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ANTIADIPOGENIC, FREE RADICAL SCAVENGING AND ANTI-INFLAMMATORY ACTIVITY OF *NIGELLA SATIVA* SEED FRACTIONS

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Keywords:

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ABSTRACT: Obesity and overweight have recently drawn greater attention due to their central role in the rapid increase of metabolic syndrome disorders. The research pursuit for exploring novel and effective therapeutic alternatives to deal with obesity ailments and oxidative stress has received immense interest. In this study, different solvent fractions of *Nigella sativa* (*N. sativa*; Black cumin) seeds were prepared, and their phytochemical analysis was done. Among different solvent fractions, the methanolic fraction of *N. sativa* (MFNS) contained a high level of polyphenols (451.77 ± 0.91 µg/mL) and flavonoids (401.24 ± 0.73 µg/mL). The GC-MS analysis of MFNS showed the presence of several glycosides, oils, and polyphenolic compounds, as mentioned in the results section. The effect of *N. sativa* seed fractions on inhibition of key digestive enzymes linked to adiposity such as α-amylase, α-glucosidase and pancreatic lipase was evaluated. The free radical scavenging activity of seed fractions was studied through standard antioxidant assays (DPPH and NO). The enzyme inhibitory effect of MFNS for α-amylase, α-glucosidase and pancreatic lipase was 81.47%, 77.12%, and 84.26%, respectively, which were much higher than the rest of the *N. sativa* fractions. The MFNS at 250 µg/mL demonstrated considerable anti-inflammatory activity on RAW 264.7 macrophages, in terms of inhibition of LPS-induced nitric oxide production (33.02%) and membrane-stabilizing activity (84.26%). In conclusion, our data suggest that MFNS of *N. sativa* seeds is rich in several bioactive phytochemicals that qualify it as an effective therapeutic alternative to attenuate free radical stress, inflammation, and obesity ailments.

INTRODUCTION: Excess energy intake and a sedentary lifestyle are the primary causes of the growing incidence of obesity and associated ailments, generally referred to as "New world syndrome". New world syndrome is similar to metabolic syndrome, including obesity, hypertension, type 2 diabetes, cardiovascular diseases (CVDs), *etc.* [1].

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Overweight and obesity lead to low-grade inflammation, oxidative stress, hormonal imbalances and certain forms of cancers [2]. Despite enormous global demand for anti-obesity drugs, very few drugs and formulations in the market are approved by the foods and drug administration (FDA) / European Medical Union (EMU). Some of the approved anti-obesity drugs like Sibutramine were later withdrawn due to considerable side effects [3].

As a result, there is a growing public inclination to favor natural product-based medications [4]. Therefore, the scientific community is exploring developing safe and cost-effective drugs/molecules from natural products [5].

In this context, the role of plant-based phytochemicals and more particularly, nutraceutical-based products gained significance [6]. Previous studies on functional foods highlighted their beneficial role in mitigating oxidative stress and other metabolic complications [7]. Indian and Chinese traditional systems of medicine have emphasized the therapeutic effects of several plants and herbs in curing many human and animal diseases [8]. Decades of scientific research have also reported the medicinal claim of certain plant-based natural and healthier alternatives to treat some metabolic disorders [9]. *Nigella sativa* (*N. sativa*) is a member of the Ranunculaceae family and a widely used medicinal plant worldwide. *Nigella* seeds and oil have a long history of usage in various recipes and traditional systems of medicines. The seeds of *N. sativa* have been widely used to treat different diseases and ailments [10]. Given the above, the present study was focused on preparing different solvent fractions of *N. sativa* seeds and evaluating their free radical scavenging efficacy, inhibitory activity on key digestive enzymes, and anti-inflammatory activity.

MATERIALS AND METHODS:

Chemicals and Reagents: Gries reagent, 2,2-diphenyl-2-picrylhydrazyl radical (DPPH), ascorbic acid, pancreatic α-amylase, pancreatic lipase, α-glucosidase, acarbose, 3,5-dinitrosalicylic acid (DNS) color reagent, orlistat, p-NPB (P-nitro phenyl butyrate), isopropyl alcohol, gallic acid, and quercetin were obtained from Sigma Aldrich, Bangalore. Folin-Ciocalteu's phenol reagent and other chemicals used in this study were of analytical grade.

Plant Material Collection and Preparation of Fractions: *Nigella sativa* seeds were collected from the local market, dried, pulverized, and soaked in maceration chambers with different solvents (Hexane, Ethyl acetate, Hydroalcoholic, Methanolic, and Aqueous) at room temperature (RT). Crude solvent fractions of *N. Sativa* were filtered and concentrated in Rota-evaporator (Heidolf). The dark brown sticky material was collected and fractionated using silica gel in column chromatography with different eluting solvents. Preliminary phytochemical analysis of hexane, ethyl acetate, methanol, and aqueous fractions was done using the standard methods of Harborne [11]. Based on TLC and phytochemical analysis, the Methanolic fraction of *Nigella sativa* (MFNS) was used for GCMS/HRMS and other experiments.

Determination of Total Phenolic Content: Total phenolic compounds present in *N. sativa* seeds was estimated by using the Folin-Ciocalteu method [12]. Briefly, the sample solution (0.6 mL) at different concentrations (ranging from 100 to 500 µg/mL) was mixed with 2.58 mL of Folin-Ciocalteu's phenol reagent. After 3 min, 0.3 mL of a saturated sodium carbonate solution was added to the mixture. The reaction mixtures were incubated at room temperature (25°C) for 20 min, and the absorbance was measured at 760 nm with a spectrophotometer. A dose-response linear regression curve was generated using the gallic acid standard absorbance. The concentration of phenolic compounds was expressed as gallic acid equivalents (mg of GAE/gm of a fraction). The estimation was performed in triplicate, and the results are expressed as mean ± SD.

Determination of Total Flavonoid Content: Total flavonoid content was estimated by the aluminium chloride method [12]. *N. sativa* seed fraction (0.5 µL) was mixed with 2.5 mL of distilled water and 150 µL $NaNO_2$ solution (5 %). The contents were vortexed for a few seconds and kept at room temperature for 5 min. Then, 300 µL $AlCl_3$ (10 %), 1 mL NaOH (1 mM) and 550 µL of distilled water were added. The solution was mixed well and kept for 15 min. The absorbance of the sample was measured at 510 nm. The total flavonoid content was calculated using the quercetin standardization curve. The results were expressed as mg of quercetin equivalent (QE) per gram of sample.

Free Radical Scavenging Assays:

DPPH Assay: Free radicals scavenging activity of *N. sativa* fraction against 2,2-Diphenyl-1-picryl hydroxyl radical (DPPH) was determined spectrophotometrically at 517 nm according to the procedure of Baliyan [13] with some modifications using vitamin C as a standard. Briefly, a 0.3 mM solution of DPPH was prepared in methanol, and 500 µL of this solution was added to 1 mL of the fraction (dissolved in 10% DMSO) at different concentrations (100-500 µg/mL). These solutions were mixed and incubated in the dark for 30 min at

room temperature. The free radical scavenging activity was calculated using the formula.

Absorbance = Control – Test × 100 / Control

Nitric Oxide (NO) Radical Scavenging Activity: Sodium nitroprusside (5 µM) in phosphate buffer (pH 7.4) was mixed with different concentrations (100-500 µg/mL) of *N. sativa* seed fractions and the tubes were incubated at 25 ºC for 120 min [14]. A control experiment was conducted with an equal amount of solvent in an identical manner. At 5 min intervals, 0.5 mL of incubation solution was taken and diluted with 0.5 mL of Griess reagent (1% sulfanilamide, 0.1% N-naphthyl ethylenediamine dihydrochloride and 2% o-phosphoric acid dissolved in distilled water). The chromophore's absorbance formed during diazotization of nitrite with sulfanilamide and resultant N-naphthyl ethylenediamine dihydrochloride was read at 546 nm. Nitric oxide scavenging activity was calculated by the following equation.

Absorbance = Control – Test × 100 / Control

Key Digestive Enzyme Inhibition Assays:

***In-vitro* Pancreatic Lipase Assay:** The ability of MFNS to inhibit the pancreatic lipase (PL) was measured using p-NPB (P-nitro phenylbutyrate) as the substrate following the procedure described by Duarte [15]. An enzyme buffer was prepared by the addition of 30 µL (10 units) of PL in 10 mM MOPS and [1 mM EDTA, pH 6.8], 850 µL of Tris-buffer (100 mM Tris HCl and 5 mM $CaCl_2$, pH7.0). 100 µL of the test sample or orlistat was mixed with 880 µL of the enzyme buffer and incubated for 15 min at 37°C. A 20 µL of substrate solution (10 mM p-NPB) was added to the reaction. The enzymatic reaction was allowed to incubate for 15 min at 37°C. The lipase activity was determined by measuring the hydrolysis of P-NPB to P-nitro phenol at 405 nm using an ELISA reader. The inhibition of enzyme activity was calculated as follows

% Lipase inhibition = 1 (Absorbance of sample × 100) / Control

***In-vitro* α-Amylase Assay:** The α-amylase activity was determined according to the method described by Karunakaranet al [16] with slight modifications. Briefly, 40 µL of α-amylase (5 U/mL) was mixed with 0.36 µL of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) and 0.2 µL of MFNS or acarbose. After incubation for 20 min at 37 °C, 300 µL of starch solution (1%) in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) was added, the mixture was re-incubated for 20 min, followed by the addition of 0.2 mL dinitrosalicylic acid (DNS). The contents were mixed well and kept in a boiling water bath for 5 min. The reaction mixture was diluted by adding 6 mL distilled water, and the absorbance was measured at 540 nm in a UV-Visible spectrophotometer. Acarbose was used as a positive control. All assays were carried out in triplicate.

% α - Amylase inhibition = 1 (Absorbance of sample × 100) / Control

***In-vitro* α-Glucosidase Assay:** The α-glucosidase inhibitory activity of MFNS was determined according to the method described by Etsassala *et al.* [17], with slight modifications. Briefly, 250 µL of MFNS or acarbose at different concentrations (100-500 µg/mL) was incubated with 500 µL of 1.0 U/mL α-glucosidase solution in 100 mM phosphate buffer (pH 6.8) at 37 °C for 15 min. Thereafter, 250 µl of p-nitrophenyl D-glucoside solution (5 mM) in 100 mM phosphate buffer (pH 6.8) was added and the mixture was further incubated at 37°C for 20 min. The absorbance of the released p-nitrophenol was measured at 405 nm and the inhibitory activity was expressed as a percentage of the control without inhibitor. All assays were carried out in triplicate and the calculation was done according to the following formula.

% α - Glucosidase inhibition = 1 (Absorbance of sample × 100) / Control

GC-MS/HRMS Analysis: Methanolic *Nigella sativa* (MFNS) fractions were analyzed using GC-MS (Gas Chromatography-Mass Spectroscopy) [18]. The analysis was conducted with a JEOL GC MATE II, GC coupled with a quadruple double-focusing mass analyzer, and a photon multiplier tube detector was used HP 5 ms Column was used (30 m-0.25 mm internal diameter, 0.25 mm film thickness). The ultrapure helium was used as a carrier gas at a 1 mL/min flow rate and a linear velocity of 37 cm/s. The injector temperature was set at 250°C. The initial oven temperature was 50°C, which was ramped up to 250°C at a rate of 10°C/min with a hold time of 3 min.

Injections of 1 mL sample were made in the split less mode. The mass spectrometer was operated in the electron ionization mode at 70 eV with the electron multiplier voltage at 1859 V. Other MS operating parameters were as follows: ion source temperature 230°C, quadrupole temperature 250°C, solvent delay 4 min and scan range 50 to 600 amu. Compounds were identified by direct comparison of the retention times and mass fragmentation pattern with those from the National Institute of Standards and Technology library (NIST) Ver.2.1 MS data library and comparing the spectrum obtained through GC–MS compounds present in the plant fractions were identified.

Cell Culture Experiments: RAW 264.7 cells, a murine macrophage cell line, were obtained from NCCS Pune and cultured in Falcon plates (100 mm), grown at 37°C in 5% CO_2 in DMEM supplemented with 10% FBS, 2 mM glutamine, and 1,000 U/mL penicillin-streptomycin [19]. The medium was changed on alternate days. The cells were passaged by trypsinization (Trypsin EDTA) to disrupt the cell monolayer at confluence while splitting RAW264.7 cells to continue cultures and plating the cells for the *in-vitro* assays.

Cell Viability Assay (MTT Assay): As described previously, the murine macrophage-like RAW 264.7 cells were cultured. Cells were seeded at a density of 10^4 cells/well in 96-well plates, grown overnight followed by the pre-treatment with different concentrations of the EETP (50, 100, 150, 200 and 250 mg/mL) for 1 h before the addition of the LPS. RAW 264.7 cells viability was measured after 24 h of exposure to the MFNS with a colorimetric assay, based on the ability of mitochondria in viable cells to reduce MTT [20]. A concentration of 0.5 mg/mL of MTT solution was added to each well. After 4 h of incubation at 37°C, the medium was discarded and the formazan blue color formed in the cells were dissolved in dimethyl sulfoxide (DMSO). Optical density at 570 nm was determined with a microplate reader (Bio-Rad).

Anti-Inflammatory Activity:

Quantification of NO in RAW 264.7 Cells: The levels of Nitic oxide production in the culture medium of 264.7 macrophages were measured using a standard procedure using the Griess reagent kit according to the manufacturer's instructions. Cells were seeded onto a 96-well plate with 2 x 10^5 cells/well and allowed to adhere overnight. Then, the medium was removed and replaced with 0.2 mL of fresh medium alone or containing 0.5 mg/mL of fractions. After 1 h of incubation, LPS stimulation was performed. LPS was added at a 1 mg/mL concentration for 24 h. The cell-free culture medium was collected, 50 µLof it was used for NO determination, and the remainder was stored at -20°C for further use. The nitrite accumulated in the culture medium was measured as an indicator of NO production, based on the Griess reaction [21].

Briefly, 50 µL of culture supernatants were gently mixed with an equal volume of sulfanilamide solution (1% sulfanilamide in 5% phosphoric acid) and incubated in the dark at room temperature (RT) for 10 min. After the incubation, 50 µL of 0.1% n-naphthyl ethylenediamine dihydrochloride (NEDA) was added to the reaction and incubated in the dark at RT for another 10 min. The absorbance at 540 nm was measured in a microplate reader (Bio-Rad max 100). Nitrite concentration, an indicator of NO production, was calculated from a $NaNO_2$ standard curve.

Membrane Stabilization Assay: The membrane stabilization method has been used to prepare blood samples of human red blood cells (HRBC) to study *in-vitro* anti-inflammatory activity [22]. The blood was collected from healthy volunteers who had not taken any Nonsteroidal anti-inflammatory drug (NSAIDs) for 3 weeks before the experiment and mixed with an equal volume of Alsever solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid, and 0.42% NaCl). All the blood samples were stored at 4°C for 24h before use. They were centrifuged at 3000 rpm for 5 min, and the supernatant was discarded. The cell suspension was washed with sterile saline solution (0.9% w/v NaCl) and centrifuged at 2500 rpm for 5 min. Centrifugation was re-peated till the supernatant was clear and colorless, and the packed cell volume (PCV) was measured. The cellular component was reconstituted to a 40% suspension (v/v) with phosphate-buffered saline (10 mM, pH 7.4) and was used in the assays.

Hypotonicity-Induced Hemolysis: To 0.5 mL of human RBC suspension, 1 mL of MFNS in

phosphate buffer, and 2 mL hyposaline was added, incubated at 37°C for 30 min and centrifuged at 3000 rpm for 20 min.

The hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm. Ibuprofen (200 µg/mL) was used as reference standard.

The percentage inhibition of hemolysis or membrane stabilization was calculated [23].

% Inhibition of Hemolysis = 100 x (OD1-OD2/OD1)

Where, OD1 = Optical density of hypotonic-buffered saline solution alone.

OD2 = Optical density of test sample in hypo-tonic solution.

Statistical Analysis: Data are expressed as mean values ± standard deviation (SD). Comparisons between control and test groups were performed with (Tukey's honest significant difference multiple range post hoc test $p < 0.05$ IBM SPSS Ver.23). The experiments were performed in triplicate.

RESULTS AND DISCUSSION:

Preliminary Phytochemical Analysis: As shown in **Table 1**, among the tested fractions, a methanolic fraction (MFNS) was found to be rich in flavonoids, polyphenols, saponins, quinones, and glycosides followed by chloroform fraction (CFNS) and aqueous fraction (AqFNS). The presence of such phytochemicals might play a decisive role in the therapeutic activity of *Nigella* seeds.

TABLE 1: PHYTOCHEMICAL ANALYSIS OF *NIGELLA SATIVA* FRACTIONS

Phytochemicals	HFNS	EAFNS	CFNS	MFNS	AqFNS
Flavonoids	-	+	+	++	+
Phenols & Tannins	-	-	-	++	+
Saponins	-	-	-	++	+
Glycosides	-	+	+	++	+
Steroids	+	-	++	+	+
Terpenoids	+	+	+++	-	-
Quinones	-	-	+	++	+
Alkaloids	++	++	-	+	+

Total Polyphenols and Flavonoids Content: The results of total polyphenols (TPC) and total flavonoids content (TFC) in different solvent fractions of *N. sativa* are presented in Table 2. The content of phenolic and flavonoid compounds extracted from biological substances is dependent on location, soil, season, and extraction conditions. The Folin-Ciocalteu method determined the TPC and expressed it as milligram gallic acid equivalent (GAE) per 100 g of dry weight. Among different fractions of *N. sativa* seed, the methanolic fraction (MFNS) showed the highest yield of polyphenols (451.77 mg/g GAE), as shown in **Table 2**. Also, MFNS had higher flavonoids (401.24 mg/g QE) compared with the rest of the solvent fractions of *N. sativa*.

TABLE 2: TOTAL POLYPHENOL CONTENT AND TOTAL FLAVONOID CONTENT OF *NIGELLA SATIVA* FRACTIONS

Fractions	Total Polyphenol Content	Total Flavonoid Content
HFNS	0.24 ± 0.04	0.11 ± 0.91
EAFNS	86.01 ± 0.27	47.52 ± 0.04
CFNS	98.3 ± 0.45	73.21 ± 1.54
MFNS	451.77 ± 0.91	401.24 ± 0.73
AqFNS	342.33 ± 1.7	146.27 ± 1.61

According to Tungmunnithum *et al.*, there is a correlation between polyphenolic, flavonoid content, and antioxidant capacities in medicinal plants since medicinal plants are generally a rich source of antioxidant compounds [24]. In the present context, the high content of polyphenols and flavonoids in MFNS might be crucial in the free radical scavenging and antioxidant capacity of *N. sativa* seeds, as demonstrated by DPPH assay Nitric oxide (NO) assays.

GC-MS & HRMS Analysis: Different fractions of *N. sativa* seeds were subjected to HRMS analysis, and their spectral data was depicted in **Fig. 1**. In

addition, GC-MS analysis of methanolic fractions of *Nigella sativa* seeds showed more than 20 phytoconstituents **Table 3**. The major components identified include thymoquinone, thymo-hydroquinone, rutin, astragalin, nigellidine, some fatty acids *etc*.

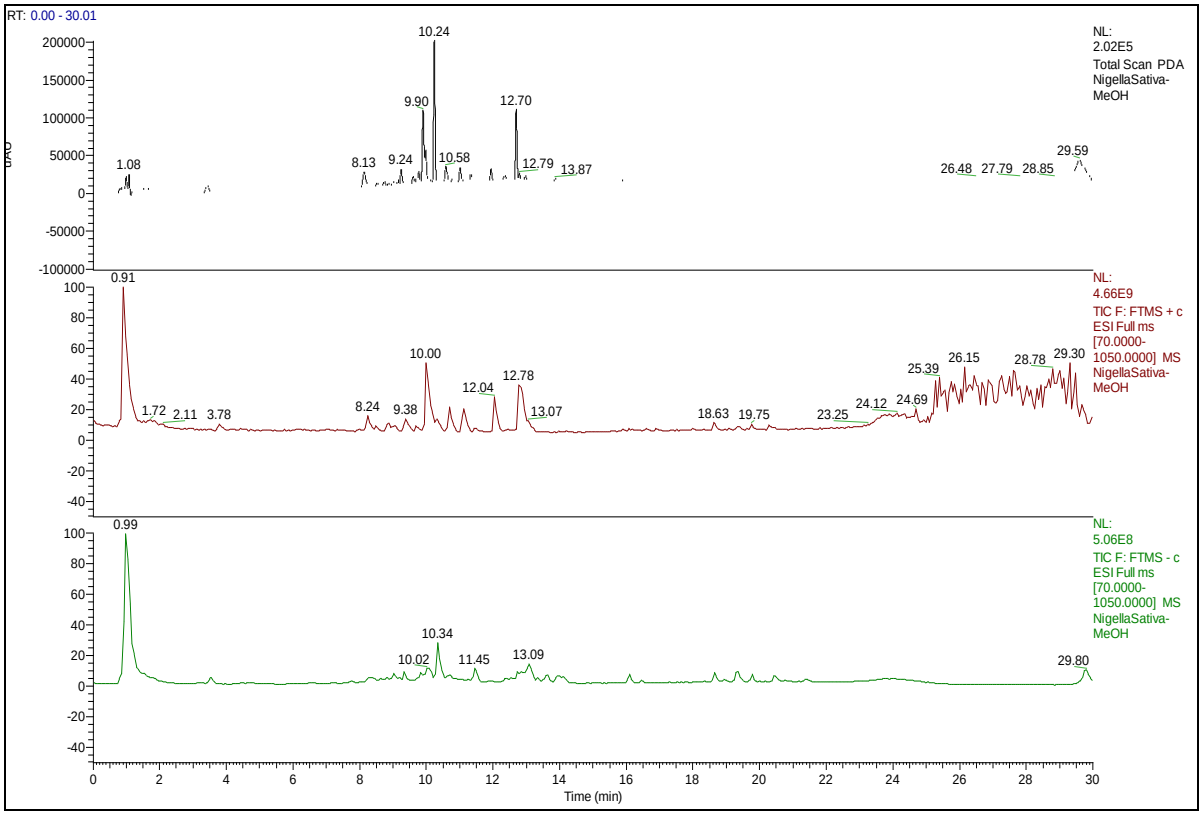

FIG. 1: HRMS ANALYSIS OF *NIGELLA SATIVA* FRACTIONS

TABLE 3: GC-MS ANALYSIS OF *NIGELLA SATIVA* FRACTIONS

S. no.	Chemical	Plant Part	Elemental Composition	Mono Isotopic Mass	M+H	M-H
1.	Valine	Seed	$C_5H_{11}NO_2$	117.0789	118.0867	116.071
2.	Leucine	Seed	$C_6H_{13}NO_2$	131.0946	132.1024	130.087
3.	Glutamic-acid	Seed	$C_5H_9NO_4$	147.0531	148.0609	146.045
4.	Carvone	Seed	$C_{10}H_{14}O$	150.1044	151.1122	149.097
5.	Thymoquinone	Seed	$C_{10}H_{12}O_2$	164.0837	165.0915	163.076
6.	Thymohydroquinone	Seed	$C_{10}H_{14}O_2$	166.0993	167.1071	165.092
7.	Arginine	Seed	$C_6H_{14}N4O_2$	174.1116	175.1194	173.104
8.	Tyrosine	Seed	$C_9H_{11}NO_3$	181.0738	182.0816	180.066
9.	Linoleic-acid	Seed	$C_{18}H_{32}O_2$	280.2402	281.248	279.232
10.	Nigellidine	Seed	$C_{18}H_{18}N_2O_2$	294.1368	295.1446	293.129
11.	Eicosadienoic-acid	Seed	$C_{20}H_{36}O_2$	308.2715	309.2793	307.264
12.	Astragalin	Seed	$C_{21}H_{20}O_{11}$	448.1005	449.1083	447.093
13.	Quercetin-3'-glucoside	Seed	$C_{21}H_{20}O_{12}$	464.0954	465.1032	463.088
14.	Rutin	Seed	$C_{27}H_{30}O_{16}$	610.1533	611.1611	609.146
15.	Isoleucine	Seed	$C_6H_{13}NO_2$	131.0946	132.1024	130.087
18.	Linoleic-acid	Seed Oil	$C_{18}H_{32}O_2$	280.2402	281.248	279.232
19.	Oleic-acid	Seed Oil	$C_{18}H_{34}O_2$	282.2558	283.2636	281.248
20.	Palmitic-acid	Seed Oil	$C_{16}H_{32}O_2$	256.2402	257.248	255.232
21.	Thymoquinone	Seed Oil	$C_{10}H_{12}O_2$	164.0837	165.0915	163.076

Effect of *N. sativa* Seed Fractions on DPPH Radicals: In-vitro DPPH assay is extensively used to evaluate various components' free radical scavenging activity. The results of the DPPH assay by different solvent fractions are presented in **Fig. 2**. Among different fractions, MFNS showed significantly higher inhibitory activity when compared with other solvent fractions in a dose

depended manner. Maximum antioxidant capacity (88.26%) was observed at the concentration of 250 µg/mL. These results are similar to previous studies by Baliyan *et al* [13]. The degree of decoloration indicates the scavenging potential of the fraction due to hydrogen proton donation.

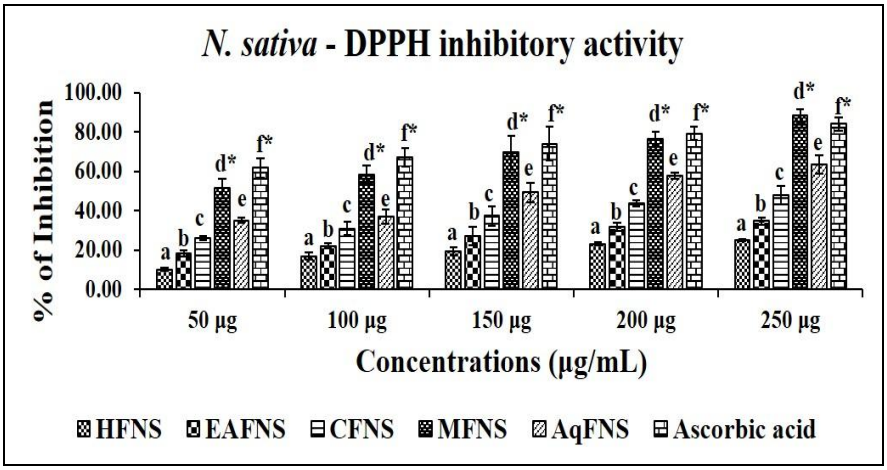

FIG. 2: EFFECT OF MFNS ON DPPH RADICALS

Nitric Oxide Scavenging Activity: Nitric oxide is a central agent in physiological and pathological conditions and, when reacted with macromolecules, may induce inflammation, muscle sclerosis, arthritis, ulcerative colitis, and other metabolic disorders [25]. The effect of *N. sativa* seed fractions against nitric oxide radicals was evaluated in this study. MFNS considerably reduced the release of nitric oxide radicals in a dose-dependent manner, and the maximum inhibition (78.66%) was noted at 250 µg/mL concentration, as shown in **Fig. 3.** Therefore, *Nigella sativa* seed fractions could alleviate nitric oxide-induced cellular derangements.

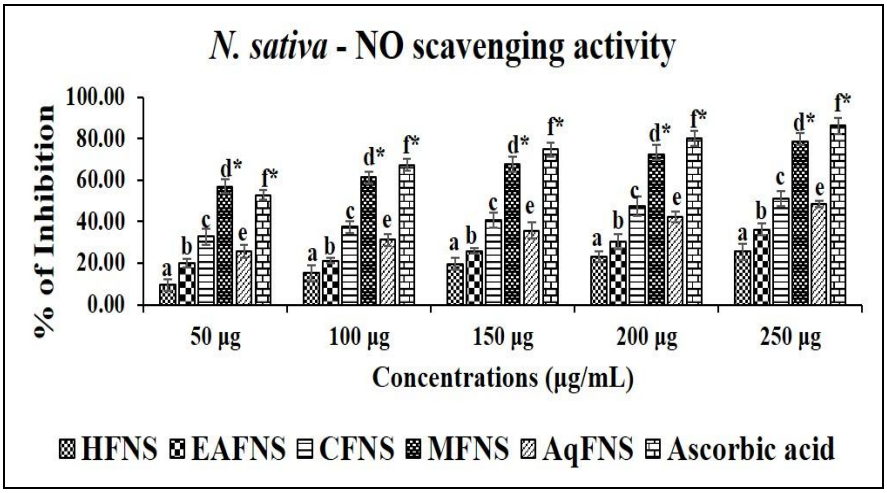

FIG. 3: EFFECT OF MFNS ON NITRIC OXIDE RADICALS

Effect of MFNS on Pancreatic Lipase Activity: Further, we evaluated the inhibitory effect of *N. sativa* seed fractions on lipase activity. In the digestive system, pancreatic lipase (PL) is the most active enzyme in the digestion of dietary fats and empowers absorption across the intestine. Control of pancreatic lipase is one of the significant marks to combat obesity and other metabolic disorders. Pancreatic lipase (PL) inhibitory activity is depicted in **Fig. 4**. MFNS has shown potent anti-lipase activity in a dose-dependent manner, which is higher than other solvent fractions. Maximum inhibition of PL (79.26%) was recorded at 250 µg/mL, which is nearer to the standard drug orlistat (81.92%). Bhardwaj *et al*., in their studies, reported the potential benefits of sitosterol derived from a plant species against hormone-sensitive lipase, adipogenesis, and lipolysis mechanism in rat adipocytes. Inhibiting lipase activity is an important therapeutic means in drug discovery to

contain obesity and adipogenesis. Since lipase inhibits lipid digestion, it reduces lipid absorption and accumulation of triglycerides in adipose and other tissues [26].

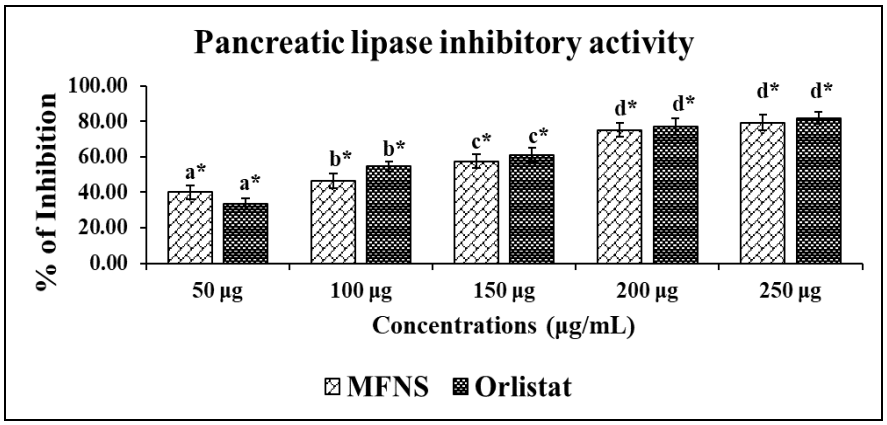

FIG. 4: PANCREATIC LIPASE INHIBITORY ACTIVITY OF MFNS. DATA ARE PRESENTED AS MEAN ± SD OF TRIPLICATE. DIFFERENT ALPHABETICAL SUPERSCRIPTS ($^{A*-D*}$) OVER THE ERROR BARS FOR GIVEN CONCENTRATIONS OF MFNS INDICATE SIGNIFICANT DIFFERENCES FROM EACH OTHER (P<0.05). MFNS: METHANOLIC FRACTION OF *NIGELLA SATIVA*

Effect of MFNS on α-Amylase and α-Glucosidase Activity: Elevation of blood sugar causes insulin resistance and diabetes, a metabolic disorder that leads to atherosclerosis, hypertension, other cardiovascular diseases, and obesity. A systematic approach for controlling diabetes would be to regulate post-prandial plasma glucose rise by inhibiting carbohydrate hydrolyzing enzymes (α-amylase, α-glucosidase) in the digestive tract. Among different solvent fractions of *N. sativa*, the MFNS showed the highest inhibition in a dose-dependent manner. **Fig. 5** explains the pancreatic α-amylase inhibitory activity of MFNS. The maximum inhibition of α-amylase (81.47%) was recorded at 250 µg/mL of MFNS, which is higher than standard drug acarbose (83.53%). **Fig. 6** explains the inhibitory activity of MFNS against the α-glucosidase activity. Among different fractions, the methanolic fraction was found to be a potential inhibitor against α-glucosidase when compared to other fractions. The maximum inhibition of 77.12% was recorded with MFNS at 250 µg/mL, close to the standard drug acarbose (79.45%). The obtained results are similar to the observations made by Salehi *et al.* and Gong *et al.*, who reported that tea polyphenols might act as anti-nutritional factors on digestive enzymes. Inhibition of amylase and glucosidase significantly slows down carbohydrates digestion and thus reduces high calorie intake. These properties of medicinal plants may be considered in alternative adjuvant therapy with other oral hypoglycemic drugs for treating metabolic disorders [27, 28].

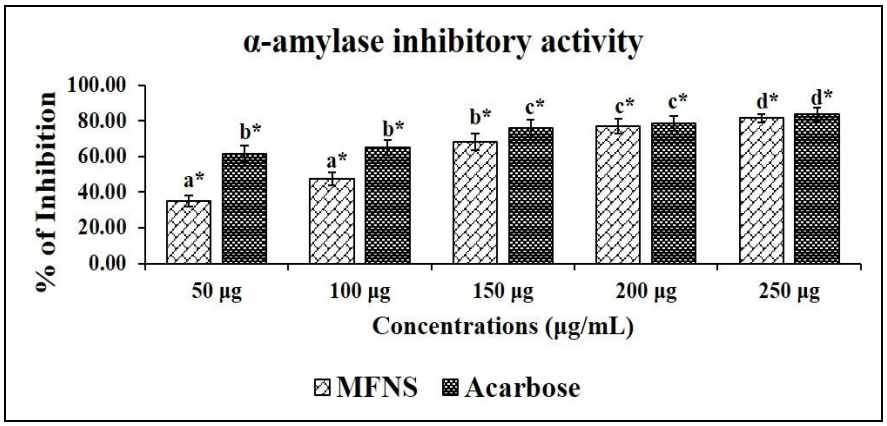

FIG. 5: A-AMYLASE INHIBITORY ACTIVITY OF MFNS. DATA ARE PRESENTED AS MEAN ± SD OF TRIPLICATE. DIFFERENT ALPHABETICAL SUPERSCRIPTS ($^{A*-D*}$) OVER THE ERROR BARS FOR GIVEN CONCENTRATIONS OF MFNS INDICATE SIGNIFICANT DIFFERENCES FROM EACH OTHER (P<0.05). MFNS: METHANOLIC FRACTION OF *NIGELLA SATIVA*

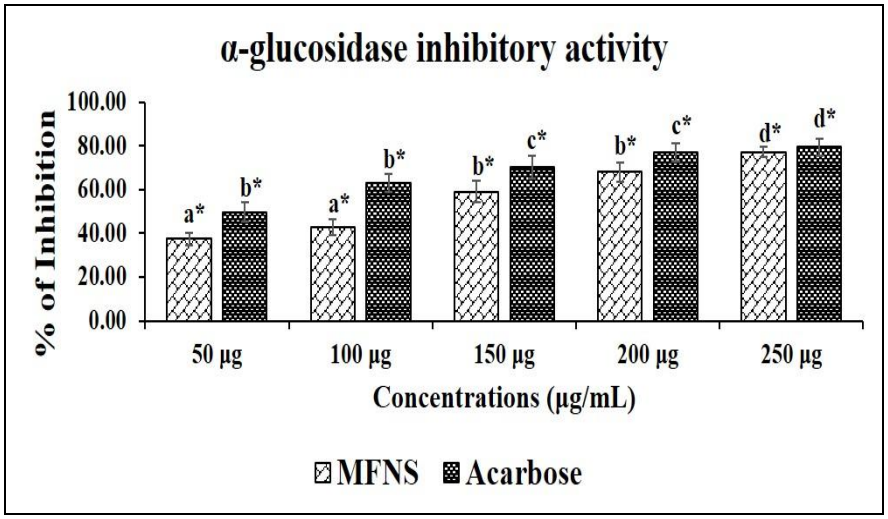

FIG. 6: α-GLUCOSIDASE INHIBITORY ACTIVITY OF MFNS. DATA ARE PRESENTED AS MEAN ± SD OF TRIPLICATE. DIFFERENT ALPHABETICAL SUPERSCRIPTS ($^{a*-d*}$) OVER THE ERROR BARS FOR GIVEN CONCENTRATIONS OF MFNS INDICATE SIGNIFICANT DIFFERENCES FROM EACH OTHER (p<0.05). MFNS: METHANOLIC FRACTION OF *NIGELLA SATIVA*

Inhibition of LPS-induced NO production: To evaluate the effects of *Nigella sativa* seed fraction on NO production, nitrite was measured as the stable metabolite accumulated in the culture supernatants.

Little production of NO occurs in unstimulated cells when compared with treated, but treatment with LPS induced a high release of NO into the culture medium. Treatment with a high concentration of MFNS resulted in considerable inhibition of the NO production compared with a low concentration of MFNS **Fig. 7**.

Thus, *Nigella sativa* seeds could contribute to good health by alleviating NO-induced inflammation and other cellular and molecular disturbances.

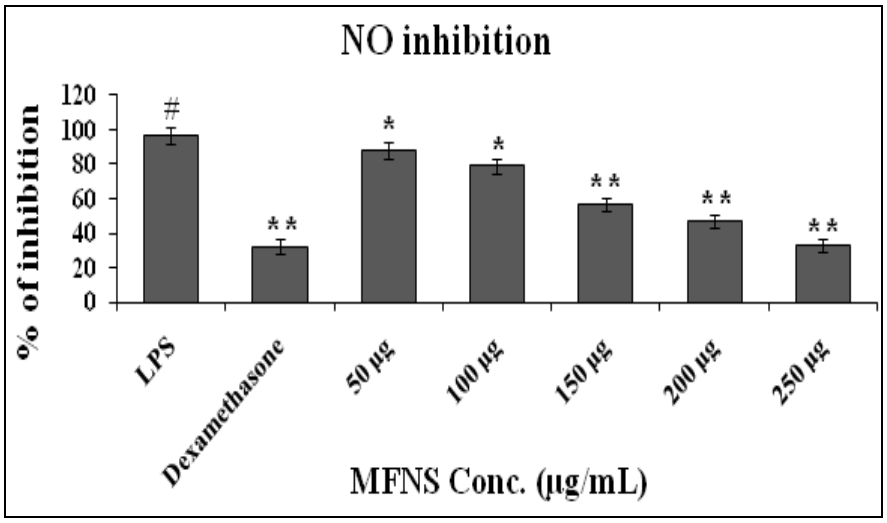

FIG. 7: EFFECT OF MFNS ON LPS-INDUCED NO PRODUCTION IN RAW 264.7 MACROPHAGES. DATA ARE SHOWN IN THE MEAN ± SD (n=3), p<0.05 SIGNIFICANTLY DIFFERENT FROM THE LPS GROUP.*INDICATES SIGNIFICANT DIFFERENCES BETWEEN CONTROL AND TREATED CELLS

Membrane Stability: The anti-inflammatory activity was determined by membrane stability test of the human red blood cells as shown in **Fig. 8.** The MFNS showed a concentration-dependent activity, and the membrane protection percent was increased with an increase in the concentration of the test samples. At a 250 µg/mL concentration, MFNS showed a maximum of 81.01 ± 5.04% inhibition of RBC hemolysis, compared with standard Ibuprofen drug, which showed 85.23± 7.15 %.

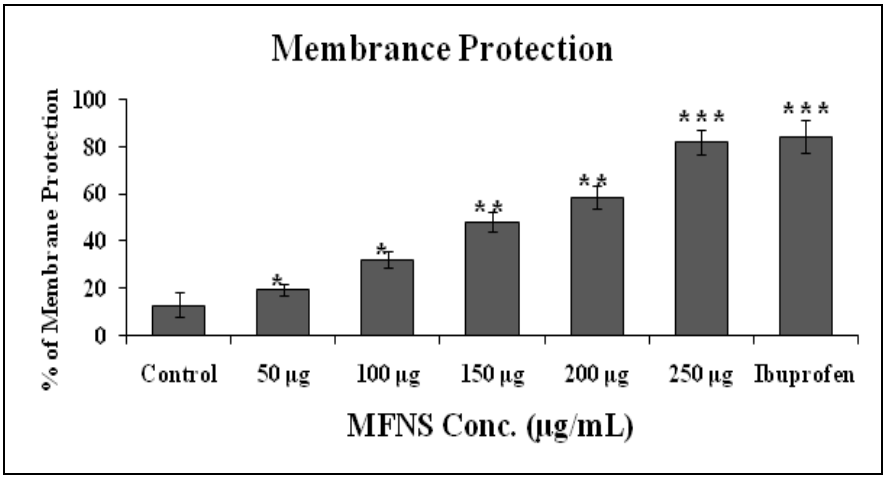

FIG. 8. REPRESENTS THE PERCENTAGE OF MEMBRANE PROTECTION OF MFNS WITH REFERENCE TO IBUPROFEN 200 µµG/ML. THE RESULTS WERE EXPRESSED AS MEAN ± SD (n=3).*INDICATES SIGNIFICANT DIFFERENCES BETWEEN CONTROL AND TREATED CELLS

CONCLUSION: The results demonstrate that MFNS showed effective and dose-dependent free radicals scavenging activity, and inhibited α-amylase, α-glucosidase and pancreatic lipase. MFNS also significantly inhibited nitric oxide production in RAW 264.7 macrophages and protected HRBC from hemolysis. Based on the above results, *Nigella sativa* seeds can be well considered as a potent anti-inflammatory, anti-adipogenic and free radicals scavenging agent.

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REFERENCES:

1. Shukla A, Baghel AS and Vyas M: Lifestyle related factors associated with Sthaulya (obesity) - A cross-sectional survey study. Ayu 2016; 37(3-4): 174-183. https://doi:10.4103/ayu.AYU_87_16.
2. Ellulu MS, Patimah I, Khaza'ai H, Rahmat A and Abed Y: Obesity and inflammation: the linking mechanism and the complications. Arch Med Sci 2017; 13(4): 851-863. doi:10.5114/aoms.2016.58928.
3. Muller TD, Bluher M and Tschop MH: Anti-obesity drug discovery: advances and challenges. Nat Rev Drug Discov 2022; 21: 201–223. https://doi.org/10.1038/s41573-021-00337-8.
4. Stanford FC, Tauqeer Z and Kyle TK: Media and its influence on obesity. CurrObes Rep 2018; 7(2): 186-192. doi:10.1007/s13679-018-0304-0.
5. Atanasov AG, Zotchev SB and Dirsch VM: Natural products in drug discovery: advances and opportunities. Nat Rev Drug Discov 2021; 20: 200–216. https://doi.org/10.1038/s41573-020-00114-z.
6. Leone A, Fico G, Bertoli S and Battezzati A. Editorial: Plant-Based Products, Phytochemicals and Glycemic Control. Front Endocrinol (Lausanne) 2022; 13: 906690. doi:10.3389/fendo.2022.906690.
7. Pasqua T, Rocca C, Lupi FR, Baldino N, Amelio D, Parisi OI, Granieri MC, De Bartolo A, Lauria A, Dattilo M, Perrotta ID, Puoci F, Cerra MC, Gabriele D & Angelone T: Cardiac and metabolic impact of functional foods with antioxidant properties based on whey derived proteins enriched with hemp seed oil. Antioxidants (Basel, Switzerland) 2020; 9(11): 1066. https://doi.org/10.3390/antiox9111066.
8. Sun W, Shahrajabian MH and Cheng Q: Natural dietary and medicinal plants with anti-obesity therapeutics activities for treatment and prevention of obesity during lock down and in post-COVID-19 Era. Appl Sci 2021; 11: 7889. https://doi.org/10.3390/ app11177889.
9. McMacken M and Shah S: A plant-based diet for the prevention and treatment of type 2 diabetes. J Geriatr Cardiol. 2017; 14(5): 342-354. doi:10.11909/j.issn.1671-5411.2017.05.009.
10. Srinivasan K: Cumin (*Cuminum cyminum*) and black cumin (*Nigella sativa*) seeds: traditional uses, chemical constituents and nutraceutical effects. Food Quality and Safety 2018; 2(1): 1–16. https://doi.org/10.1093/fqsafe/fyx031.
11. Harborne AJ: Phytochemical Methods A Guide to Modern Techniques of Plant Analysis 1984.
12. Kadam D and Lele SS: Extraction, characterization and bioactive properties of Nigella sativa seedcake. J Food Sci Technol 2017; 54(12): 3936-3947. doi:10.1007/s13197-017-2853-8.
13. Baliyan S, Mukherjee R, Priyadarshini A, Vibhuti A, Gupta A, Pandey RP and Chang CM: Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of *Ficus religiosa*. Molecules. 2022; 27: 1326. https://doi.org/ 10.3390/molecules27041326.
14. Mahmud ZA, Bachar SC, Hasan CM, Emran TB, Qais N and Uddin MMN: Phytochemical investigations and antioxidant potential of roots of *Leea macrophylla* (Roxb.). BMC Res Notes 2017; 10(1): 245. doi:10.1186/s13104-017-2503-2.
15. Duarte AM, Guarino MP, Barroso S and Gil MM: Phytopharmacological strategies in the management of type 2 diabetes mellitus. Foods 2020; 9(3): 271. https://doi.org/10.3390/foods9030271.

16. Karunakaran RS, Lokanatha O, Krishna C and Balaji M: *Tephrosia purpurea* fraction attenuates lipid accumulation and adipogenesis in 3t3-l1 adipocytes and reduces body weight in high fat diet induced obese rats. Advances in Bioscience and Biotechnology 2019; 10: 288-304.
17. Etsassala NGER, Badmus JA, Marnewick JL, Iwuoha EI, Nchu F and Hussein AA: Alpha-glucosidase and alpha-amylase inhibitory activities, molecular docking, and antioxidant capacities of *Salvia aurita* constituents. Antioxidants (Basel). 2020; 9(11): 1149. doi:10.3390/antiox9111149.
18. Nivetha K and Prasanna G: GC-MS and FT-IR Analysis of *Nigella sativa* L. Seeds. Int J Adv Res Biol Sci 2016; 3(6): 45-54.
19. Merly L and Smith SL: Murine RAW 264.7 cell line as an immune target: are we missing something? Immunopharmacol Immunotoxicol 2017; 39(2): 55-58. doi: 10.1080/08923973.2017.1282511.
20. Lenzi M, Cocchi V, Novakovic A, Karaman M, Sakac M, Mandic A, Pojic M, Barbalace MC, Angeloni C, Hrelia P, Malaguti M, & Hrelia S: *Meripilus giganteus* ethanolic extract exhibits pro-apoptotic and anti-proliferative effects in leukemic cell lines. BMC complementary and alternative medicine 2018; 18(1): 300.
21. Guo Q, Bi D, Wu M, Yu B, Hu L, Liu C, Gu L, Zhu H, Lei A, Xu X & Wang J: Immune activation of murine RAW264.7 macrophages by sonicated and alkalized paramylon from Euglena gracilis. BMC microbiology 2020; 20(1): 171. https://doi.org/10.1186/s12866-020-01782-y.
22. Yesmin S, Paul A and Naz T: Membrane stabilization as a mechanism of the anti-inflammatory activity of ethanolic root extract of Choi (Piper chaba). Clin Phytosci 2020; 6: 59. https://doi.org/10.1186/s40816-020-00207-7.
23. Schmidt A, Refaai M, Kirkley S and Blumberg N: Proven and potential clinical benefits of washing red blood cells before transfusion: current perspectives. International Journal of Clinical Transfusion Medicine 2016; 4: 79-88
24. https://doi.org/10.2147/IJCTM.S101401.
25. Tungmunnithum D, Thongboonyou A, Pholboon A, & Yangsabai A: Flavonoids and Other Phenolic Compounds from Medicinal Plants for Pharmaceutical and Medical Aspects: An Overview. Medicines (Basel, Switzerland) 2018; 5(3): 93. https://doi.org/10.3390/medicines5030093.
26. Menzel A, Samouda H, Dohet F, Loap S, Ellulu MS, Bohn T: Common and novel markers for measuring inflammation and oxidative stress *ex-vivo* in research and clinical practice-which to use regarding disease outcomes. Antioxidants 2021; 10(3): 414. https://doi.org/10.3390/antiox10030414.
27. Bhardwaj M, Yadav P and Vashishth D: A review on obesity management through natural compounds and a green nanomedicine-based approach. Molecules. 2021; 26(11): 3278. doi:10.3390/molecules26113278.
28. Salehi B, Ata A and Anil Kumar VN: antidiabetic potential of medicinal plants and their active components. Biomolecules 2019; 9(10): 551. doi:10.3390/biom9100551.
29. Gong L, Feng D, Wang T, Ren Y, Liu Y and Wang J: Inhibitors of α-amylase and α-glucosidase: Potential linkage for whole cereal foods on prevention of hyperglycemia. Food Sci Nutr 2020; 8(12): 6320-6337. doi:10.1002/fsn3.1987.

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