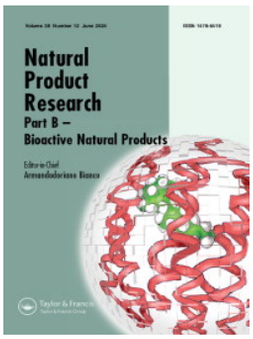

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Phytochemical investigation of *Nigella sativa* seed extract by HPTLC, HPLC and GC-MS: a comparative geographical study

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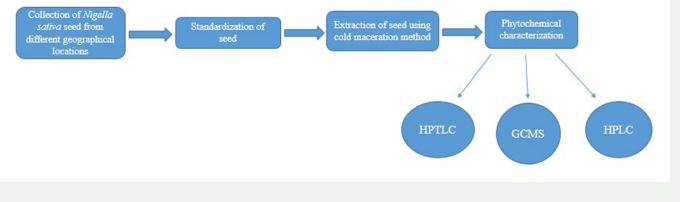
ABSTRACT

This study aimed to ensure the quality of the seed as well as determine the phytochemical composition of *Nigella sativa* seed extract (NSSE) obtained from three different geographical locations. Pharmacognostic evaluation of the seed includes preliminary phytochemical screening, physicochemical evaluation, and study of heavy metal content, in addition to HPTLC, HPLC, and GC-MS studies of the extract obtained from the seed of the *Nigella sativa* (*NS*). HPTLC fingerprinting studies revealed the presence of various bioactive compounds. HPLC analysis confirms the quantitative variation of thymoquinone (TQ) in the extracts, i.e. the maximum quantity of TQ was found in Vizag NSSE, followed by Punjab and Madhya Pradesh. GC-MS analysis reveals the presence of 33, 35, and 32 constituents in the extract obtained from Vizag, Madhya Pradesh, and Punjab, respectively. This study confirms the variation in the phytochemical composition as well as in the biomarker (Thymoquinone) content present in the collected samples.



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Nigella sativa; HPTLC; HPLC; GC MS; phytochemical evaluation; geographical variation

1. Introduction

The first Synthetic drug came into existence in 1869 until that medicinal drugs obtained from different parts of plants were used to treat and cure diseases (Jones 2011). Since the dawn of time, medicinal plants have been used to treat a variety of illnesses, and India has long had a working knowledge of these plants. The earliest known written record of the use of medicinal plants for drug preparation was discovered on a Sumerian

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clay slab from Nagpur that is thought to be about 5000 years old. This record contains 12 ways for drug preparation mentioning over 250 various plants, some of them containing alkaloids like poppy, henbane, and mandrake (Petrovska 2012).

Herbal treatment is a holistic approach that integrates emotional, mental, and spiritual approaches, whereas synthetic medicine only treats the symptoms of the diseases. Moreover, herbal medicines have very few adverse effects and greater patient compliance than synthetic drugs (Karimi et al. 2015). Since many plants are available worldwide, researchers are interested in studying them for their potential pharmacological activity, mechanism of action, efficacy, safety, and toxicological investigations. This aids in the development of novel medicines to treat illnesses, as 80% of the population in developing countries is treated with traditional medications, according to the WHO (Ekor 2014; Rini et al. 2023).

Nigella is one of numerous species that was used in folk medicine. Among nigella species, one of traditional plant is *Nigella sativa (NS)*, commonly known as 'black cumin' or 'kalonji' or 'Fennel flower', a member of the Rannunculaceae family, emerging as a miracle herb among a plethora of therapeutic agents (Ahmad et al. 2013).

It is a black seed referred to as a panacea (universal healer) by Prophet Mohammed, which is a cure for all ailments except prevention of ageing or death (Ali et al. 2018; Sarwar and Latif 2015). *NS* is an annual herbaceous hermaphrodite plant that contains non-volatile component like tannins, phenolic compounds and flavonoids along with volatile components like terpens which are responsible for various pharmacological activities (Topcagic et al. 2017).

Most of the activities of *NS* are associated with the presence of thymoquinone which is responsible for a wide range of biological activities, including anticancer (Hisham shady et al. 2023), antioxidant, anti-inflammatory, anti-hepatotoxic, analgesic, antimutagenic, anti-nephrotoxic, immunostimulant, hypoglycaemic, antiulcer, antimicrobial, and anti-parasitic properties (Babar et al. 2019, 2021; Fatima Shad et al. 2021). *NS* seed extract can also inhibit delayed stomach emptying in rats induced by cisplatin (Riyaz et al. 2017). Apart from thymoquinone molecular docking studies on *NS* seeds reveals anti-viral activity of Nigellidine that can inhibit FAS-TNF induced signalling (Banerjee et al. 2022).

Along with medicinal uses, the seeds are commercially important for their usage in food production, as a condiment, and as a way to enhance the flavour of bakery items and cheese.

This wonderful herb is widely distributed in Asia, Europe, Middle East and North Africa (Ahmad et al. 2013) but secondary metabolites differ qualitatively and quantitatively (Lumingkewas et al. 2015). The seeds of *NS* have great medicinal importance and thus it is very important to ensure its quality, purity and effectiveness, as the seeds may be adulterated with the seeds of *Allium cepa*, *Nigella damascena* and *Clitoria guianesis* intentionally or unintentionally due to resemblance in the morphology of the seeds (Margout et al. 2013).

Standardisation of herb is important to ensure its quality. Great medicinal value of *NS* seed encouraged the author to standardise the seeds collected from different geographical locations and study the impact of geographical location on the phytoconstituents obtained from the seeds of *NS* from various geographical sources in order to identify the optimal variety for wide range of purposes.

This research focuses on collection and standardisation of the seeds of *NS* from three different geographical locations in India having different climatic conditions to conduct comparative study of the phytoconstituents, as different environmental conditions directly influences the percentage and quality of the phytoconstituents even when produced in the same country. This is the first study so far to compare the phytoconstituents qualitatively as well as quantitatively in NSSE using HPTLC, HPLC and GC- MS analysis.

2. Results and discussion

2.1. Organoleptic evaluation

Organoleptic characteristics are extremely important which assists in identification of the herbal drugs. The observed organoleptic characteristics of NS seeds from various sources were listed in the (Table S1).

2.2. Physicochemical evaluation

The evaluation of physicochemical parameters involves a critical component of the quality standards of the raw materials. The results of physicochemical evaluation is depicted in (Table S2).

2.3. Preliminary phytochemical analysis

The preliminary phytochemical screening of *Nigella sativa* seeds aqueous, methanol, and petroleum ether extracts revealed the presence of different groups of secondary metabolites, including alkaloids, tannins, saponins, and others. (Table S3) displays the results of preliminary phytochemical screening.

2.4. HPTLC fingerprinting

The HPTLC analysis of NSSE revealed differentiated fingerprints which would be helpful for the authentication and quality control purposes. The NSSE obtained from Vizag shows presence of 8 peaks at the Rf value 0.037 (0.21%), 0.060 (0.43%), 0.234 (2.27%), 0.284 (16.85%), 0.434 (4.16%), 0.608 (65.73%), 0.811 (4.58%) and 0.945 (2.65%), NSSE obtained from Madhya Pradesh region reflect 6 peaks at the Rf value 0.045 (0.85%), 0.294 (18.09%), 0.421 (7.27%), 0.645 (59.29%), 0.797 (7.32%) and 0.948 (3.63%) while NSSE obtained from Punjab region shows the presence of 6 peaks at Rf value 0.037 (0.99%), 0.290 (14.54%), 0.402 (4.43%), 0.532 (37.47%), 0.656 (37.38%) and 0.802 (3.55%). These findings demonstrate the variability of the phytoconstituents, their percentage area, and the percentage variation of the biomarker i.e. TQ. The results are mentioned in the form of chromatograms (Figure S1, S2, S3 and S4). Another study, which claims that variation does exist in the phytoconstituents of the plant even when the sample was collected from extremely close geographical locations, supports our findings that phytoconstituents vary with geographical location (Toniolo et al. 2014).

2.5. HPLC analysis

The chromatographic analysis was performed to quantitate the amount of biomarker component i.e. thymoquinone (monoterpene) in all the extracts more accurately, as the quality of an extract determined by its phytoconstituents. It is also reported in the literature that TQ is responsible for most of the activities of NS and thus quantification of TQ is significant. The results of HPLC analysis depicts that monoterpene (TQ) content was found highest in NSSE obtained from Vizag region i.e. 9.32 µg/g, followed by Punjab region i.e. 7.18 µg/g and lowest TQ content was found in NSSE obtained from Madhya Pradesh region i.e. 4.50 µg/g. The chromatograms obtained i.e. (Figure S5, S6, S7 and S8) shows that there is variation in TQ content which might be due to differences in geographical location. Our findings are supported by another study which states that the monoterpene content vary with geographical location (Ahmad et al. 2020). Phytochemical variation with geographical location was also suggested by Xu et al. which states that primary and secondary metabolites in plants vary with geographical locations (Xu et al. 2022).

2.6. GC- MS analysis

The GC-MS studies was carried out for NSSE chemical profiling, which will be helpful in the identification of bioactive compounds. The GC-MS chromatogram of NSSE obtained from 3 different geographical locations revealed the presence of 33, 35, 32 peaks for bioactive compounds which were identified on the basis of their peak retention time, retention indices and mass fragmentation patterns to that of known compounds described by NIST and Wiley library. The mass spectra of the extracts are shown in (Figure S9, S10 and S11) and the results of GC-MS analysis were presented in (Table S4).

The results obtained specify that the extract from the Vizag region contains 2.06% volatile oil which is comparable to 2.04% volatile oil content in the MP region however relative percentage area of TQ in the MP extract is twice that of the extract obtained from the Vizag region. The extract obtained from the Punjab region contains only 1.07% volatile oil. It was also depicted from the results that, total 8 compounds i.e. (2-Dodecanone, Pinanediol, Dihydrojasmone, Myristic acid, TMS derivative, Tridecanoic acid, methyl ester, 6,10,14,18,22-Tetracosapentaen-2-ol, beta-Eudesmol, TMS derivative and 2-Methyl-4-(2,6,6-trimethylcyclohex-2-enyl)but-3-en-2-ol, also known as menthol), 8 compounds i.e. ((2Z)-2-Octadecenyl acetate, Methyl 14-methylpentadecanoate, cis-7-Tetradecen-1-yl acetate, (5E)-2,3,5,8-Tetramethyl-1,5,9-decatriene, Hexadecyl 2-methylpropanoate, Methyl 2-methylhexadecanoate, 1,8-Dibenzyl-3,6-diazahomoadamantan-9-one, Cholestan-3-one o-acetyloxime) and 5 compounds i.e. (Thymol, Ethyl 3-methyl-2-oxiranecarboxylate, 3α-hydroxy-5β-pregnane-20-one, n-Octadecanoic acid, methyl ester and 10,13-Eicosadienoic acid, methyl ester) were found unique in extracts obtained from the *NS* seeds obtained from NSVZ, NSMP and NSPB respectively which possess pharmacological activities. However, Myristic acid TMS derivative, beta-Eudesmol, TMS derivative found in the NSVZ extract and 1,8-Dibenzyl-3,6-diazahomoadamantan-9-one, in NSMP extract and Ethyl 3-methyl-2-oxiranecarboxylate in NSPB extract are improbable compounds that might emerge due to their strong matching with MS library. Furthermore, since methanol was used as solvent for extraction process, it is also possible that some of the esters identified in the extracts may be the artefacts like Methyl 14-methylpentadeconate (Venditti 2020).

Differences also exists in the percentages of saturated and unsaturated fatty acids, phytosterols and organic acids between the NSSE obtained from different locations. However, major component identified in all the identified was cis-9, cis-12-Octadecadienoic acid which also possess various biological activities.

It was also ascertained from the GCMS results that the relative percentage area of individual components also varying, either it is common or unique phytochemical constituent present in the extract. These findings show that differences exists in the phytochemical composition of the NSSE collected from different geographical locations. A study which found that a plant's phytochemical composition is influenced by its geographic location, also lends credence to our findings (Kumar et al. 2017). Our research confirms earlier findings that the phytochemical composition varies depending on one's location (Madawala et al. 2018). An earlier study also mentioned the differences in phytoconstituents among the six regions of Italy (Mannu et al. 2021).

3. Conclusion

The presence of various bioactive compounds makes *NS* a wonder herb with a tonne of biological activity. The majority of these biological activities are connected to thymoquinone. Present work reveals that diversity exists in the phytochemical composition in the extracts obtained from three different geographical location. It was also revealed from GC MS studies that there are 6, 7 and 4 unique phytoconstituents present in *NS*SE obtained from Vizag, MP and, PB respectively and HPLC studies indicates that TQ content also varies with geographical location. Maximum amount of TQ content was identified in the extract obtained from Vizag region, followed by MP and, PB region respectively. The variability seen in phytochemical composition might be caused due to differences in geographical location. The conditions of Vizag are not too favourable to cultivate kalonji, it might be possible that adverse conditions causes increased production of secondary metabolites to certain level.

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Disclosure statement

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