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Pharmacological screening and GC-MS analysis of vegetative/reproductive parts of *Nigella sativa* L.

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Abstract: *Nigella sativa* is an extensively utilized herbal medicinal plant. Medicinal utilization of seeds and oil of *N. sativa* was common among all the ancient medicinal systems. Previously, the vegetative part of the plant was not utilized for pharmaceutical purposes. Therefore, the current study was aimed at determining the potential of the vegetative parts (leaves, branches and stem) of the plant to reduce the pressure on the reproductive part (seeds/oil) and to get a potential alternate source of pharmaceutical materials. The experiment was carried out in split block RCD design. The dried plant was extracted through partitioning method in a series of concentrations ranging from 200-1.562mg/ml in different solvents. Phyto-chemical screening, antibacterial and anti-oxidant assays and GC-MS analysis of the potent extracts were done. The green parts of *N. sativa* were shown to contain saponins, alkaloids, terpenoids, steroids, cardiac glycosides, tannins, and flavonoids, whereas phloba-tannins were absent. Evaluation of anti-aging properties through antioxidant assays yielded significant results in all the assays. Linoleic acid, palmitic acid, stearic acid, oleic acid and glyceryl linoleate were profiled through GC-MS analysis. Based on these results, it was concluded that the vegetative parts of *N. sativa* are effective alternatives to the reproductive part/seed for anti-aging and other phytochemicals needed to meet the present day requirements of the pharmaceutical industry.

Keywords: Antioxidant, antibacterial. pharmaceuticals, phenols, radical scavenging,

INTRODUCTION

Nigella sativa (Ranunculacea) is popularly known as *black seed*. It is an annual flowering plant native to South and Southwest Asia. Its cultivation period is from November to April. The initial material for propagation of this medicinally important herb is seed which takes 20-25 days to germinate under ideal physical conditions (Chaouche *et al*., 2014). Its cultivation requires a warm climate. Unfavorable climatic conditions are responsible for neglected status of crop in Pakistan. The yield and quality of seed is adversely affected by sequential and protracted period of cold and wet weather at harvest time. Fungal diseases can easily attack during wet weather. Its seed production per hector can fluctuate sharply from one season to another due to unfavorable climatic conditions (Chandra *et al*., 2014).

Seed is utilized as spice, carminative, condiment and aromatic purposes. Several human disorders such as indigestion, diarrhea, foul breath and excessive salivation are treated using seed decoction. The seed and seed oil have been used to cure several diseases (Warrier and Nambia, 2004). It has also been used for treating chronic headache, migraine and hepatic disorders (Randhawa and Alghamdi, 2011). Usually, after harvest, the seed is collected but the vegetative part is discarded. There is multipurpose use of *N. sativa* seed in herbal medicine industry. Thus, increased production of seed is required, but this is difficult to achieve because of delicate/fragile nature of seeds and high susceptibility to fungal attack at the maturity level. The vegetative parts of *N. sativa* could be alternative source of anti-ageing and other phytochemicals. Therefore, the present research was conducted to identify and compare presence of phytochemicals and antioxidant activities of the vegetative and reproductive parts of *N. sativa.*

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MATERIALS AND METHODS

Plant material cultivation and authentication

N. sativa seeds were cultivated at Botanic garden, Lahore College for Women University Lahore (31°15′-31°45′ N and 74°01′-74°39′ E) from November 2016 to April 2017. The soil was mixed with farm yard manure and prepared within pH range 5-8. Normal plant growth was maintained through daily watering. Soil temperature range was 20-25ºC. Inter-plant distance was kept at 20 cm, whereas row distance was 30cm (Ahmad and Ghafoor, 2003). The plant parts were collected in April 2017 and authenticated by Plant Herbarium and Molecular Taxonomy Lab, Department of Botany (Lahore College for Women University Lahore). The sample was vouchered as *Nigella sativa* L. LCWU-14-06. The material was washed, dried at room temperature, ground to fine powder and sieved prior to extraction.

Extract preparation

In the extraction process, 500g of *N. sativa* was soaked in 1200ml methanol for 14 days. Following filtration, the

extract was evaporated to dryness in a rotary evaporator at 45ºC under vacuum to obtain a crude methanol extract. The crude, sticky methanol extract was dissolved in 500 mL distilled water. The mixture was then partitioned with 500mL *n*-hexane and left for complete separation of aqueous and *n*-hexane layers in a separating funnel. The *n*-hexane layer was separated, and the process was repeated till all the *n*-hexane soluble compounds were separated from the aqueous phase. The isolated *n*-hexane phase was evaporated under vacuum to yield 6.4g of this fraction. The aqueous phase was further subjected to successive partitioning using 500mL each of chloroform, ethyl acetate and *n*-butanol to yield 2.7g chloroform, 3.2g ethyl acetate and 3.8g *n*-butanol fractions. Finally, the aqueous fraction was evaporated to get 1.9g of a gummy mass.

Phytochemical analysis

Preliminary qualitative phytochemical analysis of the samples was done to check the presence or absence of secondary metabolites by using standard methods (Senguttuvan *et al*., 2014; Yadav *et al.,* 2014).

Antibacterial assay

Antibacterial assay of the extracts was done according to the method of Jahantighi *et al.,* (2016). The assay was performed against pathogenic strains i.e. *Clostridium difficile*, *Pasteurella sp*., *Pseudomonas aeruginosa* and *Xanthomonas sp*. collected from Institute of Agricultural Sciences, University of the Punjab Lahore. The isolates were sub-cultured on Nutrient Agar (NA) plates and incubated at 37°C for 24h. The assay was performed through agar disk diffusion method (Abalaka *et al*., 2012). All assays were done in triplicate.

Antioxidant assays

Antioxidant analysis was done through three different assays like DPPH assay, total antioxidant assay and total phenolic contents assay.

DPPH assay

The DPPH radical scavenging activity assay was carried out according to the method of Choi *et al*. (2000), with minor modifications. The reaction mixture at all the concentrations used was prepared by dissolving 0.2 mg/ml DPPH in DMSO with the test sample in equal concentration and kept in dark for half an hour. The scavenging activity was measured in terms of absorption at 517 nm in triplicate. The antioxidant activity was expressed as % scavenging activity (SC %) of DPPH radical. Alpha-tocopherol was used as standard at variable concentrations ranging from 200-1.562mg/ml.

Total antioxidants assay

The total antioxidants potential of the extracts was determined according to the method of Prieto *et al*. (1999), with modifications. In essence, 0.1ml of each extract was mixed with 1.9ml of reagent solution (0.6M sulphuric acid, 4mM ammonium molybdate and 28mM sodium phosphate). The reaction mixture was incubated at 95°C for 60 min and cooled to room temperature. The antioxidant activity was expressed in terms of absorption at wavelength of 695 nm. Alpha tocopherol was used as standard at variable concentrations ranging from 200 - 1.562mg/ml.

Total phenolic contents assay

Total phenolic contents were determined using the procedure described by Dewanto *et al.* (2002). The extract (0.1ml) was added to 2.8ml of 10% Na_2CO_3 and 0.1ml of 2N Folin-Ciocalteu reagent. The absorbance of the reaction mixture was measured at 725 nm in a UV visible spectrophotometer. The total phenolic contents were extrapolated from a gallic acid standard calibration curve, and expressed as gallic acid equivalents (ug/g of dry weight) using the expression.

STATISTICAL ANALYSIS

The experiment was laid out in a randomized complete block design with three replications. Analysis of variance of the results obtained from all the activities was done using statistical software SPSS 20 (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 20 Armonk, NY: IBM Corp.) One way ANOVA test was applied at 5% level of significance (Armonk, 2013).

RESULTS

Phytochemical analysis

Qualitative phytochemical analysis revealed the presence of several phytochemical compounds. Alkaloids were present in high amounts, forming deep yellow precipitates. There was a typical froth formation indicating the presence of saponins.. Rings were also quite clear, and confirmed the potential presence of terpenoids, whereas the amount of cardiac glycosides was comparatively low. Coumarins were present, as indicated by fluorescence. Anthraquinones were abundant, whereas flavonoid level was low. In addition, the levels of terpenoids and phloba-tannins were quite high. The results are presented in table 1.

Antibacterial assay

The zones of inhibition were measured on agar well containing plant extract. The assay was performed for methanol, chloroform, n-hexane, n-butanol, distilled water and ethyl acetate extracts at concentrations ranging from 1.56-200mg/ml. The zones of inhibition were measured after 24 - 48 h. The results revealed that the maximum zone of inhibition of 40±1.73 was observed against *Xanthomonas stutzeri* among all the extracts, and 100mg/ml chloroform extract produced maximum zone of inhibition for bacterial growth against *Xanthomonas*

stutzeri (36±1.26mm) among all the strains (fig. 2) At 50mg/ml, chloroform extract also exhibited maximum bacterial growth inhibition, and exactly the same pattern was noticed at all the concentrations, extracts and bacterial strains.

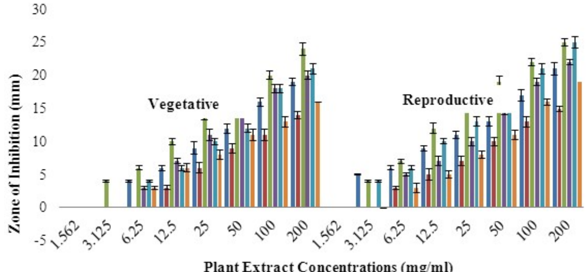

Fig. 1: Zone of Inhibition produced by various conc. of extracts against *Clostridium difficile*

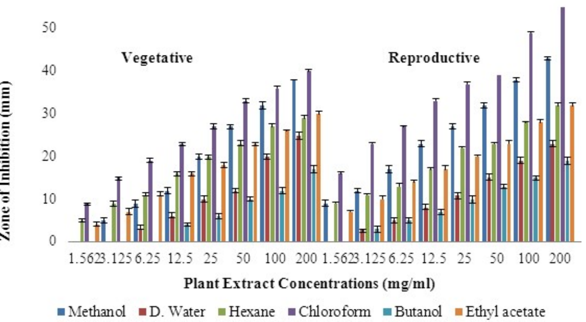

Fig. 2: Zone of Inhibition produced by various conc. of extracts against *Xanthomonas stutzeri*

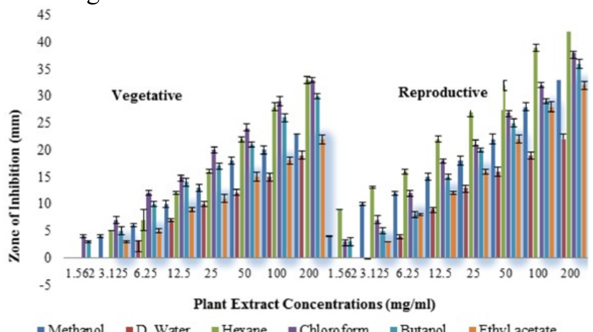

Fig. 3: Zone of Inhibition produced by various conc. of extracts against *Pseudomonas aeruginosa*

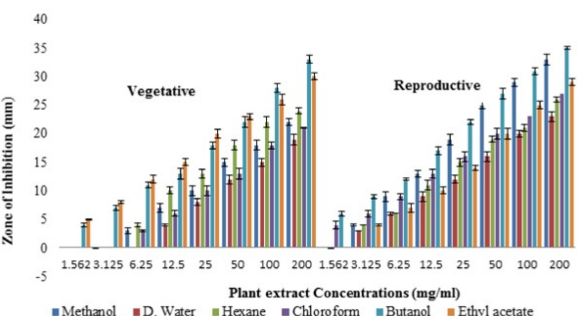

Fig. 4: Zone of Inhibition produced by various conc. of extracts against *Pasturella* sp.

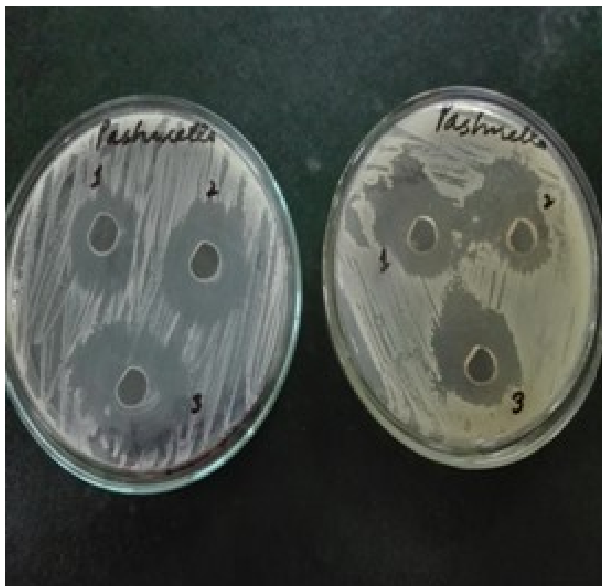

Fig. 4: Zone of Inhibition produced by various conc. of extracts against *Pasteurella sp.*

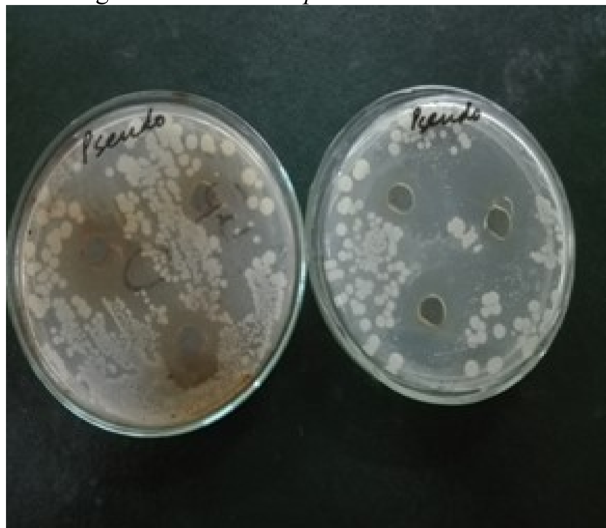

Fig. 5: Antimicrobial activity of *N. sativa* extracts against bacteria

Antioxidant assay

The collected plant materials were extracted in variable solvents viz. methanol, chloroform, water, n- hexane, petroleum ether, and n- butanol and ethyl acetate. The stock solutions were later-on serially diluted (1.562-200 mg/ml) and their antioxidant potential was determined through three different assays.

DPPH radical scavenging assay

The DPPH radical scavenging assay is the most widely used method for measuring the scavenging ability of plant extracts. Radical scavenging activity was found to increase with increasing concentrations. Methanol extract of vegetative part of *N. sativa* showed the same trend among the various dilutions, just like the standard α-tocopherol.

Table 1: Phytochemical Constituents Analysis of *Nigella sativa* Vegetative/Reproductive Parts

Phytoconstituent	Test	Observation	Results	
			Veg.	Rep.
Alkaloid	4ml HCl +0.25 plant powder, warm, filter. Filtrate+ 6 drops of Mayer Reagent	Creamy Precipitate	++	+++
Saponin	0.5 g plant powder+ 5ml distilled water, shake vigorous	Frothing	++	++
Terpenoids	2ml plant chloroform extract, filter. Filtrate + acetic acid+ 1 drop conc. H_2SO_4.	Bluish Green Ring	+++	++
Cardiac glycoside	0.5 g plant powder+2ml Glacial acetic acid+ 4 drops 1% $FeCl_3$+ 1ml Conc. H_2SO_4.	Bluish Green Colour	+	+
Coumarin	0.5 g moistened plant powder, covered with 0.1NaOH, boiling, UV examination	Fluorescence	++	+
Anthraquinone	0.5 g plant powder+ 3ml 1%HCl, Filterate+2ml benzene, shake, removal of benzene layer+ 4 drops 10% NH_4OH	Pink, Violet, Red coloration	+++	+++
Flavonoid	5g Plant powder+ 5ml petroleum ether, filter. Residue+ 20ml 80% ethanol, filter. Filtrate+ 4ml 1% KOH.	Dark yellow color	++	+++
Tannins	0.25g plant powder + 10ml D. water, boil, filter. Filtrate+ 1% Fe Cl_3	Bluish Black color	+++	+++
Phlobatannins	0.25 g plant powder + 5ml 1% aq. HCl	Red precipitates	++	+++

Table 2: Phytochemical profiling of oils from the vegetative and reproductive parts as determined through GC-MS analysis

Compound Name	Molecular Formula	Structures	Retention Time	%age Area	Similarity Index	Molecular Mass	Presence of compound Yes/No (Y/N)	
							Veg	Rep
Palmitic Acid	$C_{17}H_{34}O_2$		31.2	6.5	96	270	Y	Y
Hexadecanoic Acid	$C_{16}H_{32}O_2$		31.6	3.8	92	256	Y	N
Octadeca dienoic Acid	$C_{19}H_{34}O_2$		32.9	23.05	96	294	Y	Y
Linolenic Acid	$C_{19}H_{34}O_2$		33.04	14.6	96	294	Y	Y
Octadecenoic Acid	$C_{19}H_{36}O_2$		33.2	1.68	95	296	N	Y
Octadecanoic Acid	$C_{19}H_{38}O_2$		33.5	35.6	95	298	Y	N
Eicosadienoic Acid	$C_{21}H_{38}O_2$		35.4	1.29	92	322	Y	N
Glyceryl linoleate	$C_{21}H_{38}O_4$		35.6	0.96	89	354	Y	N

Countinue…

Phthalic Acid	$C_{16}H_{22}O_4$		40.08	2.05	95	278	Y	N
3,4 Dimethyl, 6 ethyl phenol	$C_{10}H_{14}O$		31.1	0.17	93	150.2	N	Y
Stearic Acid	$C_{19}H_{38}O_2$		33.2	2.05	95	298	N	Y
Oxacyclo-heptadec 8–en,2-one	$C_{16}H_{28}O_2$		33.5	33.5	93	252	N	Y
Oleic acid	$C_{18}H_{34}O_2$		33.6	12.2	93	282	Y	Y

Table 3: Comparative percentage DPPH values of *N. sativa* vegetative/ reproductive parts

Plant Part	Conc. (mg/ml)	Percentage DPPH Radical Scavenging Values (%)							
		α -Toco	Methanol	Distilled Water	Hexane	Chloroform	Petroleum Ether	Butanol	Ethyl acetate
Vegetative	1.562	69±1.07^{b}	67±0.5^{b}	44±0.5^{ab}	23±1^{a}	44±2.08^{ab}	9±1^{a}	78±0.5^{c}	40±0.6^{ab}
	3.125	73±0.5^{c}	69±1.07^{bc}	56±1.01^{ab}	34±0.5^{a}	50±2^{ab}	10±0.5^{a}	76±0.5	52±0.5^{ab}
	6.25	77±1.5^{c}	72±2^{c}	76±1.01^{c}	37±0.5^{ab}	52±1.2^{b}	17±0.69^{a}	70±0.5	64±1.05^{bc}
	12.5	82±0.5^{bc}	78±0.5^{c}	79±0.5^{c}	40±1.07^{a}	57±1.64^{ab}	23±1.15^{a}	64±0.5^{ab}	70±0.71^{c}
	25	87±1.05^{c}	94±0.69^{d}	82±0.57^{bc}	56±1.5^{ab}	60±1.71^{b}	30±1.5^{a}	54±1^{ab}	81±0.5^{bc}
	50	90±1^{cd}	97±1.07^{d}	85±0.51^{c}	60±1^{bc}	68±0.5^{bc}	35±1^{a}	52±0.71^{b}	84±1.05^{c}
	100	96±0.51^{d}	98±1.07^{cd}	86±0.5^{c}	71±0.5^{b}	76±0.69^{b}	49±1^{ab}	40±1.07^{a}	89±1^{bc}
	200	100±0.5^{d}	100±1.5^{d}	98±1^{c}	76±0.5^{bc}	77±1^{bc}	60±0.5^{b}	35±1^{a}	93±0.51^{c}
Reproductive	1.562	69±1.07^{d}	43±0.5^{a}	46±0.5^{b}	42±0.5^{a}	43±0.5^{a}	63±0.5^{cd}	40±0.5^{a}	56±0.5^{c}
	3.125	73±0.5^{d}	47±0.5^{a}	50±0.5^{bc}	47±0.75^{a}	49±0.51^{b}	70±1^{cd}	49±1^{b}	69±1^{c}
	6.25	77±1.5^{d}	51±1^{a}	59±1	53±1.07^{b}	53±0.75^{b}	79±0.57^{e}	57±0.57^{c}	71±1.05^{cd}
	12.5	82±0.5^{d}	60±1.05^{a}	63±0.75^{b}	60±1^{a}	60±1.5^{a}	86±1.07^{e}	68±0.75^{bc}	78±1^{c}
	25	87±1.05^{d}	68±0.5^{a}	75±1.07^{c}	69±0.5^{b}	69±0.5^{b}	90±0.5^{e}	73±1^{c}	86±0.5^{cd}
	50	90±1^{c}	74±1.07^{a}	84±1^{bc}	76±1^{a}	75±1^{a}	93±1^{d}	80±0.5^{b}	93±1.5^{d}
	100	96±0.51^{c}	87±0.5^{bc}	87±0.5^{bc}	81±0.5^{a}	83±0.5^{b}	97±0.5^{c}	87±1^{bc}	97±0.55^{c}
	200	100±0.5^{d}	94±1^{b}	99±1^{cd}	89±1^{a}	95±0.57^{c}	99±1.05^{cd}	94±0.5^{b}	100±0.75^{d}

Data represent means ± standard error (SE) of three replicates. Different letters in each column indicate significant differences between the treatments at $p<0.05$.

Table 4: Comparison of total antioxidants of *N. sativa* vegetative/reproductive parts

Plant Part	Conc. mg/ml	Absorption Values at 695 nm							
		α Tocopherol	Methanol	Distilled Water	Hexane	Chloroform	Petroleum Ether	Butanol	Ethyl acetate
Vegetative	1.562	0.4±0.00^{d}	0.04±0.00^{ab}	0.02±0.01^{a}	0.04±0.01^{ab}	0.10±0.011^{bc}	0.11±0.00^{bc}	0.14±0.01^{c}	0.06±0.00^{b}
	3.125	0.43±0.01	0.05±0.01^{a}	0.12±0.02^{b}	0.11±0.02^{ab}	0.12±0.03^{b}	0.24±0.01^{c}	0.22±0.00^{c}	0.10±0.02^{ab}
	6.25	0.46±0.02^{d}	0.10±0.00^{a}	0.23±0.03^{b}	0.21±0.01^{ab}	0.21±0.04^{ab}	0.33±0.05^{c}	0.32±0.02^{c}	0.13±0.01^{a}
	12.5	0.48±0.01^{c}	0.15±0.02^{a}	0.35±0.04^{b}	0.32±0.05^{b}	0.26±0.00^{ab}	0.43±0.01^{bc}	0.43±0.01^{bc}	0.19±0.03^{a}
	25	0.50±0.03^{c}	0.31±0.01^{ab}	0.42±0.00^{b}	0.43±0.03^{b}	0.26±0.05^{a}	0.49±0.03^{b}	0.52±0.03^{c}	0.23±0.00^{a}
	50	0.53±0.01^{c}	0.40±0.03^{b}	0.43±0.01^{b}	0.53±0.00^{c}	0.41±0.03^{b}	0.56±0.05^{c}	0.76±0.02^{d}	0.27±0.02^{a}
	100	0.58±0.00^{b}	0.76±0.01^{cd}	0.47±0.01^{ab}	0.61±0.01^{c}	0.46±0.05^{ab}	0.63±0.00^{c}	0.94±0.00^{d}	0.30±0.04^{a}
	200	0.60±0.01^{bc}	1.44±0.05^{e}	0.49±0.05^{ab}	0.64±0.02^{bc}	0.51±0.01^{b}	0.78±0.01^{c}	0.96±0.01^{d}	0.32±0.01^{a}
Reproductive	1.562	0.4±0.00^{d}	0.14±0.01^{a}	0.11±0.02^{a}	0.25±0.00^{c}	0.18±0.00^{b}	0.19±0.02^{b}	0.58±0.02^{cd}	0.11±0.00^{a}
	3.125	0.43±0.01^{c}	0.26±0.00^{b}	0.13±0.03^{a}	0.32±0.01^{bc}	0.21±0.02^{b}	0.25±0.01^{b}	0.65±0.00^{d}	0.17±0.02^{a}
	6.25	0.46±0.02^{c}	0.39±0.02^{bc}	0.16±0.01^{a}	0.43±0.03^{c}	0.26±0.01^{b}	0.31±0.03^{bc}	0.68±0.01^{d}	0.23±0.01^{b}
	12.5	0.48±0.01^{b}	0.43±0.04^{b}	0.27±0.03^{a}	0.50±0.02^{c}	0.30±0.03^{ab}	0.39±0.05^{ab}	0.70±0.02^{d}	0.29±0.03^{a}
	25	0.50±0.03^{b}	0.52±0.01^{bc}	0.29±0.04^{a}	0.52±0.01^{bc}	0.39±0.00^{ab}	0.51±0.01^{bc}	0.78±0.03^{c}	0.37±0.00^{ab}
	50	0.53±0.01^{b}	0.64±0.05^{bc}	0.32±0.01^{a}	0.58±0.10^{b}	0.46±0.04^{ab}	0.59±0.05^{b}	0.80±0.01^{c}	0.44±0.02^{ab}
	100	0.58±0.00^{b}	0.7±0.00c	0.46±0.02^{a}	0.61±0.02^{bc}	0.51±0.01^{b}	0.61±0.01^{bc}	0.86±0.01^{d}	0.50±0.04^{b}
	200	0.60±0.01^{b}	0.98±0.03^{bc}	0.55±0.00^{a}	0.67±0.01^{b}	0.58±0.00^{a}	1.05±0.00^{d}	1.00±0.02^{c}	0.52±0.01^{a}

Table 5: Comparison of total phenolic contents of *N. Sativa* vegetative/reproductive parts

Plant Part	Conc. mg/ml	ug /gram of Gallic acid						
		Methanol	Distilled Water	Hexane	Chloroform	Petroleum Ether	Butanol	Ethyl acetate
Vegetative	1.562	378 ± 0.50^{d}	386 ± 0.01^{d}	300 ± 0.15^{a}	387 ± 0.00	315 ± 0.00^{b}	368 ± 0.01^{c}	368 ± 0.00^{c}
	3.125	396 ± 1.50^{c}	401 ± 0.05^{d}	313 ± 0.00^{a}	400 ± 0.01^{d}	348 ± 0.01^{b}	387 ± 0.02^{bc}	394 ± 0.52^{c}
	6.25	397 ± 0.50^{ab}	402 ± 0.02^{b}	386 ± 0.01^{a}	411 ± 0.001^{c}	385 ± 0.02^{a}	404 ± 0.03	406 ± 0.02^{bc}
	12.5	399 ± 0.50^{a}	411 ± 0.01^{b}	439 ± 0.02^{c}	421 ± 0.02^{bc}	422 ± 0.2^{bc}	463 ± 0.04^{d}	413 ± 0.01^{b}
	25	416 ± 0.19^{a}	414 ± 1.00^{a}	447 ± 0.00^{c}	429 ± 0.03^{b}	425 ± 0.01^{b}	498 ± 0.01^{d}	432 ± 0.02^{bc}
	50	440 ± 0.19^{b}	420 ± 0.03^{a}	462 ± 0.75^{bc}	434 ± 0.00^{ab}	440 ± 0.00^{b}	501 ± 0.00^{c}	463 ± 0.01^{bc}
	100	489 ± 0.50^{bc}	421 ± 0.01^{a}	489 ± 0.01^{bc}	444 ± 0.04^{b}	446 ± 0.54^{b}	589 ± 0.03^{d}	534 ± 0.12^{c}
	200	516 ± 0.15^{ab}	458 ± 0.05^{a}	523 ± 1.00^{b}	547 ± 0.5^{bc}	527 ± 0.03^{b}	600 ± 0.02^{c}	616 ± 0.03^{c}
Reproductive	1.562	378 ± 0.50^{bc}	476 ± 1.50^{d}	365 ± 0.01^{b}	415 ± 0.10^{c}	698 ± 0.01	330 ± 0.00^{a}	439 ± 0.02^{cd}
	3.125	396 ± 1.50^{b}	469 ± 0.01^{bc}	397 ± 0.02^{b}	420 ± 0.02^{ab}	725 ± 0.02^{c}	342 ± 0.02^{a}	475 ± 0.00^{bc}
	6.25	397 ± 0.50^{ab}	474 ± 0.02^{bc}	420 ± 0.05^{b}	447 ± 0.30^{bc}	781 ± 0.03^{d}	343 ± 0.03^{a}	584 ± 0.03^{c}
	12.5	399 ± 0.50^{a}	475 ± 0.04^{d}	448 ± 0.02^{bc}	468 ± 0.03^{c}	810 ± 0.04^{e}	425 ± 0.20^{b}	589 ± 0.02^{cd}
	25	416 ± 0.19^{a}	504 ± 0.01^{c}	463 ± 0.01^{b}	478 ± 0.01^{bc}	812 ± 0.00^{c}	433 ± 0.01^{ab}	592 ± 0.03^{cd}
	50	440 ± 0.19^{a}	512 ± 1.00^{c}	489 ± 0.00^{b}	480 ± 0.00^{ab}	817 ± 0.05^{d}	472 ± 0.03^{ab}	593 ± 0.01^{bc}
	100	489 ± 0.50^{ab}	594 ± 0.01^{bc}	532 ± 0.03^{b}	489 ± 0.03^{ab}	819 ± 0.50^{d}	484 ± 0.05^{a}	609 ± 0.00^{c}
	200	516 ± 0.15^{bc}	623 ± 0.03	567 ± 0.04^{c}	493 ± 0.02^{b}	823 ± 0.01^{d}	487 ± 0.02^{a}	612 ± 0.03^{cd}

The methanol extract of the vegetative part was more effective (100±0.5), when compared to the reproductive (94±0.5). For distilled water extract at 200 mg/ml, the reproductive part was a little bit stronger (99± 0.5) than the vegetative part (97±0.5) but with very minor difference. In case of hexane extract, dilutions of the reproductive part showed higher efficiency towards free radical scavenging (89±0.5), relative to the vegetative part (76±1). Similarly, the chloroform, petroleum ether, butanol and ethyl acetate extracts and their dilutions showed greater potential against free radical production, when compared to the vegetative fractions. Among all the vegetative part extracts, methanol extract and its dilutions showed the highest effects towards free radical production whereas petroleum ether was the mildest. In case of reproductive parts extracts, ethyl acetate extract was the strongest, whereas the butanol extract and dilutions showed the least potential. The IC_{50} value of distilled water extract of the vegetative part was 3.125mg/ml, and for chloroform extract, it was 6.25mg/ml, while for petroleum ether extract, it was 100mg/ml. However, the IC_{50} of the butanol extract of the vegetative part was 50 mg/ml. For the distilled water extract of the reproductive part, the IC_{50} of the chloroform, butanol, ethyl acetate and n-hexane extracts was 3.125mg/ml (table 3)

Total antioxidant assay

The total antioxidants increased with increasing concentrations among all the extracts, similar to the alpha-tocopherol standard. The methanol extract of the vegetative part yielded maximum total antioxidants at 200 mg/ml, among all the extracts and concentrations, whereas distilled water extract of the vegetative part produced the least amount of free antioxidants at 1.56 mg/ml. The methanol extract of the vegetative part was more efficient than the seeds, whereas among all other extracts, the seeds were more efficient than the vegetative fraction. The results showed that the butanol extract had the highest antioidant activity which was quite comparable with standard alpha- tocopherol (table 4)

Total phenolic contents assay

The total phenolic contents were determined colorimetrically and expressed as gallic equivalents. Total Phenolic contents increased with increasing extract concentration. Gallic acid was used to standardize the equation and the determination of total phenolic contents among the extracts. Ethyl acetate extract of the vegetative part yielded the highest phenolic content (616 ± 0.03^{c} ug/g) at 200mg/ml, while the hexane extract was the lowest with 300 ± 0.15^{a} ug/g phenolic content at 1.56mg/ml. For the seeds, the petroleum ether extract yielded 823±0.01 ug/g of gallic acid equivalents (the highest), whereas the butanol extract was the least in phenolic content (330± 0.02ug/g) among all the solvents at 1.56mg/ml (table 5)

Gas chromatography mass spectrometry (GC-MS) analysis

Gas Chromatography Mass Spectrometry of hexane extracts of both vegetative and reproductive parts were recorded in terms of their retention times, percentage area of peaks, and similarity with the NIST 27 library, and ultimately the probable names of the compounds and their structures were deduced. Overall, 9 compounds were identified from the vegetative part, whereas 11 compounds were obtained from the reproductive part (tables 2).

The GCMS results showed the presence of palmitic acid, linoleic acid, octadecenoic acid, stearic acid, octadecadienoic acid, eicosadienoic acid and phthalic acid in variable amounts, at levels very much comparable in

both parts (vegetative and reproductive). *Nigella sativa* is tremendously used in multiple health problems due to the presence of these constituents. The level of palmitic acid in the vegetative part was 6.56% (fig. 6) whereas in reproductive part, it was 4.58%. The level of octadecenoic acid was 33.04% in the reproductive part and 35.04% in the vegetative part.

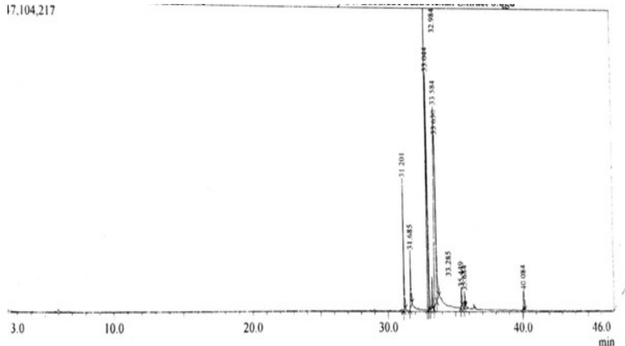

Fig. 6: GC-MS Chromatogram of *Nigella sativa* oil Vegetative part oil

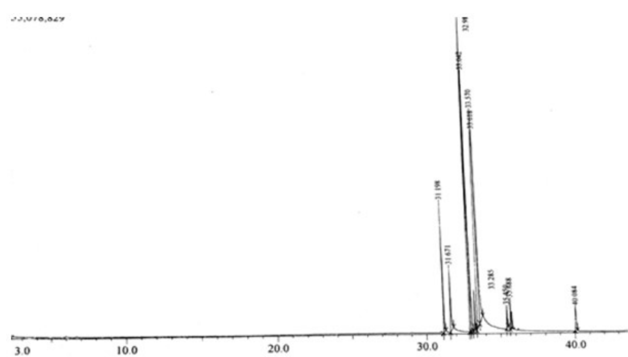

Fig. 7: GC-MS chromatogram of *Nigella sativa* oil reproductive part oil

DISCUSSION

The presence of secondary metabolites in plants is meant for therapeutic purposes. The presence of phytochemicals in the current study is similar to those reported by Gul *et al*., 2017), Ochwang *et al*., (2016) and Yadav *et al*., (2014). Phenolic compounds such as flavonoids are known to possess antioxidant, anti-carcinogenic and anti-apoptotic properties, and they also inhibit the growth of microbes (Yadav, 2014). In the literature, it has been reported that coumarins possess anti-tumor properties, and saponins exhibit anti-inflammatory and anti-tumorigenic properties (Ochwang *et al*., 2016). The anti-carcinogenic and antitumor properties of tannins have been attributed to their antioxidant potential (Becker, 1984). Cardiac glycosides, the free form of flavonoids in plants, are very beneficial for humans due to their anti-arthritic, antibacterial and antiangiogenic effects (John *et al*., 2014). Alkaloids are supposed to be major contributors to plant growth regulation and defense (Dominic *et al*., 2014). Thymoquinone is a major phytochemical component of *Nigella sativa.* Quinones have antibacterial, antioxidant, anti-plasmodial, and anti-HIV properties because of the redox properties of their carbonyl functions. Thymoquinone possesses antitumor, hepatoprotective, antioxidant and anti-inflammatory properties (Khader and Eckl, 2014).

The results of radical scavenging activity obtained for the seeds are in agreement with those reported in other studies (Ali *et al*., 2017). The trend obtained in all the extracts was quite identical with standard alpha tocopherol. Indeed, some of the extracts were superior to the standard alpha tocopherol in their scavenging potential. In the present study, it was noticed that the extracts exhibited the ability of reduction or oxidation by accepting or donating electrons, respectively. Due to their radical scavenging activity, antioxidants are quite beneficial for disease management (Djacbou *et al*., 2014). The therapeutic potential of plants and their extracts are dependent on their free radicas scavenging ability. The presence of reductones depicts the reducing ability of extracts (Sasikumar and Kalaisezhiyen, 2014). Free radicals have been very effective for the treatment of number of ailments such as AIDS, cancer, hepatitis, physiological stress and body inflammation (Hanif *et al*., 2017) The greater the number of toxic oxygen species, the greater the chances of disease occurrence (Ramakarishna *et al*., 2012). Synthetic antioxidants have hazardous side effects. Nature has overcome this problem by production of free radicals to combine with these reactive oxygen species and minimize oxidative stress **(**Sasikumar and Kalaisezhiyen, 2014).

A strong correlation between total antioxidants and total phenol contents of extracts has been reported (Chaouche *et al*., 2014). Reactive oxygen species (ROS) such as hydroxyl, peroxyl, super oxide and nitric oxides attack the primary and secondary metabolites in the body, resulting ultimately in various diseases. They (ROS) may cause food deterioration by reducing their shelf lives (Pisoschi and Negulescu, 2011). Ammonium molybdate is the key reagent in total antioxidant capacity determination, and as it is added to the reaction mixture, the color changes. The greater the color variation, the greater would be the reducing power of extracts (Phatak and Hendre, 2014).

There is always a positive correlation between phenolic contents and free radicals, and as the phenolic contents increase, the antioxidant nature of plant increases (John *et al*., 2014). Flavonoids suppress ROS formation, and hence enhance antioxidant potential of extracts (Baba and Malik, 2015). Peroxidation results in lipid destabilization, and phenolic compounds are very effective in checking the destabilization and inhibition of oxidizing enzymes (Hanif *et al.,* 2017, Harha *et al.,* 2012) Chandara *et al*., 2014). Flavonoids impart characteristic colors to plants (yellow – brown), whereas in humans, they play anti-

cancerous, anti-mutagenic, and anti-inflammatory roles (Rebaya *et al*., 2014). The structure of flavonoids and other chelating compounds depict their antioxidant nature (Stankovic *et al*., 2011).

CONCLUSION

The results of the current study indicate that the vegetative part of *N. sativa* plant could be an efficient natural alternative source of oil for developing pharmaceutical formulations against multiple ailments. In future studies, the separation of the pure compounds with bioassay-guided extraction and quantification analysis of the pure bioactive compounds are highly recommended.

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