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Quantification of Thymoquinone (TQ) and Antioxidant Properties in Hydro -ethanolic extract of *Nigella sativa*

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Abstract:- Nigella sativa L. belongs to family Ranuculaceae family, is an annual herbaceous plant which is found in the Middle East, Eastern Europe, Western Asia, and Central Asia. Because of its pharmacological and neuro protective properties, this plant has been largely studied in recent years. The goal of this study is to look at the antioxidant properties of Nigella sativa seeds hydro-ethanolic extract. N.sativa seeds were soxhlet extracted in 70% ethanol (v/v) for 48 hours at 50C°. DPPH, hydroxyl radical, and singlet oxygen testing methods were used to estimate antioxidant capabilities. Using HPLC, the presence and concentration of Thymoquinone, a bioactive component, was also determined in the hydro-ethanolic extract. The main component found in the N. sativa seed extract was shown to have exceptional antioxidant capabilities. As a result, it can be regarded an important agent in nutrition, pharmaceuticals, and the treatment of oxidative stress.

Keywords:- *Nigella sativa, Hydro-ethanolic extract, Thymoquinone, Antioxidant properties, HPLC.*

I. INTRODUCTION

The uncontrolled sequence of reduction and oxidation processes that culminates in reactive oxygen species is caused by free radicals (ROS).The oxidative stress due to these radicals is induced by various environmental factors like radiations, pathogen invasion, chemical pollutants and toxins. These radicals are to be quenched as they would lead to the development of many diseases such as cardiovascular disease, neurodegenerative diseases and other inflammatory diseases [1]. Studies on natural antioxidants that are abundant in seeds, leaves, flowers and fruits have gained significant attention. Free radical production causes oxidative stress, which may be prevented by using an antioxidant. An antioxidant is an essential molecule that has the ability to quench ROS. Recently, it has been shown that a huge number of medicinal plants across the world exhibit a wide range of biological features, including high levels of antioxidant components [2, 3].

Nigella sativa seed (Black cumin) is a Ranunculaceae family annual flowering plant. It has long been thought to have medicinal properties. It's used to treat asthma, headaches, dysentery, infections, obesity, hypertension, and gastrointestinal anticarcinogenetic, antiulcer, antibacterial, antifungal, anti-inflammatory, and antioxidant actions such quenching reactive oxygen species [4]. Thymoquinone (TQ) is N.*sativa*'s main element, and its biological action is related to its oil component [5, 6]. Natural antioxidants are also in high demand for uses in nutraceuticals and functional foods. As a result, the goal of this work is to determine the amount of TQ in *Nigella sativa* L. Hydro-ethanolic seed extract and its ability to scavenge free radicals.

II. MATERIAL AND METHODS

The Nigella sativa seeds were dried, ground, and extracted using Soxhlet with 70% ethanol as the solvent. The extract was filtered and dried with a rotary evaporator before being stored at -4°C for future analysis.

A. Determination of extraction yield

The extraction yield is a measure of the solvent efficiency from the original material. The extraction yield is calculated as percentage of the weight of the crude extract to the raw material. In the hot air oven, a part of each extract was evaporated and dried until it reached a consistent weight. The extraction yield was calculated using the below formula and result expressed in milligram of dry extract per gram of sample.

*Extraction yield (%) = (Weight of the dried Nigella sativa extract) / (Weight of the original Nigella sativa sample)*100*

B. DPPH Free radical scavenging activity

DPPH radical scavenging test [7] was used to determine the potential of *N*.sativa hydro-ethanolic extract to quench DPPH. This approach relies on the reduction of purple 1,1-diphenyl-2-picrylhydrazyl (DPPH) to a yellow-colored diphenyl-picryl hydrazine, with the residual DPPH having the highest absorption at 517nm, as determined spectrophotometrically. DPPH (3 mL) and extract at various doses (1-100g/mL), the mixtures were properly mixed before being incubated at room temperature for 15 minutes in the dark. Methanol was served as control. The absorbance of the sample and the standard were read at 517nm and compared. Increasing DPPH radical scavenging activity is shown by a reduction in the absorbance of DPPH solution. The following equation was used to determine the ability to scavenge the DPPH. As a reference, quercetin was utilised.

$$\text{DPPH radical scavenging activity} = (A_o - A_1)/A_o \times 100$$

Where: A_o is the absorbance of the DPPH and A_1 - is the Absorbance of the *N.sativa* extract.

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C. Hydroxyl radical scavenging assay:

The 2-deoxyribose assay was used to determine the effect of the *N.sativa* extract on –OH radical. Various concentrations of extract were mixed with of deoxy ribose (0.6mL 1mM) and the volume made up by buffer (phosphate 1.6mL). The reaction mixture was incubated at an ambient temperature for 10 min and 0.4mL of phenyl hydrazine hydrochloride (0.2mM) was added. Further, reaction mixture was incubated for 1h after adding TCA and TBA (each of 1mL 2.8% and 1) and heated for 10 minutes. The absorbance was measured at 532nm and negative control without any antioxidant was considered 100% deoxyribose oxidation. The percentage of hydroxyl radical scavenging activity of *N.sativa* extract was determined by comparing with the negative control. Ascorbic acid was used as the standard [3].

D. Hydrogen peroxide scavenging assay

Hydrogen peroxide radical scavenging activity of *N.sativa* extract was determined by adding hydrogen peroxide (1mL 1M) to *N*. sativa extract (1 mL), Ammonium molybdate (3%), sulphuric acid (2M) and potassium iodide (1.8 mM) was added. The mixture was titrated against sodium thiosulphate (5.09 mM). The end point was yellow to colourless. The reaction mixture without N.*sativa* extract was used as control. Ascorbic acid was used as the standard [8].

The percentage inhibition was calculated using the formula,

$\%\ \text{Inhibition} = ((V_0-V_1)/V_0) \times 100$

Where, V_0 - Volume of sodium thiosulphate used by control, V_1 – Volume of sodium thiosulphate used by *N.sativa* extract.

E. Thymoquinone (TQ) quantification by HPLC:

Thymoquinone in *N. Sativa* ethanolic extract was quantified by HPLC (High Pressure Liquid Chromatography) in a Shimadzu HPLC model followed the method [9].

F. Statistical Analysis:

All data were represented as mean ± SD of means of 3replicates. The IC_{50} were calculated using Graph Pad Prism Software (version 6).

III. RESULTS AND DISCUSSIONS

A. Extraction yield

The extraction yield of Hydro-ethanolic extract of *Nigella sativa* was calculated, the average percentage yield of 3 replicates was 34.42±0.69 %.

B. DPPH radical scavenging assay

The radical scavenging activity of antioxidant substances is commonly measured using DPPH, a stable free radical. It is based on the formation of a non-radical from the reduction of DPPH solution in the presence of a hydrogen donating antioxidant. The antioxidant capacity of the *N.sativa* hydro ethanolic extract against DPPH was raised when the concentration of the extract was increased, with an IC_{50} value of 29.5g/mL. (Fig 1: A). In the ascorbic acid standard, a parabolic pattern (1-10g/mL) was found, with linearity in scavenging activity up to 10g/mL and no substantial increase in scavenging potential above this dose. The scavenging activity increased with the rise in concentration in the current investigation. Similar tests in petroleum ether, distilled water, and methanol extract revealed a dose-dependent increase in scavenging activity [10].

C. Hydroxyl radical scavenging assay

N.sativa extract and conventional ascorbic acid both prevent hydroxyl radical-mediated deoxyribose breakdown in this assay. The extract has a lower IC_{50} value of 48.52 g/mL than the standard of 49.8 g/mL. (Fig 1: B). One of the most strong reactive oxygen species in the biological system is hydroxyl radical. It interacts with polyunsaturated fatty acid moieties in cell membrane phospholipids, causing oxidative cell damage. In a concentration-dependent manner, the extract and the standard scavenged the hydroxyl radical generated by the Fe2+ ascorbic acid and EDTA – H_2O_2 system.. This finding shows that *N.sativa* seed extract might be employed as an alternative to synthetic antioxidants in the fight against hydroxyl radical oxidation [11, 12].

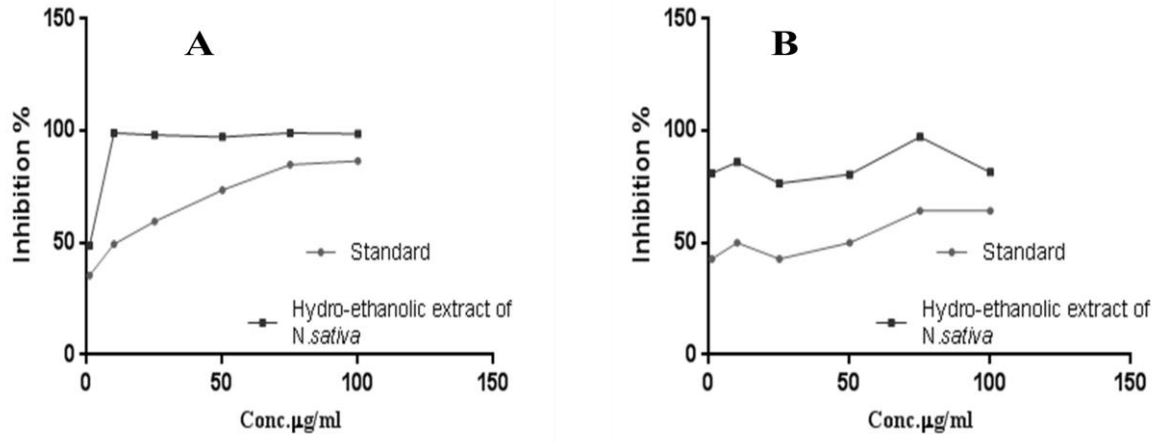

Fig. 1: (A) DPPH radical scavenging activity. (B) Hydroxyl radical scavenging activity.

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D. Hydrogen peroxide radical scavenging assay

The hydroxide scavenging assay revealed that *N.sativa* had a lower inhibition of 33.33 % than conventional Ascorbic acid, which had a higher inhibition of 46.1 %. Hydrogen peroxide (H_2O_2) is a mild oxidising agent that inactivates certain enzymes directly, generally by oxidising crucial thiol (-SH) groups. H_2O_2 controls a wide range of biological activities and plays an important role in signalling. Nejdet *et al* reported that reducing power in methanolic extract of *N.sativa* showed increased with increasing concentrations [13].

E. HPLC Quantification of Thymoquinone (TQ)

HPLC analysis was performed on the hydro-ethanolic extract of *N.sativa*. The amount of TQ was found to be 0.25% in the extract. Ashraf *et al 2018* reported depending upon the choice of solvent and extraction technique the antioxidant potential of thymoquinone and content varies [14, 15].

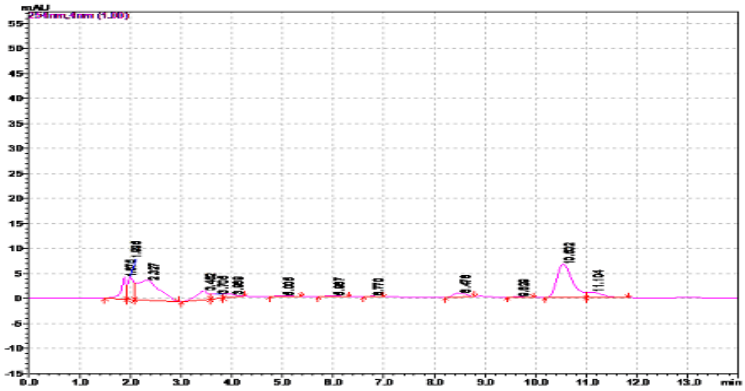

Fig.2: *N.sativa* L. seed extract HPLC peak

IV. CONCLUSION

The Antioxidant ability of the Hydro-ethanolic extract demonstrated a considerable antioxidant activity, and it also validated the existence of Thymoquinone, according to the findings of this study. TQ is the most common bioactive component utilized in the treatment of numerous ailments in the past. More bioactive chemical isolation and therapeutic value assessment in live models are needed.

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