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Phytochemical Screening and *In vitro* Antioxidant Activities of Methanolic Extract of *Nigella sativa* Seeds

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Abstract: The significance of natural flora is well known by the scientific community. The medicinal plant gifted by nature have by explored by the humans to find out their respective values in the medical field. *Nigella sativa* has been used as traditional medicine for centuries. The aim of the current study is that to examine the phytochemical screening and antioxidant capabilities of methanolic extract of *Nigella sativa* seeds. Dried plant were ground and extracted with methanol to prepare methanol crude extract. *In-vitro* pharmacological tests were conducted using these methanolic extract as per standard procedures. Phytochemical screening of plant showed detection of various medicinally bioactive compounds. Important scavenging results of methanolic extract of *Nigella sativa* obtained during scavenging of free radicals were 85% against DPPH, 87% to ABTS and 92% to β-Carotene at 3 mg/ml. Finally the results obtained in this study that *Nigella sativa* possess important antioxidants and phytochemical compounds.

Key words: Phytochemicals · DPPH · ABTS · 3mg/ml · *Nigella sativa* · *In vitro* assays

INTRODUCTION

Plants are natural factories for the production of chemical compounds, many of which are used to promote health and fight diseases and some of them are marketed as food or herbal medicines [1]. Herbal medicines are universally accepted because of the fact that medicinal plants continue to play important role in healthcare system and also used traditionally in the prevention and treatment of several diseases. The medicinal plants are rich source of secondary metabolites (phytochemicals) like alkaloids, glycosides, steroids and flavonoids, which are potential source of drugs [2]. Many phytochemicals have antioxidant activity and reduce the risk many diseases [3]. Antioxidants are substances that may protect our body cells against the effects of free radicals. Free radicals are molecules produced when our body breaks down food. They can also be produced by environmental exposures like tobacco smoke and radiation. Free radicals can damage cells and may play a role in heart disease, cancer and other diseases [4]. Oxidative damage can lead to a break down or even hardening of lipids, which is the major composition of all cell walls. This breakdown or hardening is due to lipid peroxidation and leads to death of cell or loss of normal cell function. In addition, other biological molecules including RNA, DNA and protein enzymes are also susceptible to oxidative damage. Environmental agents initiate free radical generation, which leads to different complications in body. The toxicity of lead, pesticides, cadmium, ionizing radiation, alcohol, cigarette smoke, UV light and pollution may all be due to their free radical initiating capability [5]. Antioxidants cause protective effect by neutralizing free radicals which are toxic byproducts of natural cell metabolism. The human body naturally produces antioxidants but the process is not 100% effective in case of over whelming production of free radicals and that effectiveness also declines with age [6]. Increasing the anti-oxidant intake can prevent diseases and lower health problems. Research is increasingly showing that antioxidant rich foods and herbs have health benefits. Medicinal herbs are the richest sources of antioxidants compounds [7]. *Nigella sativa* has been used as traditional medicine for centuries.

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Nigella sativa is an annual flowering plant, native to south West Asia and cultivated in middle Eastern Mediterranean region, South Europe, Syria, Turkey, Saudi Arabia, Pakistan, India. In the religion of Islam, the plant has been given a great importance because of its number of uses. As per the religion it is one of the greatest healing plants [8, 9]. The present study is an effort to present out the pharmacology and chemical constituents of the plant.

MATERIALS AND METHODS

Plant Collection: Plant "*N*igella *sativa"* seeds were collected from the market of Bannu city, Bannu, K.P.K (Pakistan) and then identified by Prof. Sultan wazir, Department of Botany, University of Science and Technology Bannu. These plants were washed, dried and then mechanically grinded of mesh size 1 mm approximately.

Extract Preparation: 200 g powder from each of the samples were taken and placed in the 70% commercial grade methanol (CH_3OH) and stirred well, then after passing of 72 hours the extracts were filtered by using qualitative whatman filter paper. In vise bath the filtrate was placed at 40°C and thus the entire methanol was evaporated, so the crude extracts of the plant were obtained and stored it in the refrigerator at 4°C for the purpose of future in-vitro studies.

Phytochemical Screening of *N*igella *sativa*: Phytochemical screening of *N*igella *sativa* was performed to recognize the existence of phytochemicals in the seeds of selected plant by using standard phytochemical methods as described by Khan *et al* (2010) [10].

DPPH Radical Scavenging Activity: The Gymfi *et al*. [11] procedure with some modifications was followed for this assay of DPPH (1, 1-diphenyle -2- picryle hydrazyl). Method was used for determination of DPPH scavenging ability of various fractions. 3 mg DPPH was dissolved in 30 mL methanol to prepare stock solution (Duraipandiyan and Lgnacimuthu, 2009) [12]. The stock solution was further diluted with methanol until reaching an absorbance less than 1.00 using the spectrophotometer at 517 nm. Scavenging calculated through the following formula. Scavenging effect (%) = [(OD of control-OD of sample) / (OD of control)] ×100

ABTS Radical Scavenging Assay: The ABTS radical scavenging activity was measured via the methodology of Re *et al.* [13] with the exception of some modifications.

β-Carotene Bleaching Assay: The β-Carotene bleaching assay was performed as given by Elzaawely *et al*. [14] and modified a little bit. The following are the equation used for β-Carotene bleaching assay.

Bleaching inhibition (%) = (β-carotene content after 2 h of assay/initial β-carotene content) × 100.

RESULTS

Phytochemical Screening of *N*igella *sativa*: The extract was subjected to preliminary phytochemical tests to find out phytoconstituents present in them. The tests were carried to detect the presence of bioactive compounds. Flavonoids, saponins, tannins, anthraquinone and terpenoids were found present while phlobatannins were found absent. The data of qualitative screening of secondary metabolites of plant are tabulated in Table 1.

***In vitro* Antioxidant Activities**

DPPH Free Radical Scavenging Assay: DPPH free radical scavenging activity is used for matching the antioxidant screening .The DPPH scavenging ability of the *Nigella sativa* methanolic extract together with the standard ascorbic acid monitored on different concentrations. Plant extract scavenging activity is very effective but below the standard ascorbic acid as shown in Figure 1.

ABTS Scavenging Activity: The free radical ABTS scavenging ability of the methanolic extract sample combined with the ascorbic acid standard was noted. Figure 2 shows that the ABTS radical scavenging potential of samples extract is lower than the standard ascorbic acid.

β -Carotene Bleaching Assay: The Figure 3 exposed the scavenging efficiency of the various fractions of *N*igella sativa determined by the β-carotene bleaching process. It is found that the scavenging ability of the sample extract is little bit lower than the standard ascorbic acid.

Table 1: Phytochemical screening of *N*igella *sativa*

Sample	Flavonoids	Saponins	Tannins	Anthraquinone	Terpenoids	Phlobatanins
NSME	+	+	+	+	+	–

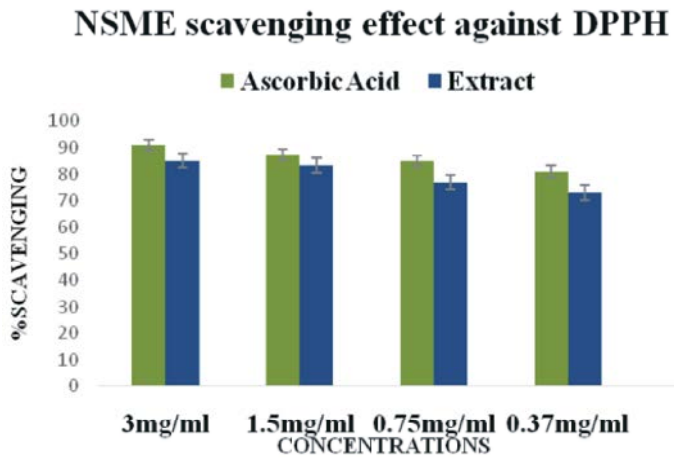

Fig. 1: DPPH free radical scavenging capability of *N*igella *sativa* methanolic extract and ascorbic acid

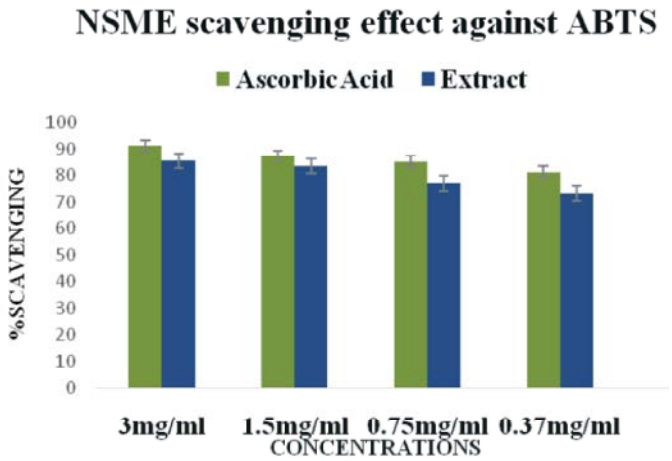

Fig. 2: Free radical ABTS scavenging ability of *N*igella *sativa* methanolic extract and ascorbic acid.

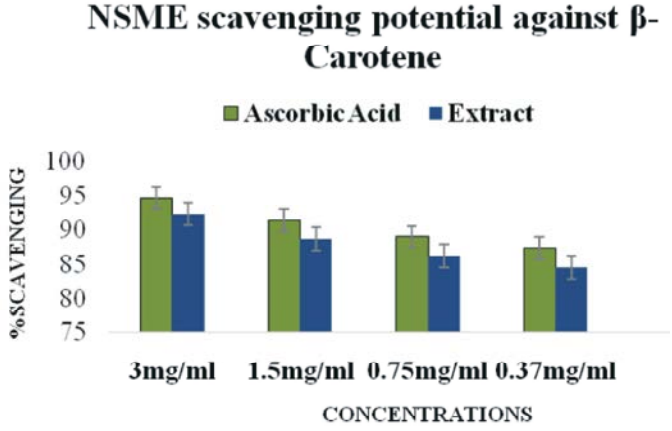

Fig. 3: β-Carotene Free radical scavenging of *N*igella *sativa* methanolic extract and ascorbic acid.

DISCUSSION

Antioxidant and Phytochemical Analysis of *N*igella *sativa* Extract: Plants are natural resource of producing large number of bioactive chemical constituents in a most proficient way and with specific selectivity. Different classes of bioactive compounds have been isolated and characterized from different medicinal plants [15].

It is a known theory in human physiology that anti-oxidants have the capability to fight against free radicals that are involved in almost all the cellular degradation processes leading to cell death [16]. *N*igella *sativa* has the highest anti-oxidant activity and contains different phytochemicals like flavonoids, saponins, tannins, anthraquinone and terpenoids. Data of the present study revealed that various fractions of plant show marked scavenging potential. Our result shows similarity with the investigation of Hagerman *et al.* and Falleh *et al.* [17, 18] reported that medicinal plants markedly scavenge free radicals. The antioxidant potential of various fractions of plant could be due the presence plant bioactive phenolic and polyphenolic compounds which significantly reduce the free radicals which cause oxidative stress. The results obtained by Duenas *et al.* [19] and Kilani *et al.* [20] also support our findings.

CONCLUSION

*N*igella *sativa* showed significant anti-oxidant activities which are might be due to the existence of bioactive compounds.

REFERENCES

1. Warrier, P.K., V.P.K. Nambiar and Ramankutty, 2004. Indian Medicinal Plants- A Compendium of 500 species. Orient Longman Pvt Ltd, Chennai, 4: 139-142.
2. Harborne, J.B, 1973. Introduction to Ecological Bio Chemistry, Second ED, Academic Press, New York, NY.
3. Agbafor, K.N. and N. Nwachukwu, 2011. Phytochemical Analysis and Antioxidant property of leaf extract of vitex doniana and mucuna pruriens. Bio. Chem. Res. Int., pp: 1-4.
4. Langseth, Marcus, G. and A. Silver, Eli, 1996. The Nicoya Convergent Margin-A region of exceptionally low heat flow. Geophysical Research Letters, 23(8): 891-894.
5. Halliwell, B. and J. Gutteridge, 2007. Free radicals in biology and medicine. (4th Edn), Oxford University Press, Oxford, USA.
6. Sies, H., W. Stahl and A.R. Sundquist, 1992. Anti-Oxidant function of vitamins, E & C, Beta-Carotene, other Cartenoids, Annuals of New York. Acad. Sci., 669: 7-20.
7. Sies, H, 2009. Oxidative stress; oxidants and anti-oxidants, Academic press, London. umbelliprenin. DARU, 17(2): 99-103.
8. The Ayurvedic Formulary of India, Part-1, 1978. Ministry of Health and Family Walfare, Government of India, New Delhi, pp: 243-244.
9. Medicinal Plants of India, 1987. Voll.11, ICMR, New Delhi, pp: 474-475
10. Khan, R.A., M.R. Khan and S. Sahreen, 2010. Evaluation of *Launaeaprocumbens* use in renal disorders: A Rat Model. J. Ethnopharmacol., 128: 452-461.
11. Gyamfi, M.A., M. Yonamine and Y. Aniya, 1999. Free-radical scavenging action of medicinal herbs from Ghana:Thonningia Sanguine on experimentally-induced liver Injuries. Gen. Pharmacol., 32: 661-667.
12. Duraipandiyan, V. and S. Ignacimuthu, 2009. Antibacterial and antifungal activity of Flindersine isolated from the traditional medicinal plant, *Toddaliaasiatica* (L.) Lam. Journal of Ethnopharmacology, 123: 494-498.
13. Re, R., N. Pellegrini, A. Proteggente, A. Pannala, M. Yong and C. Rice-Evas, 1999.Antioxidant activity applying an improved ABTS radical cation decolorusation assay. Free Radical Biology and Medicine, 26: 1231-1237.
14. Elzaawely, Abdelnaser, A., D. Tran, Xuan, Haruo, Koyama and Shinkichi Tawata, 2007. Antioxidant activity and contents of essential oil and phenolic compounds in flowers and seeds of Alpiniazerumbet Department of Bioscience and Biotechnology, Faculty of Agriculture, University of the Ryukyus, Okinawa. Food Chemistry, 104: 1648–1653.
15. Khan, W.U., R.A. Khan, M. Ahmed, N. Mushtaq, L.U. Khan, M.W. Khan, A. Nawz, S. Afreen, S. Ahmad and A. Saboor, 2015. Phytotoxic and Cytotoxic Evaluation of Methanolic Extract of *Trifolium alexandrinum*. World Applied Sciences Journal, 33(4): 542-546.
16. Vaishali, K., V. Varsha Uma Eswaran, Priya and H.R. Bhargava, 2015. A Comparative Study of the Biochemical, Antioxidative and Anti-microbial Activity of Apis and Trigona Honey Collected from Different Geographical Areas of India. World Applied Sciences Journal, 33(1): 160-167, ISSN 1818-4952 © IDOSI Publications, DOI: 10.5829/idosi.wasj.33.01.55.

17. Hogerman, A.E., K.M. Riedl, G.A. Jones, K.N. Sovik, N.T. Ritchard and P.W. Hartzfeld, 1998. High molecular weight plant polyphenolice (tannins) as biological anti-oxidants. J. Agric. Food Chemistry, 46: 1887-92.
18. Falleh, H., R. Ksouri, K. Chaieb, N. Karray-Bouraoui, N. Trabelsi, M. Boulaaba and C. Abdelly, 2008. Interspecific variability of antioxidant activities and phenolic composition inMesembryanthemum genus. Food and Chemical Toxicology, 47: 2308-2313.
19. Duenas, M., T. Hernandez and I. Estrella, 2006. Assessment of *in vitro* antioxidant capacity of the seed coat and the cotyledon of legumes in relation to their phenolic contents. Food Chemistry, 98: 95-103.
20. Kilani, S., M.B. Sghaier, I. Limem, I. Bouhlel, J. Boubaker, W. Bhouri, I. Skandrani, A. Neffatti, R.B. Ammarb, M.G. Dijoux-Franca, K. Ghedira and L. Chekir-Ghedira, 2008. *In vitro* evaluation of antibacterial, antioxidant, cytotoxic and apoptotic activities of the tubers in fusion and extracts of *Cyperus rotundus*. Bioresource Technology, 99: 9004-9008.