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STUDIES ON PHYTOCHEMICAL SCREENING - GC-MS CHARACTERIZATION, ANTIMICROBIAL AND ANTIOXIDANT ASSAY OF BLACK CUMIN SEEDS (*NIGELLA SATIVA*) AND *SENNA ALEXANDRIA* (*CASSIA ANGUSTIFOLIA*) SOLVENT EXTRACTS

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Keywords:

Nigella sativa, *Cassia angustifolia*, Phytochemical screening, Antibacterial activities and antioxidant

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ABSTRACT: As science encourage most of the medicinal plants as supreme source of unlimited chemical compounds which are of great interest in pharmacological preparations. The present investigation was aimed to study *Nigella sativa* seeds and *Cassia angustifolia* leaf solvent extract (petroleum ether, methanol and aqueous solutions) for phytochemical screening, GC-MS characterization, Antimicrobial and Antioxidant assay. Qualitative phytochemicals in both *Nigella sativa* seeds and *Cassia angustifolia* leaf contains high quantity (+++) of alkaloids, Carboxylic acid, Coumarins, Phenol, Resin, Saponin, and Steroid. Antimicrobial activity was determined with two gram positive (*Bacillus subtilis* and *staphylococcus aureus*) bacteria. Methanol extract of *Nigella sativa* seeds shown highest zone of inhibition (19.66 ± 9.29 mm) against *staphylococcus aureus*, *Escherichia coli* and *klebsiella pneumonia*. Petroleum ether extracts of both *Nigella sativa* seeds and *Cassia angustifolia* leaf extracts shown highest zone of inhibition against *klebsiella pneumonia* (20.00 ± 5.03mm and 21.66 ± 4.16 mm, respectively). From the analysis of antioxidant potential of *Nigella sativa* and *Cassia angustifolia* extracts through DPPH free radical scavenging at three different concentration (5, 10, 20 µg/ml,), *Nigella sativa* seed shown highest activity. The findings of the study revealed that both the traditional medicinal plants has potential phytoconstituents which triggers their biological activities, therefore these experimental plants can be further assayed for pharmacological preparations..

INTRODUCTION: Plants that possess vast medicinal properties or that show beneficial pharmacological effects on the human body are generally designated as medicinal plants [1]. Medicinal plants are found to be bio-resource data for pharmaceutical intermediates and chemical entities for synthetic drugs since they are in usage like modern medicines, nutraceuticals, food supplements, folk medicines *etc*.

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Medicinal plants are also a source of various life style elements including fragrances, flavors, cosmeceuticals, health beverages and chemical terpenes. Medicinal plants are traded as such in bulk raw material from many developing countries for further value addition in developed countries where they are used as therapeutic products [2]. There are different types of medicines are in use, among which herbal medicine comes from different types of plants and their parts used traditionally in folk medicine to treat various diseases and disorders [3].

Plants are an important source of natural medicines and play an important role in world health [4]. Almost all cultures from ancient times to today

have used plants as medicine. Natural products derived from higher plants which are traditionally proven medicines may form the source to search for novel drugs based on their new modes of pharmacological action [5]. Today medicinal plants are important to the global economy as they are involved in formulation of drugs with cheaper resources without side effects [6], as approximately 85% of traditional medicine preparations involve the use of plants or plant extracts [7]. Medicinal plants naturally synthesize and accumulate some secondary metabolites like; alkaloids, steroids, terpenes, flavonoids, saponins, glycosides, tannins, resins, lactones, quinines, volatile oils *etc*. Recently, the World Health Organization (WHO) estimated that 80% of the people worldwide rely on medicinal plants partially for their primary health care [1]. Therefore the present research is aimed to study the following medicinal plants with their phytochemical screening, antimicrobial, and antioxidant potential.

Nigella sativa is an indigenous herbaceous plant belongs to the Ranunculaceae family that is more commonly known as the fennel flower plant [8]. Use of the black seeds is recommended in daily use because it is regarded as one of the greatest forms of healing medium available to treat many diseases according to traditional local healers. More than 150 studies conducted since 1959 confirmed the effectiveness of *Nigella sativa* seed constituents involved in treatment of various human ailments [9]. It has been traditionally used as a natural remedy for a number of ailments that include asthma, chest congestion, hypertension, diabetes, inflammation, cough, bronchitis, headache, fever, dizziness, and influenza and for general well-being [10].

Cassia species belong to the family of Caesalpiniaceae. Caesalpiniaceae is often treated as a subfamily, Caesalpinideae, of the large family leguminasae. It is closely related to Mimosaceae and Papilionaceae, but can be distinguished by few stamens and five free petals. Caesalpiniaceae consists of trees, shrubs, and a few woody herbs found in the tropics. Due to the excellent medicinal values and well known in folk medicine for their laxative and purgative uses Cassia species is in interest for phytochemical and pharmacological research [11].

MATERIALS AND METHODS:

Materials: *Nigella sativa* seeds, *Cassia angustifolia* leaf, Gas Chromatography - Mass Spectrometry (GC-MS), gram positive bacteria (*B. subtilis* and *S. aureus*), gram negative bacteria (*E. coli* and *K. pneumonia*).

Methods:

Preparation of Plant Materials Extraction: *Nigella sativa* seeds were bought from herbs shop and *Cassia angustifolia* leaves were collected from home farm during September 2015. The plant materials were weighed and were dried under sun light for 48 hours. Seeds of *Nigella sativa* and *Cassia angustifolia* leaves were grinded to fine powder by using electrical blender. The fine powder of 20 g was weighed separately and transferred to 250 ml of different solvent (petroleum ether, methanol and distilled water) were subjected to orbital rotary shaker for 24 h at 25 ºC at a speed of 150 rpm. Then the samples were centrifuged for 15 min at 2000 rpm at room temperature and are filtered through what man no 1 filter paper. The crude extracts of petroleum ether and methanol was evaporated through rotary evaporator at 60 ºC and 70 ºC respectively under constant pressure. While, aqueous extract was evaporated on hot plate at 100 ºC for 2 hours. The Crude plant extracts were stored at 4 ºC until further usage.

Preliminary Phytochemical Screening: The medicinal extracts were subjected for preliminary screening to test for the presence or absence of phytochemical constituents by using standard methods [12, 13, 14]. 1 ml of each pure extracts were dissolved in 10 ml of Dimethyl sulfoxide (DMSO) and made the volume to 1% with distilled water and extracts are tested for the following phytoconstituents namely Alkaloids, carboxylic acid, coumarins, flavonoids, Phenols, quinines, resins, saponins, steroids, tannins, glycosides, carbohydrates and protein.

Gas Chromatographic-Mass Spectrometry (GC-MS): The published GC-MS procedure was followed [15], using HPGC (Model 6890 series). The apparatus is equipped with flame ionization detector and injector (H P 7683). The MS transfer line was established at 250 ºC using (30 × 0.25 mm, 1.0 um).

The chromatographic conditions were fixed: oven temperature was kept at 50 ºC at a rate of 2 ºC/min, using helium gas (99.9%) as a carrier gas at a constant flow rate of 22 cm/s. 1ug/ml concentration of extracts was injected (split ratio 1:30). For MS analysis coupled Agilent M Spectrometer (model 5973) was used with the help of NISTO8 Library software. Mass spectra were taken at 70 eV/200 ºC (1 scan/s).

Antibacterial Screening: The plant extract retains an inhibitory effect against the growth of a microorganism even at highest dilution MIC [16]. The Minimum Inhibitory Concentrations (MIC) was determined using the broth dilution method [17]. The extract about 1g was weighed and dissolved in 10 mL of the Dimethyl sulfoxide (DMSO) solvent to give a concentration of 10 $g.ml^{-1}$ [18]. The antimicrobial study was carried out as follows:

Test organisms namely bacteria (*E. coli* (ATCC 25922), *S. aureus* (ATCC 25932), *B. subtilis* (ATCC 6633) and *K. pneumonia* were collected from microbiology lab, Higher college of Technology, Alkhuwair, Muscat, Sultanate of Oman, which are maintained overnight respectively on nutrient broth. *In vitro* investigation of antimicrobial study agar medium through disc diffusion. The inhibitory effect of each extracts was compared with the standard antibiotics penicillin. Inhibition zone in mm around the disks were measured after 48 h of incubation at 25 ± 2 ºC. All the experiments were repeated twice, the mean values of three trials and standard deviations were calculated through descriptive statistics.

Antioxidant Study: The effect of the plant extracts on DPPH degradation was estimated according to the method described by [19, 20, 21]. Crude medicinal plant extract was diluted in pure methanol in three different concentrations (5%, 10% and 20%). By adding 2 ml of each diluted sample of crude plant extract to 0.5 ml of 0.2 mmol/l of DPPH ethanolic solution and Ascorbic acid was used as a control and each sample was maintained with three replicates. The reaction mixture was incubated in dark place for 30 min. Then, the antiradicals were measured using UV- Spectrophotometer at 517 nm. A lower IC_{50} value corresponds to the higher antioxidant activity. The ability to scavenge DPPH free radical was calculated using the following equation:

$$\text{DPPH scavenging effect} = ((A_0 - A_1)\times 100\% / A_0))$$

Where A_0 the absorbance of the control at 30 minutes, A_1 the absorbance of the sample at 30 minutes [21].

Statistical Analysis: Data reported in this study is the mean ± SD of triplicates of each experiment. Results were analyzed through simple descriptive statistics.

RESULTS:

Phytochemical Screening: Phytochemical screening is mostly studied on medicinal plants to determine presence the biologically active compounds in plants. Phytochemicals as secondary constituents gives plants with color, flavor, and also natural protection against pests. However, as the results in **Table 1** specific test for qualitative Preliminary phytochemicals in both *Nigella sativa* seeds and *Cassia angustifolia* leaves extract in different solvents as petroleum ether, methanol and distilled water contains alkaloids, Carboxylic acid, Coumarins, Phenol, Resin, Saponins, and Steroid. The alkaloids present in high quantity (+++) in both *Nigella sativa* and *Cassia angustifolia* solvents extract in petroleum ether, methanol and distilled water.

TABLE 1: PHYTOCHEMICAL ANALYSIS OF THE *NIGELLA SATIVA* SEEDS AND *CASSIA ANGUSTIFOLIA* LEAVES EXTRACTED IN DIFFERENT SOLVENT

Phytoconstituent	NS. P	NS. M	NS. W	CA. P	CA. M	CA. W
Alkaloid	+++	++	+++	++	+++	+++
Carboxylic acid	+++	++	+++	+++	++	+++
Coumarins	++	+	+++	+++	+++	+++
Flavonoid	+++	+	+++	-	+++	+++
Phenol	+++	++	+++	+	+++	+++
Quinines	+++	-	+++	++	-	+++
Resin	+++	+++	+++	+++	+++	+++
Saponins	+++	++	+++	-	+	+++
Steroid	+++	+++	+++	++	++	+++

Tannins	+++	+++	-	+	+++	+++
Glycoside	+++	-	-	+++	-	-
Protein	-	+++	++	-	+++	++
Carbohydrate (Benidict test)	+	+++	++	++	+++	+++
Carbohydrate (Moish test)	-	++	+++	+	+++	+++

Note: All the value expressed in the table is the mean of three replication. (+++: high, ++: moderate, +: low, -: not detected). Were (NS. P: *Nigella sativa* petroleum ether extract, NS. M: *Nigella sativa* methanol extract, NS. W: *Nigella sativa* distilled water extract, CA. P: *Cassia angustifolia* petroleum ether extract, CA. M: *Cassia angustifolia* methanol extract, CA. W: *Cassia angustifolia* distilled water extract).

On the other hand, in *Nigella sativa* seeds extract with petroleum ether and distilled water flavonoids are present in high quantity (+++) but with methanolic extract it was found absent (-). *Cassia angustifolia* leaf extract with petroleum ether not shown negative result (-) for flavonoids, the same plant methanol and distilled water extracts shown high quantity (+++) of flavonoids. The plants extracts also revealed the presence of phenol, resin, and steroid at high quantity (+++) in all solvent extract except *Cassia angustifolia* leaf extract with petroleum ether detected a few reduction compared with other solvents extract. Through this study, It was observed that saponins were present in high quantity (+++) except in *Cassia angustifolia* leaf petroleum ether extract (-).

Phytochemicals are certain non-nutritive plant chemicals which have some disease preventive properties [22]. Alkaloids, comprising a large group of nitrogenous compounds, are widely used as cancer chemotherapeutic agents [23, 24]. Flavonoids were well known phytoconstituents that exert a wide range of biological activities including antimicrobial, anti-inflammatory, antioxidant, cancer preventive, antiarthritic and anti-coronary [25]. The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites [26] possess biological properties such as anti-apoptosis, anti-ageing, anti-carcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities [27]. Steroids are well studied and reported to have antibacterial properties [28] and they are very important compounds especially due to their relationship with compounds such as sex hormones [29]. Quinines are not found in all the tested solvent extracts of both the plant materials. Saponins may be either triterpenoid or steroid in nature and are supposed to have immunological and pharmacological properties. Saponins were well proven to be as anti-microbial and also they inhibit moulds thereby protect plant from attacks [30]. Tannins are used medicinally as anti-diarrhoeal, hemostatic and anti-haemorrhoidal compounds [31] are found in *Nigella sativa* and *Cassia angustifolia* extracts except aqueous extract of *Nigella sativa*. Glycosides are known to lower the blood pressure according to many reports [32], found only in (+++) petroleum ether extract. Also, protein and carbohydrate which is important metabolites were detected at high quantity in methanol and distilled water.

Gas Chromatographic-Mass Spectrometry (GC-MS) Analysis: The results pertaining to GC-MS analysis led to the identification of number of compounds from the methanol and petroleum ether extract of two different plant extracts. GC-MS chromatogram showed many peaks, indicating the presence of various bioactive compounds **Table 2** and **3**. Many of the compounds reported through GCMS in the study are first time identified with the respective solvents and are found highest amount even through Preliminary phytochemical studies.

TABLE 2: BIOACTIVE COMPOUNDS OF DIFFERENT SOLVENT EXTRACTS FROM *NIGELLA SATIVA* SEEDS

Pk	Petroleum ether extract		Methanol extract		Distilled water extract	
	Compound	RT	Compound	RT	Compound	RT
1	Heptane, 3-methylheptane, Isooctane, 2-Ethylhaxane	3.150	Butanoic acid, methylene ester	3.264	Decane	15.452
2	Cis-1,3-dimethylcyclohexane	3.253	1-methylethyl	11.224		
3	Trichloroacetic acid, Dodecyl ester, Z-10-Tetradecen-1-ol acetate, 1-Hexane, Cetene, Hexadecene-1	3.402	Undecane	15.464		

4	1-methyl-2-ethylcyclopentane, 1-methyl-cis-2-ethylcyclopentane	3.459	Delta.3.Carene, Bicyclo[4.1.0]hept-3-ene, 3,7,7-trimethyl	16.488
5	Isooctane	3.573	2,5-cyclohexadiene-1, 4-dione, 2-methyl-5-(1-methylethyl), p-Cymene-2, (P-methoxyphenyl) -2-methoxyethylene	24.647
6	Trans-1,3-dimethylcyclohexane	3.711	Viridiflorene, Valence 85 Eremophila-1(10), 11-diene, Eremophilene	33.385
7	3-Ethyl-3-methylheptane	4.134		
8	Ethylcyclohexane	4.277		
9	Quinuclidine	4.346		
10		4.958		
11		5.164		
12		5.845		
13	Alpha-Thujene	6.978		
14		11.218		
15	Decane, Capric ether	15.446		

Note: Pk: Peaks, RT: Retention time.

TABLE 3: BIOACTIVE COMPOUNDS OF DIFFERENT SOLVENT EXTRACTS OF *CASSIA ANGUSTIFOLIA* LEAF

Pk	Petroleum ether extract		Methanol extract		Distilled water extract	
	Compound	RT	Compound	RT	Compound	RT
1		4.266	2-(Methylothio)-1, 3-benzoxazol	4.494	Undecane	15.446
2	Tricyclo[5.1.0.0(2,8)]oct-4-en, Tricyclo[5.1.0.0(2,8)]oct-3-en	5.158	Dodecane	15.452		
3	3-diazoacetyl-4-methoxy carbonylpyridiine, N-(methy leneamino)-2, 4-dinitroaniline	5.839				
4	Nitrophenyl azide	6.297				
5	Trichlorocyclopentane	6.371				
6		6.961				
7	Undecane	15.458				

Note: Pk: Peaks, RT: Retention time

These interpretations also clearly and strongly evidenced through antimicrobial screening. *N. sativa* petroleum ether extract showed highest profile with many peaks follows methanolic extract and the least number of peaks and compounds were noticed with aqueous extract. The more biological activity was noticed with Petroleum ether extract of *N. sativa* was noticed with more zone of inhibition while very less zone of inhibition was found with aqueous extract. Since the Bioactive compounds extracted through petroleum ether found to be more, which is evidenced through GC-MS.

Cassia angustifolia leaf extract also with petroleum ether found be more bioactive interpreted and evidenced through GCMS and Antimicrobial study followed by Methanol extract and least activity was noticed with aqueous extract. The typical Gas Chromatogram shows the relative concentrations of various compounds eluted through retention time.

Antibacterial Screening: The results in the **Table 4** shows varied inhibition zone against all the tested bacteria with *Nigella sativa* seeds and *Cassia angustifolia* leaves with different solvents. Against *Bacillus subtilis* with *Nigella sativa* seeds Petroleum ether extract, shown highest inhibition zone (16.33 mm) comparing with methanolic extract (14.66 mm) and distilled water (10 mm). On the other hand, *cassia angustifolia* leaves aqueous extract found to be highest inhibition zone (15.33 mm) compared with methanolic extract (11.66 mm) and Petroleum ether extract (9.33 mm) against *Bacillus subtilis.*

TABLE 4: ANTIBACTERIAL ACTIVITIES OF *NIGELLA SATIVA* SEEDS AND *CASSIA ANGUSTIFOLIA* LEAF EXTRACTS

Type of bacteria	Type of medicinal plant	Microorganism Standard Strains MIDZ (mm)			
		Type of Extraction			Antibiotic (Penicillin)
		P. ether	Methanol	D. water	
B. subtilis (gram positive)	N.S seeds	16.33 ± 1.52	14.66 ± 2.08	10.00 ± 9.16	9.33 ± 8.14
	C.A leaves	8.33 ± 7.37	11.66 ± 2.08	15.33 ± 3.51	7.33 ± 10.06
S. coccus (gram positive)	N.S seeds	15.00 ± 13.22	19.66 ± 9.29	12.66 ± 0.57	12 .33 ± 0.75
	C.A leaves	15.66 ± 6.65	13.66 ± 2.08	12.00 ± 1.73	12.33 ± 3.21
E. coli (gram negative)	N.S seeds	Nil	10.33 ± 10.05	6.00 ± 10.39	4.66 ± 8.08
	C.A leaves	5.66 ± 9.81	4.00 ± 6.92	9.66 ± 8.50	4.66 ± 8.08
K. pneumonia (gram negative)	N.S seeds	20.00 ± 5.03	18.66 ± 10.69	10.00 ± 0.00	3.66 ± 6.30
	C.A leaves	21.66 ± 4.16	9.00 ± 7.81	8.99 ± 7.57	11.33 ± 1.57

Note: Results of Microorganism Standard Strains MIDZ measures in millimeter (mm), and all the value expressed in the table are the (mean values ± SED) of three replications. (*B. subtilis*: *Bacillus subtilis, S. coccus: Staphylococcus aureus*, *E. coli*: *Escherichia coli, K .pneumonia: Klebsiella pneumonia*, N. S: *Nigella sativa*, C. A: *Cassia angustifolia* "Nil" no inhibition).

While, against *Staphylococcus aureus*, *Nigella sativa* seed methanol extracts shown highest activity (19.66 mm) comparing with Petroleum ether (15.00 mm) and distilled water (12.66 mm). While *Cassia angustifolia* leaves petroleum ether extraction shown highest zone of inhibition (15.66 mm) comparing with methanol distilled water extracts (13.66 mm and 12.00 mm, respectively). In contrast, using gram negative bacteria have shown different results. Against *Escherichia coli* (gram negative) bacteria with *Nigella sativa* seeds not shown any effect especially petroleum ether extracts, while methanol and aqueous extracts shown zone of inhibition.

Cassia angustifolia leaves aqueous extract has highest inhibition zone (9.66 mm). While, the same plant with petroleum ether and methanol shown less inhibition zone (5.66 mm and 4.00 mm, respectively). Against *Klebsiella pneumonia* bacteria, *Nigella sativa* seeds and *Cassia angustifolia* leaves shown highest zone of inhibition with petroleum ether extract (20.00 mm and 21.66 mm, respectively), Methanolic and distilled water showed inhibition zone (18.66 mm, 9.00 mm, 10.00 mm and 8.99 mm, respectively).

The inhibition zones (IZ) were in the range of 8.5 ± 0.35 to 22.4 ± 0.86 mm for most of the tested strains against *S. aureus* and *E. coli*. The methanol extract of C. *angustifolia* exhibited the prominent antibacterial activity against *E. coli*, *K. pneumonia*, *B. subtilis* and *S. aureus* bacteria. The *K. pneumonia* with an aqueous extract showed maximum activity against *B. subtilis* and *S. aureus*. The results exhibited by the solvent extracts against maximum growth inhibition are strongly evidenced with the phytoconstituents obtained through GCMS characterization.

In this study, *Nigella sativa* extracts was found to be more effective on both Gram +ve than Gram –ve bacteria, which is in conformity with earlier studies [33, 34]. A number of compounds derived from plants often show considerable activity against Gram +ve bacteria but not against Gram –ve species [35]. The *N. sativa* oil was also found active against multi drug resistant strains of *S. aureus*.

Earlier studies have demonstrated the effect of *N. sativa* oil against sensitive strains of *S. aureus*, *P. aeruginosa* [36, 33]. Based on the results shown [37] the methanol, petroleum ether and aqueous extracts of *C. angustifolia* exhibited varying degree of inhibitory effect against all tested pathogenic strains. The present study was supported by [38, 18].

Antioxidant Study: Radical scavengers present as antioxidants in products may directly react and quench with peroxide radicals and terminate the peroxidation chain reaction and improve the quality and stability of food product. As it is described in **Table 5** all the three concentration of *Nigella sativa* methanol extract have high antioxidant potential. On the other hand, *Nigella sativa* extract with distilled water only at 10% concentration showed high antioxidant property.

While the remaining concentration of both petroleum ether solvent extract had shown less antioxidant potential. The results obtained throughout this experiment also proven earlier with many supportive studies [21].

TABLE 5: ANTIOXIDANT ACTIVITY OF PLANT EXTRACTS

Solvent extract	Concentration (µg/ml)	Medicinal plants				Standard
		Nigella sativa		*Cassia angustifolia*		Ascorbic acid
		A.b (nm)	IC_{50}%	A.b (nm)	IC_{50}%	A.b (nm)
Petroleum ether	5	0.223	58.16	0.405	24.015*	0.533
	10	0.472	67.91	0.376	74.439	
	20	0.038	98.55	0.360	86.327	
Methanol	5	0.762	42.96*	0.117	78.048	1.471
	10	1.043	29.09*	0.252	82.868	
	20	1.405	46.62*	0.291	88.947	
Distilled water	5	0.203	61.91	0.328	38.416*	2.633
	10	1.043	29.09*	0.600	59.211	
	20	0.881	66.54	1.095	58.412	

However, *Cassia angustifolia* has only high antioxidant potential at 5% concentration with petroleum ether and aqueous extracts, and the remaining concentration showed low antioxidant activity. Comparatively *Cassia angustifolia* has less antioxidant potential than *Nigella sativa*. The stable DPPH radical has been used to evaluate antioxidants for their radical quenching capacity [39]. The antioxidant activity of the plant extracts was examined on the basis of the scavenging effect on the stable DPPH free radical activity [40].

CONCLUSION: Qualitative phytochemicals in both *Nigella sativa* seeds and *Cassia angustifolia* leaf contains high quantity (+++) of alkaloids, Carboxylic acid, Coumarins, Phenol, Resin, Saponin, and Steroid attributes that these medicinal plants are having potential bioconstituents. Methanolic extract of *Nigella sativa* seeds show highest zone of inhibition (19.66 ± 9.29 mm) against *staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumonia*. Petroleum ether extracts of both *Nigella sativa* seeds and *Cassia angustifolia* leaf extracts shown highest zone of inhibition against *Klebsiella pneumonia* (20.00 ± 5.03 mm and 21.66 ± 4.16 mm, respectively). Antioxidant assay of *Nigella sativa* and *Cassia angustifolia* extracts through DPPH free radical scavenging at three different concentration (5, 10, 20 µg/ml) proved that these medicinal plants are the potential candidates as antioxidants. Therefore the whole study proved that these traditional herbal medicinal plants need to be assayed in depth to be used in pharmacological preparations.

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CONFLICT OF INTEREST: The authors report that no conflict of interest in this work.

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