



Pharmacognostical, Phytochemical, Antioxidant and Antimicrobial Activity of Methanolic Extract of Cumin Seeds

Manisha Pradhan¹, Sarita Patel*¹, Ashish Patel²

¹Department of Chemistry, Govt. Girls PG College, Rewa, (M.P.)

²Department of Botany, Govt. Degree College Pushparajgarh, Distt. –Anuppur, (M.P.)

*Corresponding Author *E-mail*: saritapatel0787@gmail.com

ABSTRACT

Medicinal plants have bioactive compounds which are used for curing of various human diseases and also play an important role in healing. Secondary constituents contain alkaloids, flavonoids, phenol, saponins, steroids and tannins. Medicinal plants have anticancer, antimicrobial, antidiabetic, antidiuretic and anti- inflammation activities etc. The increasing interest in powerful biological activity of secondary metabolites outlined the necessity of determining their contents in medicinal plants. Jeera, commonly known as cumin is a popular herbal plant in traditional ayurvedic medicine. The dried seeds of *C. cyminum* have been traditionally used as food and medicine. The objective of this study was to investigate pharmacognostical, phytochemical features antioxidant and antimicrobial activity of methanolic extracts of *C. cyminum* seeds. The different pharmacognostical parameters were evaluated as per standard protocols with some modifications. Qualitative analysis of various phytochemical constituents and quantitative analysis of total phenol and flavonoids were determined by the well-known test protocol available in the literature. The antioxidant activity of plant extracts were determined by different *in vitro* methods such as the DPPH free radical scavenging assay, SOS activity, and reducing power assay and *in vitro* antimicrobial activity was done by well diffusion assay method. Phytochemical analysis revealed the presence of alkaloids, glycosides, flavonoids, tannins and phenolic compounds, saponins, proteins and amino acid, terpenoids and carbohydrate. The total phenolic and flavonoids content of *C. cyminum* seeds of methanolic extract was 43.66 and 38 mg/100mg respectively. The activities of seeds extract against DPPH free radical scavenging assay, SOS activity, and reducing power assay were concentration dependent. Methanolic extract exhibit maximum antibacterial activity against *E. coli* (12.66 mm zone of inhibition), and *Streptococcus mutans* (10.33 mm zone of inhibition) followed by *Pseudomonas aeruginosa* (8.33 mm zone of inhibition) and *Bacillus subtilis* (8.0 mm zone of

inhibition). The present study concluded that the crude extract of *C. cyminum* is a rich source of secondary phytoconstituents which impart significant antioxidant potential and anti microbial activity. The findings of the present study will be helpful to phytochemists, pharmacologists and pharmaceutical industries.

Keywords: *Cuminum cyminum*, Pharmacognostical, Phytochemical, Antioxidant, Antimicrobial activity.

Introduction

Infectious diseases are still a major health concern accounting for 41% of the global disease burden measured in terms of Disability-Adjusted Life Years (DALYS) [1]. One of the main causes of this problem is the widespread of acquired bacterial resistance to antibiotics in such a way that the world is facing today, a serious threat to global public health [2] in the form of not only epidemics, but also pandemics of antibiotic resistance [3, 4]. Due to this problem of resistance against antibiotics, attention is now being shifted towards biologically active components isolated from plant species communing used as herbal medicine, as they may produce a new potent source of antibacterial and antifungal activities [5, 6]. The antimicrobial properties of plants related to their ability to give several secondary metabolites of relatively complex structures possessing antimicrobial activities [7]. Antioxidants are chemical molecules that interact with free radicals, moreover neutralize their effect. Phytochemicals such as flavonoids, Tannins, Lycopene, Phenolic acids, Vitamin C serve as potential antioxidants. Phytochemicals like flavonoids, tannins, ascorbic acid, phenolic compounds, alkaloids, and saponins etc., plant showed antimicrobial properties. These phytochemicals, due to this property aids in prevention of infections and other harmful microbial diseases [8-10]. *Cuminum cyminum* (Umbelliferae), commonly known as Jeera in Hindi, Jeeragi in Kannada. It's indigenous to Nile territory. It is cultivated in Morocco, Sicily, India, Syria and China. In India, except Assam and West Bengal, it is cultivated in all states. About 90% of the world production is from India and most of it comes from states of Rajasthan and Gujarat. Cumin fruits contain 2.5 to 4.5% volatile oil, 10% fixed oil and proteins, volatile oil mainly consists of 30 to 50% cuminaldehyde, small quantities of α -pinene, β -pinene, phellandrene, cuminic alcohol, hydrated cuminaldehyde and hydro cuminine. The fruit has a sharp burning taste, astringent, carminative, tonic to the intestine, abortifacient, emmenagogue, stops epistaxis, heals corneal opacities, ulcers and styes,

cures haemoptysis, scabies, gonorrhoea, asthma, relieves cough, inflammations, enlargement of spleen; applied to boils and ulcers (yunani) [11]. The aim of this work was to determine the quality (types), quantity (amount) of bioactive compounds, *in vitro* antioxidant and *in vitro* antimicrobial activity of seeds of *Cuminum cyminum*.

Materials and methods

Collection and identification of plant material

Seeds of *Cuminum cyminum* collected from Bilaunji, Navjeevan vihar, Navanagar and Khutar, District Singrauli, (M.P.) Fresh plant materials were air dried and then homogenized to coarse powder and stored in airtight container.

Chemical reagents

All the chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India). All the chemicals used in this study were of analytical grade.

Pharmacognostical evaluation

Total ash value

Accurately weighed 5gms 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. The weight of ash was taken and the percentage of it was calculated with reference to the air-dried sample. The percentage of total ash was calculated with reference to the air-dried powder [12].

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

Loss on drying

Accurately weighed 5gms 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. The percentage of moisture content was then calculated with reference to the air-dried sample.

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

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 temperature not exceeding 450°C, cooled and weighed the obtained residue. The difference in weight represents the water soluble ash. Finally, the percentage of water soluble ash was calculated with reference to the air-dried sample [12].

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

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 percentage of acid insoluble ash was calculated with reference to the air-dried sample [12].

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

Alcoholic extractive value

Macerated 5 gm 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. Thereafter, it was filtered rapidly taking precautions against loss of the solvent. Evaporated 25 ml of the filtrate to dryness in a tarred flat bottom shallow dish dried at 105⁰ C and weighed. The percentage of ethanol soluble extractive was calculated with reference to the air dried drug [13].

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

Water soluble extract

Macerated 5 gm of the air dried coarsely seeds powder with 100 ml of chloroform water in a closed flask for 24 hours. Shaking frequently during the first 6 hours and allowed to stand for 18 hours. Thereafter, it was filtered rapidly taking precautions against loss of the solvent. Evaporated 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 [13].

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

Hot soxhlet extraction method

This technique involved gathering, correctly washing, and properly rinsing the blossoms of seeds of *C. cuminum*. They were mechanically pulverised after being shade-dried. The plant material

from seeds of *C. cyminum*, either whole or coarsely powdered, was successively extracted using solvents such as methanol. The Soxhlet apparatus' chamber was filled with powder using a "thimble" design. The solvent used for extraction was heated in flasks, and its vapours were then condensed in a condenser. The powder is extracted by touch when the condensed extractant is dropped into the thimble holding it. The liquid inside the chamber syphon drops into the flask when the liquid level in the chamber reaches the top of the syphon tube. This procedure was continued until an evaporated drop of solvent from the syphon tube did not leave any residue. The resulting extract was filtered, dried by concentration, weighed, and stored for later use [14]. The following formula is used to determine the extract's yield.

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

Phytochemical screening of the extract

A variety of phytoconstituents, including alkaloids, carbohydrates, glycosides, phytosterols, saponins, tannins, proteins, amino acids, and flavonoids were qualitatively analysed in the seeds extracts of *C. cyminum* [15, 16].

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 Folin Ciocalteu reagent was used to calculate the total phenolic content of the extracts. Gallic acid concentration (20-100 µg/ml) was produced in methanol. Concentrations of 100 µg/ml of plant extract were also made in methanol, and 0.5 ml of each sample was added to the test along with 4 ml of 7.5% sodium carbonate and 2 ml of a 10 fold diluted folin Ciocalteu reagent. The tubes were parafilm-covered, and after 30 minutes of intermittent shaking at room temperature, the absorbance at 760 nm was measured using methanol as a blank. Gallic acid's conventional regression curve was used to compute the total phenol content, and the results were given in milligrammes per gramme (mg/g) of gallic acid [17].

Total flavonoid content estimation

Rutin (20 to 100 μ g/ml) was produced in methanol at various concentrations. Test samples with a polarity of 100 μ g/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 5ml with distilled water, the mixture was let to stand for an additional 15 minutes. At 510 nm, the absorbance was calculated using water as the reference. The standard regression curve of quercetin and rutin was used to compute the total flavonoid content [17].

***In vitro* anti oxidant activity of plant extract**

DPPH assay

Free radical scavenging activity of the methanol extract of *C. cyminum* seeds, based on the scavenging activity of the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was determined by the method of Ali et al [18]. Different volume of extracts/standard (20-100 μ g/ml) was taken from stock solution in a set of test tubes and methanol was added to make the volume to 1 ml. To this, 2 ml of 0.1mM DPPH reagent was added and mixed thoroughly. Absorbance at 517 nm was determined after 30 min and the percentage inhibition activity was calculated by using the equation: % scavenging activity= $[(A_0-A_1)/A_0] \times 100$. Where A₀ is the absorbance of the control and A₁ is the absorbance of the extract. Lower the absorbance, the higher is the free radical scavenging activity. The curves were prepared and the IC₅₀ value was calculated using linear regression analysis.

Superoxide anion radical scavenging activity

1 ml of nitroblue tetrazolium (NBT) (100 μ l of NBT in 100 mM phosphate buffer, pH 7.4), 1 ml of NADH (468 μ l in 100 mM phosphate buffer, pH 7.4), solution as well as varying volumes of methanol extract of *C. cyminum* seeds (20, 40, 60, 80 and 100 μ g/ml), were mixed well with methanol. The reaction was started by the addition of 1 ml of phenazine methosulfate (PMS) (60 μ l/100 mM phosphate buffer, pH 7.4). The reaction mixture was incubated at 30°C for 15 min. The absorbance was measured at 560 nm in a spectrophotometer. Incubation without the sample (extract) was used as a blank sample. Ascorbic acid was used as the standard in comparing the different sample. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity [19]. The percentage scavenging was calculated by using the formula shown below:

$$\% \text{ Inhibition} = [(\text{Ab of control} - \text{Ab of sample}) / \text{Ab of control} \times 100]$$

Reducing power assay

Preparation of standard solution

3 mg of ascorbic acid was dissolved in 3 ml of distilled water/solvent. Dilutions of this solution with distilled water were prepared to give the concentrations of 20, 40, 60, 80 and 100µg/ml.

Preparation of extracts

Stock solutions of methanol extract of *C. cyminum* seeds were prepared by dissolving 10 mg of dried extracts in 10 ml of methanol to give a concentration of 1mg/ml. Then sample concentrations of 20, 40, 60, 80 and 100µg/ml were prepared.

Protocol for reducing power

According to this method, the aliquots of various concentrations of the standard and methanol extract of *C. cyminum* seeds (20 to 100µg/ml) in 1.0 ml of deionized water were mixed with 2.5 ml of (pH 6.6) phosphate buffer and 2.5 ml of (1%) potassium ferricyanide. The mixture was incubated at 50°C in water bath for 20 min after cooling. Aliquots of 2.5 ml of (10%) trichloroacetic acid were added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution 2.5 ml was mixed with 2.5 ml distilled water and a freshly prepared 0.5 ml of (0.1%) ferric chloride solution. The absorbance was measured at 700 nm in UV spectrometer (Systronic double beam-UV-2202). A blank was prepared without adding extract. Ascorbic acid at various concentrations (20 to 100µg/ml) was used as standard [19].

Antimicrobial activity (well diffusion assay)

Anti-bacterial activity

Preparation of dilutions of the samples

The dilutions of the samples were made for the concentration as 100µg/ml, 150µg/ml, 200µg/ml, and 250µg/ml respectively of the sample, after that volume makeup was done with distilled water till 1ml.

Preparation of nutrient agar media

28 g of nutrient media was dissolved in 1litre of distilled water. pH of media was checked before sterilization. Media was sterilized in autoclave at 121°C at 15 lbs pressure for 15 minutes. Nutrient media was poured into plates and placed in the laminar air flow until the agar was get solidified.

Well diffusion assay

The bacterial suspension of *E. coli* (MTCC 42), *S. mutans* (MTCC 389), *B. subtilis* (MTCC 736), and *P. aeruginosa* (MTCC 8076) were standardized to 10^8 CFU/ml of bacteria and kept into the shaker. Then, 100µl of the inoculum from the broth (containing 10^8 CFU/ml) was taken with a micropipette and then transferred to fresh and sterile solidified agar media plate. The agar plate was inoculated by spreading the inoculum with a sterile spreader, over the entire sterile agar surface. Four wells of 6 mm were bored in the inoculated media with the help of sterile cork-borer. Each well was filled with different concentration (100µg/ml, 150µg/ml, 200µg/ml and 250µg/ml) of samples (methanol extract of *C. cyminum* seeds). It was allowed to diffuse for about 30 minutes at room temperature and incubated for 18-24 hours at 37° C. After incubation, plates were observed for the formation of a clear zone around the well which corresponds to the antimicrobial activity of tested compounds. The zone of inhibition (ZOI) was observed and measured in mm. Zones were measured to a nearest millimeter using a ruler, which was held on the back of the inverted Petri plate. The Petri plate was held a few inches above a black, non-reflecting background. The diameters of the zone of complete inhibition (as judge by unaided eye) were measured, including the diameter of the well [20].

Results

Pharmacognostical evaluation

Table 1: Pharmacognostical evaluation of plant sample

Parameters	Value in percentage (%)
	<i>C. cyminum</i> (Cumin)
Total ash value	6.51
Loss on drying	11.23
Water soluble ash	12.58
Acid insoluble ash	0.98
Water extractive value	3.15
Alcoholic extractive value	14.12

Plant extraction

The plant material was extracted by soxhlet extraction method and the percentage yield calculated by the following formula:-

$$\% \text{ yield} = \frac{\text{Actual Yield} \times 100}{\text{Theoretical yield}}$$

Table 2: Percentage yield

Solvent	Theoretical yield (in gm)	Actual Yield (in gm)	Percentage Yield (%)
<i>C. cyminum</i> methanolic extract	110	16.36	14.87

Solubility determination

Table 3: Solubility determination of extracts

S. No.	Solvent	<i>C. cyminum</i> (Cumin)
1.	Water	Soluble
2.	Methanol	Soluble
3.	Ethyl Acetate	Soluble
4.	DMSO	Soluble
5.	Petroleum Ether	Insoluble

Phytochemical analysis

Table 4: Qualitative phytochemical analysis of extracts

S. No.	Experiment	<i>C. cyminum</i> methanolic extract
Test for Carbohydrates		
1.	Molisch's Test	+
2.	Fehling's Test	+
3.	Benedict's Test	+
4.	Bareford's Test	+
Test for Alkaloids		
1.	Mayer's Test	+
2.	Hager's Test	+
3.	Wagner's Test	+
4.	Dragendroff's Test	+
Test for Terpenoids		
1.	Salkowski Test	+
2.	Libermann-Burchard's Test	+
Test for Flavonoids		
1.	Lead Acetate Test	+
2.	Alkaline Reagent Test	+
3.	Shinoda Test	+
Test for Tannins and Phenolic Compounds		
1.	FeCl ₃ Test	+
2.	Lead Acetate Test	+
3.	Gelatine Test	+
4.	Dilute Iodine Solution Test	+

Test for Saponins		
1.	Froth Test	+
Test for Protein and Amino acids		
1.	Ninhydrin Test	+
2.	Biuret's Test	+
3.	Million's Test	+
Test for Glycosides		
1.	Legal's Test	+
2.	Keller Killani Test	+
3.	Borntrager's Test	+

Quantitative phytochemical analysis

Total phenolic content (TPC) estimation

Table 5: Total phenolic content in extracts

Extracts	Total phenolic content (mg/gm equivalent to gallic acid)
	<i>C. cyminum</i> methanolic extract
Absorbance Mean±SD	0.193±0.011
TPC (mg/gm)	43.66

Total flavonoid content (TFC) estimation

Table 6: Total flavonoid content in extracts

Extracts	Total flavonoid content (mg/gm equivalent to rutin)
	<i>C. cyminum</i> methanolic extract
Absorbance Mean±SD	0.122±0.006
TFC (mg/gm)	38

In vitro anti oxidant activity

Table 7: DPPH radical scavenging activity

Concentration	Ascorbic acid (% Inhibition)	Methanolic extract of <i>C. cyminum</i> (% Inhibition)
20	46.97	40.50
40	54.56	44.45
60	74.49	46.67
80	83.74	48.02
100	91.81	51.10
Control	0.843	1.037
IC50	25.82	91.78

Table 8: Superoxide anion radical scavenging activity

Concentration	Ascorbic acid (% Inhibition)	Methanolic extract of <i>C. cyminum</i> (% Inhibition)
20	55.06	31.77
40	61.35	35.86

60	67.63	43.34
80	72.17	49.64
100	86.72	55.60
Control	12.01	0.856
IC50	12.01	82.05

Table 9: Reducing power assay

Concentration	Absorbance of Ascorbic acid	Absorbance of Methanolic extract of <i>C. cyminum</i>
20	0.202	0.023
40	0.314	0.056
60	0.429	0.084
80	0.543	0.101
100	0.699	0.128

Antibacterial activity

Table 10: Antimicrobial activity of *C. cyminum* against *E. coli*

Concentration (µg/ml)	Plate 1 (mm)	Plate 2 (mm)	Plate 3 (mm)	Mean±SD
100	0	0	0	0±0
150	8	8	9	8.33±0.577
200	9	10	11	10±1
250	11	14	13	12.66±1.527

Table 11: Antimicrobial activity of *C. cyminum* against *S. mutans*

Concentration (µg/ml)	Plate 1 (mm)	Plate 2 (mm)	Plate 3 (mm)	Mean±SD
100	0	0	0	0±0
150	8	7	8	7.66±0.577
200	9	8	9	8.66±0.577
250	10	10	11	10.33±0.577

Table 12: Antimicrobial activity of *C. cyminum* extract against *P. aeruginosa*

Concentration (µg/ml)	Plate 1	Plate 2	Plate 3	Mean±SD
100	0	0	0	0±0
150	0	0	0	0±0
200	0	0	0	0±0
250	8	8	9	8.33±0.577

Table 13: Antimicrobial activity of *C. cyminum* against *B. subtilis*

Concentration (µg/ml)	Plate 1 (mm)	Plate 2 (mm)	Plate 3 (mm)	Mean±SD
100	0	0	0	0±0
150	0	0	0	0±0
200	7	7	7	7±0
250	8	8	8	8±0

Discussion

The present study deals with the studies on pharmacognostic, phytochemical, antioxidant and antimicrobial activity on seeds of *C. cyminum*. Raw materials were analyzed for identity, quality and purity as per the standards prescribed by WHO and Ayurvedic Pharmacopeia of India. The loss on drying of dry powder of *C. cyminum* was 11.23%. The ash value was determined by three different forms viz., total ash, water soluble ash and acid insoluble ash. 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 0.98%. The water and alcoholic extractive value of crude powder of *C. cyminum* was found to be 3.15 and 14.12%. The phytochemical analysis conducted on methanolic extract of *C. cyminum* revealed the presence of alkaloids, glycosides, flavonoids, tannins and phenolic compounds, saponins, proteins and amino acid, terpenoids and carbohydrate. Phytochemicals present in plant act as the source for the treatment of different health problem. Different phytochemical have different therapeutic value. Total phenolic content (TPC) was measures by using Folin-ciocalteau's reagent method. And total flavonoid content (TFC) of *C. cyminum* was measured by Aluminum chloride method. The TPC and TFC of the extracts were expressed as milligram of gallic acid equivalent per gram of extracts i. e. mg GAE/g extract and milligram of rutin equivalent per gram of extract i. e. mg RE/g extract respectively. The result of the present study revealed that the methanolic extract of *C. cyminum* has TPC (43.66mg GAE/g) and TFC value (38mg RE/g). Antioxidant plays a major role in protecting our body from disease by reducing the oxidative damage to cellular component caused by reactive oxygen species. Recent investigations suggest that the plant origin antioxidants with free-radical scavenging properties may have great therapeutic importance in free radical mediated diseases like diabetes, cancer, neurodegenerative disease, cardiovascular diseases, aging, gastrointestinal diseases, arthritis, and aging process. The antioxidant activity of plant extracts were determined by different *in vitro* methods such as the DPPH free radical scavenging assay, SOS activity, and reducing power assay. DPPH radical scavenging activity of methanolic extract of *C. cyminum* exhibited percent inhibition % 51.10 and its IC₅₀ value was found to be 91.78µg/ml. Similarly, SOS scavenger activity of methanolic extract of *C. cyminum* exhibited percent inhibition 55.60% and its IC₅₀ value was found to be 82.05µg/ml. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Dietary antioxidant such as ascorbic acid was used for comparison. Compounds with reducing power indicate that they are electron donors

and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants. The extract of *C. cyminum* showed good reducing capacity. Antibacterial activity was evaluated by well diffusion method against *E. coli*, *S. mutans*, *B. subtilis* and *P. aeruginosa* with concentration ranging 100, 150, 200 and 250µg/ml. *C. cyminum* extract showed the maximum antibacterial activity against gram negative bacteria i.e. *E. coli* and *S. mutans*. *C. cyminum* methanolic extract showed best zones of inhibition of 12.66mm in diameters at 250µg/ml concentration against *E. coli*. Similarly, against *S. mutans*, *P. aeruginosa* and *B. subtilis*, *C. cyminum* showed zones of inhibition of 10.33, 8.33 and 8.88mm in diameters at 250µg/ml concentration. It observe that, methanolic extract of plants contain phytochemicals including polyphenols and are reported to exhibit considerably high free radical scavenging and peroxide inhibition activity indicating its reducing character, which may in part explain the inhibition of bacterial growth.

Conclusion

The preset study verified the traditional use of *C. cyminum* for human ailments and partly explained its use in herbal medicine as rich source of phytochemicals with the presence of alkaloids, glycosides, flavonoids, tannins and phenolic compounds, saponins, proteins and amino acid, terpenoids and carbohydrate. Thus this plant can be utilized as an alternative source of useful drugs. Further studies are needed with this plant to isolate, characterize and elucidate the structure of the bioactive compounds of this plant for drug formulation.

References

1. Noah D., Fidas G. National Intelligence Council; Washington DC: 2000. The Global Infectious Disease Threat and its Implications for the United States.
2. Chopra I. Drugs for the superbugs. Microbiol. Today. 2000;27:4–6.
3. Chanda S.Y., Daravalia M.K., Rakholiya K. Fruit and vegetable peels – strong natural source of antimicrobics. Curr. Res., Technol. Educat. Topic Appl. Microbiol. Microbial Biotech. 2010;444:450.
4. Osman K.M., Marouf S.H., Samir A., AlAtfeehy N. The prevalence of multidrug resistance of various numbers of antimicrobial classes, multiple resistance patterns, and distribution of Salmonella isolates from human and avian clinical cases of diarrhea. J. Chemother. 2012;24(5):300–304.

5. Maiyo Z., Ngure R., Matasyoh J., Chepkorir R. Phytochemical Constituents and antimicrobial activity of leaf extracts of three Amaranthus plant species. *Afr. J. Biotechnol.* 2010;9(21):3178–3182.
6. Erfan A.M., Marouf S. Cinnamon oil downregulates virulence genes of poultry respiratory bacterial agents and revealed significant bacterial inhibition: An in vitro perspective. *Vet. World.* 2019;12(11):1707–1715.
7. Matasyoh J., Maiyo Z., Ngure R., Chepkorir R. Chemical composition and antimicrobial activity of the essential oil of *Coriandrum sativum*. *Food Chem.* 2009;113(2):526–529.
8. Chang, C.J.; Tzeng, T.-F.; Liou, S.-S.; Chang, Y.-S.; Liu, I.-M. Acute and 28-day subchronic oral toxicity of an ethanol extract of *Zingiber zerumbet* (L.) Smith in rodents. *Evid.-Based Complementary Altern. Med.* 2012, 2012, 608284.
9. Diaz-Sanchez, S.; D'Souza, D.; Biswas, D.; Hanning, I. Botanical alternatives to antibiotics for use in organic poultry production. *Poult. Sci.* 2015, 94, 1419–1430.
10. Haque, M.A.; Jantan, I. Recent Updates on the Phytochemistry, Pharmacological, and Toxicological Activities of *Zingiber zerumbet* (L.) Roscoe ex Sm. *Curr. Pharm. Biotechnol.* 2017, 18, 696–720.
11. Shivakumar SI, Shahapurkar AA, Kalmath KV, Shivakumar B. Antiinflammatory activity of fruits of *Cuminum cyminum* Linn. *Der Pharmacia Lettre.* 2010;2(1):22-4.
12. Dev, U. K., Hossain, M. T., & Islam, M. Z. (2015). Phytochemical investigation, antioxidant activity and anthelmintic activity of *Mikania micrantha* leaves. *World J. Pharm. Res.* 4(5), 121-133.
13. Bhutia, S. (2020). Fluorescence analysis and extractive values of some Ethno-medicinal plants of Sikkim Himalaya region. *Advance Pharmaceutical Journal*, 5(1), 31-35.
14. Pradhan A, Jain P, Pal M, Chauhan M, Jain DK. Qualitative and quantitative determination of phytochemical contents of hydroalcoholic extract of *Salmalia malabarica*. *Pharmacologyonline* 2019; 1:21-6.
15. Dutta R, Sharma MK, Khan A, Jha M. Phytochemical and in vitro antioxidant assay of *Fumaria officinalis* leaf extract. *Journal of Advanced Scientific Research* 2020; 11(03):176-82.

16. Jain DK, Gupta S, Jain R, Jain N. Anti-inflammatory Activity of 80% Ethanolic Extract of *Acorus calamus* Linn. Leaves in Albino Rats. *Research Journal of Pharma Technology* 2010; 3(3): 882-884.
17. Joshi S, Parkhe G, Aqueel N, Dixit N, Jain DK. Estimation of total phenolic, total flavonoids and total protein content of hydroalcoholic extract of *Anacyclus pyrethrum*. *Pharmacologyonline*. 2019; 1:27-33.
18. Ali, M. S., Amin, M. R., Kamal, C. M. I., & Hossain, M. A.. In vitro antioxidant, cytotoxic, thrombolytic activities and phytochemical evaluation of methanol extract of the *A. philippense* L. leaves. *Asian Pacific Journal of Tropical Biomedicine* 2013. 3(6), 464-469.
19. Choi CW, Kim SC, Hwang SS, Choi BK, Ahn HJ. Antioxidant activity and free radical scavenging capacity between Korean medicinal plants and flavonoids by assay –guided comparison. *Plant Sci*. 2002;163:1161–8.
20. Singh, A. R., Bajaj, V. K., Sekhawat, P. S., & Singh, K. (2013). Phytochemical estimation and antimicrobial activity of aqueous and methanolic extract of *Ocimum sanctum* L. *J Nat Prod Plant Resour*, 3(1), 51-8.