Indian Journal of Natural Products and Resources
Vol. 14(1), March 2023, pp.
DOI:

Impact of geographical location on the polyphenolic content of *Nigella sativa* seed extract and the relative anti-inflammatory and antioxidant potential thereof: A comparative study

Singhal Rini[1]*, Verma Shweta[1], Singh Vijender[2] and Bajaj Sakshi[3]

[1]Department of Pharmacy, IFTM University, Moradabad, Uttar Pradesh 244102, India

[2]Rakshpal Bahadur Pharmacy Institute, Greater Noida 201306, India

[3]Delhi Institute of Pharmaceutical Sciences and Research, Delhi Pharmaceutical Science and Research University, PushpVihar, New Delhi 110017, India

Received 22 November; revised received 19 February 2023; accepted 24 February 2023

The herb *Nigella sativa* belonging to the family Ranunculaceae is a well-investigated plant that has a wide range of pharmacological effects. The study aimed to evaluate and compare the antioxidant and anti-inflammatory potential as well as phytochemical screening in terms of total phenolic and flavonoid content of *N. sativa* seeds collected from various locations in India to establish the impact of geographical location on phytoconstituents of the seeds. The total phenolic and flavonoid content in methanolic extracts were evaluated using the Folin-ciocalteu reagent and $AlCl_3$ colourimetric method respectively. The antioxidant activities of the extracts were evaluated using the DPPH method, while the anti-inflammatory activity of the extracts was determined *in vitro* using the bovine serum albumin denaturation assay. The extract of the seed from Vizag had the highest concentration of polyphenols, followed by those from Madhya Pradesh and Punjab. The study confirmed variations in the quantity of the phytoconstituents of *N. sativa* seeds of different geographical origins and their consequential impact on the antioxidant and anti-inflammatory activity indicating that the geographical location exerts a vital effect on the same.

Keywords: Anti-inflammatory, Antioxidant, Fennel flower, *Nigella sativa*, Polyphenols

IPC code; Int. cl. (2021.01)-

Introduction

Medicinal plants have been used for a range of diseases since ancient times, and according to the WHO, traditional medications are used to treat 80 per cent of the population in underdeveloped nations[1]. Furthermore, as compared to synthetic drugs, medicinal plants used to make herbal medicine have fewer negative effects and so, researchers are interested in investigating numerous plants for their prospective pharmacological activity, mechanism of action, efficacy, safety, and toxicological investigations which help for the development of innovative medicine to cure ailments, as an abundance of herbs is available around the world.

There are 18 species in the genus Nigella that are used in traditional medicine. One species of the Nigella genus, *Nigella sativa* (NS), also known as fennel flower, black cumin, kalonji, upakunchika (Sanskrit), or habbatulbarakah (Arabic), is a popular spice and a member of the Rannunculaceae family[2,3].

Traditionally, the seeds of NS were widely used to treat a variety of diseases such as asthma, cough, bronchitis, headache, rheumatism, fever, headache, kidney and liver disorders, influenza, and eczema[4-5]. The recent research on NS seed oil and extract indicates its usage in rheumatoid arthritis, asthma, inflammatory diseases, diabetes, and digestive diseases[6-7]. These beneficial effects developed a commercial interest in the pharmaceutical industry to produce novel medicines that can treat a variety of diseases.

A review of the literature reveals that studies on the phytochemistry of Nigella species have largely ignored the phenolic composition and other elements that influence the quantity of polyphenols, focusing instead on the biological activities of volatile or fatty oils.

Polyphenols are secondary plant metabolites which are biosynthesized through phenylpropanoid and shikimic acid pathways which are typically involved in defence against ultraviolet radiation or pathogen

*Correspondent author
Email: rini.pharma20@gmail.com

aggression. It is thought that they help plants to adapt when they are under stress from environmental changes[8].

In recent decades, Polyphenols attracted attention in the field of nutrition due to their antioxidant and anti-inflammatory properties[9]. Expanding research studies suggest that consuming polyphenols may be essential for maintaining good health by controlling metabolism, weight, chronic disease, and cell proliferation[10-11].

Polyphenols have a variety of pharmacological effects and can be used in the treatment of diabetes[12], cancer[13], osteoporosis[14], cardiovascular[15], and neurodegenerative diseases[16]. They exert anti-inflammatory, immunomodulatory, and anti-rheumatoid effects via influencing tumour necrosis factor, mitogen-activated protein kinase, nuclear factor kappa-light-chain-enhancer of activated B cells, and c-Jun N-terminal kinases[17]. It also has vasodilator properties and can improve lipid profiles and slow the oxidation of low-density lipoproteins[18]. Polyphenols are thought to be potential pharmacological modulators of neuroinflammation via the Keap1/Nfr2/ARE pathway, which provides a strong rationale for treating neurodegenerative disorders[19]. Polyphenols, in combination with other micronutrients, reduce oxidative stress parameters and bone resorption, potentially lowering the risk of osteoporosis[20]. Thus, it shows that polyphenol content is very important, and from a literature search, it was also revealed that the polyphenolic content of the plant can be greatly influenced by a variety of factors, including processing, storage, and the degree of ripeness at harvest[21]. In addition, polyphenolic content in food is also significantly influenced by environmental and edaphic factors, including soil type, sun exposure, rainfall, etc.[22].

Therefore, the present study was conducted to address the knowledge gap regarding variation in polyphenolic content. The study aimed to determine the geographical impact on the quantity of polyphenol content, anti-inflammatory, and antioxidant activities. Hence, this study paves a path for the selection of NS seed based on geographical location and its utilization for further investigations.

Materials and Methods

Plant material

The seeds of NS were collected from Neemuch (Madhya Pradesh NSMP), Ludhiana (Punjab NSPB) and Rajahmundry (Vishakhapatnam NSVZ) in the month of March 2021 and were identified by RHMD, CSIR- National Institute of Science Communication and Information Resources. The voucher specimen NISCAIR/RHMD/2021/ 4142-43-1, NISCAIR/ RHMD/2021/4142-43-2, NISCAIR/ RHMD/ 2021/4142-43-3 has been deposited in the herbarium for future reference. Fig. 1. Shows a picture of the collected seed.

Sample preparation and extraction

The air-dried and finely grounded seeds of NS were extracted by cold maceration using absolute methanol. On the incubator shaker, extraction was conducted for 72 h. After that, Whatman filter paper was used to filter the extract. After filtration, the solvent was evaporated under vacuum to dryness using rotavapor (Buchi) and the product was stored at +4°C until analysis[23]. Fig. 2 explains the schematic representation of the extraction of phytoconstituents.

Preliminary phytochemical screening

Preliminary qualitative phytochemical tests have been carried out on NS seed extract (NSSE) to ensure the presence or absence of various phytoconstituents like flavonoids, glycosides, alkaloids, saponins, carbohydrates, lipids, phenols, and tannins[24].

Fluorescence analysis

The fluorescence analysis technique is employed to determine the presence of chromophores in the extracts as well as for the qualitative evaluation of herbal drugs. Fluorescence analysis is based on the principle that organic molecules typically absorb light

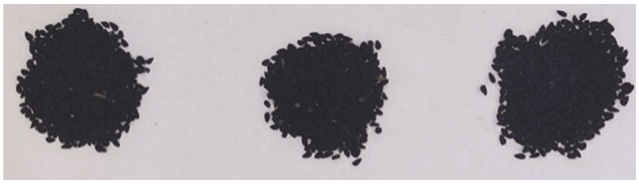

Fig. 1 — Picture of *Nigella sativa* seeds collected from various locations.

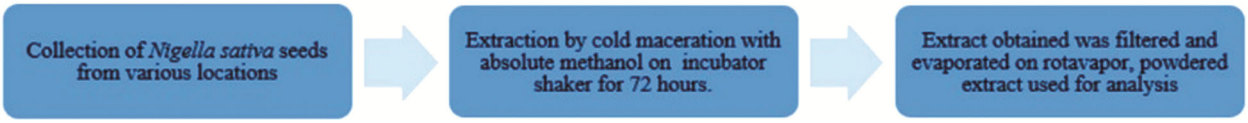

Fig. 2 — Schematic figure representing extraction of *Nigella sativa* seed.

within a certain range of wavelengths and many of them reemit similar radiations when the drug is treated with different chemical reagents[25]. When NSSE was treated with various chemical reagents, the wide range of coloured fluorescence that was produced in a UV fluorescence cabinet during the day as well as, in both short (254 nm) and long (366 nm) wavelengths, was carefully observed to identify fluorescence compounds.

Total phenolic content (TPC) determination

The main contributors to antioxidant activity are phenolic molecules, which are thought to have the greatest potential to neutralize free radicals[26].TPC of NSSE was determined by colourimetric assay using Folin-ciocalteu reagent (mixture of tungsten and molybdate). The gallic acid in different concentrations with the same solvents as in the test was used as the standard to prepare the calibration curve. This method requires alkaline conditions which enable the phenolic component to transfer the electrons to phosphomolybdic/phosphotungstic acid complexes. These electron transfers, let a colour change, which is then visible at 765 nm in the visible spectrum[27]. Briefly, 0.2 mL of 10% Folin-ciocalteu reagent was added to 0.5 mL of NSSE. The solution was shaken well. To make the solution alkaline, 4 mL of 1M sodium carbonate was added. The solution was again mixed properly and allowed to stand for 2 h in dark and absorbance was taken at 765 nm. The same procedure was repeated for gallic acid (standard) to form a standard calibration curve[28].

Total flavonoid content (TFC) determination

Flavonoid is a prevalent class of polyphenolic compounds and is responsible for antioxidant activity. The antioxidant capacity depends on the molecular structure (eg. position of the hydroxyl group) of flavonoids[29]. TFC of NSSE from different geographical origins was estimated using the aluminium chloride ($AlCl_3$) colourimetric method. This method is based on the principle that $AlCl_3$ forms acid-stable complexes with the keto group which is present at position C-4 as well as the hydroxy group present at position C-3 and C-5 in flavone and flavanol. Additionally, $AlCl_3$ also combines with ortho-dihydroxyl groups present on the A or B ring of flavonoids to form acid labile complexes[30]. Briefly, 0.1 mL of extract was taken, and 1.5 mL of methanol was added to it. After 5 min, 0.1 mL of 10% $AlCl_3$ was added and a quick spin was given to the solution. Then 0.1 mL of 1M sodium acetate was added. The solution was mixed well and incubated for 45 min. Absorbance was taken at 415 nm and quercetin in different concentrations using the same solvents was used as the standard to prepare the standard calibration curve[31].

Determination of antioxidant activity (DPPH Assay)

DPPH radical is used in this assay to estimate the total antioxidant compound's capacity to quench the DPPH radical and prevent its harmful effects. Delocalized spare electron over DPPH radical makes it stable and thus, prevents dimer formation. The antioxidant component present in the extract reduces DPPH to its non-radical form and the colour changes from dark purple to colourless or pale yellow[32].

To determine antioxidant activity, NSSE in different concentrations were added to 2 mL of 0.1 mM methanolic solution of DPPH. The solutions were then incubated in dark for 30 min and absorbance was determined against blank at 517 nm to calculate the percentage radical scavenging activity (% RSA). Finally, The IC50 was calculated using the equation obtained after plotting radical scavenging activity against extract concentration on a graph[33].

Determination of anti-inflammatory activity

Protein denaturation is a process in which the tertiary and secondary structure of proteins gets disoriented due to external stress factors[34]. The anti-inflammatory activity of NSSE was determined in *in vitro* settings using heat-induced Bovine serum albumin (BSA) denaturation assay. This assay's implementation is justified by the fact that albumin protein denaturation produces antigens that trigger type III hypersensitive reactions and inflammation[35].

To assess anti-inflammatory activity, 5 mL of the reaction mixture was prepared which consists of 0.2 mL of 1% bovine serum albumin (BSA), 4.78 mL of phosphate-buffered saline (PBS, pH 6.4), and 0.02 mL (in various concentration i.e. 25, 50, 100, 150, and 200 µg/mL) of NSSE or 0.2 mL of reference drug (Diclofenac). The mixture was then mixed properly and incubated in a water bath at 37°C for 15 min. The incubation mixture was then heated at 70°C for 5 min and absorbance was measured at 660 nm after cooling. All the experiments were performed in triplicate and results were calculated as per cent inhibition of BSA denaturation using the formula[36].

$$\% \text{ inhibition of BSA denaturation} = 100 \text{ X } (1 - A2 / A1)$$

where, A1 is the absorption of the control sample i.e. phosphate buffer, and A1 is the absorption of the test sample (NSMP, NSPB, NSVZ).

Results

Preliminary phytochemical screening

Alkaloids, carbohydrates, flavonoids, tannins, terpenoids, and Phenolic compounds were all present in the methanolic extract of NS seeds from all the locations. In all samples, the differences in the results were not significant, but there were variations in the colour intensity. The outcomes are displayed in Table 1.

Fluorescence analysis

The variations in colours were observed in daylight, short wavelength and long wavelength carefully after treating NSSE with various reagents. The results are presented in Table 2.

Total phenolic content

TPC was estimated by gallic acid and expressed as mg gallic acid equivalent, (mg GAE/g) of extract. Total phenolic content was found to be highest in the NSSE sample collected from Vizag i.e. 5.22 mgGAE/g followed by M.P (3.38 mgGAE/g) and Punjab (0.197 mgGAE/g) which was calculated using equation y = 0.0023x + 0.1093 (R^2 = 0.992) established from gallic acid standard calibration curve as shown in Fig. 3a. Where y = absorbance of the sample at 765 nm and x= concentration in NSSE.

Table 1 — Results of phytochemical screening

Phytochemical test	NSVZ Methanolic extract	NSMP Methanolic extract	NSPB Methanolic extract
Alkaloids	+	+	+
Carbohydrates	-	-	-
Phenols	+	+	+
Flavonoids	+	+	+
Terpenoids	+	+	+
Tannins	+	+	+

Note: + indicates presence and – indicates absence of phytochemical

Table 2 — Fluorescence analysis of NS seeds with different reagents

Extract	Visible/Daylight	UV- 254 nm	UV- 366 nm
Aqueous	Pale yellow	Light green	Light blue
Pet ether	Peach	Lemon	Cobalt blue
Methanol	Pale yellow	Parrot green	Blue
Acetone	Pale yellow	Light green	Cobalt blue
Ethyl acetate	Pale yellow	Pale green	Light blue
Chloroform	Light yellow	Lime	Sky blue
Ammonia	Coffee	Malachite green	Army green
10 % NaOH	Coffee	Dark green	Brownish black
Conc. HNO_3	Coffee	Dark green	Brownish black
1M H_2SO_4	Lemon	Pale green	Cobalt blue
I M HCl	Pale yellow	Pale green	Cobalt blue

*The variations in colour observed were the same for NSVZ, NSMP, NSPB

Total flavonoid content

TFC of methanolic extract of NS was expressed as mg quercetin equivalents/ g of extract. TFC was found to be highest in the sample collected from Vizag i.e., 1.43 mg QE/g followed by Madhya Pradesh (1.06 mg QE/g) and Punjab (0.45 mg QE/g) which was calculated using equation y = 0.011x + 0.0074 (R^2 = 0.998), established from quercetin standard calibration curve which is shown in Fig. 3b. Where y is the absorbance of the sample at 415 nm and x is concentration in NS seed extracts.

DPPH radical scavenging assay

U.V.Visible spectra for various concentrations of NSSE clearly reflect the antioxidant potential of extracts as indicated in the graph. In DPPH assay ascorbic acid was used as standard and the IC50 value was calculated for ascorbic acid from the equation y= 0.4102X + 48.265 as shown in Fig. 4a. It was found to be 4.22±.01 µg/mL while IC50 values of NSVZ, NSMP and NSPB were found to be 37.82±.04, 51.83±.07, and 154.46±1.09 µg/mL respectively as shown in Fig. 4b.

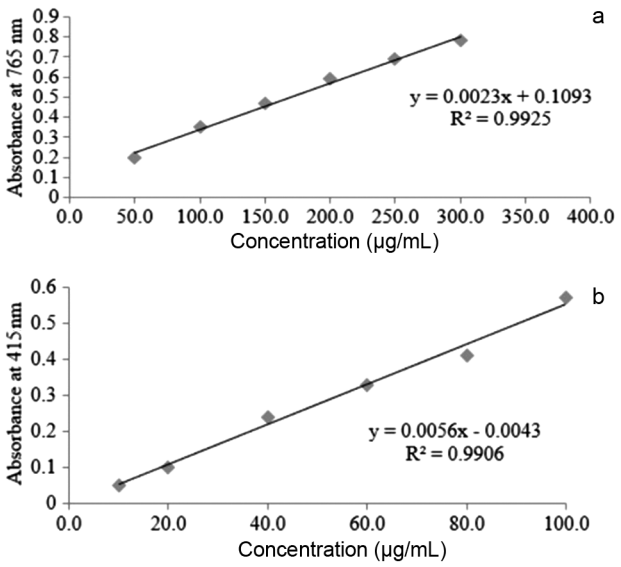

Fig. 3 — Standard calibration curve for, a) gallic acid for TPC; and b) quercetin for TFC.

Anti-inflammatory activity

Protein denaturation is a well-known contributor to inflammation. So, the *in vitro* anti-inflammatory activity of all the NSSE was determined using a bovine serum albumin protein denaturation assay and the result is shown in Fig. 5. As per the results, NSSE from the Vizag region showed the best anti-inflammatory activity followed by NSMP and NSPB.

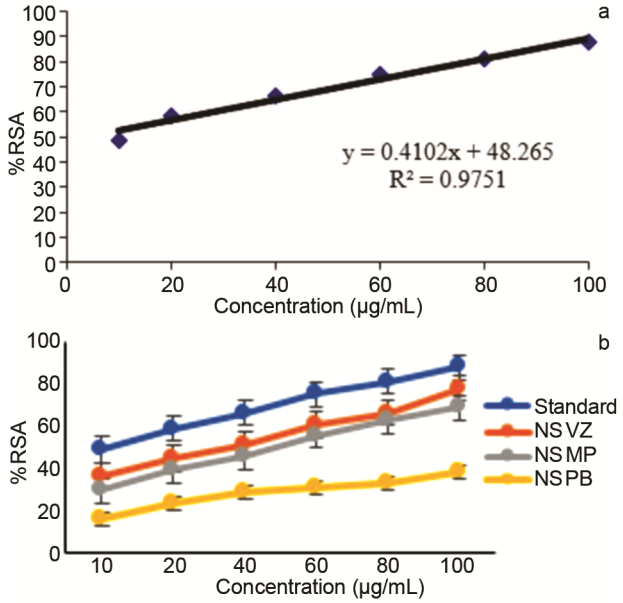

Fig. 4 — a) Standard calibration curve for Ascorbic acid; and b) DPPH activity of methanolic NSSE from different locations in comparison to AA. Results is expressed as mean±SD.

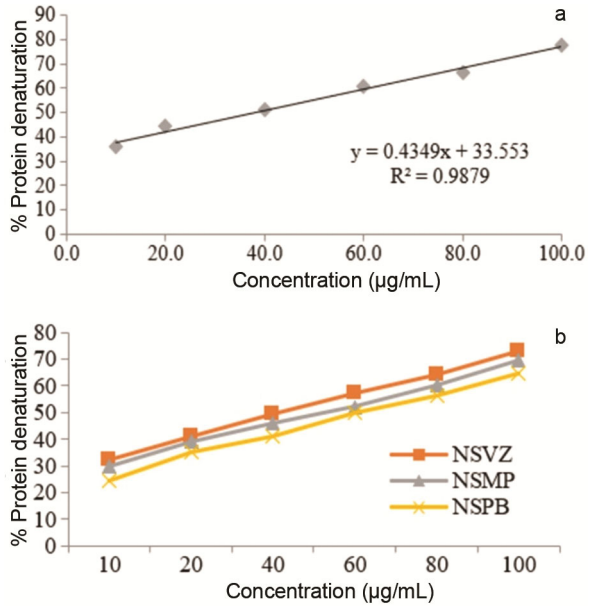

Fig. 5 — a) Standard calibration curve for Diclofenac; and b) Percentage protein denaturation of methanolic NSSE from different locations. Results are expressed as mean±SD.

IC50 value was calculated from individual regression equation and found to be 37.90±.06 µg/mL for diclofenac (standard). It was found to be 44.76±.08, 53.13±.06, and 57.67±.04 µg/mL for NSVZ, NSMP, and NSPB respectively.

The correlation between TPC, TFC, and antioxidant activity

Numerous studies have shown a positive relationship between total phenolic, flavonoid, and antioxidant activity[37]. Fig. 6 depicts the significant correlation between total phenolic (DPPH, $R^2 = 0.978$) and flavonoid content (DPPH, $R^2 = 0.9115$) with antioxidant activity. By comparing the correlation coefficients (R-values), it was also possible to conclude that TPC and TFC play a significant role in the antioxidant activity of NSSE.

Discussion

Regional cultivars of NS seeds can be grown from sea level to 2500 m and the quality of NS seeds can be affected by climatic factors or regions (Altitude). The purpose of this study is to determine the effect of geographical region on NS seed polyphenol content (TPC and TFC) and relative antioxidant and anti-inflammatory activity[38].

To establish the variation, seeds of NS were gathered from three different commercially cultivated agro-climatic regions in India. In the results, we observed a marked difference in the TPC and TFC levels as well as in *in vitro* activities.TPC of extracts

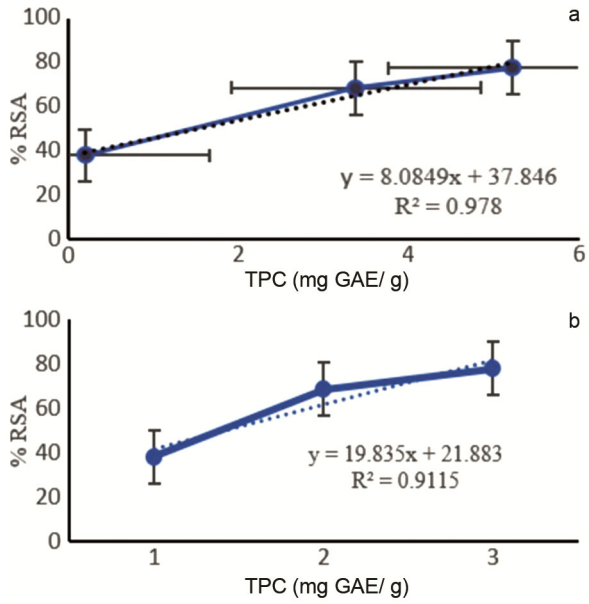

Fig. 6 — a) % RSA versus TPC; and b) % RSA versus TFC respectively. The coefficient correlation values for TPC ($R^2 = 0.978$) and TFC ($R^2 = 0.9115$) was observed.

of sample collected from Vizag, MP, and Punjab was calculated as 5.22, 3.88 and 0.197 mg GAE/gm respectively while TFC was calculated as 1.43, 1.06 and 0.45 mg QE/g in NSVZ, NSMP and NSPB respectively. These results indicate, that the best TPC and TFC were found in the sample collected from the region of Vizag, and TPC as well as TFC varied with geographical location.

Our results showed that NSSE of the Vizag location were rich in TPC and TFC as well as possessed greater antioxidant and anti-inflammatory efficacy as compared to NSSE of other locations. Variation in the phenolic content due to the geographical location was reported by Adhikari *et al.*, The authors studied and concluded that geographical location exerted an effect on TPC, TFC, and antioxidant activity of the *Withania somnifera* fruit extract[39].

Toma *et al.* observed TPC (4.12±0.02 mg GAE/g) and TFC (2.01±0.01 mg QE/g) in NS seeds collected from Arad country, Romania[8]. Ahmed *et al.*, have quantified the biomarker thymoquinone (TQ) from various geographical regions and concluded that variation exists between the concentrations of TQ gathered from various locations. The differences in concentration were explained with the reasons altitude variations, and phytochemical degradations due to reasons like improper storage, shipping etc.[40].

Kabir *et al.* also reported variation in TQ content in Indian (Imported to Japan from India) and Bangladeshi NS seeds but the reasons for variation were not discussed in the research[41]. A previous study by Sen*et al.* also states the variation in antioxidant activity of NS seed collected from various regions of Turkey may be due to variations in environmental conditions or genetic background[42].

As per the findings and literature, it is asserted from this study that the concentration of TPC and TFC in the sample is correlated with antioxidant and anti-inflammatory activity. Higher concentration of polyphenols in plant leads to higher antioxidant and anti-inflammatory activity[43]. The present results are found to be similar with another reported scientific investigation, which states a positive correlation of TPC and TFC with anti-inflammatory and antioxidant activity[44]. All these observations support the finding that TPC and TFC vary with geographical locations and quantity of polyphenol in plant is relative to their anti-inflammatory and antioxidant potential.

Conclusion

Plants contain a variety of secondary metabolites that give them their pharmacological activity. These secondary metabolites vary with environmental conditions. India is known for its extreme seasonal variations with other environmental fluctuations. Therefore, we collected NS seed from different locations in India and investigated for polyphenol content. The result of the present study showed the presence and variation in polyphenol content among the samples. These differences reflected in TPC and TFC values are relative to the *in vitro* anti-inflammatory and antioxidant activities. Among the samples, the best polyphenol content was found in the sample collected from Vishakhapatnam region followed by Madhya Pradesh and Punjab. These variations in the study can be attributed to differences associated with the locations or climatic condition where the seeds were grown, hence indicating that the geographical location exerts a vital effect on the active constituent's content as well as on the antioxidant and anti-inflammatory potential of NSSE.

Conflict of interest

The authors has no conflicts of interest.

References

1 Ekor M, The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety, *Front Pharmacol*, 2014, **10**, 177.

2 Madhulika S, Vijayalaxmi A and Narasimha V, Physicochemical and preliminary phytochemical analysis of Upakunchika (*Nigella Sativa* Linn) seed in different solvent extracts, *Int Ayu Med J*, 2017, **5**(12), 4292-4297.

3 Yimer E M, Tuem K B, Karim A, Ur-Rehman N and Anwar F, *Nigella sativa* L. (black cumin): A promising natural remedy for wide range of illnesses, *Evid-Based Complement Altern Med*, 2019. doi: 10.1155/2019/1528635

4 Ediriweera E R, Rathnayaka R L, Premakeerthi W M and Weerasinghe K D, Efficacy of Sri Lankan traditional decoction of Katuwelbatu Deduru Katukadi in treatment of Kaphaja Shira Shula (chronic sinusitis), *Ayu*, 2010, **31**, 58.

5 Meziti A, Meziti H, Boudiaf K, Mustapha B and Bouriche H, Polyphenolic profile and antioxidant activities of *Nigella sativa* seed extracts *in vitro* and *in vivo*, *Int J Biotechnol Bioeng*, 2012, **6**(4), 109-117.

6 Kooti W, Hasanzadeh-Noohi Z, Sharafi-Ahvazi N, Asadi-Samani M and Ashtary-Larky D, Phytochemistry, pharmacology, and therapeutic uses of black seed (*Nigella sativa*), *Chin J Nat Med*, 2016, **14**, 732-745.

7 Toma C C, Olah N K, Vlase L, Mogoşan C and Mocan A, Comparative studies on polyphenolic composition, antioxidant and diuretic effects of *Nigella sativa* L. (black cumin) and Nigella damascena L. (lady-in-a-mist) seeds, *Molecules*, 2015, **20**, 9560-9574.

8 Mutha R E, Tatiya A U and Surana S J, Flavonoids as natural phenolic compounds and their role in therapeutics: An overview, *Future J Pharm Sci*, 2021, **7**, 1-3.

9 Yahfoufi N, Alsadi N, Jambi M and Matar C, The immunomodulatory and anti-inflammatory role of polyphenols, *Nutrients*, 2018, **10**, 1618.

10 Rudrapal M, Khairnar S J, Khan J, Dukhyil A B, Ansari M A, *et al*., Dietary polyphenols and their role in oxidative stress-induced human diseases: Insights into protective effects, antioxidant potentials and mechanism (s) of action, *Front Pharmacol*, 2020, **13**, 283.

11 Cory H, Passarelli S, Szeto J, Tamez M and Mattei J, The role of polyphenols in human health and food systems: A mini-review, *Front Nutr*, 2018, **5**, 87.

12 Aryaeian N, Sedehi S K and Arablou T, Polyphenols and their effects on diabetes management: A review, *Med J Islam Repub Iran*, 2017, **31**, 134.

13 Briguglio G, Costa C, Pollicino M, Giambo F, Catania S, *et al.*, Polyphenols in cancer prevention: New insights, *Int J Food Sci Nutr*, 2020, **1**, 1.

14 Đudarić L, Fužinac-Smojver A, Muhvić D and Giacometti J, The role of polyphenols on bone metabolism in osteoporosis, *Food Res Int*, 2015, **77**, 290-298.

15 Michalska M, Gluba A, Mikhailidis D P, Nowak P, Bielecka-Dabrowa A, *et al.*, The role of polyphenols in cardiovascular disease, *Med Sci Monit*, 2010, **16**, RA110-9.

16 Bhullar K S and Rupasinghe H P, Polyphenols: Multipotent therapeutic agents in neurodegenerative diseases, *Oxid Med CellLongev*, 2013, **2013**, 891748.

17 Maity S and Wairkar S, Dietary polyphenols for management of rheumatoid arthritis: Pharmacotherapy and novel delivery systems, *Phytother Res*, 2022, **36**, 2324-2341.

18 Quiñones M, Miguel M and Aleixandre A, Beneficial effects of polyphenols on cardiovascular disease, *Pharmacol Res*, 2015, **68**, 125-131.

19 Ontario M L, Siracusa R, Modafferi S, Scuto M, Sciuto S, *et al.*, Potential prevention and treatment of neurodegenerative disorders by olive polyphenols and hydrox, *Mech Ageing Dev*, 2022, **203**, 111637.

20 Kang N N, *Oxidative stress and the risk of osteoporosis: The role of dietary polyphenols and nutritional supplements in postmenopausal Women*, (University of Toronto, Canada), 2012.

21 Karlund A, Moor U, Sandell M and Karjalainen R O, The impact of harvesting, storage and processing factors on health-promoting phytochemicals in berries and fruits, *Processes*, 2014, **2**, 596-624.

22 Pandey K B and Rizvi S I, Plant polyphenols as dietary antioxidants in human health and disease, *Oxid Med Cell Longev*, 2009, **2**, 270-278.

23 Mohammed Y H, Ghaidaa J M and Imad H H, Analysis of bioactive chemical compounds of *Nigella sativa* using gas chromatography-mass spectrometry, *J Pharmacogn* Phytother, 2016, **8**, 8-24.

24 Trease G E and Evans W C, *Pharmacognosy*, 12th edn, (Bailliere Tindall, A division of Cas-sell Ltd.: Eastbourne BN. 2130N), 1983, 104-158.

25 Tewari D, Sah A N, Bawari S, Sharma H and Mangal A K, Pharmacognostical evaluation and HPTLC fingerprinting identification of Ficuspalmata Forssk.(Bedu) from Western Himalaya, *Curr Bioact Compd*, 2018, **14**, 180-190.

26 Sánchez-Rangel J C, Benavides J, Heredia J B, Cisneros-Zevallos L and Jacobo-Velázquez D A, The folin–ciocalteu assay revisited: Improvement of its specificity for total phenolic content determination, *Anal Methods*, 2013, **5**(21), 5990-5999.

27 Ford L, Theodoridou K, Sheldrake G N and Walsh P J, A critical review of analytical methods used for the chemical characterisation and quantification of phlorotannin compounds in brown seaweeds, *Phytochem Anal*, 2019, **30**(6), 587-599.

28 Bajaj S and Wakode S, Evaluation of total phenolic, flavonoid, *in vitro* antioxidant potential and anti-inflammatory activity of successive extracts of swertiaalata, *Bull Env Pharmacol Life Sci*, 2017, **6**, 70-78.

29 Bhaigyabati T H, Devi P G and Bag G C, Total flavonoid content and antioxidant activity of aqueous rhizome extract of three Hedychium species of Manipur valley, *Res J Pharm Biol Chem Sci*, 2014, **5**, 970-976.

30 Tristantini D and Amalia R, Quercetin concentration and total flavonoid content of anti-atherosclerotic herbs using aluminum chloride colorimetric assay, In *AIP Conference Proceedings*, 2019, 2193 (1), 030012). AIP Publishing LLC.

31 Do Q D, Angkawijaya A E, Tran-Nguyen P L, Huynh L H, Soetaredjo F E, *et al.*, Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophila aromatic, *J Food Drug Anal*, 2014, **22**, 296-302.

32 Gangwar M, Gautam M K, Sharma A K, Tripathi Y B, Goel R K, *et al.*, Antioxidant capacity and radical scavenging effect of polyphenol rich Mallotusphilippenensis fruit extract on human erythrocytes: An *in vitro* study, *Sci World J*, 2014, **2014**, 279451.

33 Shashni S and Sharma S, Antioxidant activities of dried wild rosehips (*Rosa moschata*) of Kullu Valley, Northwestern Indian Himalaya, *Indian J Nat Prod Resour*, 2022, **13**, 339-345.

34 Dharmadeva S, Galgamuwa L S, Prasadinie C and Kumarasinghe N, *In vitro* anti-inflammatory activity of *Ficusracemosa* L. bark using albumin denaturation method, *Ayu*, 2018, **39**, 239.

35 Heendeniya S N, Ratnasooriya W D and Pathirana R N, *In vitro* investigation of anti-inflammatory activity and evaluation of phytochemical profile of *Syzygium caryophyllatum*, *J Pharmacogn Phytochem*,2018, **7**, 1759-1763.

36 Grabowska K, Podolak I, Galanty A, Załuski D, Makowska-Wąs J, *et al.*, *In vitro* anti-denaturation and anti-hyaluronidase activities of extracts and galactolipids from leaves of impatiens parviflora DC, *Nat Prod Res*, 2016, **30**, 1219-1223.

37 Aryal S, Baniya M K, Danekhu K, Kunwar P, Gurung R, *et al.*, Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from Western Nepal, *Plants*, 2019, **8**(4), 96.

38 Malhotra S K, Nigella, In *Handbook of herbs and spices*, (Woodhead Publishing), 2012, 391-416.

39 Adhikari L, Kotiyal R, Pandey M, Bharkatiya M, Sematy A, *et al.*, Effect of geographical location and type of extract on total phenol/flavon contents and antioxidant activity of different fruits extracts of *Withania somnifera*, *Curr Drug Discov Technol*, 2020, **17**, 92-99. doi: 10.2174/1570163815666180807100456.

40 Ahmad R, Ahmad N, Mohd A, Aijhisi F, Alamer M H, *et al.*, Variation in *Nigella sativa* quality and its standardization via instrumental analysis: A study based on geographical origin, *Not Bot Horti Agrobot*, 2020, **48**, 1141-1154.

41 Kabir Y, Akasaka-Hashimoto Y, Kubota K and Komai M, Volatile compounds of black cumin (*Nigella sativa* L.) seeds cultivated in Bangladesh and India, *Heliyon*, 2020, **6**, e05343.

42 Sen N, Kar Y and Tekeli Y, Antioxidant activities of black cumin (*Nigella sativa* L.) seeds cultivating in different regions of Turkey, *J Food Biochem*, 2010, **34**, 105-119.

43 Liu Y, Chen P, Zhou M, Wang T, Fang S, *et al.*, Geographic variation in the chemical composition and antioxidant properties of phenolic compounds from *Cyclocarya paliurus* (Batal) Iljinskaja leaves, *Molecules*, 2018, **23**, 2440.

44 Ben Salem M, Affes H, Athmouni K, Ksouda K, Dhouibi R, *et al.*, Chemicals compositions, antioxidant and anti-inflammatory activity of *Cynarascolymus* leaves extracts, and analysis of major bioactive polyphenols by HPLC, *Evid-Based Complement Altern Med*, 2017, **2017**, 4951937.