

# Optimization and Characterization of Essential Oil from Black Pepper (*Nigrum*) Seeds Using the Distillation Extraction Method for Perfume Additives

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**Abstract:** The black pepper seed oils (*Piper nigrum*) were highly concentrated substances extracted from seeds. The study aims to utilize the seed as a source of oil for cosmetics and perfume applications since the *Piper nigrum* seed is inexpensive and grows abundantly in our country. It is an annual herbaceous plant. The characteristics of the seed are; that the color is black after drying and the particle size for my experiment was 500, 750, and 1000 micrometers. The conditions for extracting the seed were 1 m and 30-250 minutes. The seed produced a colorless, clear aromatic essential oil, which was used in the production of several cosmetics and perfumeries. The seeds were extracted by steam distillation because they had high conversion efficiency and necessarily for oil because of perfume properties addition. The distillation was carried out under controlled temperature and pressure. The optimum extraction temperature was 105°C, 127.5°C, 150°C. The oil yield was 4.83% under the optimal conditions and the minimum yield was 1.67%. The characteristic of the *Piper nigrum* seed was a strong pleasant aroma. These oils were often used for their cosmetics and their therapeutic or odoriferous properties, in a wide selection of products such as foods and medicines. Cosmetics oil is one of the most time- and effort-consuming processes. The cosmetic composition mainly includes pigment fatty acids, oleic acid 22.94%, hexanoic acid, and octanoic acid 8.61%. The antibacterial properties of the oils were 60% higher efficiency. The acid value of the oil was 5.3 by using NaOH titration with ethanol solution. The aroma odor was changed into a good odor by the addition of Lemmon oil. Cosmetic products were homogeneous and stable during application.

**Keywords:** *Piper nigrum* Seed, Composition, Temperature, Oleic Acid, Yield

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## 1. Introduction

Essential oils (EOs) of plants have been extensively used in food, cosmetics, healthcare, and agriculture industries. Essential oils are hydrophobic liquids containing volatile aromatic compounds and are usually extracted from plant tissues by different methods such as steam distillation, soxhlet extraction, and hydro-distillation. [17]

A variety of plants have a high content of feasible oils. These plants are local crop plants that require little maintenance and growth in almost all parts of our country. [18]

Most plant extractive Essential oils are several bioactive constituents such as phenolics, flavonoids, terpenes, and sterols. These constituents have been evaluated for their

biological and antibacterial effects [12]. *Piper nigrum* L. is an aromatic plant that belongs to the Piperaceae family. This plant is currently cultivated in tropical regions. [9]. Ethiopia is a potential country resourceful for essential oils in the production of cosmetics. "Essential oil" or "etheric oil" means a volatile oil and is obtained from plant and animal bodies by different methods, from the essential oil which is got from spices, such as; pepper oil, cinnamon oil, lemon leaves, clove oil, fennel, black pepper, orange, ginger, etc. Pepper seed oil is commonly used in medicine, pharmacy, condiments, cosmetics, and perfumes [2]. Black pepper oils are volatile, fragrant, and pleasant-tasting oils obtained from the seeds. It has great applications in pharmaceuticals, food, perfumery, and cosmetics [4].

Essential oil from black pepper constitutes approximately 1.2 to 6.7% of the yield of essential oils. The volatile oil constituent's piper amide and nerolidol exhibited insecticidal activities. Black pepper is rich in minerals, vitamins, and nutrients [13]. The chemical composition of 100 g of black pepper seeds includes carbohydrate 66.5 g, Protein 10 g, and fat 10.2 g, as well as a relatively high concentration of minerals such as calcium (400 mg), magnesium (235.8-249.8 mg), potassium (1200 mg), phosphorus (160 mg), and the lower concentration of sodium, iron, and zinc. [7]. these minerals are essential elements for the day-to-day activities of humans. In addition, black pepper also has a significant concentration of vitamins such as Vitamins C, B1, B2, and B3 [8]. Nigerian black pepper had a tannin concentration ranging from 2.11 to 2.80 mg /100 g [1].

Black pepper is composed of 37.4% carbohydrate, 25.5% proteins, 23.6% fibers, 4.7% moisture, and 5.3% fat, as well as minerals, including 0.66%, potassium (K), 0.20% calcium (Ca), 0.16% phosphorus and 0.16% magnesium (Mg) [3].

The main volatile flavor compounds in black pepper are terpenes, and black pepper oils contain nitrogen-containing compounds [5]. Essential oils extracted from the plant by acting as antifungal, antibacterial, insecticidal, or antiviral components and are also thought to promote the dispersion of seeds and pollen by attracting insects [19]. Essential oils are composed of terpenes and aromatic polypropanoid compounds derived from the acetate-mevalonic acid pathway, respectively. However, the essential oil composition can vary with the stage of development of the plant [14].

The cosmetic composition mainly includes pigments, fatty binders, and fillers. Perfume products must be homogeneous and stable during application [10]. Pepper seed is an excellent source of essential cosmetic oil. It grows abundantly in our country. The physical properties of the seed, like those of other grains and seeds, are essential for the application of perfume for clothing, skincare, and processing or determining the behavior of the product [6].

Black pepper oil is also used in perfume. However, extracting the black pepper oil requires an appropriate method due to the rigid structure of the black pepper seed [18].

Black pepper essential oils contain aromatic polypropanoid compounds and terpenes, which are obtained from the shikimic acid and acetate-mevalonic acid pathways. (Black pepper), belonging to the Piper Araceae family, which is cultivated mainly in tropical and subtropical countries [15].

Black pepper is commonly used in different fields such as agronomics, pharmaceuticals, food, and cosmetics [8]. Black peppers have a wide variety of compounds like antioxidant, antibacterial, antifungal, anti-inflammatory, and antitumor properties and insecticidal agents [20]. The extraction process of black pepper oil has increased attention, due to the wealth of beneficial components of essential oil composition. There are different techniques to extract the oil from black pepper including solvent extraction, hydrodistillation, steam distillation, and so on. Hydrodistillation is the primary method for obtaining essential oils [19]. Hydrodistillation is available in larger-scale production for economic reasons [11]. For the hydro distillation process, the distillation column and construction materials are the main sensitive to extract from such raw materials. The optimal conditions of the hydrodistillation method (material size, sodium chloride concentration, soak time concentration, extraction time, ratio of material to water, air flow rate) and chemical composition of essential oils collected from seeds of black pepper (*Piper nigrum* L.). These raw materials and fruits are grown in Ethiopia. [16].The extract of essential oils of pepper is widely used in our country for condiments, food flavors, and cosmetics. After the extraction process, the chemical constituent extracts were characterized by GC-MS. [12].

## 2. Materials and Methods

### 2.1. Materials and Equipment

#### 2.1.1. Materials

The main raw materials used during the experimental work were Nigrum seeds, filter paper, aluminum foil, Na<sub>2</sub>SO<sub>4</sub>, NaOH, NaCl (Mg / KOH), Lemmon oil, Gram-positive bacterial cells, ethyl acetate, hexane, alcohol (ethanol and methanol), mineral oil, ethanol, 150-mL of nutrient agar, the other chemicals used in my experimental work at my university.

#### 2.1.2. Equipment

For my experimental work, I used equipment, storage, and separator tanks, distillation units, cutters, beakers (3), 250-ml beakers (3), beaker, tongs, volumetric flask, decanters, weight machine, GC\_MS, FTIR, condenser, measuring cylinders, burette, stirring rod, Petri dish, thermometer, 110°C.



**Figure 1.** Raw materials collected and oil extracted from black pepper seeds (Ethiopian Biodiversity Institute & Addis Ababa Institute of Technology, <http://eid.aau.edu.et/>, [www.ebi.gov.et](http://www.ebi.gov.et), 2018. G.C).

## 2.2. Raw Material Collection and Preparation

Raw black pepper (*Nigrum*) seeds collected from Shewarobit far from 90 km, Figure 1; shows that the raw materials collected from the Shewa North zone (Debre Berhan) shewarobit and Kemisse in the semitropical zone of the Amhara region based on information from the Ethiopian Biodiversity Institute. The lab works were also performed at Addis Ababa University, the leather industry development institute, and Debrebrhan University. Due to the reason of assessability of the equipment, and potential of the seed was highly planted.

## 2.3. Proximate Analysis of *Nigrum* Seeds and Leaves with Integrated Ethanol as a Solvent

### 2.3.1. Moisture Content

The seeds and the moisture content left were done by using an oven at a temperature of 105°C for one day for better removal of moisture. The raw seed was pretreated before being extracted with ethanol by extractive distillation. Ethanol to distilled water, solvent ratio 3:1.

### 2.3.2. Ash Content

The weight of the crushed *Nigrum* seed is known before it is inserted into the furnace to determine the ash content.

## 2.4. Optimization of the Extraction Process with Ethanol Solvent

The solvent of the extracted oil was used at a temperature of 127.5°C, a particle size of 750 micro mm, and a time of 4:30 h to extract a high oil yield. From Table 1; when the time factor was increased, the maximum amount of yield was recorded. The same temperature of the extractive distillation was 127.5°C, at this time the maximum yield or amount of oil in ml was extracted at this particle size. The ethanol was selected with its ratio of for seed to ethanol 1:5 and for leaves. 1:6 and 1:7. The ethanol extractive method was good for optimization. The solvent ratio of ethanol to distilled water 5:1 is good for extraction [6].

In extractive distillation, The ethanol solvents were used for the extraction of the seed and leaves in a ratio of 1:3, 1:5, and 1:6. The amount of yield is to be maximum with ethanol extraction, the experimental setup was settled with simple extractive distillation in the laboratory setup.

## 2.5. Statistical Analysis of the Experimental Data

The ethanol solvent was diluted with distilled water from 97% pure ethanol to 75%, indicating that the career of the distillation unit is so not suitable for extraction due to the formation of a zoetrope.

The result of oil was obtained using Mat Lab formulation and data investigated.

Oil amount =  $(x)^{0.5} + [0.49 * \text{water/raw seed materials}]$ .

The residue of seed =  $[(x)^{0.5} - 0.5 * x]$ .

Where x is the ethanol or solvent that has 75% alcohol

content with diluted distilled water. The remaining amount 25% solvent is distilled water. Optimizations of oil using this extraction method were performed to save time and other parameters with this solvent. Perfume Oil Characterization.

The *Nigrum* oils were characterized by FTIR, GC\_MS, and acid values. The physicochemical properties of the oil analysis by GC-MS and the acid value of NaOH were important indices of physicochemical properties, and the antibacterial properties were also evaluated.

### 2.5.1. Characterization of Physicochemical Properties

The color of the oil that was clear, and colorless indicates the quality of the oil. And the method of extraction was selectable. Indicated that FTIR spectra were stretching unsaturated fatty acids, aromatics, and amines. From the GC\_MS result, oleic acid, 9, 12-octadecadienoic acid [Z, Z], octadecanoic acid, 9-octadecenoic acid, (E) -cis-13-octadecenoic, n - hyperadecanoic acid.

### 2.5.2. Antibacterial Properties of Oil

Microorganisms were obtained from the Department of Microbiology of the Ethiopian Biodiversity Institute. Figure 2 indicates that the bacterial cultured cell was propagated in a Petri dish with nutrient agar for microorganism growth with an optimal temperature of 37 degrees centigrade. The staphylococcus aureus bacteria have been selected. the essential oil products characterized by the Staphylococcus bacteria. The indifferent sample was collected for skincare and perfumes. The bacteria colonies were countered as shown in Figure 2.



**Figure 2.** Microorganisms (*Staphylococcus aureus* and *Bacillus subtilis*, from Ethiopian biodiversity institute, <http://etd.aau.edu.et/www.ebi.gov.et2018G.C>).

### 2.5.3. Acid Value

After all these things were done, the acidity of the oil was a one-factor effect for the perfume additives, Table 4; indicated that I checked the acidity of the essential oil of black pepper. For the titration procedure, Table 4; shows the result and the amount of oil added, it took 10gm or 16ml of *nigrum* oil mixed accurately, weighed by using 60°C in 16 ml mixture of equal volume alcohol

$$\text{Acid value} = V * N * 5.61 / W.$$

## 3. Results and Discussion

### 3.1. Proximate Analysis of the Seeds and Leaves of Black Pepper (*Nigrum*)

At a temperature of 105°C, the seeds of *Nigrum* were

dried. At this temperature, Nigrum was dried. The moisture content was as follows:  $((4\text{kg}-3.86\text{kg})/4\text{kg}) \times 100\% = 3.5\%$ , the moisture content of the leaves was as follows:  $((3.5\text{kg}-3.246\text{kg})/3.5\text{kg}) \times 100\% = 7.257\%$ . It would be prepared from samples of the extracted oil. Seeds are seasonally from November to December.

Otherwise, the raw seeds were not fully cropped.

### 3.2. Black Pepper Oil Extraction Process

The maximum amount of oil for this particle for the first

run by using 105 oc the result was 10.72ml from 250gm samples until the time run up to 4:30hr. In general, for all sizes, the separation of oil from some nonvolatile and water was done by density separation method using a decanter. The optimum oil was extracted from the size of 750 $\mu\text{m}$  based on the quantity and yield. The temperature selection should be between 100-200°C from the essential oil standard temperature. The size is also from 0.5mm up to 2.5mm from the standard of the oil particle size to get the best size for essential oil.

**Table 1.** Design expert analysis of the yield of the extracted oil from laboratory results using design expert (Department of Chemical Engineering, Addis Ababa Institute of Technology, 2018 G.C).

Code terms of design expert				
	Code terms	Particle size	Temperature [°C]	time[hr.]
Low code	-1	500micro.m	105	3:30
Center code	0	750micro.m	127.5	4:00
High code	1	1000micro.m	150	4:30

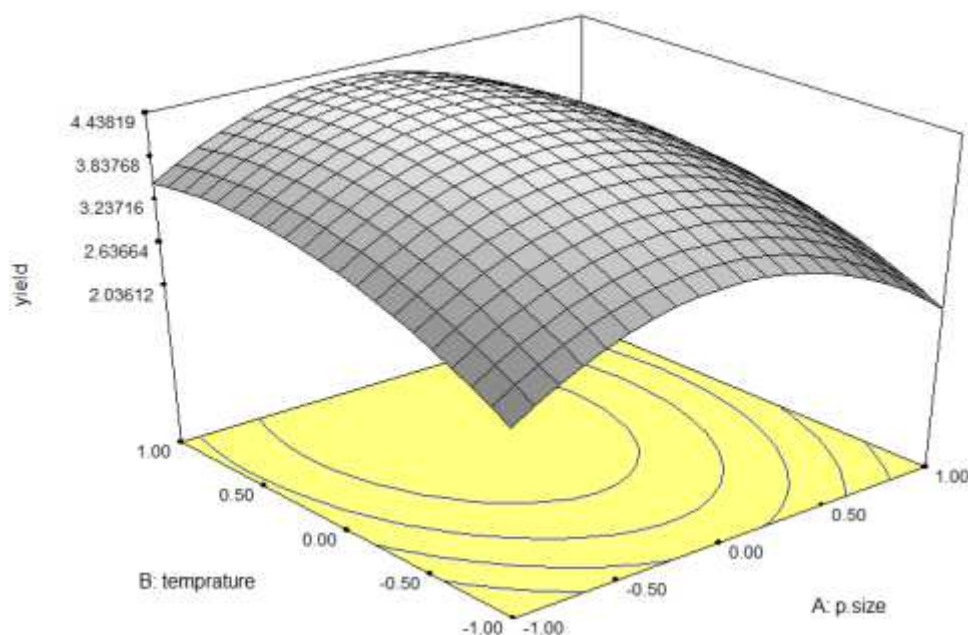
Std	Run with no	Factor A. P. size,(mm)	factor B. temp.(°C)	factor, C. time (hr.)	Yield (%)
1	1	-1.00	-1.00	-1.00	1.67
2	25	0.00	-1.00	-1.00	2.407
3	29	1.00	-1.00	-1.00	1.8
4	14	-1.00	0.00	-1.00	3.21
5	17	0.00	0.00	-1.00	3.716
6	27	1.00	0.00	-1.00	2.15
7	26	-1.00	1.00	-1.00	3.17
8	8	0.00	1.00	-1.00	3.7
9	13	1.00	1.00	-1.00	2.5
10	12	-1.00	-1.00	0.00	2.28
11	16	0.00	-1.00	0.00	2.8
12	30	1.00	-1.00	0.00	2.27
13	20	-1.00	0.00	0.00	3.25
14	6	0.00	0.00	0.00	4.7
15	4	1.00	0.00	0.00	2.33
16	7	-1.00	1.00	0.00	3.36
17	9	0.00	1.00	0.00	4.09
18	2	1.00	1.00	0.00	3.07
19	19	-1.00	-1.00	1.00	2.7
20	32	0.00	-1.00	1.00	3.35
21	18	1.00	-1.00	1.00	2.45
22	28	-1.00	0.00	1.00	3.3
23	22	0.00	0.00	1.00	4.83
24	31	1.00	0.00	1.00	2.6
25	3	-1.00	1.00	1.00	3.48
26	24	0.00	1.00	1.00	4.15
27	11	1.00	1.00	1.00	3.15
28	5	0.00	0.00	0.00	4.67
29	15	0.00	0.00	0.00	4.3
30	10	0.00	0.00	0.00	4.45
31	23	0.00	0.00	0.00	4.27
32	21	0.00	0.00	0.00	4.56

### 3.3. Statistical Analysis of Factors for the Extraction of Black Pepper Oil

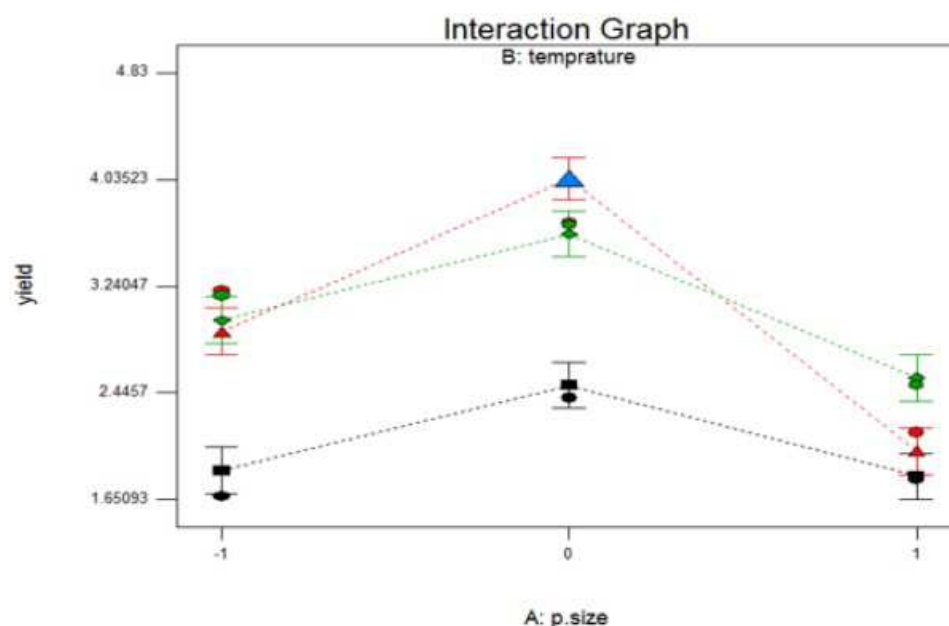
The maximum yield of oil was performed in the lab work with an efficient amount of factor effect distillation process. From the above table 1, the yield of essential oil was extracted resulting from different factor integration such as temperature. Contact time, and particle size of the black

pepper seed. Figure 3 indicates that the optimum design points were observed including temperature, particle size, and time on the counter curve, so the optimum point for the graph yield content was 4.7226 with a particle size of 750 micros.m at a temperature of 127.5°C. From Figure 3, the product was the maximum yield, so the amount of oil in the graph of the optimum variables was as shown below in Figure 3.





**Figure 3.** 3D surface graph with the interaction of the three factors. (Design expert from Addis Ababa, Ethiopia) University institute of technology, department of chemical engineering, <http://etd.aau.edu.et/> 2018 G.c).



**Figure 4.** Interaction graph of temperature, particle size, and time on the yield of oil.

From the above Figure 4, the three-factor plot time for the given extraction result highly affected the yield with the particle size of 750microm, at 4:30h, the high amount of yield extracted was about 4.83%. In Figure 4, the particle size of the *Nigrum* seed also affected the extracted oil, the optimum point of the size was 750microm and the yield also performed well about 4.04% under the time of 3:30h. It also the maximum temperature of the distillation at the optimum point of particle size and time, the *Nigrum* seed oil was highly extracted, and the amount of yield was also high. At the temperature of 127.5°C and the time of 4:30 h and p.size 750, the oil yield was 4.5%.

### 3.4. Comparison of Black Pepper Seed Oil with Its Leaf Oil

**Table 2.** To leave the oil design expert using box- Hi-cken method (Addis Ababa institute of technology, <http://etd.aau.edu.et/> 2018, G.C).

Std.	Run	temperature	Particle size	Time	Yield
1	16	105	500	1.00	2.3
2	4	150	500	1.00	3.46
3	13	105	1000	1.00	2
4	9	150	1000	1.00	2.25
5	8	105	750	0.00	2.1
6	17	150	750	0.00	2.4
7	14	105	750	2.00	2.6

Std.	Run	temperature	Particle size	Time	Yield
8	2	150	750	2.00	2.7
9	11	127.5	500	0.00	3
10	3	127.5	1000	0.00	2.5
11	7	127.5	500	2.00	3.4
12	5	127.5	1000	2.00	2.9
13	15	127.5	750	1.00	3.79
14	1	127.5	750	1.00	3.79
15	6	127.5	750	1.00	3.79
16	12	127.5	750	1.00	3.79
17	10	127.5	750	1.00	3.79

### 3.5. Optimization Process Variables

The *piper Nigrum* oil was extracted with steam distillation to obtain clear and quality the essential oil. From Table 2, the comparison of raw black pepper seed and leaves was investigated with how much oil yield, the optimal oil yield, and the predicted combination of parameters were as follows:

temperature 136°C, particle size, 723.35 micrometers, and time 4:30 hr. Under these conditions, the yield was predicted at 4.51% with a desirability value of 0.949. To validate the optimum conditions predicted by the model using desirability, ramp, and triplicate experiments were conducted using the optimized process conditions, and the mean percentage value of yield of 4.2% was obtained and the experiment results were related to the data obtained from the optimization analysis using desirability functions. Therefore, the study shows that black pepper seed oils can be used for skincare cosmetics with these optimal variables.

### 3.6. Fourier Transform Infra-red (FTIR) Spectroscopy

Following figures 5 and 6; indicating the FTIR results, I was done at Addis Ababa University, Faculty of Chemistry. The peak of the curve indicates the functional groups that exist. When we saw the peaks, there were different components displayed.

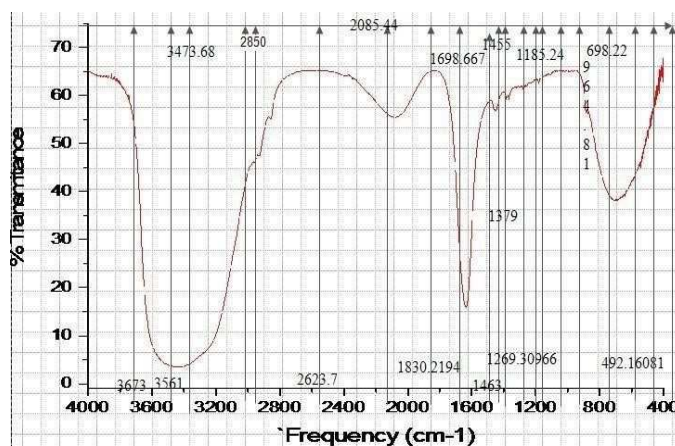


Figure 5. FTIR graph analysis of functional group results (Addis Ababa University, department of chemistry, FTIR laboratory results, <http://etd.aau.edu.et/> 2018 G.C).

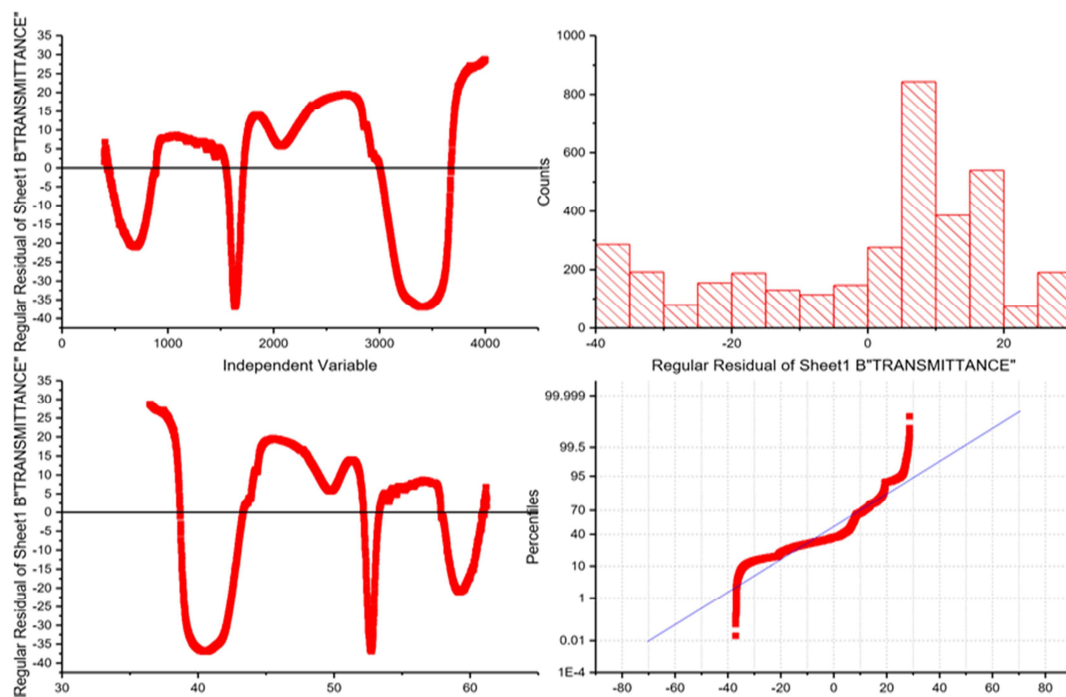


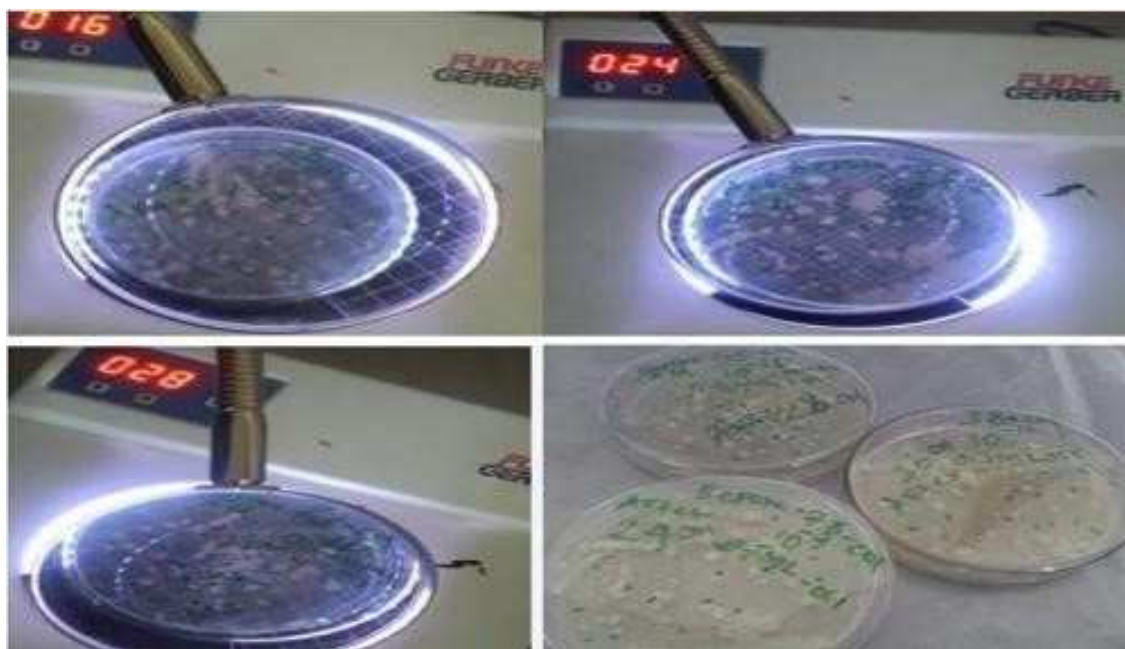
Figure 6. The linear trace frequency and absorbance, the wavelength of the oil, and the transmittance of the oil. (<http://etd.aau.edu.et/>).

The functional group depends on the factors or chemicals that would be too NaCl added to the samples. From the above figure 5, based on the peaks, functional groups, unsaturated fatty acids C=C stretch, O=C-H stretch, in both aldehydes in the region 2830-2695  $\text{cm}^{-1}$ , the carbonyl stretch C=O of a carboxylic acid appears as an intense band from 1760-1690  $\text{cm}^{-1}$ . N-H stretch from 3400, 3250  $\text{cm}^{-1}$ . 1° amine: two bands from 3400-3300 and 3330-3250  $\text{cm}^{-1}$ . 2° amine: one band from 3350-3310  $\text{cm}^{-1}$ . 3° amine: no bands in this region. N-H band (primary amines only) from 1690-1715  $\text{cm}^{-1}$ ,  $\text{R}_2\text{C}=\text{CH}_2$  Acids, fatty acids (aromatic amines) from 1335-1250  $\text{cm}^{-1}$ , C-N stretch (aliphatic amines) from 1250 to 1020  $\text{cm}^{-1}$ . N-H wag (primary and secondary amines only) from 910-665  $\text{cm}^{-1}$ . The region from 900-650  $\text{cm}^{-1}$ . Aromatics, alkyl halides, carboxylic acids, amines, and amides, figure 6 showed moderate or strong absorption bands (bending

vibrations) and transmittance in this region. All spectra contain peaks attributable to unsaturated fatty acids in 1690  $\text{cm}^{-1}$  to -1715 and peaks attributable to asymmetric stretching of unsaturated C-H for fatty acids, showing the acidity of the oil is highly concentrated.

### 3.7. Anti-bacterial Properties of Black Pepper Oil

The oil had anti -microorganism properties, one of these micro spices was the bacterial cell, and the bacterial cell should be specified under gram-positive and gram-negative spices. In my work, Grampositive bacterial spices were identified because these spices attack our skin, but not all bacteria attack our body. From Figure 7: the number of colonies was injured or missed by the addition of black pepper (Nigrum seed).



**Figure 7.** The number of bacterial colonies after the addition of oil (the laboratory result using nutrient agar at Addis Ababa University, Department of bioengineering, The source of bacterial cells was from the Ethiopian Biodiversity Institute. <http://etd.aau.edu.et/>, [www.ebi.gov.et](http://www.ebi.gov.et) 2018G.C).

**Table 3.** Differences in bacterial cells before oil addition and after oil addition in the Department of Bioengineering, source: <http://etd.aau.edu.et/>, and [www.ebi.gov.et](http://www.ebi.gov.et) 2018 G.C.

Petri dish sample and colonies	Amount of oil added to a petri dish	After adding the oil, the remaining no. colonies	Bacterial cells counted after Addition
$10^{-11}$ , 38	0.3ml	16	$16 \times 10^{11}$
$10^{-10}$ , 65	0.4ml	24	$24 \times 10^{10}$
$10^{-9}$ , 78	0.5ml	28	$28 \times 10^9$
$10^{-8}$ , 135	0.5ml of leave oil	53	$53 \times 10^8$

Sample and colonies	Bacterial cells counted after the addition,	The missed or injured cell after addition
$10^{-11}$ , 38	$16 \times 10^{11}$	$22 \times 10^{11}$
$10^{-10}$ , 65	$24 \times 10^{10}$	$41 \times 10^{10}$
$10^{-9}$ , 78	$28 \times 10^9$	$50 \times 10^9$

When we compared the three results in Table 3. The third was efficient. For the leaf oil, I used 108 Petri dishes before adding, in Figure 7, no colonies, 135 were counted and cells were  $135 \times 10^8$  after adding 0.5ml of left oil, it became  $53 \times 10^8$

cells counted and the missed or injured cells were 82 colonies when I calculated the ratio to become 60.074%, to conclude the antibacterial effect of leaves oil and seed oil were almost the same.

**Table 4.** Acidity test of the oil using NaOH titration in the department of chemistry at Addis Ababa University; <http://etd.aau.edu.et,2018G.c>.

Exp.no	Amount of 0.1N, NaOH	Amount of oil	Acid value
1	9.45ml,	16ml or 10gm,	5.3
2	10.16ml,	10gm or 16ml,	5.7
3	9.7ml	16ml	5.57

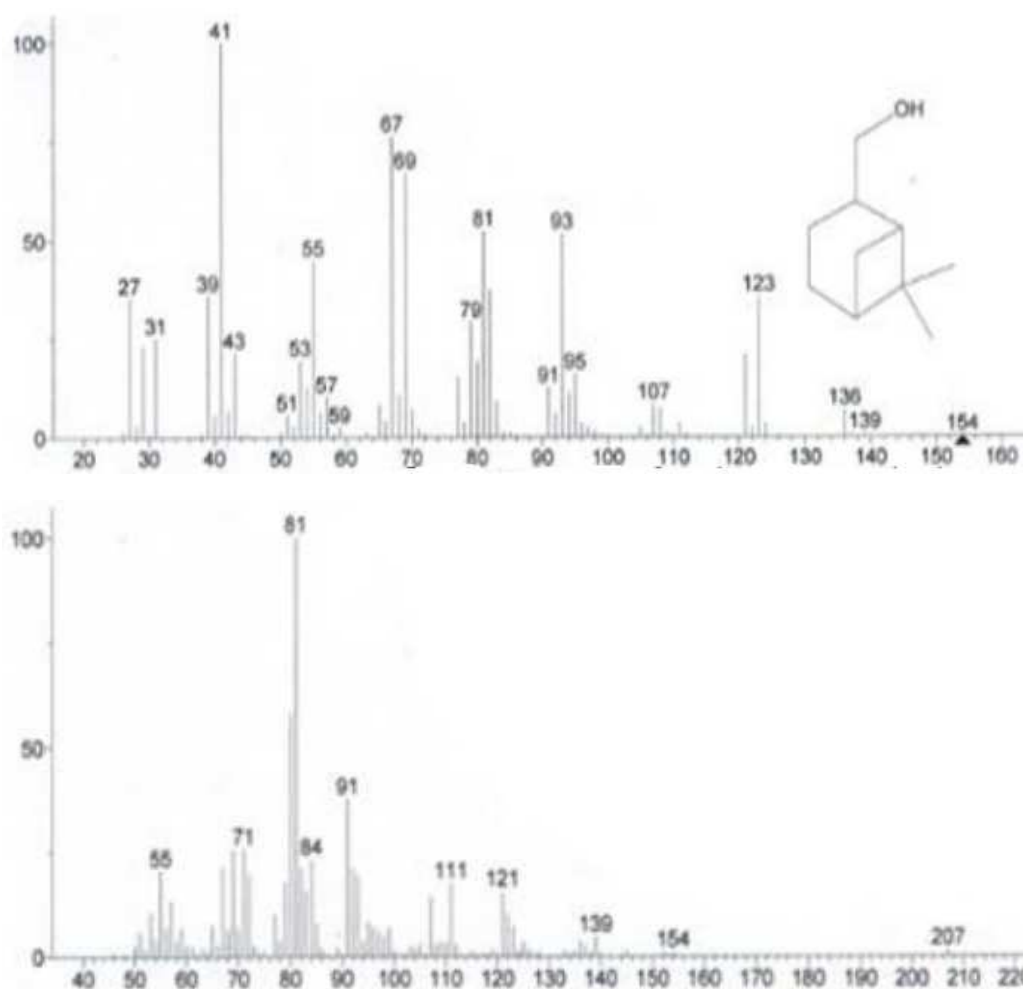
### 3.8. Acidity Properties of Black Pepper Seed Oil

From the above Table 4; three results were observed with a pink color indication after titration, and I took the average value. The acid value was 5.5. From this, I concluded that the *piper nigrum* oil had no admirable quality or attributes as a skincare application. The additives have different

components depending on the GC-MS result.

### 3.9. GC-MS / Gas Chromatography-Mass Spectroscopy

The GC-MS result experiment was done in the Leather Industry Development Institute in Addis Ababa Akaki Kaliti sub-city. Figure 8: shows GC-MS analysis performed on the samples taken from optimum conditions.

**Figure 8.** The GC- MS result of black pepper seeds and cosmetics (Nigrum), (leather industry development institute in Addis Ababa Akaki Kaliti; <http://etd.aau.edu.et, elidilab@gmail.com, www. elidior.org, 2018 G.C>).**Table 5.** GC-MS / Gas Chromatography-Mass Spectroscopy result (elidilab@gmail.com, [www.elide.org](http://www.elide.org),2018 G.C).

No	Component	Pk	(CAS number)	Composition area%c
1	(1R,5S,E)-2-methyl-4-[2,2,3-trimethyl-6-methylidenecyclohex-2-en-1-yl]but-2-enal	71	71999	0.80
2	Cis-13-octadecenoic acid	39	39303	13.33
3	Alpha, -terpineol	55	55633	3.69
4	p-mentha-4,8-diene,5-methyl,cyclohexene,1 isopropenyl-2,4-dimethyl	67	67228	0.14
5	a-phellandrene epoxide	69	69438	0.36
6	Oleic acid	80	80337	21.94
7	Ledene oxide	93	93799	22.54



From the above Figure 8, the GC-MS result, the amount of oleic acid was a large amount of the composition area covered. From the result, the percentage of oleic acid was 21.94% with high composition and Ledene oxide at 22.54%. More than 180 components were displayed. When we saw the component composition in Table 5; the maximum percent was covered by unsaturated fatty acids, alcohols, and phenolic compounds. The GC\_MS result component was one of the perfume oil compounds. 9, 12 octadecadienoic acid, [Z, Z], Octadecanoic acid, 9-octadecenoic acid, (E)-cis-13octadecenoic acid 13.33%, n--Hexadecanoic acid 8.61%, 4,4,8-trimethyltricyclo[6.3.1.0(1,5) dodecane-2,9diol, ledene oxide-(I) 3.61%, (z)-2,6,6trimethyl-.alpha.-(1-propenyl)-2-cyclohexane-1methanol, 4(1,3-dimethyl-3-cyclohexyl)-1 3.69%, 6heptadien-4-ol, were high percent covered. So as I concluded, the oil was used for perfume and had been a money advantage for the human being.

## 4. Conclusions

This study provides useful information for the optimization and characterization of the process of steam extraction of *Piper nigrum* seeds. The highest total extract yield and the highest content of target compounds with antibacterial properties were obtained with the optimal temperature of essential oils. The minimum amount of liquid (ethanol) and distilled water solvent ratio was 1:2, and the solid-to-liquid ratio n was 1:3. The minimum amount of oil for the result of extraction was 1.67gm or 6.63ml of oil at which the solvent concentration was not much diluted and the operation temperature nearest to the water boiling temperature. The characterization of the samples was by FTIR, and GC\_MS analysis. The samples were taken under optimal conditions, using the extracted oil at a temperature of 127.5°C, a particle size of 750 micros.m, and a time of 4:30 h. The results of the GC\_Ms analysis were mostly unsaturated fatty acids from oleic acid, linalool, hexanoic acid, hexadecanoic acid, and octadecanoic acid, and for the optimization of process variables, the results were closely related to a model, the optimized value of the oil yield 4.2%. Generally, the oil has some applications such as perfume, skincare, pharmaceuticals, and condiments for additives and for bacterial infection body to directly apply to the infected body.

## Data Availability

Data used to support the study findings are included in the article.

## Conflict of Interest

All the authors do not have any possible conflicts of interest.

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