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Evaluation Of Flavonoids Content, Phenolic Compounds And AntioxidantProperties Of *Nigella Sativa* Seed Extracts Using Different Solvents

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ABSTRACT
Black cumin is one of the most important seed spice belongs to Ranunculaceae family, used in several cooked and processed products in India due to its antimicrobial and antioxidant properties. It is commonly used as a form of traditional medicine in Middle-Eastern countries due to having high antioxidant potential and immunity boosting properties. So, this study was planned to prepare the seed extracts by using different solvents and evaluating their antioxidant activity along with the determination of total flavonoids and phenolic compounds. The analysis revealed that the highest antioxidant activity (58.08%) of methanol seed extract was obtained and the highest level of total phenolic compounds was found in ethyl acetate extract as (148.59 mg/GAE/g). Moreover, the ethyl acetate extract showed the highest flavonoids content (870.0 mg/g QE) respectively. Thus, the findings showed that a high level of natural antioxidants can be derived from *Nigella sativa* seed extracts by methanol solvent.
Keywords: Black cumin, Immunity boosting, Antioxidant potential, Flavonoids, Phenolic compounds, Seed extracts

1. INTRODUCTION
Nigella sativa, a member of the family Ranunculaceae, is a blooming annual herbaceous plant commonly known as black caraway, kalonji, kalajeera or roman coriander in various parts of the world (Allah *et al.,* 2021). It is native to Southern Europe, North Africa and Southwest Asia and widely grown or consumed throughout India, particularly in Punjab, Bihar, Himachal Pradesh, Madhya Pradesh, Bengal, Assam, Rajasthan and Maharashtra (Lal *et al.,* 2020). It has linear, finely split leaves and reaches a height of 20–30 cm and flowers have five to ten petals and are pale blue or white in color (Ramadan, 2015). Hangargekar *et al.,* (2020), claimed that black seeds in Indian and Middle Eastern cuisines are widely used as spices because of their spicy bitter flavor and aroma. Kinki, (2020) stated that depending on the region, the seed contains volatile oils (0.40-0.45%), non-volatile oils (33.20- 40%), protein (16.00-20.85%), carbohydrates (31.0-33.9%), fibre (5.50-7.94%), alkaloids, tannins, saponins, iron, calcium, potassium, magnesium, zinc, and copper (1.79-3.44%), as well as vitamins A, C, thiamine, niacin, pyridoxine and folate (Khazdair *et al.,* 2021).. Black cumin contains several beneficial bioactive compounds, including: thymoquinone, dithymoquinone (nigellone), thymohydroquinone, carvacrol, p-cymene, terpineol, longifolene, t-anethole, and pinene, among others, which are the most notable active compounds (Nyemb *et al.,* 2022).

Thymoquinone, the active component of black cumin, is the most prevalent, responsible for its pharmacological effects (Fidan *et al.,* 2019). Apart from this, Nigella seeds add flavor to many different foods, such as vegetables, bread, curries, pickles, and p ulses, when dried and

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roasted (Amin *et al.,* 2016). Black cumin seeds are staple in the Bengali spice blend known as panch phoron (Kiralan *et al.,* 2014), although they can also be used on their own. However, black cumin has traditionally been used for mummification purposes in Egypt. Ayurveda and Unani, two forms of ancient Indian medicine, have historically included black cumin in their regimens (Sharma *et al.,* 2005).

Fig.1 *Nigella sativa* Plant and Flowers

It has been claimed by Yimer *et al.,* (2019) that *Nigella sativa* is a promising natural remedy for a variety of ailments due to its high content of traditional, medicinal, and biological principles, as well as its oil, containing functional food and nutraceutical compounds. Sultana *et al.,* (2018) further reported the most important bioactive compounds thymoquinone and other phytochemicals include sterols, saponins, phenolic compounds, alkaloids and volatile oils of different composition. Several scientific studies have been conducted to assess the biological and therapeutic properties of black cumin (Vaz *et al.,* 2018). In addition to its therapeutic and pharmacological potential, thymoquinone is a primary bioactive constituent of black cumin (Ziaei *et al.,* 2012).

Fig. 2 *Nigella sativa* seeds

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The seeds of black cumin contain a diverse array of chemicals that are physiologically active (Salem *et al.,* 2005). These have been shown to exhibit analgesic, anti-inflammatory, anti-epileptogenic, antidiabetic, anticancer, antioxidant, antimicrobial, antischistosomiasis, immunomodulatory, cardiovascular supportive, gastroprotective, hepato-protective, and nephron-protective activities, amongst others, which provide particular therapeutic benefits in treating a variety of ailments(Wako *et al.,* 2020). Nephron-protective activity has also been shown to be a potential benefit (Ahmad *et al.,* 2013).

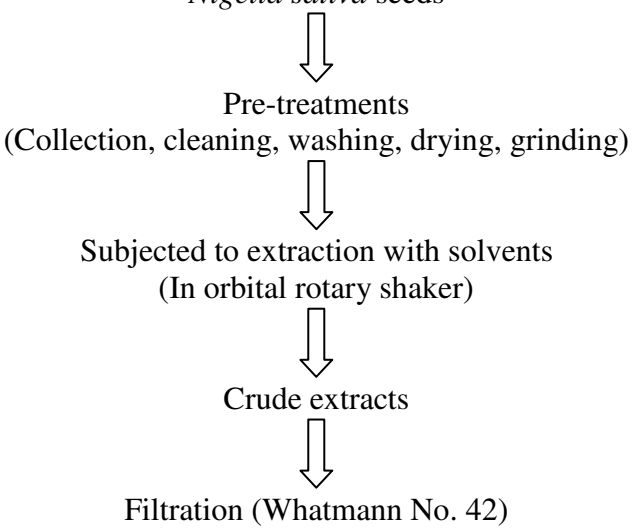
2. MATERIALS AND METHOD

2.1. ***Apparatus and Material:*** 100ml of measuring cylinder, 5ml of measuring cylinder, 100ml of beaker, funnel, test tubes, dropper, reagent bottles, electric grinder, rotavapor, UV-Vis Spectrophotometer.

2.2. ***Chemicals and reagents:*** Methanol, acetone, ethanol, petroleum ether, ethyl acetate, distilled water, Folin-ciocalteu reagent, gallic acid, querectin, 10% aluminium chloride, 5% sodium nitrite solution, 1M NaOH, 20% sodium carbonate solution, DPPH (1,1-Diphenyl-2-picryl hydrazyl), ascorbic acid.

2.3. ***Sample collection:*** The seeds of *Nigella sativa* L. (AN -1 Variety) were purchased in the month of October in the year 2021 from the National Research Centre of Seed Spices (ICAR-NRCSS), Tabiji, Ajmer, Rajasthan, India. Seeds quality was visually observed. Fresh, mature seeds were cleaned, washed and blotted on blotting paper for 2 days at room temperature. The shade dried seeds were ground to fine powder using an electric grinder (Sujata supermix,900W). The powdered sample was used to determine the physico-chemical profile, proximateanalysis, minerals and nutrients composition.

2.4. ***Preparation of Nigella sativa seed extracts:*** The variety of extracts from the sample was prepared using six types of solvents: methanol, ethanol, distilled water, acetone, ethyl acetate,and petroleum ether. 10gm of sample was macerated with 100ml (Sample to solvent ratio 1:10g/ml) of each solvent (six solvents) subjected to Orbital rotary shaker for 72 hours at 32ºC with speed of 200rpm. After centrifuging for 15 minutes at 2000 rpm at room temperature, the samples were filtered through filter paper (Whatmann No. 42). Then the extracts were evaporated till dryness in vacuum by using Rota vapor at 40ºC under constant pressure. In the meantime, 25 minutes of aqueous extract was evaporated on a hot plate at 100ºC. We obtained crude viscous extracts of plant and stored them at 4°C for further use.



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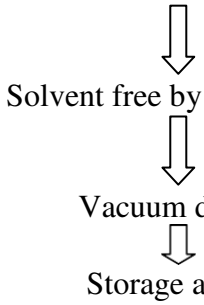

Fig. 3 Flow diagram for seed extracts

3. Proximate Analysis

Proximal analysis for moisture, crude fat, protein, dietary fiber and ash was performed according to respective methods of the official Methods of Analysis of the Association of Official Analytical Chemists (AOAC) followed by Saleh *et al.* (2018) with slight modifications. The carbohydrate of *Nigella sativa* seeds was calculated by subtracting the total of protein, fat, moisture, fiber and ash from 100. All the analytical procedures were performed at least in triplicates in order to ensure the results. The values were expressed as the mean ± standard deviation.

4. Total Phenolic Content

Gallic acid was used as a standard to determine the total phenolic content using the Folin-Ciocalteu procedure. This procedure involved preparing a fresh Gallic acid stock solution (mg/ml). We added 0.5ml of Folin-Ciocalteu reagent (diluted tenfold, 1:10 v/v) to an aliquot (1ml) of a suitable diluted extract. After 5 minutes, 2ml of 20% sodium carbonate solution (Na_2CO_3) was added to the tubes, and the final volume of the tubes was filled to 10ml with distilled water. At room temperature, the solution was left to stand for a period of 90 minutes. The UV-VIS spectrophotometer was used to test for the presence of total polyphenols at 765nm versus a blank. Through the use of the calibration curve with gallic acid, the total phenolic content was quantified in aliquots and reported as milligrammes of gallic acid equivalent per one hundred grammes of dry weight (mg GAE/100g). All the samples were performed in triplicates for precision and values were expressed as mean ± standard deviation.

5. Total Flavonoids Content

The total flavonoid concentration was calculated using the aluminium chloride colorimetric method, with quercetin serving as the standard compound. In a volumetric flask, 1 ml of test sample added with 4 ml of distilled water (10ml volume). After waiting for 5 minutes, 0.3 millilitres of a solution containing 5% sodium nitrite and 0.3 millilitres of a solution containing 10% aluminium chloride were added. After 6 minutes of incubation at room temperature, 1ml of 1M NaOH was added and final volume was made up to 10ml with distilled water. Using a calibrated UV-VIS spectrophotometer, the absorbance of the sample was measured in comparison to that of the blank at 510 nm. Flavonoid concentration was measured in terms of mg quercetin equivalents per gram (mg QE/100g), and all the samples were run through the process three times to ensure accuracy. The results were then expressed as the mean value ± standard deviation.

6. Determination of Antioxidant Activity

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1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay was used to evaluate the ability of extracts of black cumin seed powder to scavenge free radicals. 0.1mM fresh stock solution of DPPH was prepared by dissolving in methanol. The DPPH solution (3ml) was added to different extracts (1ml) with ethanol in different concentrations (10, 20, 30 µl/ml). A dilution procedure was utilized to prepare the concentrations of six different solvents (ethanol, methanol, water, petroleum ether, ethyl acetate, and acetone) used in the plant extracts. The mixture was vigorously agitated before being let to stand at room temperature in full darkness for 30 minutes. The UV-VIS Spectrophotometer (Varian Microsystems U.K.) was used to observe the solution's absorbance reduction at 517 nm. The experiment was carried out in triplicate and ascorbic acid was used as the standard chemical. The antioxidant activity was calculated by using the following equation:

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

Where A_0 denotes the absorbance of the control reaction and A_1 denotes the absorbance with the test or standard sample present, respectively.

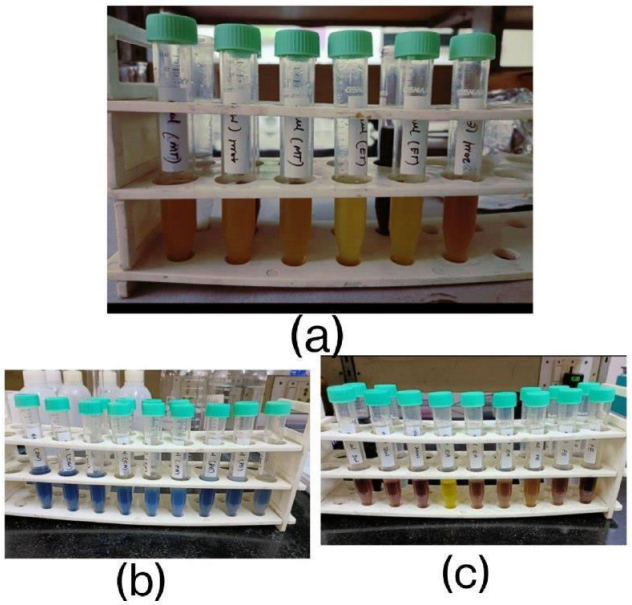

Fig.4 (a) Flavonoid compounds (b) Antioxidant activity (c) Phenolic compounds

7. RESULTS AND DISCUSSION

The physical parameters of black seeds were observed visually and the proximate analysis of black cumin seeds i.e. moisture content (7.05 %), crude fat (45.09%), crude protein (18.57%), crude fiber (7.30%), ash (4.30%) and carbohydrate content (17.50%) were determined by using the standard procedures shown in table 1. The different parameters such as antioxidant activity, total phenolic compounds and total flavonoids content of prepared black cumin seed extracts by using various solvents, were also evaluated.

Table: 1. Proximate analysis of black cumin seed powder

Sr. No.	Nutritional components	Composition (%)
1.	Moisture	7.05±0.52

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2.	Crude Protein	18.57±1.98
3.	Crude Fat	45.09±0.84
4.	Crude fiber	7.30±1.04
5.	Ash	4.30±0.47
6.	NFE	17.50±0.3

*****_Values represented as mean ± SD and data performed in triplicate_ (**Source**: Kaushik and Barmanray, 2022)

Table 2. Depicted the antioxidant activity of various seed extracts of black cumin as follows: methanol seed extract (58.08%), ethanol seed extract (44.06%), ethyl acetate seed extract (39.71%), acetone seed extract (35.98%), petroleum ether seed extract (25.45%) and aqueous extract (20.81%). The analysis revealed that the highest antioxidant activity (58.08%) of methanol seed extract and lowest (20.81%) antioxidant activity of aqueous extract were obtained as shown in table 2.

Table 2. DPPH Radical Scavenging Activity of black cumin seed extracts

Sr. No.	Solvent type	DPPH radical activity inhibition (%)
1	**Ethanol**	44.06±1.33
2	**Methanol**	58.08±1.50
3	**Acetone**	35.98±1.52
4	**Petroleum ether**	25.45±0.67
5	**Ethyl acetate**	39.71±1.25
6	**Water**	20.81±2.23

*_Values represented as mean ± SD and data performed in triplicate_

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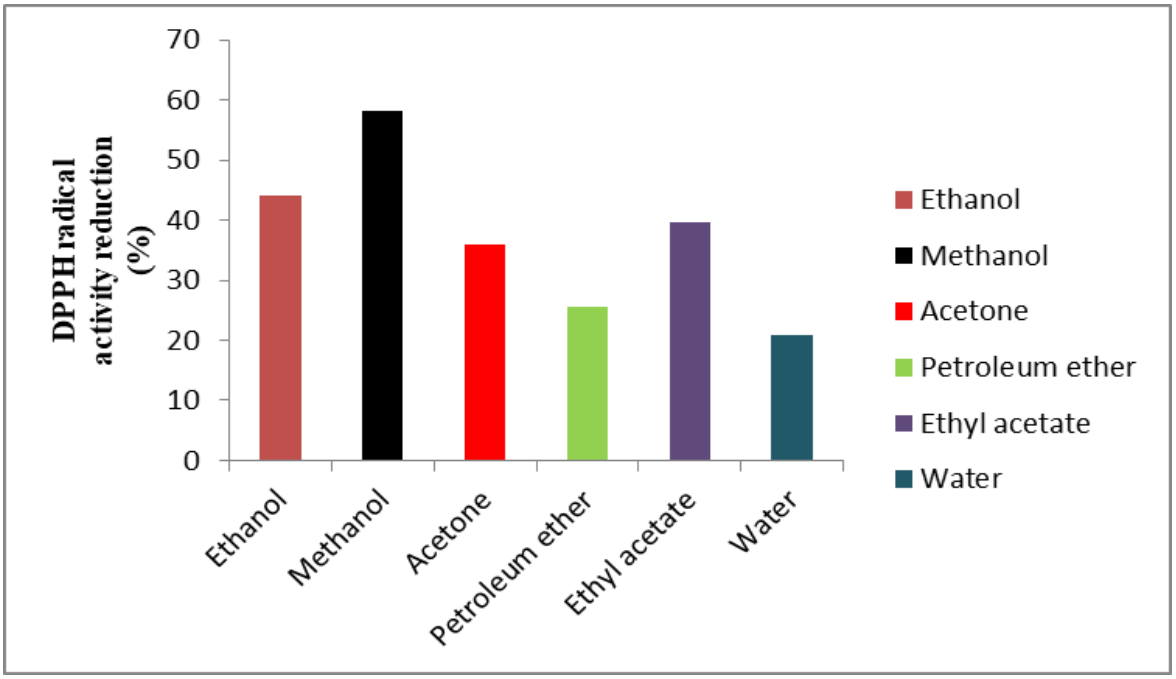

Fig. 5. Effect of different solvents on inhibition of *Nigella sativa* seed extracts using 1,1-Diphenyl-2-Picrylhydrazyl (DPPH)

Table 3. Total Phenolic compounds of black cumin seed extracts

Sr. No.	Solvents used	Total Phenolic Compounds (mg/g GAE)
1	**Ethanol**	138.59±2.76
2	**Methanol**	44.57±0.89
3	**Acetone**	54.02±1.08
4	**Petroleum ether**	58.99±1.18
5	**Ethyl acetate**	148.59±2.96
6	**Water**	41.75±0.83

**Values represented as mean ± SD and data performed in triplicate*

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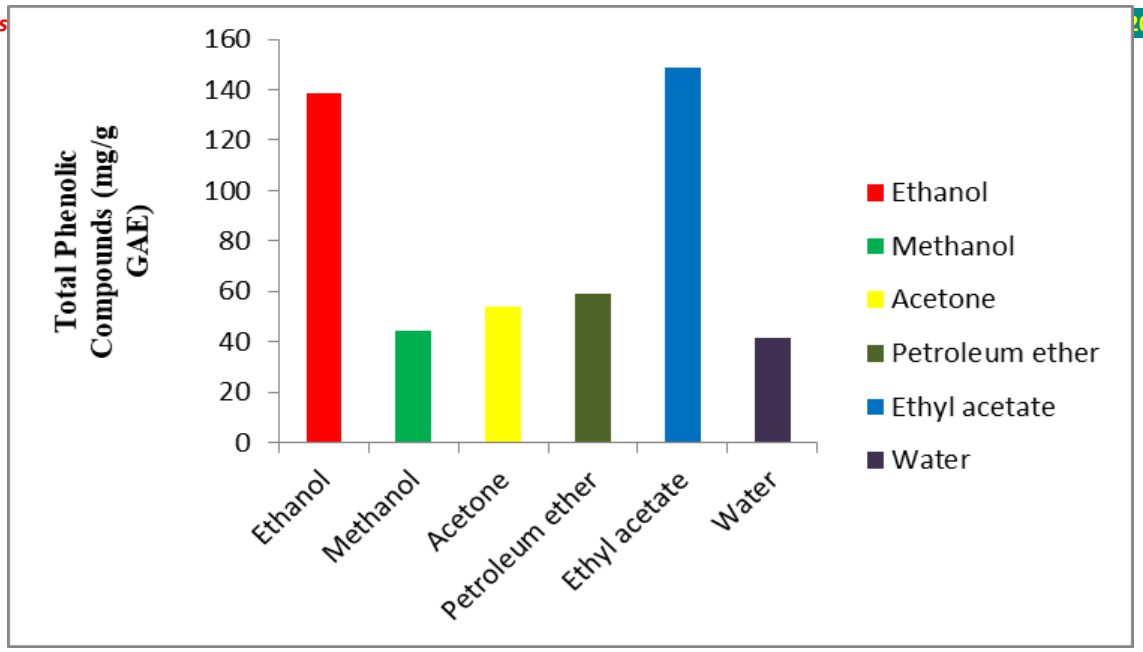

Fig.6 Total Phenolic compounds of black cumin seed extracts using different solvents

Table 4. Total Flavonoid compounds of black cumin seed extracts

Sr. No.	Solvents used	Total Flavonoid compounds(mg/g QE)
1	Methanol	77.91±0.66
2	Ethanol	413.33±1.46
3	Acetone	368.33±1.36
4	Petroleum ether	658.33±2.05
5	Ethyl acetate	870.0±2.56
6	Water	520.0±1.72

**Values represented as mean ± SD and data performed in triplicate*

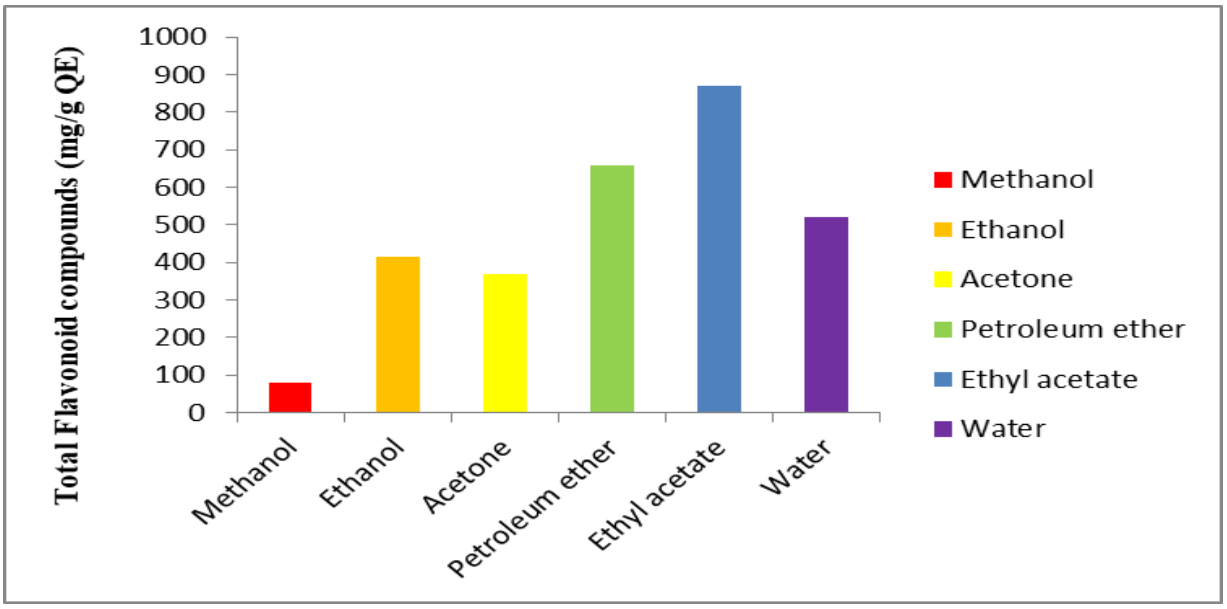

Fig.7 Total Flavonoid compounds of black cumin seed extracts

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CONCLUSION

The use of natural plant extracts has become more popular in illness prevention and treatment due to their high tolerance and few adverse effects. The *Nigella sativa* plant is well-known and has been utilized in traditional medicine for many decades due to its efficiency in managing a variety of medical conditions; it is regarded as a "wonder herb." There are numerous indications that the black seed possesses potent antioxidant potential, according to the extensive scientific literature and the study also showed the presence of higher antioxidant potential in methanolic seed extract. The presence of phenolic compounds in ethanolic extract is highly noticeable while flavonoids were highly present in methanolic extract. This study's findings are expected to serve as a benchmark for similar efforts in the future.

Disclosure Statement: No potential conflict of interest was reported by the authors.

REFERENCES

Ahmad, A., Husain, A., Mujeeb, M., Khan, S. A., Najmi, A. K., Siddique, N. A. and Anwar, F. (2013). A review on therapeutic potential of *Nigella sativa*: A miracle herb. *Asian Pacific journal of tropical biomedicine*, *3*(5), 337-352.

Allah Ditta, H. M., Aziz, A., Hussain, M. K., Mehboob, N., Hussain, M., Farooq, S. and Azhar, M. F. (2021). Exogenous application of black cumin (Nigella sativa) seed extract improves maize growth under chromium (Cr) stress. *International Journal of Phytoremediation*, *23*(12), 1231-1243.

Amin B. and Hosseinzadeh H. (2016). Black Cumin (Nigella sativa) and Its Active Constituent, Thymoquinone: An Overview on the Analgesic and Anti-inflammatory Effects. *Planta medica,* 82(1-2),8–16.

Fidan, Hafize and Stankov, Stanko and Daraba, Aura and doğan, Hülya and Alexieva, Iordanka and Stoyanova, Albena and Ercisli, Sezai. (2019). Phytochemical composition of black cumin (Nigella sativa L.) seeds from Turkey as a unconventional source for the food industry. *Journal of the Brazilian Chemical Society* 1. 1-9.

Hangargekar, C. B., Quazi, R. S., Khan, M. S. A., Joshi, A. A. and Hangargekar, P. B. (2020). A Review on kalonji (*Nigella sativa*): A seed of blessing. *Internaional Journal Research and Analytical Reviews,* 590-596.

Huchchannanavar, S., Yogesh, L. and Prashant, S. (2019). The black seed Nigella sativa: a wonder seed. *International Journal of Chemical Studies*, *7*(3), 1320-1324.

Kaushik, N. and Barmanray, A. (2022). A Study on Physico-chemical Properties and Nutritional Profile of Indigenous Cultivar-Black cumin (*Nigella sativa* L.). *International Journal of Food and Nutritional Sciences,* 379-391.

Khazdair, M. R., Ghafari, S. and Sadeghi, M. (2021). Possible therapeutic effects of Nigella sativa and its thymoquinone on COVID-19. *Pharmaceutical biology*, *59*(1), 694-701.

Kinki, A. B. (2020). Physico-Chemical Characteristics of Released and Improved Black Cumin (Nigella Sativa L.) Varieties. *World Scientific Research*, *7*(1), 1-4.

Kiralan, M., Özkan, G., Bayrak, A. and Ramadan, M. F. (2014). Physicochemical properties

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, S Iss 3, Dec 2022

and stability of black cumin (Nigella sativa) seed oil as affected by different extraction methods. *Industrial Crops and Products*, *57*, 52-58.

Lal, G., Meena, S. and Lal, S. (2020). Nigella (Nigella sativa L.), a novel herb can cure many diseases: A review. *International J. Seed Spices*, *10*(1), 1-10.

Nyemb, J. N., Shaheen, H., Wasef, L., Nyamota, R., Segueni, N. and Batiha, G. E. S. (2022). Black Cumin: A Review of its Pharmacological Effects and its Main Active Constituent. *Pharmacognosy Reviews*, *16*(32), 107.

Ramadan, M. F. (2015), Nutritional value, functional properties and nutraceutical applications of black cumin (Nigella sativa L.): an overview, *International journal of food science & technology*, *42*(10), 1208-1218.

Saleh, F. A., El-Darra, N., Raafat, K. and El Ghazzawi, I. (2018). Phytochemical analysis of Nigella sativa L. Utilizing GC-MS exploring its antimicrobial effects against multidrug-resistant bacteria. *Pharmacognosy Journal*, *10*(1).

Salem, M. L. (2005). Immunomodulatory and therapeutic properties of the Nigella sativa L. seed. International immunopharmacology, 5(13-14), 1749-1770.

Sharma, P. C., Yelne, M. B., Dennis, T. J. and Joshi, A. (2005). Database on Medicinal plantsused in Ayurveda and Sidha. *New Delhi: CCRAS, Dept. of AYUSH, Ministry of Health and Family Welfare, Govt. of India*, *1*, 265-6.

Sultana S., Das B., Rudra B.C., Das G. and Alam M.B. (2018). Effect of Date of Sowing on Productivity of Black Cumin. *International Journal of Current Microbiology and Applied Sciences,* 7(1): 1796-1800.

Vaz, N. P., De Oliveira, D. R., Abouelella, G. A. and Khater, H. F. (2018). The black seed, Nigella sativa (Ranunculaceae), for prevention and treatment of hypertension. *JN Govil and Bhardwaj N*, *48*, 221-244.

Wako, F. L. (2020). Black cumin (Nigella sativa L.) Production: A Mini Review. 1-12.

Yimer, E. M., Tuem, K. B., Karim, A., Ur-Rehman, N. and Anwar, F. (2019). *Nigella sativa* L. (black cumin): a promising natural remedy for wide range of illnesses, *Evidence Based Complementary and Alternative Medicine,* 2019.

Ziaee, T., Moharreri, N. and Hosseinzadeh, H. (2012). Review of pharmacological and toxicological effects of Nigella sativa and its active constituents. *Journal of Medicinal Plants*, *11*(42), 16-42.