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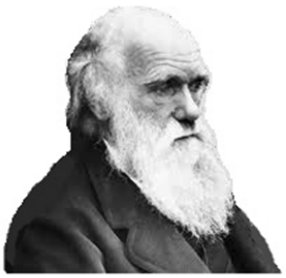

World News of Natural Sciences

An International Scientific Journal

WNOFNS 52 (2024) 82-97 EISSN 2543-5426

Evaluation of phytochemical, analgesic, acute toxicity and moisture content of *Cumin cyminum*

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ABSTRACT

The purpose of this study was to evaluate the analgesic, phytochemical screening, acute toxicity, and moisture content activity of the crude ethanolic seed extract of *Cuminum cyminum* and its n-hexane, chloroform, carbon tetrachloride and aqueous fraction. The ethanolic extract was used to the moisture content was found 43% evaluate analgesic, phytochemical screening, and moisture content activities. The analgesic activity was measured by acetic acid induced writhing test. The extract showed highly significant ($p < 0.001$) analgesic activity with % inhibitions of writhing response at doses 250 mg/kg was 24.67% and 500 mg/kg was 36.95%. The moisture content was found to be 43%. The results of the study clearly indicate the present of analgesic, phytochemical screening and moisture content properties of the extract and its fractions. Phytochemical analysis revealed the presence of alkaloids, saponins, phenols, terpenoids and glycosides. Screening of analgesic property on mice by acetic acid induced method for crude extracts and its different fractions of *Cuminum cyminum.* At zero-hour test samples, control (1% Tween-80 solution in saline) and Diclofenac sodium were administered orally by means of a long needle with a ball shaped end. After 40 minutes acetic acid (1%) was administered intra-peritoneally to each of the animals of all the groups. The forty minutes interval between the oral administration of test materials and intra-peritoneal administration of acetic acid was given to assure proper absorption of the administered samples. Five minutes after the administration of acetic acid, number of squirms or writhing were counted for each mouse for ten minutes. Each mouse of all groups was observed individually for counting the number of writhing they made in 15 minutes commencing just 5 minutes after the Intra-peritoneal administration of acetic acid solution. Full writhing was not always accomplished by the animal, because sometimes the animals started to give writhing but they did not complete it. This incomplete writhing was considered as half-writhing. Accordingly, two half writhing were taken as one full writhing. In conclusion the ethanolic extract of *C. cyminum* seeds showed predominately showed significant analgesic activity.

(Received 24 October 2023; Accepted 15 November 2023; Date of Publication 17 November 2023)

***Keywords*:** cumin, phytochemical, analgesic, toxicity, moisture content, *Cuminum cyminum*

1. INTRODUCTION

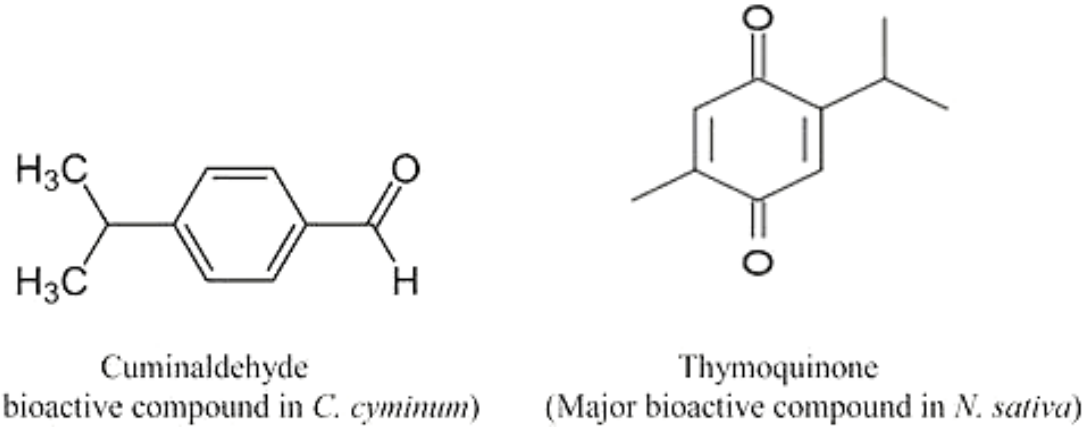
Plants are the gifts of nature. Today the large numbers of drugs in use are derived from plants, like morphine from *Papaver somniferum*, Ashwagandha from *Withania somnifera*, Ephedrine from *Ephedra vulgaris*, Atropine from *Atropa belladonna*, Reserpine from *Rauwolfia serpentina* etc. The medicinal plants are rich in secondary metabolites (which are potential sources of drugs) and essential oils of therapeutic importance. The important advantages claimed for therapeutic uses of medicinal plants in various ailments are their safety besides being economical, effective and their easy availability.

A considerable number of definitions have been proposed for medicinal plants. According to the WHO, "A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemopharmaceutical semi-synthesis." When a plant is designated as 'medicinal', it is implied that the said plant is useful as a drug or therapeutic agent or an active ingredient of a medicinal preparation. "Medicinal plants may therefore be defined as a group of plants that possess some special properties or virtues that qualify them as articles of drugs and therapeutic agents and are used for medicinal purposes". Wound healing is a process that is fundamentally a connective tissue response initial stage of this process involves an acute inflammatory phase followed by synthesis of collagen and other intracellular macromolecules, which are later remodeled to form a scar. The dried ripe seeds of *Cuminum cyminum* are commonly known as cumin seeds in English, Jeera in Hindi and Jeeragi in Kannada is a daily used spice in India. Cumin seeds belong to the family Umbellifer.

The review of literature reveals that cumin seed has a potent medicinal value. Volatile oil extracted from cumin is known to possess 49 compounds. There are 16 hydrocarbon and 32 oxygenated compounds. The main compounds are culminal and safranal. The other 9 compounds are monoterpenes, sesquiterpenes, aromatic aldehydes and aromatic oxides etc. It contains 2.5 to 4.5% volatile oil, 10% fixed oil and proteins. Volatile oil mainly consists of 30 to 50% cuminaldehyde, small quantities of alpha pinene, beta pinene, phellandrene, cuminic alcohol, hydrated cuminaldehyde, hydrocuminine and terpenes mainly monoterpenes and sesquiterpenes. The presence of sesquiterpenes in the plant material is known to possess a potent antiulcer activity. It is known to possess anticarcinogenic, hepatoprotective, antidiabetic antibacterial antiepileptic and antioxidant activities. One of the main constituents of the seeds of cumin is terpenoids.

The free terpenoids present in tridax procumbens have been reported to be a pro-healing activity. In view of these, has been designed the present work to study the possible effect of extract and different fractions of cumin seeds wound healing activity. The Literature survey revealed that no scientific investigation has been made regarding the wound healing activity of seeds of cumin. The cumin seeds have traditionally been used in the Southeast Asian and Middle East countries for the treatment of diseases such as asthma, bronchitis, rheumatism, and other inflammatory diseases. *Cuminum cyminum* has extensively been used because of its therapeutic potential and possesses a wide spectrum of activities, namely, diuretic, antihypertensive, antidiabetic, anticancer, immune-modulatory, antimicrobial, anthelmintic,

analgesic and anti-inflammatory, spasmolytic, bronchodilator, gastroprotective, hepatoprotective, and renal protective properties. Traditionally, seeds of cumin seeds are widely used for asthma, diabetes, hypertension, fever, inflammation, bronchitis, dizziness, rheumatism, skin disorders, and gastrointestinal disturbances. It is also used as a liver tonic, digestive, antidiarrhoeal, emmenagogue, and to control parasitic infections and boost immune system. In the present investigation, an attempt was made to screen the seeds of cumin wound healing activity. Cumin seeds are nutritionally rich; they provide high amounts of fat (especially monounsaturated fat), protein, and dietary fiber. Vitamins B and E and several dietary minerals, especially iron, are also considerable in cumin seeds. Cumin aldehyde, cymene, and terpenoids are the major volatile components of cumin. Cumin has a distinctive strong flavor. Its warm aroma is due to its essential oil content. Its main constituent of aroma compounds is cuminaldehyde. (Figure 1) and cuminic alcohol. Other important aroma compounds of roasted cumin are the substituted pyrazines, 2-ethoxy-3-isopropylpyrazine, 2-methoxy-3-secbutylpyrazine, and 2-methoxy-3-methylpyrazine. Other components include γterpinene, safranal, cymene, and β-pinene.



Cuminaldehyde
(Major bioactive compound in *C. cyminum*)

Thymoquinone
(Major bioactive compound in *N. sativa*)

Figure 1. Chemical structures of cuminaldehyde and thymoquinone.

2. RESULT/EXPERIMENTAL (MATERIAL AND METHODS)

Plant collection

The Cumin seeds (*Cuminum cyminum)* were selected for this investigation. These were collected and identified from various areas of North Badda, Dhaka. Nutritional analysis of the plants involves the following three major steps (for crude extraction): (a) Collection and proper identification of the seeds, b) Preparation (drying and pulverization), and c) Extraction of desired crude drugs.

Drying and pulverization

About 1 kg of *Cuminum cyminum seeds* were grinded into small pieces with the help of an electric blending machine. These small pieces were shed dried for 15 days. After complete drying, the dried pieces were then pulverized into a coarse powder with the help of a blending machine and were stored in an airtight container for further use. The materials used are Dried

plant, ethanol, 1% Tween solution, diclofenac, feeding needles, syringe, mice holder, and test material of crude seed sample (ethanolic extract) fraction of *Cuminum cyminum*. In cold extraction the coarse powder of cumin seeds about 500 gm was submerged in Ethanol, the common solvents for extracting most of the constituents present in the herbal materials. Solvent is given at a ratio of 50 ml/10 gm. Flat bottom 2.5 litter reagent bottles were used for this purpose which were kept at room temperature and allowed to stand for 15 days with occasional shaking and stirring. When the solvent became concentrated, the liquid alcohol content was filtered through cotton and then through filter paper (Whatman filter paper no. 1). Then the solvents were allowed to evaporate. Thus, the highly concentrated and crystalline crude extracts were obtained.

Determination of Moisture Content

Dried seeds sample were taken and grinded into powder with the help of an electric blending machine. Moisture content of different samples is determined by oven drying methods. The different samples are heated under specific conditions and the loss of weight is used to calculate the moisture content of the sample. The moisture content is highly dependent on the type of oven used, conditions of the oven, time, and temperature of drying.

Figure 2. Digital picture of the air oven used for sample drying.

About 5 gm of each raw sample was taken and placed into a weighed porcelain basin. The porcelain basins were placed into the air-oven for drying purposes to determine the moisture content as weight difference at 60 °C for 2 hours. After drying for 2 hours the porcelain basins containing the samples were removed from the oven and kept in desiccators for sometimes and again weighed. Drying was continued at 60 °C until the weight of the porcelain basins containing the dried samples was increased. After completion of drying the porcelain

basins containing the samples were finally weighed and the moisture content of the samples was determined by the following equation using oven-drying procedure

% of Moisture content = (Final moisture / sample weight) × 100

where,
Final Moisture = [porcelain basin + sample weight(dry)] – [porcelain basin +sample weight]

Determination of analgesic activity

Analgesic activity of the ethanolic extract of the seed *Cuminum cyminum* was tested using the model of acetic acid induced writhing in mice. The acetic acid induced writhing method is an analgesic behavioral observation assessment method that demonstrates a noxious stimulation in mice.

The test consists of injecting the 0.7% acetic acid solution intra-peritoneally and then observing the animal for specific contraction of body referred as 'writhing'. A comparison of writhing is made between positive control (Diclofenac), and test sample given orally 40 minutes prior to acetic acid injection. If the sample possesses analgesic activity, the animal that received the sample will give lower number of writhing than the control, i.e., the sample having analgesic activity will inhibit writhing. Diclofenac is used as reference standard drug. It is a widely used drug and has analgesic, antipyretic, and anti-inflammatory actions at different steps of pharmacological investigation with mild adverse effects. To prepare suspension of the test samples at the doses of 250 and 500 mg/kg body weight, 125 mg and 250 mg of samples were measured respectively. The extract was triturated in unidirectional manner by the addition of small amount of tween-80. After proper mixing of extract and tween 80 the distilled water was slowly added. The final volume of the suspension was made 5.0 ml. For the preparation of diclofenac at the dose of 25 mg/kg-body weight, 12.5 mg of diclofenac was taken, and a suspension of 5.0 ml was made.

Experimental animal

Figure 3. Swiss Albino mice used for analgesic test.

Young Swiss-albino mice aged 4-5 weeks, average weight 23-30 gm were used for the experiment. The mice were purchased from Jahangirnagaar University, Dhaka, Bangladesh. They were kept to standard environmental condition for one week for adaptation after their purchase and fed rodent food and water *ad libitum.*

Experimental animals were randomly selected and divided into four groups denoted as group-I, group-II, group-III, group-IV consisting of 5 mice in each group. Each group received a particular treatment i.e., control, positive control and the two doses of the extract. Each mouse was weighed properly, and the doses of the test samples and control materials were adjusted accordingly.

Table 1. Experimental profile to assess the effect of crude extract on acetic acid induced writhing of mice

	Group	**Identification**	**Dose (mg/kg)**	**Route Of Administration**
Acetic acid (0.7%)	Group I	Writhing inducer	0.1 ml/10g of body weight	I.P.
Diclofenac sodium	Group II	Standard Group	50	Oral
Ethanolic extract	Group III	Test Sample	250	Oral
Ethanolic extract	Group IV	Test Sample	500	Oral

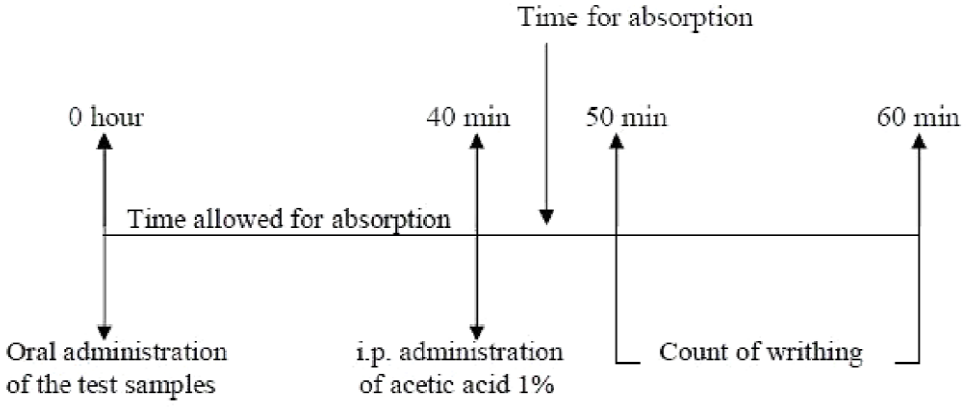


Figure 4. Schematic diagram of the induction and counting of writhing.

Here is schematic representation of procedure for screening of analgesic property on mice by acetic acid induced method for crude extracts and its different fractions of *Cuminum*

cyminum. At zero-hour test samples, control (1% Tween-80 solution in saline) and diclofenac sodium were administered orally by means of a long needle with a ball shaped end. After 40 minutes acetic acid (1%) was administered intra- peritoneally to each of the animals of all the groups.

The forty minutes interval between the oral administration of test materials and intra-peritoneal administration of acetic acid was given to assure proper absorption of the administered samples. Five minutes after the administration of acetic acid, the number of squirms or writhing were counted for each mouse for ten minutes.

Counting of Writhing

Each mouse of all groups was observed individually for counting the number of writhing they made in 15 minutes commencing just 5 minutes after the Intraperitoneal administration of acetic acid solution. Full writhing was not always accomplished by the animal, because sometimes the animals started to give writhing but they did not complete it. This incomplete writhing was considered as half-writhing. Accordingly, two half writhing were taken as one full writhing.

Table 2. Animal profile and dose used in acetic acid induced writhing of mice

Group	Administered (Dose)	Mice No.	Weight (gm)	Amount admin. (ml)	Writhing of mice.	Mean Writhing
I Control	Vehicle	C1	20	0.20	29	31.2
		C2	21	0.21	34	
		C3	20	0.20	27	
		C4	22	0.22	31	
		C5	22	0.22	35	
II Standard	Diclofenac Na (25 mg/kg)	S1	22	0.22	10	8.4
		S2	21	0.21	8	
		S3	23	0.23	9	
		S4	22	0.22	7	
		S5	20	0.20	8	
III Test group-I	Extract (250 mg/kg)	T1	24	0.24	24	25.2
		T2	27	0.27	27	
		T3	25	0.25	25	

		T4	28	0.28	28	
		T5	22	0.22	22	
IV Test group-II	Extract (500 mg/kg)	T1	25	0.25	25	23.8
		T2	26	0.26	26	
		T3	23	0.23	23	
		T4	24	0.24	24	
		T5	21	0.21	21	

Evaluation of Acute Toxicity

Acute toxicity describes the adverse effects of a substance that result either from a single exposure or from multiple exposures in a short space of time (usually less than 24 hours). To be described as acute toxicity, the adverse effects should occur within 14 days of the administration of the substance. An adverse effect is "any effect that results in functional impairment and/or biochemical lesions that may affect the performance of the whole organism or that reduce the organ's ability to respond to an additional challenge".

Consequently, a chemical that enters the organism via the oral route during a restricted time and produces any adverse effect with little delay is orally and acutely toxic. However, the term acute oral toxicity is most often used in connection to lethality and LD_{50} determinations. If the dose dependent lethality incidence is determined in a precise manner, it is usually expressed as an LD_{50}. This is defined as the statistically derived dose that, when administered in an acute toxicity test, is expected to cause death in 50% of the treated animals in each period. Acute toxicity is distinguished from chronic toxicity, which describes the adverse health effects from repeated exposures, often at lower levels, to a substance over a longer time period (months or years). The purposes of acute toxicity testing are to obtain information on the biologic activity of a chemical and gain insight into its mechanism of action. The acute toxicity of a test substance can be assessed by different methods such as Fixed-dose method, Toxic-class Method and Up and Down method.

There is evidence that the toxic effect of a substance may interfere with its pharmacological activities. So, it is necessary to determine the LD_{50} value of the crude extract before evaluate the pharmacological activities and ensure that the responses obtained by different tests are correct because of pharmacological effect not toxic effect. In the present study, the acute toxicity of *C. cuminum* is determined by fixed dose method.

The Fixed-Dose Procedure

The fixed-dose procedure was first proposed by the British Toxicology Society in 1984. After an international validation study involving 20 reference chemicals tested in 31 laboratories from 11 different countries, the procedure was incorporated into the OECD (Organization for Economic Co-operation and Development) guidelines (guideline 420) in 1992. The recent OECD Guidelines (guideline 423) is based on a stepwise procedure with the

use of a minimum number of animals per step; sufficient information is obtained on the acute toxicity of the test substance to enable its classification. The substance is administered orally to a group of experimental animals at one of the defined doses (500, 1000, 1500, and 2000 mg/kg).

The substance is tested using a stepwise procedure, each step using five animals of a single sex (normally females). Depending on the results of the first test, either no further testing is needed, or a higher or lower dose is tested. If mortality occurs, retesting at a lower dose level is necessary. If no signs of toxicity occur at the initial dose, it is necessary to retest at a higher dose level. The results are thus interpreted in relation to animal survival and evident toxicity, and it becomes possible to assign the chemical to one of the OECD classification categories.

Healthy, young, female adult Swiss-albino mice, average weight 20-40 gm were used for the experiment. The mice were purchased from the animal Research Branch of the International Centre for Diarrheal Disease and Research, Bangladesh (ICDDR, B). They are kept in standard environmental condition for one week in the animal house of the Pharmacy Discipline, Khulna University, Bangladesh for adaptation. The animals were provided with standard laboratory food and tap water and maintained at natural day night cycle.

Study Design

The animals were randomly selected, marked to permit individual identification, and kept in their cages for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions. Animals were allowed to fast prior to dosing withheld for 3-4 hours. According to fixed dose procedure there were four fixed doses (500, 1000, 1500, and 2000 mg/kg) are available to be started. Since there is no acute toxicological data on this plant were available, so here I started with the lowest dose 500 mg/kg between, to minimize the death of mice. Each mouse was weighed properly, and the dose of the test sample was prepared accordingly.

Preparation of doses of extract

Preparation of dose of 500 mg/kg body weight

To prepare suspension of the test sample at the dose of 500 mg/kg body weight, 500 mg of extract was measured. The extract was triturated in unidirectional manner by the addition of small amount of tween-80. After proper mixing of extract and tween-80, distilled water was slowly added. The final volume of the suspension was made 10 ml. To stabilize the suspension, it was shaken well by vortex mixer. As the dose is 500 mg/kg, the mice were administered (0.01 × body weight) ml solution.

Preparation of dose of 1000 mg/kg body weight

To prepare suspension of the test sample at the dose of 1000 mg/kg per body weight 1000 mg of extract was measured and dissolved in 10 ml of distilled water. As the dose was 1000 mg/kg, the mice of this group were administered (0.01 × body weight) ml solution.

Preparation of dose of 1500 mg/kg body weight

To prepare suspension of the test sample at the dose of 1500 mg/kg per body weight 1500 mg of extract was measured and dissolved in 10 ml of distilled water. As the dose was 1500 mg/kg, the mice of this group were administered (0.01 × body weight) ml solution.

Preparation of dose of 2000 mg/kg body weight

To prepare suspension of the test sample at the dose of 2000 mg/kg per body weight 2000 mg of extract was measured and dissolved in 10 ml of distilled water. As the dose was 2000 mg/kg, the mice of this group were administered (0.01× body weight) ml solution. At first the starting dose 500 mg/kg was given orally by means of a feeding needle to five animals which were initially weighted and marked. Then individual observations were started during the first 30 minutes and periodically during the first 24 hours. After 24 hours, if the animals were alive and did not show any adverse effect then the next dose was administered to another three animals.

3. RESULTS AND DISSCUSSION

Phytochemical test result

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. They are non-essential nutrients, meaning that they are not required by the human body for sustaining life. Testing of different chemical groups present in the extracts represents the preliminary phytochemical studies.

Table 3. Result of phytochemical group test

Serial	Groups	Status
i.	Reducing sugar	-
ii.	Tannins	+
iii.	Saponin	+
iv.	Flavonoids	+
v.	Steroids	+
vi.	Alkaloids	+
vii.	Glycosides	-
viii.	Terpenoids	+
ix.	anthraquinone	+
x.	Phytosterol	+

+ indicates Presence & - indicates Absence of chemical groups.

Small quantity of freshly prepared ethanol extract of seed of *Cuminum cyminum* was subjected to preliminary quantitative phytochemical investigation for detection of phytochemicals such as alkaloids, carbohydrates, glycosides, phytosterols, proteins, flavonoids, tannins, saponins, phenols, terpenes, fats & fixed oils using the standard methods.

The following reagents were used for the different chemical group test (Evans, 1989). Mayer's Reagent: 1.36 gm mercuric iodide in 60 ml of water was mixed with a solution containing 5 gm of Potassium iodide in 20 ml of water. Wagner's Reagent: 2 gm of iodine and 6gm of Kl were dissolved in 100 ml of water. Hager's Reagent: 1g of picric acid was dissolved in 100 ml of water to prepare Hager's reagent. Dredendroff's b Reagent: 1.7 gm basic Bismuth nitrate and 20 gm tartaric acid were dissolved in 80 ml water. The solution was mixed with a solution which contained 16 gm potassium iodide and 40 ml water. Fehling's Solution A: 34.64 gm copper sulphate was dissolved in a mixture of 0.50 ml of sulfuric acid and sufficient water to produce 500 ml. Fehling's solution B: 176 gm of sodium potassium tartrate and 77 gm of sodium hydroxide were dissolved in sufficient water to produce 500 ml. Equal volume of above solution were mixed at the time of use. Benedicts Reagent: 1.73 gm cupric sulphate, 1.73 gm sodium citrate and 10 gm anhydrous sodium carbonate were dissolved in water and the volume was made up to 100 ml with water. Molisch Reagent: 2.5 gm of pure –naphthol was dissolved in 25 ml of ethanol. Liebermann-Burchard Reagent: 5 ml acetic anhydride was carefully mixed under cooling with 5 ml concentrated sulfuric acid. This mixture was added cautiously to 50 ml absolute ethanol while cooling. In *Cuminum cyminum* seed extract revealed the presence of alkaloids, flavonoids, tannins, glycosides, fat, and fixed oil, saponin and phytosterol, saponin, anthraquinone. The presence of these compounds influenced pharmacological activities such as analgesic activity, nutritional analysis and antihyperglycemic, hypolipidemic, and hepatoprotective activity. Detection of s Ferric Chloride Test: 5ml solution of the extract was taken in a test tube. Then 1 ml of 5% Ferric chloride solution was added. Greenish black precipitate was formed and indicated the presence of tannins

Detection of Saponin

Froth Test: Extracts were diluted with distilled water to 20 ml, and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam marked the presence of saponins. Foam Test: 0.5 gm of extract was shaken with 2 ml of water. Foam was produced which remained for 10 minutes and confirmed the presence of saponins.

Detection of Phytosterols

Salkowski's Test: Extracts were treated with chloroform and filtered. The filtrates were treated with a few drops of concentrated sulphuric acid, shaken, and allowed to stand. Appearance of golden yellow color showed the presence of triterpenes. Liebermann-Bur chard's test: Extracts were treated with chloroform and filtered. The filtrates were treated with a few drops of acetic anhydride, boiled, and cooled and then conc. sulphuric acid was added. The formation of brown ring at the junction confirmed the presence of phytosterols.

Detection of tannins

Ferric Chloride Test: 5 ml solution of the extract was taken in a test tube. Then 1 ml of 5% Ferric chloride solution was added. Greenish black precipitate was formed and indicated the presence of tannins.

Detection of glycosides

Extracts were hydrolyzed with dil. HCl, and then subjected to test for glycosides. Legal's Test: Extracts were treated with sodium nitroprusside in pyridine and sodium hydroxide. The formation of pink to blood red color indicated the presence of glycosides. Modified Bontrager's Test: Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes.

The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. The formation of rose-pink color in the ammoniacal layer showed the presence of glycosides.

Detection of alkaloids

Both the extracts were dissolved individually in dilute Hydrochloric acid and the solutions were filtered. Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). The formation of a yellow-colored precipitate indicated the presence of alkaloids. Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide).

The formation of brown/reddish precipitate indicated the presence of alkaloids. Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). The presence of alkaloids was confirmed by the formation of yellow colored precipitate. Dragendroff's Test: Filtrates were treated with Dragendroff's reagent (solution of potassium bismuth iodide).

The formation of red precipitate indicated the presence of alkaloids.

Detection of Anthraquinones.

About 5g of seed extract 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 anthraquinone was confirmed by formation of violet color.

Detection of Steroid

Salkowski reaction. A 2 gm of dry extract was shaken with chloroform then sulphuric acid was added slowly to the chloroform layer by the side of the test tube. Formation of red color indicate the presence of steroid. The test result of the qualitative phytochemical investigation of ethanol and n- hexane extract of cumin seed are shown in the table 3. In the study, tannin, glycosides, saponin, alkaloid, phytosterol, steroid and anthraquinone were present.

Analgesic activity test

Acetic acid induced writhing reflex

The effect of ethanolic extract of *Cyminum cuminum* on acetic-acid induced writhing in mice showed dose dependent decrease in number of writhing. Extract at doses 250 and 500 mg/kg produced 24.67 % ($P<0.01$) and 36.95 % ($P<0.001$) decrease in number of writhing respectively compared to the control group. Diclofenac sodium (25 mg/kg) also showed significant 68.79 % ($P<0.0001$) decrease in number of writhing.

Table 4. Statistical evaluation of effect on acetic acid induced writhing reflex in mice

Group	No. of mice	Total writhing	Mean writhing	% Writhing	% Inhibition of writhing	SD	SEM	t- test (p values)
Control	5	157	31.4	100	_	5.54	2.48	_
Standard (25 mg/kg)	5	49	9.8	31.21	68.79	2.77	1.24	5.37 (P<0.0001)
Extract (250 mg/kg)	5	126	25.2	74.80	24.67	2.64	1.71	3.21 (P<0.001)
Extract (500 mg/kg)	5	119	23.8	76.2	36.95	5.13	1.99	1.97 (P<0.001)

SD = Standard deviation, SEM = Standard Error Mean

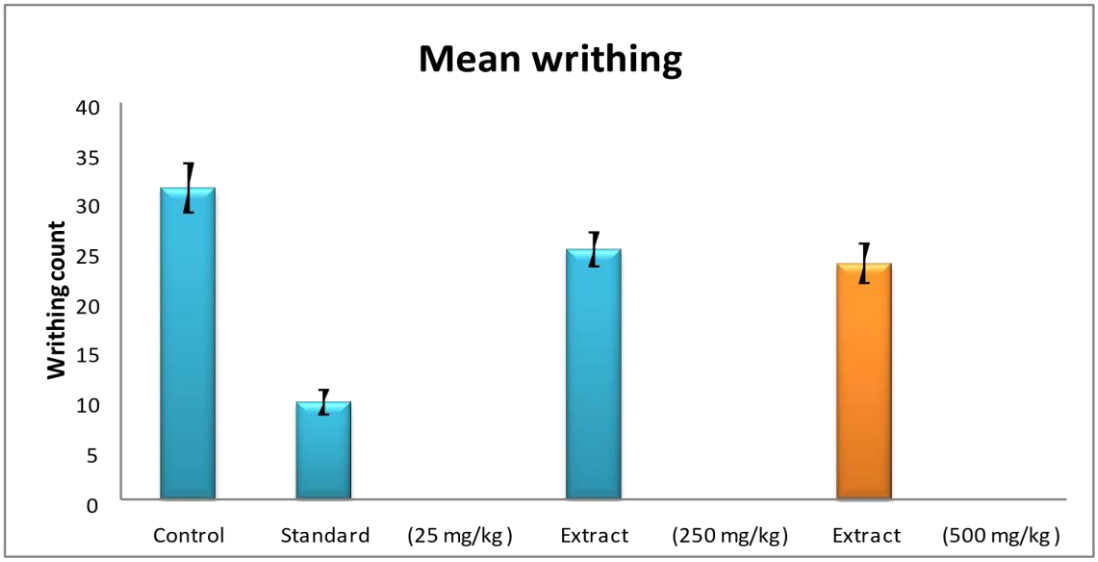


Figure 5. Percentage of writhing inhibition of acetic acid induced writhing in mice by the standard drug (diclofenac sodium) & *Cyminum cuminum*

Weight of porcelain basin = 36.32 gm

Weight of sample = 5 gm

Weight of porcelain basin+ sample = 41.32 gm

Weight of porcelain basin+ sample (dry) = 39.1 7gm

Final moisture = Weight of porcelain basin + sample - Weight of porcelain basin + sample(dry) = 41.32 - 39.17 = 2.15

% of moisture content = (Final moisture /sample weight) × 100 = 43%

Moisture is necessary for most physiological reactions in plant tissue and if it is lack, life does not exist. The moisture content was found 43%

4. CONCLUSIONS

The result obtained in this study indicate that the ethanolic seeds extract of *Cuminum cyminum and* n-hexane, chloroform, carbon tetrachloride and aqueous fractions possess bioactive principles that have significant analgesic activity in different in vivo animal model systems. The medicinal value of the plant seed extract may be related to their constituent phytochemicals. So, further investigations are needed to isolate and identify the active component present in the seed extract and its various fractions, and their efficacy needs to be done. It will help in the development of new novel and safe drugs for the treatment of various disease. In revealed the presence of *Cuminum cyminum* alkaloids, flavonoids, tannins, glycosides, saponin and terpenoids, phytosterol, saponin. The presence of these compounds influenced pharmacological activities such as analgesic activity, nutritional analysis and antihyperglycemic, hypolipidemic, and hepatoprotective activity. In the case of nutritional analysis, the moisture content of seed of *Cuminum cyminum* is 43%. In the analgesic activity test using acetic acid induced writhing method extract at dose of 500 mg/kg exhibited significant ($P<0.05$) inhibition of writhing reflex by 59.80 to 43.40 (±S.E.M) in *Cuminum cyminum* while the standard drug Diclofenac Na inhibition was found to be reduced in 59.80 to 20.20 (±S.E.M). As malnutrition is a great problem in all over the world, so it is concluded that both the investigated plants do have much potentiality in medicine as well as for nutritional requirements, however further research is needed.

ACKNOWLEDGEMENT

We will like to thank Professor Farida Begum, the Head of Pharmacy Department, Dhaka International University Bangladesh and my Lecturer Nowrin Feridious for their support and guidance in completing our Project on the topic Evaluation of Phytochemical, Analgesic, Acute Toxicity and Moisture Content of Cuminum cyminum seeds. It was a great learning experience. I will like to take this opportunity to show my profound appreciation to all my group members Fahim Mostafa, Boishaki Ahmed. The project would not have been successful without their cooperation and inputs.
Chimezie Mike Azubugwu.

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