total ash value:

Accurately weighed 5 g of powdered seeds of *C. cyminum* were taken in a dried silica crucible. It was incinerated at 600˚C temperature, until free from carbon and then cooled. Using the air-dried sample as a guide, the weight of the ash was measured and its percentage was computed. The air-dried powder was used as a reference to compute the percentage of total ash. [14].

$$\%\ Ash\ content = \frac{Weight\ of\ crucible + ash - Weight\ of\ crucible}{Weight\ of\ crucible + sample - Weight\ of\ crucible} \times 100$$

Loss on drying:

Accurately weighed 5 g of powdered seeds of *C. cyminum* were taken in a crucible. It was kept in a hot air oven at 105 – 110˚C, until free from moisture. Next, the percentage of moisture content was computed using the sample that had been air-dried.

$$LOD\ \% = \frac{Wt.\ of\ petridish + crude\ drug - After\ drying\ Wt.\ of\ petridish + sample}{Weight\ of\ crude\ drug} \times 100$$

Water soluble ash:

The total ash obtained was boiled with 25 ml of water for few minutes, filtered and the insoluble matter was collected on ashless filter paper. Then, it was washed with hot water, ignited in silica crucible for 15 minutes at a temperature not exceeding 450˚C, cooled and weighed the obtained residue. The water-soluble ash is represented by the weight differential. In the end, the air-dried sample was used to compute the proportion of water soluble ash. [14].

$$\%\ Water\ soluble\ ash\ = \frac{Weight\ of\ crucible + ash - Weight\ of\ crucible}{Weight\ of\ crude\ drug} \times 100$$

Acid insoluble ash:

The total ash obtained was boiled for 5 minutes with 25 ml of 2 N HCl, filtered and the insoluble matter was collected on ashless filter paper. Then, it was washed with hot water, ignited in silica crucible for 15 minutes at temperature not exceeding 450˚C, cooled and weighed the obtained residue. The air-dried sample was used to compute the proportion of acid insoluble ash. [14].

$$\%\ Acid\ soluble\ ash\ = \frac{Weight\ of\ crucible + ash - Weight\ of\ crucible}{Weight\ of\ crude\ drug} \times 100$$

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Alcoholic extractive value:

Macerated 5 g of the air dried coarsely seeds powder with 100 ml of 95 % ethanol in a closed flask for 24 hours, shaking frequently during the first 6 hours and allowed to stand for 18 hours. After that, it was quickly filtered while being careful not to lose the solvent. Evaporated 25 ml of the filtrate to dryness in a tarred flat bottom shallow dish dried at 105˚C and weighed. The medication that had been air dried was used to calculate the percentage of ethanol soluble extractive. [15].

$$Alcohol\ soluble\ extractive\ value\ = \frac{Weight\ of\ residue}{Weight\ of\ the\ drug} \times 100$$

Water soluble extract:

Macerated 5 g of the air dried coarsely seeds powder with 100 ml of chloroform water in a closed flask for 24 hours. After allowing it to stand for eighteen hours, shake often for the first six hours. After that, it was quickly filtered while being careful not to lose the solvent.. vaporated 25 ml of the filtrate to dryness in a tarred bottom flat bottom shallow dish dried at 105°C and weighed. The percentage of water soluble extractive value was calculated with reference to the air dried drug [15].

$$Water\ soluble\ extractive\ value\ = \frac{Weight\ of\ residue}{Weight\ of\ the\ drug} \times 100$$

Hot Soxhlet extraction method:

This method comprised collecting, appropriately cleaning, and thoroughly rinsing the blooms of the *C. cyminum* seeds. They were mechanically pulverized after being shade-dried. The plant material from seeds of *C. cyminum*, either whole or coarsely powdered, was successively extracted using solvent methanol. The Soxhlet apparatus chamber was filled with powder using a "thimble" design. The solvent used for extraction was heated in flasks, and its vapors were then condensed in a condenser. The powder is extracted by touch when the condensed extractant is dropped into the thimble holding it. When the liquid level in the chamber reaches the top of the syphon tube, the liquid inside the chamber syphon falls into the flask. Until no residue was left behind after a solvent drop from the syphon tube evaporated, this process was repeated. The resulting extract was filtered, dried by concentration, weighed, and stored for later use [13]. The following formula is used to determine the extract's yield.

$$Yield\ (\%)\ = \frac{Weight\ of\ residue\ obtained}{Weight\ of\ the\ plant\ material\ taken} \times 100$$

Figure: Extraction by using soxhlet method

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Phytochemical screening of the extract:

Phytochemical analysis of seed extract of methanol was carried out by qualitative test according to standard methods. The extracts were screened for alkaloids, glycosides, flavonoids, tannins, saponins, resins, and phenols.

Test for alkaloids:

Dragendroff's test: 1 ml Dragendroff's reagent added to 2 ml extract. White precipitation formation suggested the presence of alkaloids.

Mayer's test: To the 1 ml test solution, Mayer's reagent (Mercuric-potassium iodide) was added. Creamy precipitation showed the presence of alkaloids.

Wagner's test: 2 ml of Wagner's reagent was added to a diluted extract solution. Formation of reddish brown precipitate indicated the presence of alkaloids.

Test for cardiac glycosides:

Keller-Kiliani Test: To the 2 ml of extract, 2 ml glacial acetic acid added. Mix it well. After mixing, 2 drops of ferric chloride were added followed by conc. H_2SO_4 along the side wall of the test tube. A reddish-brown coloured ring at the interface indicated the presence of cardiac glycosides.

Test for flavonoids:

Shinoda test: Magnesium chips were added to the extract followed by addition of conc. HCl. Flavonoids were indicated by a reddish-pink color.

Ethyl Acetate Extract: To the test solution, add a few drops of ferric chloride solution, an intense green color was formed to show the presence of flavonoid.

Test for tannins:

Lead acetate test: 1% lead acetate was added to 2 ml of extracts. The presence of tannins was shown by a yellow precipitation.

FeCl3 test: Few drops of 5% $FeCl_3$ were added to 2 ml of extracts. A grey or black color indicated the presence of tannins.

Test for saponins:

Foam test: 5 ml distilled water added to the 3 ml of extract. The production of foam suggested the presence of saponins.

Test for resins:

Precipitate test: About 10 ml of distilled water was added to 5ml of spice extract. A precipitate indicating the presence of Resins.

Test for phenols

Ferric chloride test: To a 2 ml of spice extract, 1 ml of ferric chloride ($FeCl_3$) solution was added. Deep blue black color indicates the presence of phenols.

Quantification of secondary metabolites :

For the purpose of estimating the quantity of phytoconstituents contained in plant extracts, quantitative analysis is a crucial instrument. TPC and TFC are established for this. TPC and TFC levels were determined using a conventional technique using extracts from the seeds of *C. cyminum.*

Total phenolic content estimation:

The extracts' total phenolic content was determined using the Folin-Ciocalteu reagent. Methanol produced gallic acid at concentrations between 20 and 100 µg/ml. Plant extract concentrations of 100 µg/ml were likewise prepared in methanol, and 0.5 ml of each sample was introduced to the test, along with 2 ml of a Folin Ciocalteu reagent that had been diluted 10 times and 4 ml of 7.5% sodium carbonate. After shaking the tubes intermittently for 30 minutes at room temperature and covering them with parafilm, the absorbance at 760 nm was measured with methanol serving as a blank. The total phenol content was calculated using the standard regression curve for gallic acid, and the findings were expressed in milligrams per gram (mg/g) of gallic acid [16].

Total flavonoid content estimation:

Rutin was synthesized in methanol at different doses (20 to 100µg/ml). Test samples with a polarity of

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100µg/ml or close to it were created. A sample that had been diluted to 0.5 ml was combined with 2 ml of distilled water before being added to 0.15 ml of a 5% NaNO2 solution. After waiting for 6 minutes, 0.15 ml of a 10% AlCl3 solution was added. The combination was then given 5 minutes to stand before receiving 2 ml of a 4% NaOH solution. After reducing the final volume to 5 ml with distilled water, the mixture was let to stand for an additional 15 minutes. Water was used as the reference to calculate the absorbance at 510 nm. The standard regression curve of quercetin and rutin was used to compute the total flavonoid content [16].

Results and Discussion:

Pharmacognostical evaluation:

Table 1: Pharmacognostical evaluation of plant sample

Parameters	Value in percentage (%)
	C.cyminum (Cumin)
Total ash value	6.47
Loss on drying	11.34
Water soluble ash	12.48
Acid insoluble ash	0.94
Water extractive value	3.25
Alcoholic extractive value	14.21

Plant extraction:

The plant material was extracted by soxhlet extraction method and the percentage yield calculated by the following formula:$Yield\ (\%) = \frac{Actual\ Yield}{Theoretical\ yield} \times 100$

Table 2: Percentage yield

Solvent	**Theoretical Yield (in gm)**	**Actual Yield (in gm)**	**Percentage Yield (%)**
***C. Cyminum* extract**	110	16.27	14.79

Solubility determination:

Table 3: Solubility determination of extracts

Sr. No.	**Solvent**	***C.cyminum* (Cumin)**
1.	Water	Soluble
2.	Methanol	Soluble
3.	Ethyl acetate	Soluble

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Preliminary Phytochemical analysis:

A preliminary phytochemical analysis of *C. cyminum* was performed in methanolic extract by using various qualitative tests. The results of the tests are shown in Table 4.

Table 4: Preliminary phytochemical analysis of *C. cyminum* methanolic extract

Sr. No.	Name of phytochemicals	Specific test followed	Sample 1	Sample 2
1.	Alkaloids	Dragendroff's test	++	+
		Mayer's test	++	++
		Wagner's test	++	++
2.	Cardiac glycosides	Keller kiliani test	++	+
3.	Flavonoids	Shinoda test	++	+
4.	Tannins	Lead acetate	+	+
		5% FeCl3	+	+
5.	Saponin	Foam test	+	++
6.	Resins	Precipitate test	+	+
7.	Phenols	Ferric chloride test	++	-
Dark color visibly observed (++), light color visibly observed (+), Absent (-)				

Table 5: Calibration curve of gallic acid

Sr. No.	Concentration (µg/ml)	Absorbance (760 nm)
0	0	0
1	5	0.19
2	10	0.42
3	15	0.63
4	20	0.84
5	25	1.03

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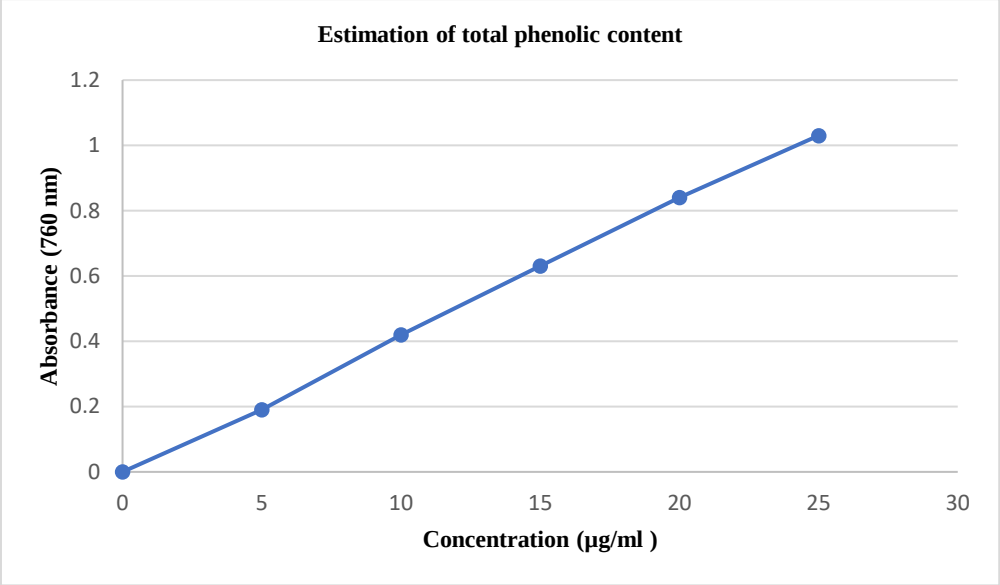

Table 6: Calibration curve of quercetin acid

Sr. No.	Concentration (µg/ml)	Absorbance (760 nm)
0	0	0
1	5	0.35
2	10	0.62
3	15	0.91
4	20	1.21
5	25	1.52

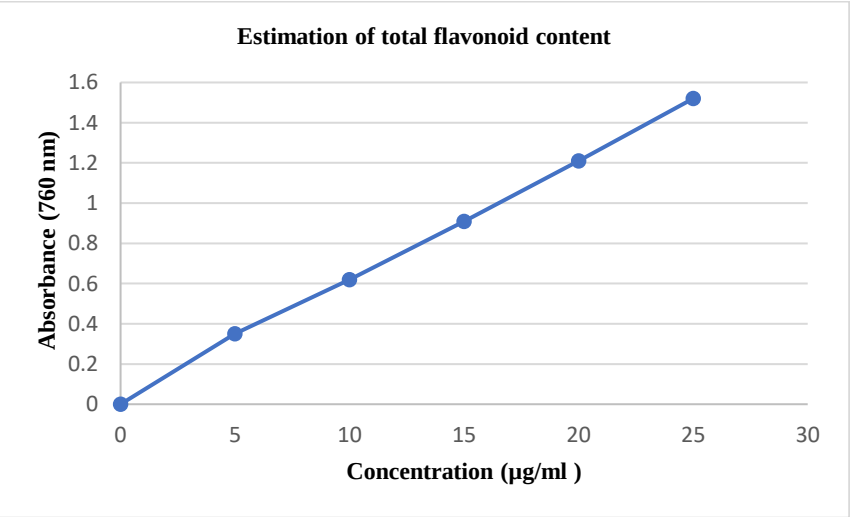

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Table 7: Total phenolic and flavonoid content

Sr. No.	Extract	Total phenol (mg/100 mg)	Total flavonoid (mg/100 mg)
1.	*C. Cyminum* methanolic extract	0.607 %	0.565 %

Discussion:

One of the most important methods for determining if a plant has pharmacologically active ingredients is phytochemical analysis. The present study deals with the studies on pharmacognostical and phytochemical activity on seeds of C. cyminum. Raw materials were analyzed for identity, quality and purity as per the standards prescribed by WHO and Ayurvedic Pharmacopoeia of India. The percentage yield of *C. cyminum* methanolic extract was found to be 14.79%. The loss on drying of dry powder of *C. cyminum* was 11.34%. Three distinct kinds of ash—total ash, water soluble ash, and acid insoluble ash—were used to calculate the ash value. The total ash of crude powder of *C. cyminum* was found to be 6.51%, water soluble ash was 12.58% and acid insoluble ash was 0.94%. The water and alcoholic extractive value of crude powder of *C. cyminum* was found to be 3.25% and 14.21%. Total phenolic content (TPC) was measured by using Folin-ciocalteau's reagent method. And total flavonoid content (TFC) of *C. cyminum* was measured by the Aluminum chloride method. The total phenolic and flavonoid content were determined by established methods and were found to be 0.607 mg/100mg and 0.565 mg/100mg in gallic acid and quercetin respectively. Phytochemicals present in plants act as the source for the treatment of different health problems. Different phytochemicals have different therapeutic values. The phytochemical analysis conducted on methanolic extract of *C. cyminum* revealed the presence of Alkaloids, Glycosides, Steroids, Flavonoids, Tannins, Saponins, Resins and Phenols were present in the spice *C. cyminum* extracts.

Conclusion:

The present study verifies the presence of phytochemicals such as alkaloids, cardiac glycosides, flavonoids, tannins, saponin, resin and phenolic compounds. The powder which we have crushed shows a higher amount of alkaloid and phenolic content. This may be because there might be some adulteration. Future research is required to confirm whether it is adulterated and if at all then the chemical content of adulterant.

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