COMPARATIVE ANTIOXIDANT ACTIVITY STUDY OF *NIGELLA SATIVA* L. EXTRACTS

Authors: *Smita Panditrao Shelke, [1] Abhishek Jibhau Dukale[1], Sujata Raosaheb Badhe[1] , Tanvi Jayesh Gavande[1].

1. *Gokhale Education Society's Sir Dr. M. S. Gosavi College of Pharmaceutical Education and Research, Nashik-05.*

*** Corresponding author:**

Smita Panditrao Shelke

Assistant Professor,

Address: Gokhale Education Society's Sir Dr. M. S. Gosavi College of Pharmaceutical Educationand Research, Nashik-05.

Contact No: 9890213783

Email.ID: shelkesmitap@gmail.com

ABSTRACT: *The aim of this study was to standardize Nigella sativa L. seed extracts with the goal of making natural medicines more palatable to today's health-conscious populace. The maceration process and Soxhlet extraction were used to produce the ethanol extracts from Nigella sativa seeds. The ethanolic extract made by Soxhlet extraction has higher concentrations of active ingredients (thymoquinone) than the extract made by maceration extraction, according to antioxidant studies of the extract conducted using 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assays.*

Keywords: Nigella sativa L., Soxhlet Extraction, Maceration, Antioxidant Activity, DPPH.

INTRODUCTION: *Nigella sativa* L., or N. sativa, is another name for black cumin, which has been used for a long time, in particular in Southeast Asia and the Middle East. This plant is a member of the Ranunculaceae family of annual herbaceous plants[1]. N. sativa (NS) has been utilized for a long time in Arabian, Indian, and Chinese cultures, as well as in traditional medical procedures such as Unani and Ayurveda, because of its various benefits[2]. Though the Unani system asserts that cumin seeds help treat inflammation, ascites, jaundice, piles, a high fever, paralysis, and eye disorders, Ayurveda says that NS balances Vata, Kapha, and Pitta[3]. Moreover, they are carminative, laxative, stomachic, and galactagogue[4]. The chemical composition of N. sativa is incredibly rich and complex.Thymoquinone is the primary active ingredient[5].

Standardization of Herbs: The process of standardizing herbal medicines involves creating a set of requirements or fundamental characteristics, standardized parameters, and exact qualitative and quantitative values that convey an assurance of quality, efficacy, safety, and repeatability[6].

Antioxidants are a vital component that safeguards health. Antioxidants reduce the risk of chronic diseases including cancer and heart disease, according to scientific study[7]. In this study, the DPPH (1,1diphenyl-2-picryl hydrazyl) radical scavenging method was used to assess the antioxidant activity of an ethanolic Nigella sativa extract using two different approaches[8]. Stronger quantities of Nigella sativa's alcoholic extract have a stronger antioxidant value when compared to the reference standard ascorbic acid[9]. The radical scavenging activity of DPPH (2, 2'-diphenyl-1-picrylhydrazyl) is based on the reduction of a DPPH solution in methanol by an antioxidant molecule that acts as a hydrogen donor and forms the non-radical form of DPPH[10].

Inhibition (%) = [(Abs Control-Abs Sample)/Abs Control] X 100

Where; A Control: the absorbance of the control solution and A Sample: absorbance of the sample at 517 nm and IC50: the sample concentration needed to scavenge half of the DPPH radicals[11.]

MATERIALS AND METHODS: The seeds were bought at a local herbal medicine market in Nashik. After being cleansed, they were ground into a fine powder and stored in a dark area for later use[12.] The analytical grade solvents and calibrated instruments were employed.

Morphology studies: Specific characteristics in the morphology of Nigella sativa (N. sativa) seeds enable identification and potential therapeutic benefits[13]. The seeds have an angular, triangular, and flat shape; they are 0.2 cm long and 0.1 cm wide. They are thick, crescent-shaped, have a strong bitter flavor, and a smooth, black texture [15.] The thin, cuticle-coated layers of dense, lignified parenchymal cells that make up the transverse section (T.S.) of Nigella sativa seeds reach the papillae.[16]
The endoderm follows the epidermis in two layers and is composed of multiple layers of parenchymal cells, the outermost of which carries pigments.[17] In the center of the slice is a little embryo[18].

Extraction of seeds of N. sativa: Soxhlet extraction: Using a Soxhlet system, 500 ml of 100% ethanol (99.9% v/v) was used to extract 200g of ground crude powder over six hours[19.] The ethanol extracts (SE) were concentrated by steam distillation and then stored at 4°C until they were needed once again[20.] **Maceration:** The organic solvent extracts were made using the sequential maceration method with extractants of increasing polarity, including methanol, ethanol, chloroform, and n-hexane[21.] 200 g of N. sativa seed powder were macerated with 100 ml of each solvent for a whole day at room temperature22. After passing the extracts through Whatman No. 1, condensing them using the steam distillation method, and allowing them to settle at room temperature, crude extracts (ME) were produced[23.]

To verify the quality of the medications and identify those that are genuine, phytochemical analysis was performed [24.]
Test for Alkaloids (Wagner's Reagents): 2.0 ml of each extract and 1.5 milliliters of 1% hydrochloric acid (HCL) were mixed together in a test tube[6]. The contents of the test tube were warmed over a water bath before a few drops of Wagner's reagent were added. The formation of an orange precipitate confirmed the presence of alkaloids.
Test for Flavonoids : For each sample, 2.0 ml of ferric chloride hexahydrate (fecl3−6H2O) solution was added few drops at a time. The presence of flavonoids is indicated by the formation of a vibrant green color.
Test for Phenols: For each sample, two drops of a 5% FeCl3–6H2O solution were added to a 2.0 ml volume. A deep blue-black hue stated the presence of tannins.

Test for Cardiac Glycosides: For each sample, 2.0 ml was filled with a few drops of a 5% FeCl3–6H2O solution. There was a deep blue-black hue that suggested the presence of tannins. The shade of blue in CH3COOH reveals the existence of cardiac glycosides[25.] Table1 reports on the phytochemical assessment of NS seed extracts.

Table 1: Phytochemical screening of NS seed Extracts.

Sr. No	Phytochemical Component	Observation
1	Alkaloids	+
2	Flavonoids	+
3	Phenols	-
4	Cardiac Glycosides	-

Antioxidant Activity: The sample solutions were prepared by dissolving ethanol extract by soxhlet extraction method and maceration method in methanol solvent with different concentrations.[26] Samples were blended with DPPH solution to create the reaction mixture, which was then left in the dark for 30 minutes before the absorbance at 715 nm was measured. The extracts were incubated at room temperature for 30 minutes, and the absorbance at 517 nm was measured against a blank solution. The reaction color changed from purple to yellow to indicate the antioxidant capacity, which was then estimated for percentage inhibition.

Both extracts showed significant antioxidant, DPPH radical scavenging activity. The percentage inhibition was estimated using an equation. The inhibitory concentration (IC50) is defined as the concentration required to inhibit 50% of DPPH (I% = 50). The inhibition % was computed [27] and reported in table 2.

Table 2: Antioxidant activity: Comparative inhibition of standard antioxidant ascorbic acid, ethanolic extract by soxhlet extraction (SE) and Ethanolic Extract by maceration (ME).

Sr. No.	Concentration (µg/ml)	Percentage inhibition		
		Ascorbic Acid	Ethanolic Extract (SE)	Ethanolic Extract (ME)
1	10	56	47	28
2	20	63	50	32
3	30	73	55	40

RESULTS AND DISCUSSION: Specific characteristics in the morphology of *Nigella sativa* seeds enable identification and potential therapeutic benefits. The soxhlet apparatus and sequential maceration approach was used by methanol, ethanol and n-hexane as extractants. The necessary filtrates were isolated from solid residues, pre-concentrated, and dried, yielding crude extracts. The phytochemical screening conducted on *Nigella sativa* extracts revealed the presence of alkaloids and flavonoids

According to an antioxidant study, as compared to an extract obtained by maceration (ME) and ascorbic acid as a control, the Nigella sativa ethanolic extract via soxhlet apparatus (SE) exhibited higher DPPH radical scavenging activity, indicating its ability to battle free radicals and oxidative stress.

CONCLUSION: The goal of the study was to standardize *Nigella sativa* L. seed extracts in order to increase the adoption of natural medicines among people who are concerned about their health. In comparison to maceration extraction, the N. sativa ethanolic extract by soxhlet extraction showed high DPPH radical scavenging activity, indicating larger amounts of active components, particularly thymoquinone.

REFERENCES:

[1] V Vahita. (2023).Black Cumin (Nigella sativa L.): Studies on Phytochemistry, Health Benefits, Molecular Pharmacology, and Safety. Acta Scientific Microbiology. 6.938–44.

[2] Medicinal plants of India [Internet]. Asia-pacific Biotech News. 2007. Available from: https://www.worldscientific.com/doi/abs/10.1142/S0219030307000730

[3] Hossain, M. S., Sharfaraz, A., Dutta, A., Ahsan, A., Masud, M. A., Ahmed, I. A.,& Ming, L. C. (2021). A review of ethnobotany, phytochemistry, antimicrobial pharmacology and toxicology of Nigella sativa L. Biomedicine & Pharmacotherapy, 143, 112182.

[4] Hossain, M. S., Sharfaraz, A., Dutta, A., Ahsan, A., Masud, M. A., Ahmed, I. A., & Ming, L. C. (2021). A review of ethnobotany, phytochemistry, antimicrobial pharmacology and toxicology of Nigella sativa L. Biomedicine & Pharmacotherapy, 143, 112182.

[5] Dinagaran, S., Sridhar, S., & Eganathan, P. (2016). Chemical composition and antioxidant activities of black seed oil (Nigella sativa L.). International Journal of Pharmaceutical Sciences and Research, 7(11), 4473.

[6] Kumari, R., & Kotecha, M. (2016). A review on the standardization of herbal medicines. International journal of pharma sciences and research, 7(2), 97-106.

[7] Zehiroglu, C., & Ozturk Sarikaya, S. B. (2019). The importance of antioxidants and place in today's scientific and technological studies. Journal of food science and technology, 56, 4757-4774.

[8] Baliyan, S., Mukherjee, R., Priyadarshini, A., Vibhuti, A., Gupta, A., Pandey, R. P., & Chang, C. M. (2022). Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of Ficus religiosa. Molecules, 27(4), 1326.

[9] Aumeeruddy MZ, Aumeeruddy-Elalfi Z, Neetoo H, Zengin G, Fibrich B, Rademan S, Van Staden AB, Szuman K, Lambrechts IA, Lall N, Mahomoodally MF. Biological, phytochemical, and physico-chemical properties of two commercial Nigella sativa seed oils: A comparative analysis. Istanbul Journal of Pharmacy. 2019;48(3):89-99.

[10] Kedare SB, Singh RP. Genesis and development of DPPH method of antioxidant assay. Journal of food science and technology. 2011 Aug;48:412-22.

[11] Kumara, P., Sunil, K., & Kumar, B. A. (2018). Determination of DPPH Free Radical Scavenging Activity by RP-HPLC. Rapid Sensitive.

[12] Deepak Kadam, D. K., & Lele, S. S. (2017). Extraction, characterization and bioactive properties of Nigella sativa seedcake.

[13] Badalamenti, N., Modica, A., Bazan, G., Marino, P., & Bruno, M. (2022). The ethnobotany, phytochemistry, and biological properties of Nigella damascena–A review. Phytochemistry, 198, 113165.

[14] Forouzanfar, F., Bazzaz, B. S. F., & Hosseinzadeh, H. (2014). Black cumin (Nigella sativa) and its constituent (thymoquinone): a review on antimicrobial effects. Iranian journal of basic medical sciences, 17(12), 929.

[15] Ferizi, R., Ramadan, M. F., & Maxhuni, Q. (2023). Black Seeds (Nigella sativa) Medical Application and Pharmaceutical Perspectives. Journal of Pharmacy and Bioallied Sciences, 15(2), 63-67.

[16] Rajasekhar, S., & Kuldeep, B. (2011). A Review-Pahrmacognosy and pharmacology of Nigella Sativa. International Research Journal of Pharmacy, 2(11), 36-39.

[17] Aftab Ahmad, A. A., Asif Husain, A. H., Mohd Mujeeb, M. M., Khan, S. A., Najmi, A. K., Siddique, N. A., ... & Firoz Anwar, F. A. (2013). A review on therapeutic potential of Nigella sativa: a miracle herb.

[18] Panahi, M., Namjoyan, F., & Shakerin, Z. (2011). Evaluation of antioxidant effects of nigella sativa extract on the ultra structure of neural tube defects in diabetic rats's offspring.

[19] Sharma, D., Kosankar, K. V., & Lanjewar, A. M. (2017). Extraction and Chemical Tests on Nigella sativa L. Collected from Vidarbha Region of India.

[20] Benkaci-Ali, F., Akloul, R., Boukenouche, A., & Pauw, E. D. (2013). Chemical composition of the essential oil of Nigella sativa seeds extracted by microwave steam distillation. Journal of Essential Oil Bearing Plants, 16(6), 781-794.

[21] Abubakar, A. R., & Haque, M. (2020). Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. Journal of Pharmacy and Bioallied Sciences, 12(1), 1-10.

[22] Shafodino, F. S., Lusilao, J. M., & Mwapagha, L. M. (2022). Phytochemical characterization and antimicrobial activity of Nigella sativa seeds. PloS one, 17(8), e0272457.

[23] Zelelew, D., & Gebremaria, A. (2018). Optimization of extraction and pharmacological activities of essential oil from black cumin (Nigella sativa L.) seeds using clevenger distillation. Current Research in chemistry, 10(1), 1-9.

[24] Jabeen, N. S., Jagapriya, L., Balasubramanian, S., Devi, K., & Jeevanandam, J. (2018). Phytochemical analysis of nigella sativa L. seeds aqueous extract by gas chromatography-mass spectroscopy and Fourier-transform infrared. In Phytochemistry (pp. 587-604). Apple Academic Press.

[25] Tiji, S., Benayad, O., Berrabah, M., El Mounsi, I., & Mimouni, M. (2021). Phytochemical profile and antioxidant activity of Nigella sativa L growing in Morocco. The Scientific World Journal, 2021(1), 6623609.

[26] Kasiramar, G. (2019). Significant Role Of Soxhlet Extraction Process In Phytochemical Mintage Journal Of Pharmaceutical & Medical Sciences Significant Role Of Soxhlet Extraction Process In Phytochemical.

[27] Muthoni Guchu, B., Machocho, A. K. O., Mwihia, S. K., & Ngugi, M. P. (2020). In vitro antioxidant activities of methanolic extracts of Caesalpinia volkensii Harms., Vernonia lasiopus O. Hoffm., and Acacia hockii De Wild. Evidence-Based Complementary and Alternative Medicine, 2020(1), 3586268.