ISSN 1996-5052

Current Research in Chemistry

Knowledgia SCIENTIFIC
A Place to Publish Outstanding Research

http://knowledgiascientific.com

OPEN ACCESS Current Research in Chemistry

ISSN 1996-5052
DOI: 10.3923/crc.2018.1.9

Research Article
Optimization of Extraction and Pharmacological Activities of Essential Oil from Black Cumin (*Nigella sativa* L.) Seeds using Clevenger Distillation

[1]Demis Zelelew and [2]Amha Gebremariam

[1]Department of Chemistry (Organic Chemistry), College of Natural and Computational Sciences, Wachemo University, P.O. Box 667, Hossaina, Ethiopia
[2]Department of Biology (Microbiology), School of Natural and Computational Sciences, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia

Abstract

Background and Objectives: Recent statistics have shown a rise in the margin of consumption and utilization of essential oils. It has been recognized as some of the most promising compounds in the development of novel products. This study aimed to extract optimum amount of essential oil and investigation of antimicrobial activity of the oils from black cumin seeds. **Materials and Methods:** Extraction was carried out by use of steam distillation method in a Clevenger-type apparatus and the oils was established by using standard methods and the disc dilution method used for antimicrobial activity of oils and the test results were compared with standard antibiotics. **Results:** From the results obtained that, it was discovered that the cumin seeds could give the maximum yields of essential oil to be 40.4% w/w can be extracted by steam distillation at the optimum condition of temperature 96°C and time of 60 min. The seed essential oil was also tested for antimicrobial activity of the seeds oil against six pathogenic bacteria of *E. coli*, *B. cereus*, *S. aureus*, *S. typhimurium*, *L. monocytogenes* and *E. faecalis* show a maximal diameter of inhibition zone 19, 10, 12, 12,14 and 11 mm, respectively. The MIC values of these active plants seed extracts obtained in this study were lower than MBC values this suggests the seed extract were bacteriostatic at lower concentration and bactericidal at higher concentrations. **Conclusion:** Hence, the oil from cumin seed showed the highest antimicrobial activity against on all these pathogens bacteria and crude seed extracts are a mixture of different active compounds against bacterial strains. Therefore, the seed oil can be utilized for industrial and pharmacological purpose as well.

Key words: Optimization , essential oils, antimicrobial activity, clevenger distillation, black cumin seed

Citation: Demis Zelelew and Amha Gebremariam, 2018. Optimization of extraction and pharmacological activities of essential oil from black cumin (*Nigella sativa* L.) seeds using clevenger distillation. Curr. Res. Chem., 10: 1-9.

Corresponding Author: Demis Zelelew, Department of Chemistry (Organic Chemistry), College of Natural and Computational Sciences, Wachemo University, P.O. Box 667, Hossaina, Ethiopia Tel: +251-910656788

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Mankind has used many plants for medicinal purposes for thousands of years. Natural products engender from microbes, plants or animal sources and about 25% of the drugs prescribed worldwide come from plants. In many developing countries, like Ethiopia, one of valuable natural resources is medicinal plant[1,2]. The benefit of plant resources to humanity, besides basic needs, contributes to economic advantages in food industries, flavors, fragrances, health care product and so forth. For thousands of years to present day, people have been using plants as medicine due to their therapeutic values. Essential oil are aromatic oily liquids which are secondary metabolites of plants. These oils originated from aromatic plants may be found in all plant organs or leaves, fruits, peel, fruit stem or seeds[2]. There is wide area of use of essential oils in the pharmaceutical industry and cosmetic industry, in addition to chemical industry such as detergent, toothpaste, soap and aromatherapy traditionaly[3]. Herbs and spices are invaluable resources, useful in daily life as food additives, flavours, fragrances, pharmaceuticals, colors or directly in medicine. Therefore, products from herbs, especially the *Nigella sativa* L., it is known as "Tikure azimude" in Ethiopia, are the right choice in treating certain kinds of ailments or diseases without introducing side effects to our body if consumed accordingly based on scientific findings and research[4].

Black cumin seed has been employed for thousands of years as a spice, food preservative and curative remedy for numerous disorders[5,6]. *Nigella sativa* is known to have beneficial effects on a wide range of diseases, anti-asthmatic, anti-tumor, antiviral, anti-bacterial[7], anti-inflammatory, gastroprotective, anti-malaria, anti-hypertensive, anti-diabetic, anti-atherosclerotic, protective and anti-oxidant, nutritional, anti-cholesterol[7,8]. The seeds are still believed to increase heat in the body, making metabolism more efficient. As a nutritional supplement in modern times, black cumin seed is used to treat respiratory conditions. However, the significant variations in chemical composition of *N. sativa* seed essential oil have previously been described to the influence of origin of the plant[9], extraction[10] and agronomic techniques used, e.g., sowing date, irrigation regime or fertilizer type. The steam distillation method is preferred for essential oil extraction because it is cheap, flexible, versatile and does not lead to decomposition of the essential oil and has potential for commercialization due to its reliability in producing mass oil production. But it is appropriate to improve the traditional distillation method because of the energy wasting. The extraction using traditional steam distillation method cannot give the highest purity and quality of *Nigella sativa* essential oil[11,12]. In essential oil industry, steam distillation is the primary method to extract the essential oil from herbs, spices, medicinal and aromatic plants for commercial product[9-13]. However, not much research has been done to identify the optimal condition for extraction of oil. In the best knowledge of the authors, limited research study has been found to investigate the effect of distillation method parameters on essential oil yield and anti-microbial activities against pathogenic bacteria.

Thus, this study showed the effect of traditionally used medicinal plant of *Nigella sativa* seeds which contributes to scientific evidence on studied pathogenic tested bacterial. Due the development of antimicrobial resistance by the using of antibiotics among pathogenic bacteria, a natural products extracted from the plants can be used as alternative strategies to reduce pathogenic bacteria from foods and patients. The potential use of alternative antibiotics in drug-resistant bacteria from various plant extracts have been studied by many researches. The main aim of the present study is to optimize the rate of extraction by using steam distillation and determination of the antimicrobial activity of the black cumin (*Nigella sativa* L.) seeds oil against pathogenic bacteria in order to justify its industrial and medicinal properties.

MATERIALS AND METHODS

Description of the study area: Hossana, the capital city of Hadiya zone of North West Ethiopia, is 235 km South from capital city, Addis Ababa, Located at 7°33' 32" N Latitude and 37°51' 58" E Longitude, the climate of the city is subtropical, with a mean annual temperature of 17.1°C and annual average rainfall ranging from 920.4-1436.5 mm. The highest temperature is experienced between January and March and the lowest between July and September. Based on 2007 census results the regional average growth rate of 2.9%, the population of Hossana town was estimated to be 83,046 in 2013. Based on the 2007 national census conducted by the Central Statistical Agency of Ethiopia (CSA).

Experimental materials and chemicals: Chemicals of analytical grade purity and distilled water were used in the preparation of reagents. The instruments used for this work were round bottom flask, rotary evaporator, basket heater, distillation unit, thermometer, horizontal condenser, measuring cylinder, conical flask, separating funnel and Gram-positive and negative bacterial species. These micro-organisms were provided from the Department of Microbiology, Pasteur Institute, Addis Abeba, Ethiopia and standards of Tetracycline and Cefazolin antibiotic discs also was used in this study.

Sampling technique and sample collection: In Ethiopia, *Nigella sativa* occurs in all regions and agro-ecologies at different altitudinal ranges. But the markets were assessed for the suitability of proper sampling and the merchants around Hossana town are orally interviewed on the origin of their seed. Mature black cumin (*Nigella sativa* L.) seeds samples were purchased, first by selecting the shops using convenient sampling technique from local herbal shops from Hossana area. Samples of collected plant seed were prepared, packaged and stored according to the herbarium rules and regulations[14]. They were authenticated by the Botanic Department of the national biodiversity institute. The voucher specimens are deposited at the herbarium of this institute.

Preparation of black cumin seeds extract: The seeds of *N. sativa* were fine powered and steam distilled in a Clevenger apparatus at different time and temperature conditions. The essential oil was collected, dried over anhydrous sodium sulphate, stored in brown bottles and finally kept in refrigerator for further analysis. The collected sample of mature black cumin seeds is cleaned and pith is manually separated from the outer colored part of the seeds. That is because of the reason that the majority of the oil in oil sac present in them. The collected seed 30 g materials were dried completely under laboratory conditions of 23-24°C and darkness[15]. Immediately prior to the extraction process, the seeds were ground in a blender to produce a powder with grounded using mortar and pestle and allowed to pass through a 0.2 mm sieve and subjected immediately to oil extraction.

Extraction and isolation of essential oil by steam distillation: Steam distillation involves bubbling steam through the plant seed and the experiment was conducted in a modified Clevenger's Apparatus[15,16]. It consist of one round bottom flask of 1000 mL which is connected with another two way round flask which holds raw material. The top flask is connected with condenser through the connecter. The separating funnel is used for the separation of essential oil and water. About 100 g of pre-treated black cumin seed sample is taken in a distillation flask. To that 200 mL of water is added. Heat is supplied to the distillation unit by temperature controlled basket heater. At the initial stage, experiment is carried out at a temperature of 88°C for 60 min time period. The distillate is collected in a conical flask. This distillate has two layers, one dense layer and other less dense layer. This is then separated using a separating funnel. The less dense upper layer is the black cumin oil. Then the cumin seeds oil was separated from water by chloroform extraction. The distillate was poured into a 250 mL separating funnel and 2 g of sodium chloride was added in it because sodium chloride helps to minimize emulsions during the extractions by making the organic layer less soluble in the aqueous layer. The separating funnel was stoppered, shaken and vented for four or five times. About 15 mL chloroform was used to rinse the apparatus and it was allowed to drain into the separating funnel. The black cumin seeds oil was extracted from the upper organic layer by inverting the separating funnel and shaking it back and forth gently. The layers were allowed to separate before draining the lower aqueous layer. The top most layer was collected and the extraction was repeated by using another 10 mL of chloroform. The chloroform extracts were combined in a single beaker and kept in a water bath to vaporize the chloroform present in the extract. After the chloroform had evaporated completely, cumin oil remained and it was collected and stored for further analysis[17] and the extracted essential oil was used further for chemical characterization (constituents) and agar disc diffusion test and determination of Minimum Inhibitory Concentration (MIC)[18].

Preparation of test organisms and sensitivity test: Standard culture of six strains namely, *E. coli*, *B. cereus*, *S. aureus*, *S. typhimurium*, *L. monocytogenes* and *E. faecalis* were obtained from Ethiopian Paster Health Research Institute, Addis Ababa, Ethiopia. Muller Hinton agar medium was prepared and the test organism was grown at 37°C for 24 h. About 38 g of Muller Hinton Agar was dissolved in 1000 mL distilled water. Then, the solution was autoclaved under 121°C for 15 min and the standard 0.5 McFarland was prepared in saline solution.

Yield of black cumin seed essential oil yield: The yield of the oil extracted using each of the methods of extraction was calculated using basis of dried weight of the plant seed (w/w) using the formula below. Light yellow colored, apparently colorless oil, with a somewhat lemon like and pleasant odor, was obtained. The extracted oil was separated from distillate and dried over the minimum amount of anhydrous sodium sulfate to remove traces of moisture[19,20]. The percentage oil yield is expressed as follows:

$$\text{Essential oil yield (\%)} = \frac{\text{Weight of essential oil (g)}}{\text{Weight of plant sample (g)}} \times 100 \quad (1)$$

Moisture content determination: A crucible was washed and dried in the oven, after cooling in the desiccators and weighed

(W1). About 2.0 g of the sample was carefully weighed in the crucible and the weight was taken as W2. The crucible containing the sample was then placed in an oven at temperature of 105°C for 1 h. It was cooled and weighed. The crucible was then introduced into the oven again and process of cooling and weighing continued at intervals until a constant weight was obtained (W3)[20]. Percentage moisture content was calculated as:

$$\text{Moisture content (\%)} = \frac{W2 - W3}{W2 - W1} \times 100 \quad (2)$$

Analysis of antimicrobial activity of the seed oil: Antimicrobial activities from steam distilled-essential oils from black cumin seed were performed against various micro-organisms by agar disc diffusion method[21,22]. The overnight cultures were used for the antimicrobial activity of the essential oil used in this study. Susceptibility tests were performed by Bauer-Kirby[23] disc diffusion by using Mueller Hinton Agar (Merck 1.05437). The *E. coli* (ATCC 25922), *B. cereus*, *S. aureus* (ATCC 25923), *S. typhimurium* (ATCC 14028), *L. monocytogenes* (ATCC 7644) and *E. faecalis* (ATCC 29212) were used as test micro-organisms. These standard strains were inoculated into Luria-Bertani (LB) broth (Merck) and incubated for 18 h at 37°C in a shaking incubator. Then, 100 µL of each bacterial solution revived in this way were evenly spread on Muller Hinton agar plates by using sterilized cotton swabs. The plates were left at room temperature for 15-20 min to allow the agar surface to dry. Sterilized blank antibiotic susceptibility discs (0 and 6 cm in diameter) were arrayed on the plates dish using a disc dispensing system (Oxoid). Steril blank discs were impregnated with 10 µL of the oil. Plates were incubated at 37°C, for 18-24 h in an incubator. The formation of clear inhibition zone around the wells of about >7 mm diameters were taken as significant susceptibility measurement. The experimental study was laid down in completely randomized design with three replications. The mean value and standard deviation value was used for analysis. The results were expressed as susceptible/resistant according to the criteria developed by National Committee for Clinical Laboratory Standards[24] and Manual of Antimicrobial Susceptibility Testing guidelines[25]. The dishes were incubated at 37°C for 18-24 h for microbial. Then the relative anti-bacterial potency of the given preparation was calculated by comparing its zone of inhibition with that of the standard drug antibiotics of Tetracycline (10 µg/disc) and Cefazolin (10 µg/disc) antibiotic discs. After the incubation period, the colonies were enumerated and were used to find out the growth inhibition by using the following formula below. The lowest concentrations of the extracts capable of inhibiting the complete growth of the bacteria being tested were expressed as Minimum Inhibitory Concentration (MIC) values:

$$\text{Inhibition (\%)} = \frac{1 - T}{C} \times 100 \quad (3)$$

where, T is mg mL^{-1} of extract and C is mg mL^{-1} of control.

Determination of minimum inhibitory and bactericidal concentration: The minimum bactericidal concentration was performed to test the antibacterial activity of active extract by the using disc dilution method. The MIC was defined as lowest concentration able to kill any microbe. The Minimum Inhibitory Concentrations (MICs) of the essential oil are determined according to the method reported by Pinto[26] and Savadoro *et al.*[27]. Dilutions of the essential oil of *N. sativa* L. were prepared in sterile nutrient broth to get a final concentration of 16, 8, 4, 2, 1, 0.5 and 0.25 µL, respectively. To each of these dilutions, a loop full of bacterial culture adjusted to 0.5 McFarland standards was inoculated in Mueller Hinton Agar (MHA) and all the tubes were incubated at 37°C for 24 h. After incubation, loop full from each tube was inoculated onto nutrient agar plates. The antibacterial activity was evaluated by measuring the diameter of the inhibition zone formed around the disc and the diameters were interpreted according to the CSFA[28]. The plate without growth was recorded as MIC. The diameter of the inhibition zones was measured in milliliter. All tests were performed in triplicate[18-28]. Two control tubes were maintained for each test batch. These included antibiotic containing control and organism control. The lowest concentration (highest dilution) of the extract that produced no visible bacterial growth (no turbidity) when compared with the control tubes were regarded as MIC. However, the MBC was determined by sub-culturing the test dilution on to a fresh drug free solid medium and incubated further for 18-24 h. The highest dilution that yielded no signal bacterial colony on the solid medium was taken as MBC.

Statistical analysis: Experiments were carried out in triplicates. The antibacterial activity of the extract data was collected and the data were analyzed according to the analysis of variance one way ANOVA using SAS Statistical Package Program was used to check the presence of significant difference at 95% confidence. The significant differences between the group means were separated using the LSD (Least Significant Difference) test was performed to examine significant differences among inhibition zone diameters and components of essential oils.

RESULTS AND DISCUSSION

The extraction of natural products is an essential tool for evaluation of raw plant materials as well as for the quality control of natural products. In plant screening process it is essential to have simple, rapid and specific extraction procedure, which allows the quantitative determination of the analysts[29]. Volume of essential oil obtained from a particular plant material was different for different temperatures and at a particular temperature for different time of heating. Volume of essential oil obtained is less in comparison to hydrosol of the same plant material. Sahoo *et al.*[15] and Celeghini *et al.*[29] observed the increase in volume of essential oil with increase in temperature by keeping time of heating constant and vice versa. The data in Fig. 1 showed the effect of temperature on oil yield. In the next phase of experiment, the extraction of cumin oil from black cumin seed is carried out by changing the time at an interval of from 15-75 min time period, while the other parameters temperature and solid to solvent ratio kept constant. It is observed that with increase of distillation temperature the oil yield increases and it is maximum at 96°C. Further increase in distillation temperature the oil yield decreases that is because of the fact that at higher temperature charring of cumin seed takes place at the bottom of the flask.

This result provided much more yield of the essential oil from other literature percentage yield under conditions mentioned above. So it has potential for commercialization due to its reliability in producing mass oil production. It could be seen that in Fig. 2 no oil was extracted from the system within the first 80 min but after that, extraction of essential oil started and it kept increasing with as the time of extraction was also increasing. It was observed that volume of essential oil obtained was negligible before the components of the oil reach to their boiling point. After reaching to their boiling points essential oil was obtained and further it was speculated that if increase more temperature proliferation in volume can be achieved. According to the Fig. 2, the yield of the oil obtained it was observed that with increase of distillation time the oil yield increased and it was maximum at 60 min durations. Further increase in time had no effect on oil yield. The effect of distillation time on oil yield was shown in Fig. 2. This case had also indicated the dependency of extraction process on temperature as the amount of oil was found to vary as the temperature of the extraction process was varied. With this relative high percentage of oil yield of 40.4% w/w in the present study, the processing of the oil for industrial as well edible purposes would be of economic importance.

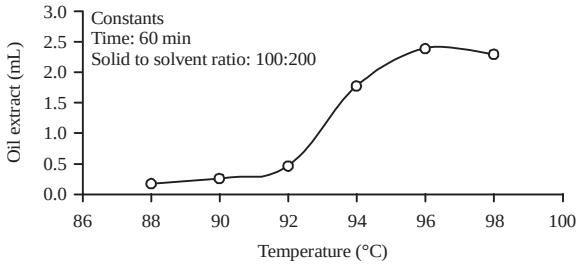

Fig. 1: Effect of temperature on oil yield

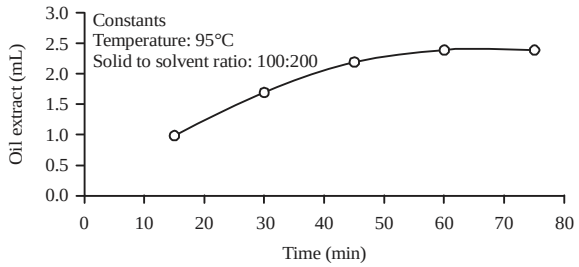

Fig. 2: Effect of time on oil yield

Actually, based on the information obtained from the literature, steam distillation extraction was, generally, dependent on the temperature and pressure of the process[30]. This can be explained by considering the fact that at low temperatures, steam goes up into the plant material slowly and its pressure build up is not sufficient enough to extract the oil out of the seed matrix. However, if left for a longer period of time, the oil will eventually break out of the seed matrix and, thus, be extracted. That is why it was noticed from the extraction process carried out that at a temperature of 95°C, there was no yield initially but after some time, the steam being formed rather more rapidly and pressure being obviously higher, the oil extraction began and it was continuous thereafter. Though the distillation procedure allowed only the separation of volatile compounds (essential oils), which to a greater or lesser extent were transformed under the influence of the elevated temperature[31]. It was palpable from the values of the oil yields obtained using the given quantity of black cumin seed that large amount of the raw material would be required for large scale extraction of the oil.

Antimicrobial investigation of the seed oil: The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including bacteria and fungi[32]. In the present study, the antibacterial activity of the essential oils of *N. sativa* L. against various

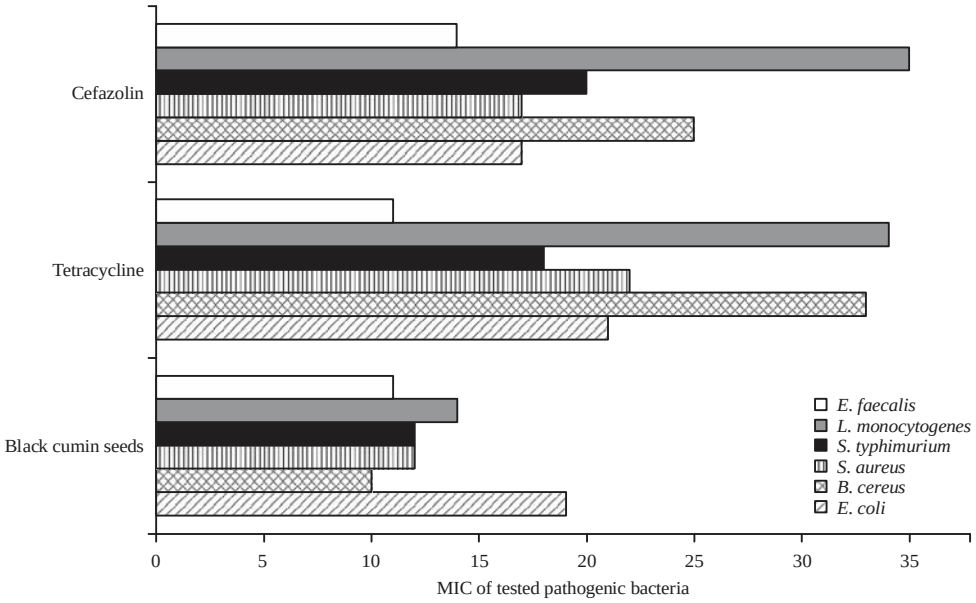

Fig. 3: Mean minimum inhibition zone (mm) of cumin seeds oil and selected standard antibiotics against tested pathogenic bacteria

Table 1: Antibacterial activity of black cumin seeds essential oils and standard antibiotics against different six bacteria

	E. coli	*B. cereus*	*S. aureus*	*S. typhimurium*	*L. monocytogenes*	*E. faecalis*	LSD
Black cumin seeds	19.0±0.1^{Ba}	10.00±0.4^{Efd}	12.00±0.44^{Dc}	12.00±0.3^{Cc}	14.00±0.7^{Cb}	11.00±1.3^{Bcd}	1.85***
Tetracycline	21.0±0.3^{Ab}	33.00±0.7^{Aa}	22.00±0.1^{Ab}	18.00±0.9^{Bc}	34.00±0.22^{Aa}	11.00±0.5^{Bd}	1.34***
Cefazolin	17.0±0.3^{Cd}	25.00±0.7^{Bb}	17.00±0.12^{Bd}	20.00±0.8^{Ac}	35.00±0.21^{Aa}	14.00±0.54^{Ae}	1.81***
LSD	1.15±1.0***	1.35±0.34**	1.14±0.23***	1.17±0.8***	1.67±0.13***	1.09±0.77**	

Lowercase letters: Comparison between essential oils, Uppercase letters: Comparison between bacteria, *p<0.05, **p<0.01, ***p<0.001

Gram-positive and Gram-negative bacteria has been investigated. For the evaluation of the essential oils of *N. sativa* L., the disc diffusion method, the determination of MIC and the bacterial growth in the presence and the absence of the essential oils have been studied. All the essential oils of the black cumin seeds, according to the disc diffusion test results were presented in Table 1 and Fig. 3. According to the results of the study, black cumin seeds oil against the tested bacteria (Gram-positive and negative) showed strong antimicrobial activity on all strains. It has showed the highest activity especially on *E. coli* (19 mm). It was found that this activity was 11.76% higher compared to that of Cefazolin (17 mm). It showed a lower activity against the *B. cereus* with the diameter of inhibition of 10 mm. Other oils have demonstrated antimicrobial effects of different levels. The higher resistance of Gram-negative bacteria to external agents has been earlier documented and it is attributed to the presence of lipopolysaccharides in their outer membranes, which make them inherently resistant to antibiotics, detergents and hydrophilic dyes. Results of the present study are consistent with the literature data reported by Alhaj *et al.*[33]. The obtained results on the antibacterial activity of the essential oils of *N. sativa* L. has manifested clear inhibition zone of at least 10 mm and above for all the tested strains. The essential oils had inhibitory effects on the six bacteria tested and cumin essential oils were most effective against *E. coli.* The obtained results indicated that the essential oil of N*. sativa* L. possesses a wide inhibition activity spectrum on pathogenic bacteria for humans.

In the present study, the antibacterial activity of the essential oils of *N. sativa* L. against various Gram-positive and Gram-negative bacteria has been investigated. which is in conformity with earlier studies. A number of compounds derived from plants often show considerable activity against Gram (+) bacteria but not against Gram (-) species[33,34]. For many years, many researchers have investigated the antimicrobial properties of essential oils derived as by-products of the cumin seed against bacteria. According to present study black cumin seeds oil had proven to have significant antimicrobial properties. This oil was very effective against both *L. monocytogenes* and *E. coli.* All essential oils had inhibitory effects on the 6 bacteria tested and

Table 2: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of black cumin seeds oil extract against selected microbial strains

Test microbial strains	Gram type	MIC (mg mL^{-1})	MBC (mg mL^{-1})
E. coli (ATCC 25922)	-	5.5	7.1
B. cereus (ATCC 14579)	+	7.1	7.9
S. aureus (ATCC 25923)	+	6.0	7.5
S. typhimurium (ATCC 14028)	-	5.8	8.1
L. monocytogenes (ATCC 7644)	-	10.5	12.0
E. faecalis (ATCC 29212)	-	7.7	8.9

cumin essential oils were most effective against *E. coli* and *S. typhimurium*. This antibacterial activity may be indicative the presence of metabolic toxins or broad spectrum antibacterial compounds. This was in agreement with previous reports by the several researchers. Furthermore; Olila *et al.*[35] has reported that the essential oil of *N. sativa* showed high inhibitory activity against a range of bacteria resistant to antibiotics.

In the study conducted, it was observed that the amount of black cumin seeds oil was directly proportional to the antimicrobial effect. It was as effective as cefazolin and Tetracycline on some micro-organisms. It has been found that most of the cumin essential oils used here have antimicrobial activity on micro-organisms. This study demonstrated that *Nigella* seed oil has more potent antibacterial activity compared to commonly antibiotic ampicillin. Hence, there is potential for the use of *Nigella* seed oil to combat drug resistant strains of bacteria thereby having medicinal value. Parekh and Chanda[36] reported different antibacterial action, antifungal and inflammatory properties of medicinal plants based on various parameters to ensure their activity and efficacy. Among these properties of medicinal plants, some of them have facilitated in isolation and characterization of the active compounds for the development of drugs for therapeutics. According to Arya *et al.*[37], human mortality rate was mainly due to infections caused by *S. aureus*, *P. aeruginosa*, *K. pneumonia*, *E. coli* and *S. sonnie*. Also, treatment of infections caused by these bacterial strains is challenging. Hence, the challenge to discover newer and effective drugs is increasing. Vasek *et al.*[38] examined the effectiveness of black cumin seeds essential oil to inhibit the growth of wild food-borne spoilage and pathogenic bacterial strains. They have observed that essential oil of seed had antibacterial activities on 100.00% of Gram-negative and 40.74% of Gram-positive. In addition, all strains of *E. coli* were sensitive to this essential oil. This result was somewhat consistent with current study. Due to the development of antimicrobial resistance by using of antibiotics among pathogenic bacteria, a natural products extracted from the plants seeds can be used as alternative strategies to reduce pathogenic bacteria from foods and patients[39-42]. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values obtained for extracts against the bacterial strains varied from plant extract to the other. The MIC and MBC test was conducted for oil extract that is used in this study, i.e., *E. coli* in 5.5 and 7.1*, B. cereus* in 7.1 and 7.9*, S. aureus* in 6.0 and 7.5*, S. typhimurium* in 5.8 and 8.1*, L. monocytogenes* in 10.5 and 12.0 and *E. faecalis* in 7.7 and 8.9 mg mL^{-1}, respectively as shown in Table 2.

The MIC value of oil extracts range between 5.5 and 10.5 mg mL^{-1} on *E. coli* and *L. monocytogenes*. Generally, it was suggested the crude plant seed extracts are a mixture of different active compounds against bacterial strains. The MIC values of these active plants extracts obtained in this study were lower than MBC values suggesting that plant extract were bacteriostatic at lower concentration and bactericidal at higher concentrations. These observations may be attributed may be by the nature of biologically active components (Alkaloids, anthraquinone, saponins and tannins) which could be enhanced in optimal operating parameters. It has been documented that these components are well known for antimicrobial activity[41,42].

CONCLUSION AND RECOMMENDATIONS

The optimal extraction of the essential oil from black cumin seed have shown that the maximum yield obtained in this work were 40.4% w/w can be extracted by steam distillation at the optimum condition of temperature of 96°C and time of 60 min. The antibacterial activity of the essential oils of *N. sativa* has been investigated against pathogens bacteria using a disc-diffusion method show a maximal diameter of inhibition zone of 19, 10, 12, 12, 14 and 11 mm, respectively. The MIC values of these active plants seed extracts obtained in this study were lower than MBC values this suggests the seed extract were bacteriostatic at lower concentration and bactericidal at higher concentrations. Thus, the oil from cumin seed showed the highest anti-microbial activity against on all these pathogens bacteria. Therefore, the plant seed oil can be utilized for industrial and pharmaceutical purpose as well.

SIGNIFICANCE STATEMENT

This study discovered the maximum extraction of oil using clevenger distillation unit was used to get the essential oil. To obtain a series of high quality extraction from *Nigella sativa* seed, the factors that influence the rate of extraction was study to get high yield of essential oil. So, it is

appropriate to improve the traditional distillation method because of the energy wasting. Due to extraction using traditional steam distillation method cannot give the highest purity and quality of *Nigella sativa* essential oil. Nowadays, in Ethiopia, essential oil is gaining popularity as an herbal medication as it gave a lot of benefit to overcome the disease. This study will help the researcher to uncover the critical areas of traditionally used medicinal plant of *Nigella sativa* seeds which contributes to scientific evidence on studied pathogenic tested bacterial. Based on the knowledge on the constituent of our local *Nigella sativa* and the potential commercial value essential oil as a whole, it is appropriate for further the research into producing and commercialize a new product from these invaluable herbs.

ACKNOWLEDGMENT

This work was supported financial by grants of Wachemo University. Research and community service office that allowed ours to undertake the research work. We also acknowledge Grace Agri food laboratory service PLC for allowed ours to use laboratory for partial test free of charge and also technicians for their expert technical advice. There has been no significance financial support for this work that could have influenced its outcome.

REFERENCES

1. Ozguven, M. and N. Sekeroglu, 2007. Agricultural practices for high yield and quality of black cumin (*Nigella sativa* L.) cultivated in Turkey. Acta Horticult., 756: 329-337.
2. Bakkali, F., S. Averbeck, D. Averbeck and M. Idaomar, 2008. Biological effects of essential oils-A review. Food Chem. Toxicol., 46: 446-475.
3. Raeissi, S. and C.J. Peters, 2005. Liquid-vapor and liquid-liquid-vapor equilibria in the ternary system ethane+ limonene+ linalool. J. Supercritical Fluids, 33: 201-208.
4. Ali, B.H. and G. Blunden, 2003. Pharmacological and toxicological properties of *Nigella sativa*. Phytother. Res., 17: 299-305.
5. Toncer, O. and S. Kizil, 2004. Effect of seed rate on agronomic and technologic characters of *Nigella sativa* L. Int. J. Agric. Biol., 6: 529-532.
6. Hosseinzadeh, H., B.S. Fazly Bazzaz and M.M. Haghi, 2007. Antibacterial activity of total extracts and essential oil of *Nigella sativa* L. seeds in mice. Pharmacologyonline, 2: 429-435.
7. Kamal, A., J.M. Arif and I.Z. Ahmad, 2010. Potential of *Nigella sativa* L. seed during different phases of germination on inhibition of bacterial growth. J. Biotech. Pharm. Res., 1: 9-13.
8. Meddah, B., R. Ducroc, M. El-Abbes Faouzi, B. Eto and L. Mahraoui *et al*., 2009. *Nigella sativa* inhibits intestinal glucose absorption and improves glucose tolerance in rats. J. Ethnopharmacol., 121: 419-424.
9. Nickavar, B., F. Mojab, K. Javidnia and M.A.R. Amoli, 2003. Chemical composition of the fixed and volatile oils of *Nigella sativa* L. from Iran. Zeitschrift Naturforschung C, 58: 629-631.
10. Stoyanova, A., E. Georgiev, A. Wajs and D. Kalemba, 2003. A comparative investigation on the composition of the volatiles from seeds of *Nigella sativa* L. from Bulgaria. J. Essent. Oil Bear. Plants, 6: 207-209.
11. Milojevic, S.Z., T.D. Stojanovic, R. Palic, M.L. Lazic and V.B. Veljkovic, 2008. Kinetics of distillation of essential oil from comminuted ripe juniper (*Juniperus communis* L.) berries. Biochem. Eng. J., 39: 547-553.
12. Amenaghawon, N.A., K.E. Okhueleigbe, S.E. Ogbeide and C.O. Okieimen, 2014. Modelling the kinetics of steam distillation of essential oils from lemon grass (*Cymbopogon* Spp.). Int. J. Applied Sci. Eng., 12: 107-115.
13. Hunter, M., 2009. Essential Oils: Art, Agriculture, Science, Industry and Entrepreneurship: A Focus on the Asia-Pacific Region (Agriculture Issues and Policies). Nova Science Publishers, New York, ISBN: 9781607418658, Pages: 780.
14. Cochran, W.G., 1977. Sampling Techniques. 3rd Edn., Willey Eastern Ltd., New Delhi, India, ISBN: 0852261217, Pages: 428.
15. Kumar, K.S., 2010. Extraction of essential oil using steam distillation. BTech Thesis, Department of Chemical Engineering, National Institute of Technology, Rourkela.
16. Kabuba, J. and R. Huberts, 2009. Steam extraction of essential oils: investigation of process parameters. Can. J. Chem. Eng., 87: 915-920.
17. Mukhtar, H., M.K. Shabbiri, R. Nadeemi and W. Mumtazi, 2012. Physicochemical analysis and determination of various chemicals constituents of essential oils in Rosa cent foli. University of Agriculture, Faisalabad, Pakistan.
18. Geraci, A., V. Di Stefano, E. di Martino, D. Schillaci and R. Schicchi, 2016. Essential oil components of *Nigella sativa* seed and antimicrobial activity. Natural Product Research, pp: 1-7.
19. Manzoor, M., F. Anwar, T. Iqbal and M.I. Bhanger, 2007. Physico chemical characterization of *Moringa concanensis* seeds and seed oil. J. Am. Oil Chem. Soc., 84: 413-419.
20. Sikdar, D.C., R. Menon, K. Duseja, P. Kumar and P. Swami, 2016. Extraction of citrus oil from orange (*Citrus sinensis*) peels by steam distillation and its characterizations. Int. J. Technical Res. Applic., 4: 341-346.
21. Coyle, M.B., 2005. Manual of Antimicrobial Susceptibility Testing. Vol. 25, American Society for Microbiology Press, Washington, DC., pp: 39.

22. Ottaviani, D., I. Bacchiocchi, L. Masini, F. Leoni, A. Carraturo, M. Giammarioli and G. Sbaraglia, 2001. Antimicrobial susceptibility of potentially pathogenic halophilic vibrios isolated from seafood. Int. J. Antimicrobial Agents, 18: 135-140.
23. Bauer, A.W., W.M. Kirby, J.C. Sherris and M. Turck, 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol., 45: 493-496.
24. NCCLS., 2002. Performance standards for antimicrobial susceptibility testing: Twelfth informational supplement. NCCLS Document M100-S12, National Committee of Clinical Laboratory Standards (NCCLS), Wayne, PA., USA.
25. Cheesbrough, M., 2006. District Laboratory Practice in Tropical Countries. Cambridge University Press, UK., Pages: 434.
26. Pinto, E., C. Pina-Vaz, L. Salgueiro, M.J. Goncalves and S. Costa-de-Oliveira *et al*., 2006. Antifungal activity of the essential oil of *Thymus pulegioides* on *Candida*, *Aspergillus* and dermatophyte species. J. Med. Microbiol., 55: 1367-1373.
27. Savadogo, A., C.A. Ouattara, I.H. Bassole and A.S. Traore, 2004. Antimicrobial activities of lactic acid bacteria strains isolated from Burkina Faso fermented milk. Pak. J. Nutr., 3: 174-179.
28. Cavallo, J.D., H.C. Chardon, C. Chidiac and P. Choutet, 2006. Comite de l'antibiogramme de la societe francai sedemicrobiologie. Communique.
29. Celeghini, R.M.S., A.P. Silva, I.M.O. Sousa and M.A. Foglio, 2006. Evaluation of *Artemisia annua* L. clean-up methods for artemisinin quantification by HPLC. Rev. Bras Plant Med., 8: 119-122.
30. Kabuba, J.T., 2009. Steam extraction of essential oil: Investigation of process parameters. Master's Thesis, University of Johannesburg, South Africa.
31. Zekovic, Z.P., Z.D. Lepojevic and I.O. Mujic, 2009. Laurel extracts obtained by steam distillation, supercritical fluid and solvent extraction. J. Nat. Prod., 2: 104-109.
32. Erdogrul, O.T., 2002. Antibacterial activities of some plant extracts used in folk medicine. Pharm. Biol., 40: 269-273.
33. Alhaj, N.A., M.N. Shamsudin, H.F. Zamri and R. Abdullah, 2008. Extraction of essential oil from *Nigella sativa* Using supercritical carbon dioxide: Study of antibacterial activity. Am. J. Pharmacol. Toxicol., 3: 225-228.
34. Tegos, G., F.R. Stermitz, O. Lomovskaya and K. Lewis, 2002. Multidrug pump inhibitors uncover remarkable activity of plant antimicrobials. Antimicrob. Agents Chemother., 46: 3133-3141.
35. Olila, D., Olwa-Odyek and J. Opuda-Asibo, 2001. Antibacterial and antifungal activities of extracts of *Zanthoxylum chalybeum* and *Warburgia ugandensis*, Ugandan medicinal plants. Afr. Health Sci., 1: 66-72.
36. Parekh, J. and S. Chanda, 2007. Antibacterial and phytochemical studies on twelve species of Indian medicinal plants. Afr. J. Biomed. Res., 10: 175-181.
37. Arya, M., P.K. Arya, D. Biswas and R. Prasad, 2005. Antimicrobial susceptibility pattern of bacterial isolates from post-operative wound infections. Indian J. Pathol. Microbiol., 48: 266-269.
38. Vasek, O.M., L.M. Caceres, E.R. Chamorro and G.A. Velasco, 2015. Antibacterial activity of *Citrus paradisi* essential oil. J. Nat. Prod., 8: 16-26.
39. Okwemba, R.I., P.K. Tarus, A.K. Machocho, A.W. Wanyonyi and I.M. Waweru *et al*., 2018. Isolation, characterization and biological activity of organic extractives from *Calodendrum capense* (Lf) Thunb. (Rutaceae). J. Med. Plants Res., 12: 139-163.
40. Barkatullah, I.M., A. Rauf and K. Inyat-Ur-Rahman, 2012. Physicochemical characterization of essential and fixed oils of *Skimmia laureola* and *Zanthoxylum armatum*. Middle-East J. Med. Plants Res., 1: 51-58.
41. Burits, M. and F. Bucar, 2000. Antioxidant activity of *Nigella sativa* essential oil. Phytother. Res., 14: 323-328.
42. Singh, G., P. Marimuthu, C.S. de Heluani and C. Catalan, 2005. Chemical constituents and antimicrobial and antioxidant potentials of essential oil and acetone extract of *Nigella sativa* seeds. J. Sci. Food Agric., 85: 2297-2306.