



Research Article

EVALUATION OF PHYTOCHEMICALS, TOTAL PHENOLS, ANTIOXIDANT AND ANTHELMINTIC ACTIVITY OF HOT WATER EXTRACTS OF *CUMINUM CYMINUM* SEEDS

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ABSTRACT

In traditional medicine, cumin seeds were used as stomachic, diuretic, carminative, stimulant, astringent and abortifacient properties. Its seeds are used commonly for herbal drink preparation in Kerala and consumed as part of diet. The present study was carried out to investigate the phytochemical profile, total phenol content, antioxidant and anthelmintic activity of hot water extracts of seeds of *Cuminum cyminum*. Phytochemical analysis showed the presence of saponins, steroids, alkaloids, phenols and tannins. The total phenol content in 5 µg/ µl hot water extract is 50 µg/ µl phenol expressed as catechol equivalent. The radical scavenging potential showed dose dependent increase on increasing the concentration of cumin seed extracts 1-5 µg. IC₅₀ value of seeds was 1.82 µg/µl. Anthelmintic activity also showed dose dependant increase.

Keywords: *Cuminum cyminum*, Phytochemical analysis, Anthelmintic activity, Antioxidant activity

INTRODUCTION

India is the largest producer of medicinal plants. Medicinal plants are the richest bioresource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. Traditional medicine using plant extracts continues to provide health coverage for over 80% of the world's population, especially in the developing world¹. Plant-derived substances have recently become of great interest owing to their versatile applications and also minimal toxicity, cost effectiveness and pharmacological activity and provide an easy remedy for many human ailments as compared to the synthetic drugs². Therapeutic efficacy of medicinal plants depends upon the quality and quantity of chemical constituents.

Plants are the source of many important phytochemicals. The most important are phenols, flavonoids, alkaloids, cardiac glycosides, saponins, terpenoids, steroids, and tannins. These compounds are well known to possess biological and pharmacological activity against various chronic diseases such as cancer and cardiovascular and gastrointestinal disorders³. The primary benefits of using plant derived medicines are that they are relatively safer than their synthetic alternatives offering profound therapeutic benefits and more affordable treatment⁴.

Two important property of plant phytochemicals is their antioxidant and anthelmintic activity. An antioxidant can be broadly defined as any substance that delays or inhibits oxidative damage to a target molecule removes or scavange free radicals or molecule possessing unpaired electrons. Thus this property can prevent oxidative damage to proteins, lipids and DNA and chronic diseases such as cancer, diabetes, aging and other degenerative diseases in humans⁵. Helminthiasis is a common infection in humans and animal causing blood loss, injury to organs, intestinal or lymphatic obstruction⁶. The most

among helminths, the colon parasites including the tapeworm, pinworm and whipworm are parasitic worms that spend part of their life cycle in the large intestine. Infection of these parasites can cause significant damages and discomforts. These parasites can cause nutrient deficiencies and other health problems such as fatigue, anemia, nausea and diarrhea. Anthelmintic herbs work by stopping the parasitic worms form reproducing or preventing their growth and has no side effects.

Hot water extracts of Cumin seeds is evaluated in this present study. Cumin is used in traditional medicines to treat many diseases. The previous pharmacological studies revealed that *Cuminum cyminum* exerted antimicrobial, insecticidal, anti-inflammatory, analgesic, antioxidant, anticancer, antidiabetic, antiplatelet aggregation, hypotensive, bronchodilatory, immunological, contraceptive, anti-amyloidogenic, anti-osteoporotic, aldose reductase, alpha-glucosidase and tyrosinase inhibitory effects, protective and central nervous effects⁷. Seeds of Cumin are used for preparing medicated drinking water in Kerala. The main objective of the present study is to phytochemical analysis, total phenol quantification, the antioxidant and anthelmintic potential of hot water extract of *Cuminum cyminum* L. seeds

MATERIALS AND METHODS

Preparation of crude plant extract

Plant material consisting of *Cuminum cyminum* (seeds) was purchased from the local market The dried seeds were powdered using a mixer grinder. About 10 g of dried, ground plant materials were soaked separately in water (100 ml) for one week. The soaked material was stirred and heated to boiling point and stirred using sterilized glass rod. The final extracts were passed through Whatman filter paper No.1. The extracts were dissolved in distilled water to make a concentration of 1 mg/ml.

Phytochemical Analysis

The plant extract was diluted with distilled water for phytochemical analysis of primary and secondary metabolites using standard procedures^{8,9}

Total phenolic content

The concentration of phenolics in seed extract was determined using spectrophotometric method¹⁰. The diluted working solutions of the test extracts were prepared in water. The reaction mixture was prepared by mixing 5 µl, 2.5 µl, 1µl of hot water extract, Make up the volume to three ml of distilled water, added 0.5 ml of Folin-Ciocalteu's reagent and 2 ml 20% Na₂CO₃. Blank was concomitantly prepared, containing 3ml water, 0.5ml Folin-Ciocalteu's reagent and 2ml of 20% of Na₂CO₃. The samples were thereafter incubated in a dark for 30min. The absorbance was determined using spectrophotometer at λ_{max} = 650 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of catechol and the calibration line was constructed(Figure 1). Based on the measured absorbance, the concentration of phenolics was read (µg/µl) from the calibration line; then the content of phenolics in extracts was expressed in terms of catechol equivalent (µg of CE/µg of extract).

Antioxidant activity

The ability of the seed extracts to scavenge DPPH free radicals was assessed by the standard method¹¹. The stock solution of extracts were prepared in water to achieve the concentration of 1 mg/ml. Dilutions were made to obtain concentrations of 5, 2.5, 1µg/ml. Diluted solutions (1 ml each) were mixed with 1 ml of methanolic solution of .002% DPPH. After 30 min incubation in darkness at room temperature (23°C), the absorbance was recorded at 517 nm Labtronics NT 290 Spectrophotometer. Control sample contained all the reagents except the extract. Percentage inhibition was calculated using equation 1, whilst IC₅₀ values were estimated from the % inhibition versus concentration plot. The effective concentration of sample required to scavenge DPPH radical by 50% (IC₅₀ value) was obtained by linear regression analysis of dose-response curve plotting between % inhibition and concentrations¹².

The optical density was recorded and % inhibition was calculated using the formula given below

$$\text{Percent (\%)} \text{ inhibition of DPPH activity} = \left\{ \frac{(A-B)}{A} \right\} \times 100$$

Where, A = optical density of the blank and B = optical density of the sample.

Anthelmintic activity

The anthelmintic assay was carried out as per the Ayaiyeoba *et al* method¹³. Adult earthworms (*Pheretima posthuma*), were used to evaluate anthelmintic activity *in vitro*. Earthworms of 3-5 cm in length and 0.1-0.2 cm in width (same type) were collected from Kerala Agricultural University, Mannuthy. Test samples of the extract was prepared at the concentrations, 25,50,75 mg/ml in distilled water and three worms i.e. *Pheretima posthuma*, of approximately equal size were used for all the experimental protocol were placed in each nine cm Petri dish containing 25 ml of above test solution of extracts. Albendazole (25 mg/ml and 50mg/l) was used as reference standard as advocated earlier. All the test solution and standard drug solution were prepared freshly before starting the experiments. Observations were made for the time taken for

paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms was recorded after ascertaining that worms neither moved when shaken vigorously.

RESULTS AND DISCUSSION

Phytochemical analysis is very useful in the evaluation of active biological components of medicinal plants. The qualitative analysis of the powdered Cumin seeds showed the presence of secondary metabolites such as alkaloids, Total phenols and Tannins, steroids, saponins and the primary metabolites (Table 1). Phytochemicals such as saponins, flavonoids, tannins, steroids and alkaloids have anti-inflammatory effects¹⁴. Phenols and alkaloids have antimicrobial, antidiarrhoeal, anthelmintic activities¹⁵. Alkaloids acts as an analgesic drug and can increase nutrient absorption and blood circulation, hypoglycemic activities reduce pain and stimulate nerve system as it has narcotic effect¹⁶. Saponins helps in controlling cholesterol, antidiabetic properties¹⁷ in addition to its anticancer and anthelmintic activity¹⁸. The steroids have antidiarrhoeal activities¹⁹.

Presence of total phenols was detected in Cumin seeds. The phenolic compounds are heterogeneous groups of substances that are present in almost all plants, inside the vacuole, cytoplasm or consisting the cell wall²⁰. Natural antioxidant mainly come from plants in the form of phenolic compounds such as flavonoid, phenolic acids, tocopherols etc. The total phenol content play a very important role in the protection of the plants against the deleterious effects of UV rays and also against certain phytopathogenic microorganisms²⁰. It is also well known that phenolic compounds contribute to the quality and nutritional value and also provide health beneficial effects²¹. Phenols are found to be useful in the preparation of antimicrobial compounds²². The Folin-Ciocalteu reagent is sensitive to reducing compounds including polyphenols, thereby producing a blue colour upon reaction²³. This is confirmed by the presence of blue colour in the extracts in comparison to the catechol which is taken as the standard. The phenol possess biological properties such as antiapoptosis, antiaging, anticarcinogen, antiinflammation, antiatherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities²⁴. They are also found in many medicinal plants, and herbal medicines containing these compounds have often been used in pharmacy.

The DPPH activity of hot water extract was calculated from 1 µg, 2.5 µg and 5 µg extract. All the concentrations showed antioxidant activity (Figure 2). Table 3 shows the results of the free radical (DPPH) scavenging activity in % inhibition. The radical scavenging potential showed dose dependent increase on increasing the concentration of cumin extracts 1-5 µg. IC₅₀ value was 1.82µg/µl. Antioxidants due to their scavenging activity are useful for the management of diseases. DPPH stable free radical method is a sensitive way to determine the antioxidant activity of plant extracts²⁵. Phenolic compounds are known for their antioxidant activity²⁶. Such activity is related to their redox properties in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides product 2,2-diphenyl-1-picryl hydrazine. A freshly prepared DPPH solution is of deep purple colour with absorption maximum at 517 nm and in the presence of antioxidant, this colour disappears due to quenching of DPPH free radicals and converting them into a colourless²⁷. Hence DPPH is usually used as a substance to evaluate the antioxidant activity

Table 1: Preliminary phytochemical screening of hot water extracts of *Cuminum cyminum* seeds

Sl No	Plant Constituents	Test /Reagent	Observations	Aqueous Extract
1	Carbohydrates	Molisch Test	Violet Ring observed	+
2	Proteins	Biuret Test:	Violet to pink colouration	+
3	Amino Acids	Ninhydrin Test:	Blue to violet colouration	+
4	Alkaloids	Mayer's Test	White precipitate	+
5	Steroid	Salkowski reaction:	Chloroform layer appears red and acid layer shows greenish yellow fluorescence	+++
6	Saponins	Foam test	Persistent Foam	+
7	Phenols and Tannins	Folin Test	Blue Colouration	++++
		Bromine water	Decolouration of Bromine water	+
		Acetic acid	No red Colouration	-

'+' low, '++' Average, '+++ High, '-' Nil

Table 2 : Total Phenol Quantification of hot water extracts of *Cuminum cyminum*

Concentration Plant Extract µg/ µl	Phenol concentration µg/µl CE
5	50
2.5	27
1	18.2

Table 3: Antioxidant activity of the hot water extracts of *Cuminum cyminum*

Concentration Plant Extract µg	Optical Density Initial	Optical Density Extract colour	Optical Density (Initial- Extract Colour)	Percentage of Inhibition	IC ₅₀ µg/µl
Control	0.340				1.82
5	0.122	0.098	0.024	92	
2.5	0.102	0.048	0.054	84	
1	0.203	0.018	0.185	45	

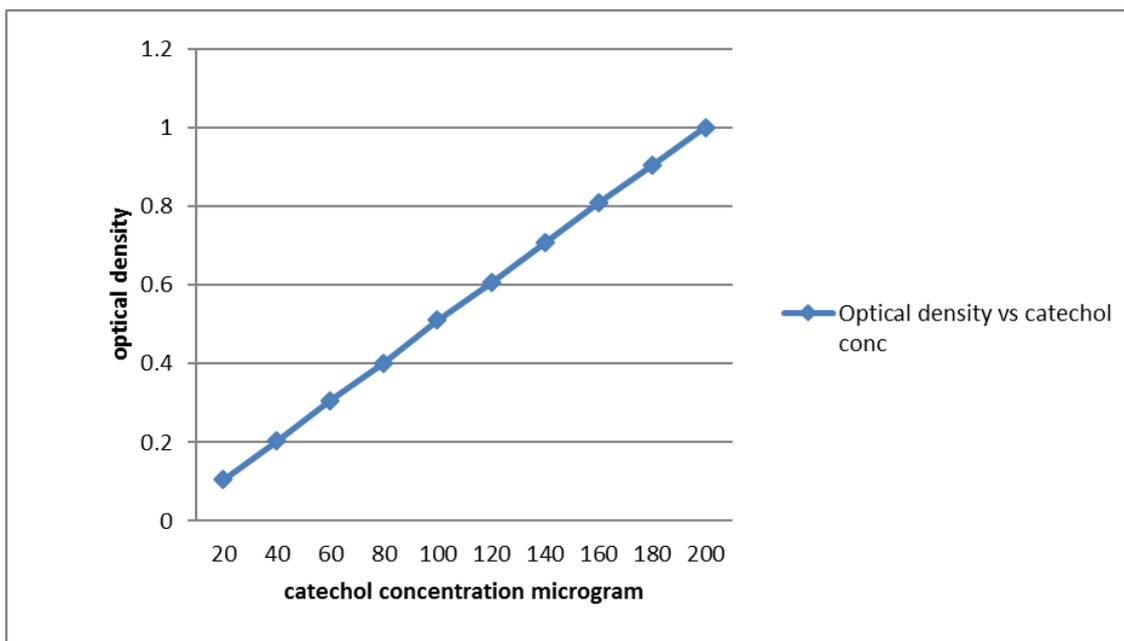


Figure 1 : Catechol calibration curve

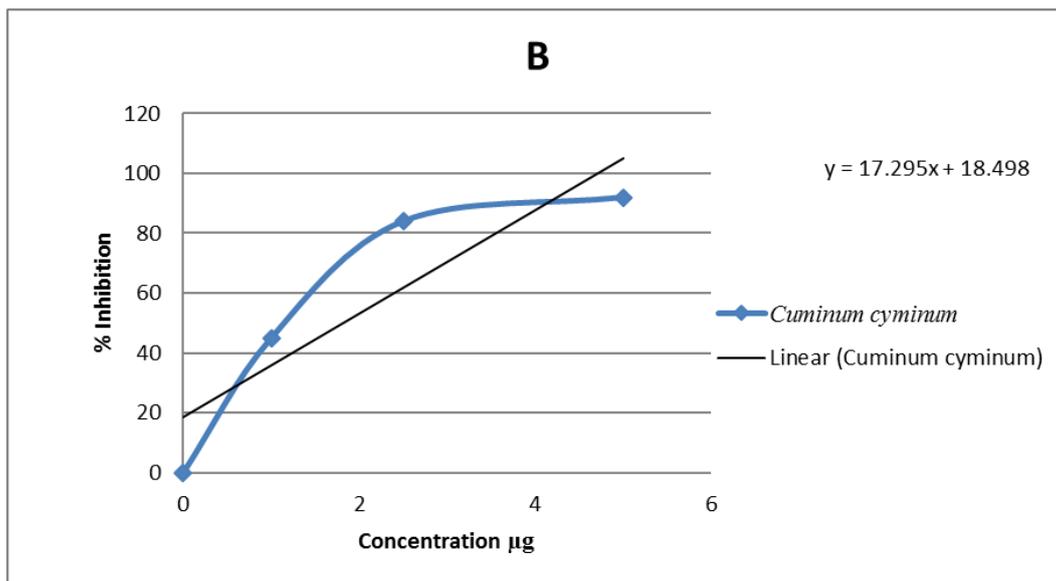


Figure 2: DPPH free radical scavenging activity of hot water extracts of *Cuminum cyminum*

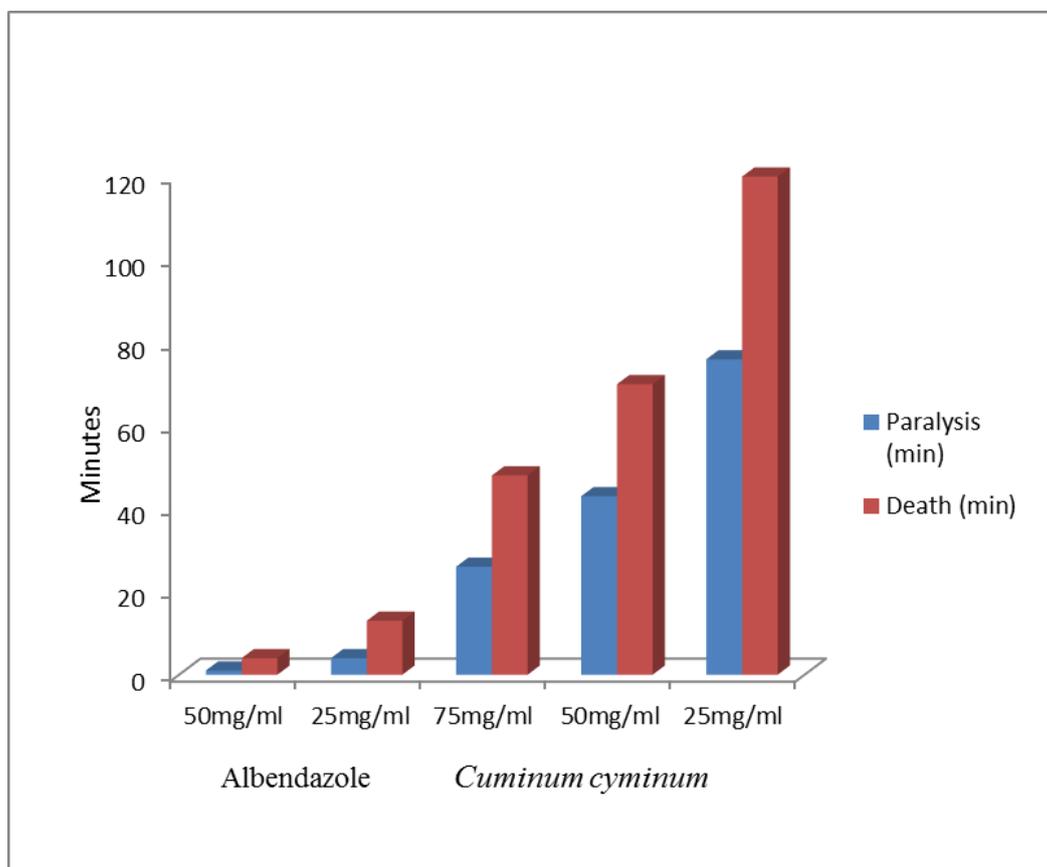


Figure 3: Anthelmintic activity of hot water extract of *Cuminum cyminum*

Hot water extract of *Cuminum cyminum* 25, 50, 75 mg/ml were tested for anthelmintic activity (Table 5). The standard used was 25 and 50 mg/ml of albendazole. It was observed that 75 mg/ml concentration extract showed promising effect (Figure 3). The anthelmintic activity of hot water extracts was studied against the Indian adult earthworm *Pheretima posthuma* due to its anatomical and physiological resemblance to intestinal round worm parasite of human beings²⁷. Development of resistance in helminths against conventional anthelmintics and serious side effects of is the foremost problem in treatment of helminths

disease⁶. Henceforth, it is important to look for alternative strategies against gastrointestinal nematode parasites, and plants for their anthelmintic activity.

It can be concluded that plant phytochemicals play a crucial role as therapeutic agents against human diseases. The therapeutic value of cumin seeds is usually consumed as part of diet and used for making herbal drinking water. There is a need for further studies in order to isolate the active ingredients that are responsible for its potential anthelmintic activity and to

elucidate the mechanism of action of these active ingredients to open the new door for natural anthelmintics.

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