Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(8):432-436

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Antidiabetic and phytochemical screening of *Nigella sativa*

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ABSTRACT

Nigella sativa was screened for phytochemical constituents such as alkaloids, flavonoids, tannins, phenols, saponins, carbohydrates, glycosides, sterols, terpenoids and proteins. The antidiabetic activity of N.sativa by Alpha-Amylase and Alpha-Glucosidase assay method showed that the Ethyl Acetate extract had the highest percentage of inhibition against α-Glucosidase than α-Amylase, which reveals the hypoglycemic nature of N.sativa. Investigation of Total antioxidant activity by the phosphomolybdenum method suggested that Ethyl Acetate extract of N.sativa has significantly higher activity than n-Butanol extract at low concentrations than Ascorbic acid. The results confirm the antidiabetic activity of N.sativa seeds extract and suggest that because of its antioxidant affect it may be useful in helping out with the diabetic complications.

Keywords: Nigella sativa, α-Amylase, α-Glucosidase, Phosphomolybdenum method, Thymoquinone.

INTRODUCTION

Nigella sativa Linn. (Family-Ranunculaceae) commonly known as Kalaunji, Kalika, Ajaji, Kalvanjika, Kunchika and black cumin, is a small elegant herb, mostly found and cultivated throughout India [1]. It is very popular in various traditional systems of medicine like Unani and Tibb, Ayurveda and Siddha. Seeds and oil have a long history of folklore usage in various systems of medicines and food. The seeds of *N.sativa* have been widely used in the treatment of different diseases and ailments [2].

Black cumin seeds are used as a carminative, aromatic, stimulant, diuretic, anthelmintic, galactagogue and diaphoretic. They are used as a condiment in curries. A tincture prepared from the seeds is useful in indigestion, loss of appetite, diarrhoea, dropsy, amenorrhoea and dysmenorrhoea and in the treatment of worms and skin eruptions. Externally the oil is used as an antiseptic and local anesthetic. Roasted Black seeds are given internally to arrest vomiting [3].

Anti-Diabetic studies on laboratory animals have reported that *N.sativa*, its Extract and thymoquinone, the most widely studied active principle, are quite safe and effective against diabetes [4, 5, 6, 7, 8, 9, 10, 11, 12]. Significant hypoglycemic activity has been reported and is thought to be due to the essential oil present. Clinical studies have confirmed these results suggesting the antidiabetic action of the plant extract [13, 14, 15, 16]. .*Nigella sativa* oil and extracts are also proved to have anti-oxidant properties determined by many assays such as DPPH Assay and Free Radical Scavenging effects [17, 18].

EXPERIMENTAL SECTION

Collection and identification of medicinal plant seeds

The seeds of *Nigella sativa* (Klonji) were purchased, dried and grinded using mechanical blender and packed in polythene bags for further extractions. The plant species were identified by Dr. G. Kathiravan, Associate Professor, Department of Biotechnology, Vels University, Chennai, Tamilnadu, India. The voucher specimen VUCC0010 was given to *N. sativa* (Klonji) and deposited in the Herbarium of Vels University, Chennai, Tamilnadu, India. The plant name has been verified with www.theplantlist.org.

Extract Preparation

Soxhlet extraction assembly was used for this purpose. Each of 25 g dried and powdered seeds were mixed with 200 ml Ethyl Acetate and n-Butanol successively and continuous extraction was done for 5 to 6 h. After that extracts were filtered, the organic extracts were dried in a rotary evaporator at reduced pressure. The extract was used for further studies.

Phytochemical screening

Chemical tests were carried out to evaluate the presence of the phytochemicals such as Alkaloids, Flavonoids, Saponins, Tannins, Cardiac glycosides, Phenols, Terpenoids, Carbohydrates, Amino acids and Sterols in the extract using standard procedures described by Sofowora [19]; Trease and Evans [20].

Alpha-Amylase inhibition assay

The method adopted was slightly modified from Apostolidis *et al*., (2007) [21]. 250µl of 0.02M sodium phosphate buffer containing α-amylase solution (1mg/mL) and 500µl of samples of varying concentration were incubated at 37°C for 10 min. After pre incubation, 250 µL of 0.5% starch solution in 0.02 M sodium phosphate buffer was added. The reaction was incubated at room temperature for 10 min. 1 mL of DNSA color reagent was added to stop the reaction. The test tubes were then incubated in boiling water bath for 10 min and cooled to room temperature. The assay was performed in duplicates. The absorbance was read at 540 nm. Percentage of inhibition was calculated using the below formula:

%Inhibition = [(A540Blank − A540Extract)] * 100 / (A540 Blank)

The IC 50 values were determined from plots of percent inhibition versus log inhibitor concentration and were calculated by non linear regression analysis from the mean inhibitory values. Acarbose was used as the reference alpha glucosidase inhibitor. All tests were performed in duplicate.

Alpha-Glucosidase inhibition assay

The assay procedure is modified from Pistia-Brueggeman and Hollingsworth (2001) [22]. Alpha-glucosidase (20µl, 1.25u/ml) was premixed with 200µl of samples of various concentrations and made up in a 50mM phosphate buffer at pH 6.8 and incubated at 37°C for 10 minutes. 1Mm pNPG dissolved in phosphate buffer was added to initiate the reaction and incubated at room temperature for 20 minutes. The reaction was terminated by adding 20µl of 1M Na_2Co_3 and final volume was made up to 1ml with phosphate buffer. The absorbance was read at 405nm. The assay was done in duplicates. The IC50 values were calculated. Percentage of inhibition was calculated using the below formula:

%Inhibition = [(A405Blank − A405Extract)] * 100/ (A405 Blank)

The IC 50 values were determined from plots of percent inhibition versus log inhibitor concentration and were calculated by non linear regression analysis from the mean inhibitory values. Acarbose was used as the reference alpha glucosidase inhibitor. All tests were performed in duplicate.

Total anti-oxidant activity

The total antioxidant capacity of the extracts was evaluated by the phosphomolybdenum method according to the procedure described by Prieto *et al*., (1999) [23].An aliquot of 500 µl of extracts with varying concentrations was combined with 4.5 ml of reagent solution (0.6 M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate) and boiled at 95°C for 90 minutes and the absorbance was measured at 695 nm. Ascorbic acid

equivalents were calculated using standard graph of ascorbic acid. The values were expressed as equivalents of Ascorbic acid in µg per mg of extracts.

RESULTS AND DISCUSSION

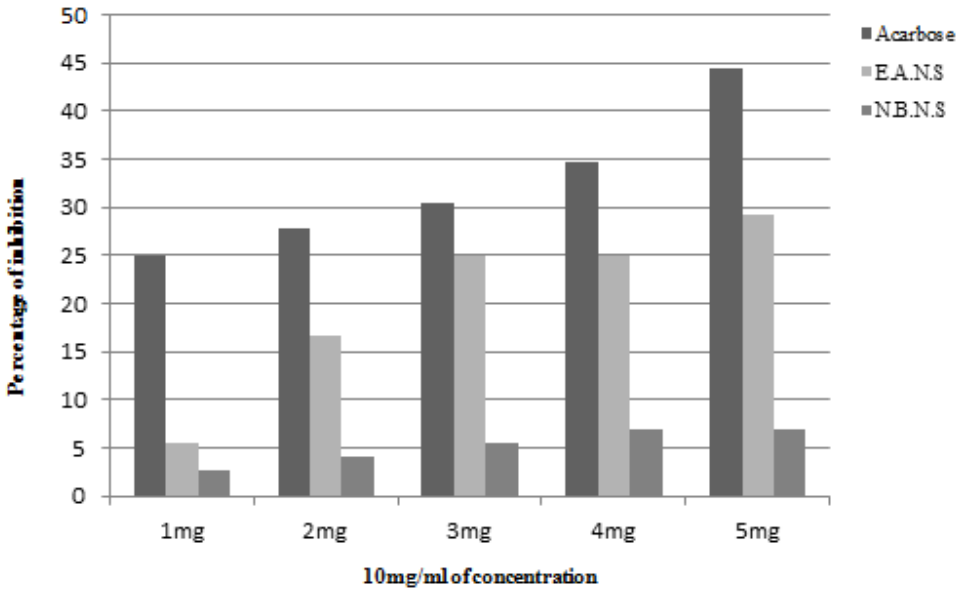
The preliminary phytochemical screening tests for the Ethyl Acetate and n-Butanol extract of *Nigella sativa* (Table 1) revealed the presence of carbohydrates, alkaloids, flavonoids, tannins, sterols, glycosides, phenols, saponins, proteins and terpenoids. Any of these secondary metabolites, singly or in combination with others could be responsible for the anti-diabetic activity of the plant.

Table 1. Result of phytochemical screening of Nigella sativa

S.No	Phytochemicals	Ethyl Acetate Extract	n-Butanol Extract
1	alkaloids	-	-
2	carbohydrates	+	+
3	flavanoids	+	+
4	glycosides	+	+
5	saponins	+	+
6	tannins	+	+
7	terpenoids	+	-
8	phenols	-	-
9	proteins	+	+
10	steroids	+	+

+ = present
- = absent

Figure: 1 Alpha-Amylase assay of Ethyl Acetate and n-Butanol Extracts of *Nigella sativa*



Alpha-Amylase and Alpha-Glucosidase enzymes have been recognized as therapeutic targets for modulation of postprandial hyperglycemia since they are known to reduce postprandial hyperglycemia by partially inhibiting the enzymatic hydrolysis of complex carbohydrates and hence may delay the absorption of glucose. Acarbose, voglibose and miglitol are synthetic inhibitors used either alone or in combination with insulin secretogogues for patients with type II diabetes. However, theses inhibitors are reported to cause several side effects. Hence, several other safer natural inhibitors are reported from plant resources [24].

On checking the a-amylase inhibitory activity of Ethyl Acetate (E.A.N.S) and n- Butanolic (N.B.N.S) extracts of *Nigella sativa*, we found that the extract showed only low inhibitory activity against a-amylase when compared with

standard Acarbose. The maximum inhibition shown was only 29.16% and the concentration was 5mg/mL (Fig. 1). On further increasing the concentration the extract showed a decreased inhibitory activity.

Figure: 2 Alpha-Glucosidase assay of Ethyl Acetate and n-Butanol Extracts of *Nigella sativa*

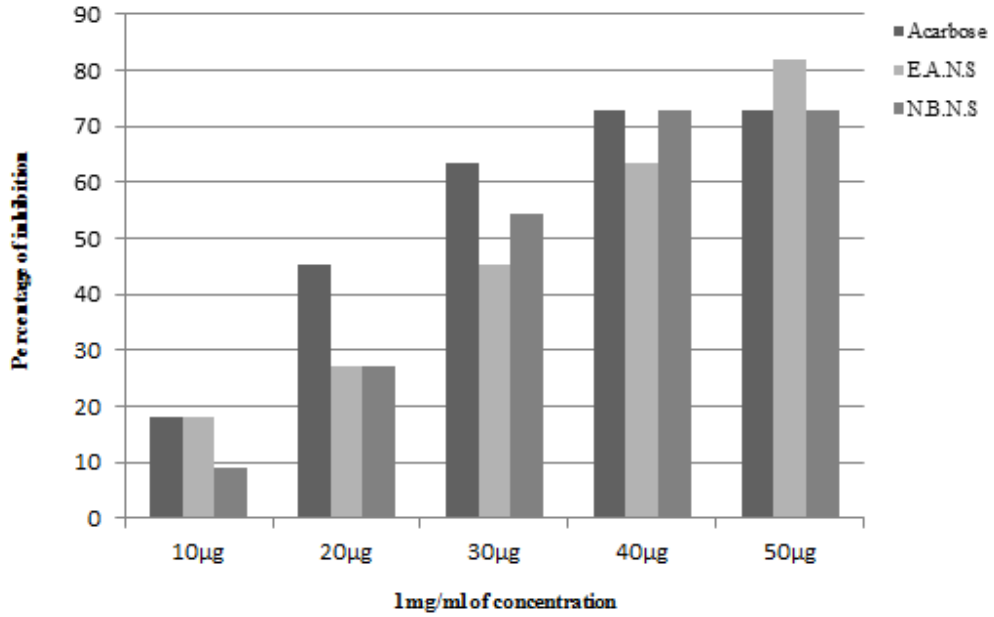
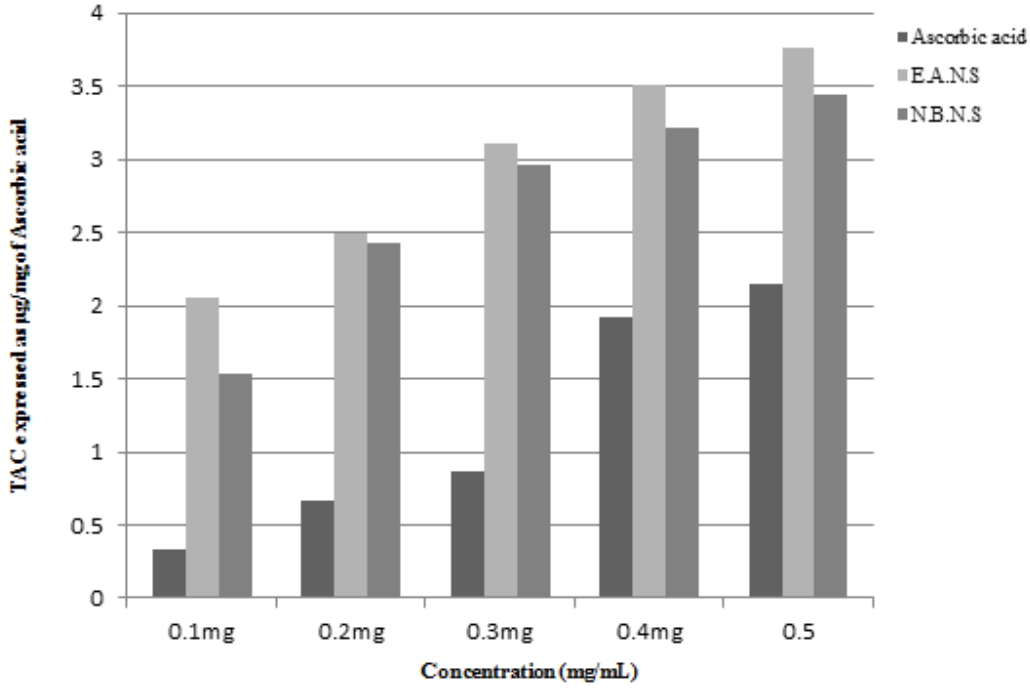


Figure: 3 Phophomolybdenum assay of Ethyl Acetate and n-Butanol Extracts of *Nigella sativa*



The a-glucosidase inhibitory activities of both the extracts of *Nigella sativa* were determined and the values obtained were compared with standard Acarbose (Fig. 2). The enzyme inhibition of Ethyl Acetate extract (E.A.N.S) was in the dose-dependent manner and the IC50 value was 3.25 ± 0.05µg/ml and of standard Acarbose was

2.35±0.03µg/mL. Plant compounds were reported to modulate the enzymatic breakdown of carbohydrate by inhibiting amylase and glucosidase enzymes.

The total antioxidant capacity (TAC) was based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of green phosphate/ Mo (V) complex at acid pH. It evaluates both water-soluble and fat-soluble antioxidants (total antioxidant capacity). The significantly higher absorbance values of Ethyl Acetate and n-Butanol extracts than Ascorbic acid(Fig. 3) at lower concentrations indicate higher TAC (expressed as ascorbic acid equivalent) suggesting that both the extracts, especially at low concentrations, has high redox potentials and can acts as reducing agent, hydrogen donor and singlet oxygen quencher [25].

CONCLUSION

The present study clearly exhibits the antidiabetic potential of Nigella sativa through inhibitory effects on Alpha-amylase and Alpha-glucosidase enzymes, which are main targets for present day medicines for the metabolic syndrome, Diabetes mellitus. The presences of many phytochemicals are having the potential to be antidiabetic in nature. The Ethyl Acetate extract had good inhibition effect on Alpha-glucosidase than Alpha-amylase enzyme than the n-Butanolic extract. Even the Total antioxidant capacity of both the extracts was higher than the standard Ascorbic acid value proving the high antioxidant capacity.

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