
Phytochemical Extraction and Screening of Bio Active Compounds from Black Cumin (*Nigella Sativa*) Seeds Extract

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Abstract: This study is interested to now a day's attracting attention of the researchers; which is natural products and their derivatives because of they are becoming sources to important drugs and the pharmaceutical industries have come to consider traditional medicine as a source of bioactive agents that can be used in the preparation of synthetic medicines and helpful also as nutritional values which are very interesting in their eco-friendliness and free of the toxicity. Base on this, the current investigation is directed to the detection of the bioactive Black cumin which is one of the miraculous plant having multifarious roles in its phytochemical constituents and nutritional values, treating digestive tract conditions including gas, colic, diarrhea, dysentery, constipation, and hemorrhoids. S powdered *Nigella sativa* seed was used for crude oil extracts by using different solvents. In this manner, the results of investigation of qualitative phytochemical analysis conducted on the crude cumin seeds extract revealed the presence of bioactive compounds in the petroleum ether, ethyl acetate and methanol extracts which are known to exhibit medicinal as well as physiological activities. Identification and separations were taken by TLC and CC. Finally four potentially active phytochemicals have been obtained from methanol extracts; are alkaloids, phenol, flavonoids and steroids. Thus we can scientifically conclude that the cumin seeds extract could be seen as an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit.

Keywords: Bioactive, Black Cumin, Eco-Friend, Toxicity, Phytochemicals, Traditional Medicine

1. Introduction

In the plant kingdom there is a remedy for every disease. Two hundred and fifty years ago, there were few or no synthetic medicines. The plants were the main source of drugs for the world's population. Today, 75% of the world's population, the poor 3/4ths, still relies on those plants and other tools of traditional medicine. Plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well-being [1]. Medicinal plants are the richest bio resource of drugs for traditional systems of medicine, nutraceuticals, food supplements, modern medicines, pharmaceutical intermediates, folk medicines and chemical entities for synthetic drugs. World Health Organization (WHO) has suggested that medicinal plants would be the best source to obtain variety of drugs. Since the use of medicinal plant based drugs contain least or no side effects they are considered to be great importance to the health of individuals

and communities. WHO estimates that 80% of the people in developing countries of the world rely on traditional medicine for their primary health care, and about 85% of traditional medicine involves the use of plant extracts. This means that about 3.5 to 4 billion people in the world rely on plants as sources of drugs [2].

Plant products derived from barks, flowers, roots, leaves, seeds, fruits are the part of phytomedicines. Bio active constituents of plants known as phytochemical components such as tannins, carbohydrates, alkaloids, terpenoids, phenolic compounds, steroids and flavonoids are responsible for various pharmacological activities of the plants. These phytochemical compounds are synthesized by primary or secondary metabolism of living organisms. However, a number of these secondary metabolites have been noted for their antimicrobial activity. They are extensively used in agriculture, human therapy, veterinary and related scientific

research etc.[2].

Black cumin (*Nigella sativa* L.) is considered as a miracle herb due to its wonderful power of healing. The *Nigella sativa* seeds have been widely used for the treatment of different diseases and ailments. Seeds exhibit a wide spectrum of biological and pharmacological activities which include antihypertensive, antidiabetic, diuretics, anticancer, immunomodulator, analgesic, antioxidant, antimicrobial, anti-inflammatory, spasmolytic, bronchodilator, hepatoprotective, pulmonaryprotective, nephro-protective, gastro-protective, antioxytotic and anticonvulsant properties etc [3]. Due to its miraculous power of healing, *N. sativa* has got the place among the top ranked evidence based herbal medicines [4].

Nigella sativa is commonly known as black cumin, black caraway or small fennel in English, "Habat-ul-Sauda" (seed of blessing)" in Arabic, *siyah daneh* in Persian and etc. *Nigella sativa* is cultivated in many countries in the world like Middle Eastern Mediterranean region, South Europe, Saudi Arabia, Turkey, Syria, Pakistan and India. *Nigella sativa* (a member of family Ranunculaceae) is an annual flowering plant with finely divided leaves and 20-90 cm in height. The delicate flowers have 5-10 petals [5].

Keeping in view the importance, the seeds of *Nigella sativa* were standardized as per standard methods available in WHO and Pharmacopoeial guidelines for herbal drugs. Therefore, present work is interested to natural products chemical constituents because they are the eco-friendly and sources to any important now a day's drugs. So it is designed to perform the physicochemical and phytochemical investigations of *Nigella sativa* seeds [5].

There exist limited studies on the *Nigella sativa* oil, its properties and the contents of fatty acids and tocopherols. Black seed oil is reported to be beneficial in the control or management of African sleeping sickness due to its content of over a hundred components such as aromatic oils, trace elements, and vitamins. Reduce the risk to illness and disease by strengthening immune system and protecting the body. Recent reports however suggest that thymoquinone present in the oil might be the active components [6]. Millions of people in the Mediterranean region and on the Indian subcontinent use the oil from the seed of *N. sativa* daily as a natural protective and curative remedy. The seeds are very rich and diverse in chemical composition [7].

Many active compounds have been isolated, identified and reported so far in different varieties of black seeds. The most important active compounds are thymoquinone, thymohydroquinone, dithymoquinone and thymol etc. Black seeds also contain some other compounds in trace amounts. Seeds contain two different types of alkaloids; i.e. isoquinoline alkaloids e.g. nigellicimine and nigellicimine-N-oxide, and pyrazol alkaloids or indazole ring bearing alkaloids which include nigellidine and nigellicine [8].

The seeds of *N. sativa* contain protein, fat, carbohydrates, crude fiber and total ash. The seeds are also containing good amount of various vitamins and minerals like Cu, P, Zn and Fe etc. The seeds contain carotene which is converted by the liver to vitamin A. The seeds reported to contain a fatty oil rich in unsaturated fatty acids, mainly linoleic acid, oleic acid, eicodadienoic acid and dihomolinoleic acid [9].

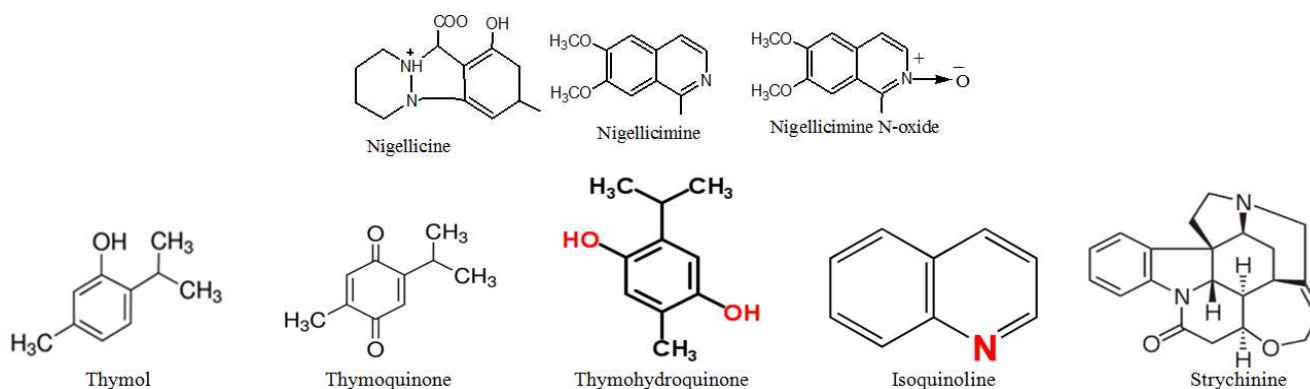


Fig. 1. Chemical structures of some major compounds isolated from *Nigella Sativa*.

2. Material and Method

2.1. Apparatus and Material

Materials used are 100ml of measuring cylinder, 5mL of measuring cylinder, 5000mL of volumetric flask, 500mL of round bottom flask, 100mL of beaker, 50mL of beaker, funnel, test tube, dropper, reagent bottles, mortar and pestle, Rota vapor, Thin Layer Chromatography, Column Chromatography, ruler.

2.2. Chemical and Reagent

Concentrated sulfuric acid, petroleum ether, ethyl acetate, methanol, chloroform, glacial acetic acid, ferric chloride, 20% of NaOH solution, dilute HCl, 5% of ferric chloride, distilled water, acetic anhydride, 10% alcoholic ferric chloride solution, Wagner's reagent.

2.3. Sample Collection

Initially the black cumin (*Nigella sativa*) seed was

purchased from sikela market in Arbaminch city. First the dirty part from black cumin seed was removed by using our hand and dried at room temperature, then grinded in to fine powder by using mortar and pestle.

2.4. Extraction

Powdered *Nigella sativa* seed was macerated in 400ml of petroleum ether, for 24 hrs with occasional shaking. The result was extracted and filtered by using filter paper (What Man No 1.5 Whatman Ltd., England). The petroleum ether extract were evaporated to dryness in vacuum by using Rota vapor at 40° C to yield 3.5 gm of crude extract (2.8 %W/W). The mark from petroleum ether extract would be soaked with 400ml of ethyl acetate for 24hrs. The ethyl acetate would be filtered out connected by using Rota vapor at 40° C to yield 7gm of crude extract (5.6%W/W). The mark from ethyl acetate extract would be soaked with 400ml of methanol for 24 hrs. The methanol extract was filtered out and connected to Rota vapor at 40 centigrade to yield crude extract. The mark was soaked again with the same amount of methanol for the same period of time. The total weight of crude methanol extract after evaporated using Rota vapor at 40° C to yield 20gm of crude extract (16 %W/W). After dried and weighed the above three crude extract had been apply to phytochemical analysis and from the above three crude extract only methanol used for column chromatography and thin layer chromatography [11].



Fig. 2. Black cumin (*Nigella Sativa* Linn.) seed.

2.5. Phytochemical Screening

The crude extract was tested for the presence of bioactive compounds by using following standard methods [10], [11] and [12].

2.5.1. Test for Alkaloids (Wagner's Reagents)

A. Petroleum Ether Extract

To the 2ml of extract, 1.5ml of 1% HCl was added. After heating the solution in water bath, 6 drops of Wagner's reagent was added. Formation of orange precipitated indicates the presence of alkaloid.

B. Ethyl Acetate Extract

To the 2mL of extract, 1.5mL of 1% HCl was added. After heating the solution in water bath, 6 drops of Wagner's

reagent was added. Formation of orange precipitated indicates the presence of alkaloid.

C. Methanol Extract

To the 2mL of extract, 1.5ml of 1% HCl was added. After heating the solution in water bath, 6 drops of Wagner's reagent was added. Formation of orange precipitated indicates the presence of alkaloid.

2.5.2. Test for Steroids

A. Petroleum Ether Extract

To 2 mL of extract, 5ml of chloroform and 2 mL acetic anhydride was added followed by concentrated H₂SO₄ reddish brown coloration of interface indicates the presence of steroids.

B. Ethyl Acetate Extract

To 2 mL of extract, 5ml of chloroform and 2 mL acetic anhydride was added followed by concentrated H₂SO₄ reddish brown coloration of interface indicates the presence of steroids.

C. Methanol Extract

To 2 ml of extract, 5ml of chloroform and 2 ml acetic anhydride was added followed by concentrated H₂SO₄ reddish brown coloration of interface indicates the presence of steroids.

2.5.3. Tests for Phenol

A. Petroleum Ether Extract

To 2mL of extract, 5% ferric chloride solution was added. Deep blue black color indicates the presence of phenol.

B. Ethyl Acetate Extract

To 2mL of extract, 5% ferric chloride solution was added. Deep blue black colour indicates the presence of phenol.

C. Methanol Extract

To 2mL of extract, 5% ferric chloride solution was added. Deep blue black colour indicates the presence of phenol.

2.5.4. Test for Terpenoids

A. Petroleum Ether Extract

Treat extract in chloroform with few drops of concentrated sulphuric acid, shaken well and allow to stand for some time, formation of yellow colored lower layer indicate the presence of terpenoids.

B. Ethyl Acetate Extract

Treat extract in chloroform with few drops of concentrated sulphuric acid, shaken well and allow to stand for some time, formation of yellow colored lower layer indicate the presence of terpenoids.

C. Methanol Extract

Treat extract in chloroform with few drops of concentrated sulphuric acid, shaken well and allow to stand for some time, formation of yellow colored lower layer indicate the presence of terpenoids.

2.5.5. Test for Flavonoids

A. Petroleum Ether Extract

To the test solution, add few drops of ferric chloride solution, intense green color was formed to show the presence of flavonoid.

B. Ethyl Acetate Extract

To the test solution, add few drops of ferric chloride solution, intense green color was formed to show the presence of flavonoid.

C. Methanol Extract

To the test solution, add few drops of ferric chloride solution, intense green color was formed to show the presence of flavonoid.

2.5.6. Test for Tannins**A. Petroleum Ether Extract**

Some amount of extract was dissolved in distilled water to this solution 2 mL of 5% ferric chloride solution was added. Formation of blue green indicates presence of tannins.

B. Ethyl Acetate Extract

Some amount of extract was dissolved in distilled water to this solution 2 mL of 5% ferric chloride solution was added. Formation of blue green indicates presence of tannins.

C. Methanol Extract

Some amount of extract was dissolved in distilled water to this solution 2 mL of 5% ferric chloride solution was added. Formation of blue green indicates presence of tannins.

2.5.7. Tests for Saponins**A. Petroleum Ether Extract**

The extract was diluted with distilled water and shaken in graduated cylinder for 15 minutes. The formation of layer of foam indicates the presence of saponins.

B. Ethyl Acetate Extract

The extract was diluted with distilled water and shaken in graduated cylinder for 15 minutes. The formation of layer of foam indicates the presence of saponins.

C. Methanol Extract

The extract was diluted with distilled water and shaken in graduated cylinder for 15 minutes. The formation of layer of foam indicates the presence of saponins.

2.5.8. Test for Cardiac Glycosides**A. Petroleum Ether Extract**

To 2 mL of test solution, 3 mL of glacial acetic acid and 1 drop of 5% ferric chloride were added in a test tube. Add carefully 0.5 mL of concentrated sulphuric acid by the side of the test tube. Formation of blue color in the acetic acid layer indicates the presence of Cardiac glycosides.

B. Ethyl Acetate Extract

To 2 ml of test solution, 3 ml of glacial acetic acid and 1 drop of 5% ferric chloride were added in a test tube. Add carefully 0.5 mL of concentrated sulphuric acid by the side of the test tube. Formation of blue color in the acetic acid layer indicates the presence of Cardiac glycosides.

C. Methanol Extract

To 2 ml of test solution, 3 ml of glacial acetic acid and 1 drop of 5% ferric chloride were added in a test tube. 0.5 ml of concentrated sulphuric acid were carefully added by the side of the test tube. Formation of blue color in the acetic acid layer indicates the presence of Cardiac glycosides.

2.6. TLC Profile

Thin layer chromatographic plate (5 × 20 cm) 0.5 mm Thickness was used. The sample of methanol extract crude oil was dissolve in methanol and spotted manually using a capillary tube. The Plate was developed in petroleum ether: ethyl acetate: methanol with different ratio as solvent system. After development, sprayed with ninhydrin solution with sulfuric acid to visualize the presence of spots. The presence of spot with trailing was revealed. This was purified with column chromatography[13].

2.7. Column Chromatography

Fractionation with column chromatography, columns of silica gel (230 to 400mL, 20 by 1.5cm) was washed with 75mL of the mixed solvents of petroleum ether and ethyl acetate (2:8) before the sample had been applied. A 10gm of dried methanol extract and 25.6gm of silica gel were measured and packed in to column with petroleum ether and then eluted with solvents starting from combination of low polarities and increasing their polarities as shown table below. Total of 30 fractions was collected. The purity of each fraction had been tested by TLC.

3. Result and Discussion

Extracts of Black cumin seed were isolated successfully by using different solvents. The results are presented in Table 1.

Table 1. The percentage yield of different extracts of black cumin (*Nigella sativa*) seed.

S.No.	Solvent	Color of extracts	Yield of the extract (gm)	Percentage yield (%w/w)
1	Petroleum ether	Light brown	3.5	2.8
2	Ethyl acetate	Light brown	7	5.6
3	Methanol	Dark brown	20	16

In phytochemical screening of the present study carried out in the black cumin (*Nigella sativa*) revealed the presence of medicinal active constituents.

Table 2. Results of phytochemical screening of petroleum ether, ethyl acetate and methanol seed extract of *Negella sativa*.

S.No.	Name of the phytochemical	Petroleum ether	Ethyl acetate	Methanol
1	Alkaloids	-	-	+
2	Flavonoids	-	-	+
3	Phenol	-	-	+
4	Tannins	+	+	-
5	Glycosides	-	-	+
6	Steroids	+	+	+
7	Saponins	-	-	-
8	Terpenoids	++	+	-

Key:

+indicates presence of the Phytoconstituents

++ indicates present in more quantity of the Phytoconstituent

-indicates absence of the Phytoconstituents

The phytochemical active compounds of black cumin were

qualitatively analyzed for seeds and the results are presented in Table 2. In these screening process alkaloids, glycosides, saponins, phenol, tannins, sterols, flavanoids, and terpenoids shows different types of results in different solvents extracts. Among these phytochemicals, Alkaloids, Flavonoids, Glycosides and Phenols were absent in all solvent extracts except methanol, whereas saponins were absent in all solvent extracts. Tannins and Terpenoids are present in all solvent except methanol. Steroids were present in all solvent extract. So Alkaloids, Flavonoids, Phenol compounds, Glycosides and Steroids were present in methanol extract.

In the present study, phytochemical screening for all three extracts showed significant indication about the presence of metabolites; Alkaloids, Tannins, Flavonoids Terpenoids, Phenol and steroids were found to be present in all the sequential extracts of *Black cumin seed* whereas saponins were absent in all solvents; summarized in Table 2. These detected phytochemical compounds are known to have beneficial importance in medicinal as well as physiological activities. In this manner isolating and identifying these bioactive compounds, new drugs can be formulated to treat various diseases and disorders.

A. Alkaloids which are one of the largest groups of phytochemicals have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity.

B. Tannins are used medicinally in anti-diarrheal, haemostatic and anti-haemostatic and anti-haemorrhoidal compounds and may be responsible for its broad spectrum anti-microbial against bacteria, fungi and viruses.

C. Steroids possess antibacterial properties and they are very important compounds especially due to their relationship with compounds such as sex hormones.

D. Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection have been found to be antimicrobial substances against wide array of microorganism *in vitro*. They also are effective antioxidant and show strong anticancer activities,

E. Terpenoids are essentially lipids, known for their aromatic qualities. Different functions have been described to terpenoids including growth regulating, colour, odour and anti-microbial activity and also responsible for anti-bacterial and anti-fungal activity.

F. Phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites. They possess biological properties such as anti-apoptosis, anti-aging, anti-inflammation, cardiovascular protection and improvement of endothelial function.

G. Glycosides are known to lower the blood pressure.

Table 3. Rf values of TLC solvent systems for methanol extract of (*Nigella sativa*) seed before purification.

S.No	Solvent system	Ratio	No. of spot detected	Rf value
1	Solvent system I	3:7	No	0
2	Solvent system II	1:2:7	No	0

S.No	Solvent system	Ratio	No. of spot detected	Rf value
3	Solvent system III	2:3:5	3	0.05, 0.25, 0.80
4	Solvent system IV	3:4:3	2	0.10, 0.81
5	Solvent system V	6:2:2	4	0.09, 0.25, 0.81, 0.92
6	Solvent system VI	5:2:3	3	0.1, 0.25, 0.92
7	Solvent system VII	6:3:1	2	0.25, 0.81

Thin layer chromatographic studies a large number of solvent systems were tried to achieve a good resolution. Finally, the solvents petroleum ether, ethyl acetate and methanol used as solvent systems for TLC studies of the Methanol extract of *Black cumin*. Solvent system I consist of Ethyl acetate: Methanol (3:7), no separately detected spots and its Rf value became 0. In solvent system II which consists of Petroleum ether: Ethyl acetate: Methanol (1:2:7), no separately detected spots and its Rf value became 0. In solvent system III Petroleum ether: Ethyl acetate: Methanol (2:3:5), 3 spots were detected Rf values 0.05, 0.25 and 0.80. In solvent system IV Petroleum ether: Ethyl acetate: Methanol (3:4:3), 2 spots were visible Rf values 0.10 and 0.81. In solvent system V Petroleum ether: Ethyl acetate: Methanol (6:2:2), 4 spots were obtained having Rf values 0.09, 0.25, 0.81 and 0.92. In solvent system VI Petroleum ether: Ethyl acetate: Methanol (5:2:3), 3 spots were obtained having Rf values 0.1, 0.25, 0.92. In solvent system VII, Petroleum ether: Ethyl acetate: Methanol (6:3:1), 2 spots were obtained having Rf values 0.25 and 0.81.

Table 4. The Results of CC eluted with solvents of increasing polarity.

S.No	Ratio (Petroleum ether/ethyl acetate/methanol)	Fractions	Volume (ml)
1	10:0:0	F1	10
2	9:1:0	F2	10
3	8:2:0	F3	10
4	7:3:0	F4	10
5	6:4:0	F5	10
6	5:5:0	F6	10
7	4:6:0	F7	10
8	3:7:0	F8	10
9	2:8:0	F9	10
10	1:9:0	F10	10
11	9:0:1	F11	10
12	8:0:2	F12	10
13	7:0:3	F13	10
14	6:0:4	F14	10
15	5:0:5	F15	10
16	4:0:6	F16	10
17	3:0:7	F17	10
18	2:0:8	F18	10
19	1:0:9	F19	10
20	0:0:10	F20	10
21	0:10:0	F21	10
22	0:9:1	F22	10
23	0:8:2	F23	10
24	0:7:3	F24	10
25	0:6:4	F25	10
26	0:5:5	F26	10

S.No	Ratio (Petroleum ether/ethyl acetate/methanol)	Fractions	Volume (ml)
27	0:4:6	F27	10
28	0:3:7	F28	10
29	0:2:8	F29	10
30	0:1:9	F30	10

TLC profiling of Methanol extracts gave an impressive results that directing towards the presence of number of phytochemicals. Different phytochemicals gave different Rf values in different solvent system. This variation in Rf values of the phytochemicals provides a very important clue in understanding of their polarity and also helps in selection of appropriate solvent system for separation of pure compounds by column chromatography. Mixture of solvents with variable polarity in different ratio used for separation of pure compound from plant extract. The selection of appropriate solvent system for a particular plant extracts was achieved by analyzing the Rf values of compounds in different solvent system.

According to table 4 the variation in Rf values (calculated as $Rf = \frac{\text{Spot distance}}{\text{Solvent distance}}$) of the phytochemicals provides a very important clue in understanding of their polarity and also helps in selection of appropriate solvent system for separation of pure compounds by column chromatography. The Methanol Extracts of *black cumin (Nigella sativa)* were subjected to column chromatography over silica gel (230 to 400ml, 20 by 1.5 cm). The column was eluted with solvents of increasing polarity.

Table 5. Rf values of TLC for methanol extract of (*Nigella sativa*) seed after elution.

S.No	Ratio (Petroleum ether/ethyl acetate/methanol)	Fractions detected with single spots	Rf values
1	6:2:2	F4	0.25
2	6:2:2	F5	0.25
3	6:2:2	F16	0.79
4	6:2:2	F17	0.79
5	6:2:2	F19	0.81
6	6:2:2	F20	0.81
7	6:2:2	F24	0.92
8	6:2:2	F25	0.92

Fractionation with column chromatography, Column was eluted with solvents of increasing polarity and a total of 30 fractions would be collected as shown in table above. The purity of each fraction has checked be tested by TLC. Fraction 4, 5, 16, 17, 19, 20, 24 and 25 shows single spot with Rf value given in the table 5. Rf values of fraction 4 and 5 were the same. Fraction 16 and 17 were the same. Fraction 19 and 20 were similar, also fraction 24 and 25 were identical. Then totally 4 single spots present. Using phytochemical screening test fraction 4 and, 5 (steroids), fraction 16 and 17 (phenols), fraction 19 and 20 (Flavonoids) and fraction 24 and 25 (Alkaloids) were present. Therefore four different types of Phytochemicals are reported in this study.

The Methanol extract of *Nigella sativa* seed was subjected to column chromatograph for the separation of compounds.

Using phytochemical screening, the petroleum ether: Ethyl acetate: Methanol (6:2:2) fraction of Methanol extract (F16 and F17) gave deep blue black compound which indicates presence of phenol. In the same ratio of solvent fraction of methanol extract (F24 and F25) gave orange precipitate which indicates presence of alkaloids, (F19 and F 20) gave intense green color was formed which indicates the presence of flavonoids, (F4 and F5) gave reddish brown which indicate the presence of steroids.

4. Conclusion

In recent years, ethno-botanical and traditional uses of natural compounds, specially of plant origins received much attention as they are well tested for their efficiency and generally believed to be safe in human use. They obviously deserve scrutiny on modern scientific lines such as phytochemical investigations, biological evaluations, toxicity studies and investigations of molecular mechanism of actions of isolated phyto constituents. In the process of controlling food borne pathogens, antimicrobials from plant origin have more potential and effective [14]. In this manner, in this study powdered *Nigella sativa* seed was used for crude oil extracts by using different solvents based on effectiveness solvents by their ratios. The crude extract was tested for the presence of bioactive compounds by using different standard methods. The results of investigation revealed the presence of medicinally important phytochemical constituents in the petroleum ether, ethyl acetate and methanol extracts of Black cumin (*Nigella sativa*) seeds, such as; alkaloids, Tannins, Steroids, Phenol, Terpenoids, Glycosides and flavonoids. TLC profiling of Black cumin seed extract give an idea about the presence of various phytochemicals. Different Rf (Retention factor) value of various phytochemicals provide valuable clue regarding their polarity and selection of solvents for separation of phytochemicals. Finally four potentially active phytochemicals have been obtained from methanol extracts; alkaloids, phenol, flavonoids and steroids. The purity of these phytochemicals was checked by TLC and it was confirmed.

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