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Research Article

ANTIOXIDANT AND ANTHELMINTIC ACTIVITY OF *BRASSICA NIGRA*

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A R T I C L E I N F O

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A B S T R A C T

Aim: To determine phytochemical screening, total phenolic quantification, antioxidant and anthelmintic activity of hot water extract of *Brassica nigra* L.
Background: Kerala is endowed with abundant plants and these plants contain numerous photochemicals. The rising prevalence of chronic diseases world-wide and the corresponding rise in health care costs cause interest among researchers to find nontoxic compounds from plants used for culinary purposes.
Methodology: Preliminary phytochemical analysis and total phenol quantification of hot water extract of *Brassica nigra* was done by standard procedures. The antioxidant activity was evaluated by DPPH radical scavenging activity. Anthelmintic activity was checked against Indian earthworm *Pheretima posthuma*.
Results: In the present study, hot water extract of *Brassica nigra*, consist of phytochemicals like carbohydrate, amino acid, protein, alkaloid, steroids, saponins, phenols and tannins. The extract also confirmed DPPH radical scavenging activity. In anthelmintic studies, Albendazole was used as standard drug. In 100 mg/ml of *Brassica nigra* hot water extract, earthworms get paralysed after 4 min and died after 7 min. The antioxidant and anthelmintic effect of *Brassica nigra* may be due to presence of secondary metabolite like alkaloids or tannins.
Conclusion: The result of the present study clearly pointed out that the hot water extract of *Brassica nigra* can produce antioxidant and anthelmintic activity. However, further investigation of *Brassica nigra* is necessary for assessing its potential in the treatment of various diseases.

INTRODUCTION

Medicinal plants have been used in traditional medicine since prehistoric times. These plants contain numerous photochemicals with known or unknown activity; however, the effect of using a whole plant as medicine is uncertain. Spices and herbs are distinguished as the resource of natural antioxidants and thus play a significant function in the chemoprevention of diseases and aging (Abid *et al*., 2017). In recent times, focus on plant research has increased all over the world. In future more synchronized research is needed to correlate botanical and phytochemical properties because food antioxidants augment the body's natural resistance to oxidative damage.

Free radicals are synthesized in the human body due to exposure to pollutants and industrial chemicals (Lobo *et al*., 2010). Helminthiasis is infestation with intestinal parasitic worms in humans and animals. Synthetic drugs which are available also possess side effects like abdominal pain, dizziness and also drug resistance (Srivastava *et al*., 2017).

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These factors cause an upsurge of interest to use herbal drugs with no side effects. But these medicinal plants need to be screened for their *in vitro* and *in vivo* activity before putting them in use.

Brassica nigra L. (Family: Brassicaceae) commonly known as mustard has both edible as well as curative value. The seeds are usually thrown into hot oil or ghee, after which they pop, releasing a characteristic nutty flavour. The oil obtained from seeds used as cooking oil in India. The plant is used in the treatment of rheumatism, epilepsy, snakebite, and toothache, carcinoma and throat tumours. Mustard seeds were used for treating vermicide, throat complaints etc. The seeds are bitter in taste which can be used to treat skin diseases. (Alam *et al.,* 2011). The main purpose of the present study is to explore phytochemistry, total phenol quantification; antioxidant and anthelmintic potential of hot water extract of *Brassicanigra*L. seeds.

MATERIALS AND METHODS

Preparation of crude plant extract

Brassica nigra L. (seeds) was purchased from the local market. The dried seeds were powdered using a mixer grinder. About 10 g of dried, ground plant materials were soaked

separately in water (100 ml) for one week. The soaked material was stirred and heated to boiling point and stirred using sterilized glass rod. The final extracts were passed through Whatman filter paper No.1. The extracts were dissolved in distilled water to make a concentration of 1 mg/ml.

Phytochemical Analysis

The plant extract was diluted with distilled water for phytochemical analysis of primary and secondary metabolites using standard procedures (Bhandary *et al*., 2012).

Total phenolic content

The concentration of phenolics in seed extract was determined using spectrophotometric method (Bray and Thorpe, 1954). The diluted working solutions of the test extracts were prepared in water. The reaction mixture was prepared by taking 5 µl, 2.5 µl , 1µl of hot water extract and made up the volume to three ml of distilled water, added 0.5 ml of Folin-Ciocalteu's reagent and 2 ml 20% Na_2CO_3. Blank was concomitantly prepared**,** containing 3 ml water, 0.5ml Folin-Ciocalteu's reagent and 2ml of 20% of Na_2CO_3. The samples were thereafter incubated in a dark for 30min. The absorbance was determined using spectrophotometer at 650 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of catechol and the calibration line was constructed (Fig 1). Based on the measured absorbance, the concentration of phenolics was read (µg/µl) from the calibration line; then the content of phenolics in extracts was expressed in terms of catechol equivalent (µg of CE/µg of extract).

Antioxidant activity

The ability of the seed extracts to scavenge DPPH free radicals was assessed by the standard method (Braca *et al*., 2002). The stock solution of extracts were prepared in water to achieve the concentration of 1 mg/ml. Dilutions were made to obtain concentrations of 5, 2.5, 1µg/ml. Diluted solutions (1 ml each) were mixed with 1 ml of methanolic solution of .002% DPPH. After 30 min incubation in darkness at room temperature (23^{o}C), the absorbance was recorded at 517 nm Labtronics NT 290 Spectrophotometer. Control sample contained all the reagents except the extract. Percentage inhibition was calculated, whilst IC_{50} values were estimated from % inhibition versus concentration plot.

The effective concentration of sample required to scavenge DPPH radical by 50% (IC_{50} value) was obtained by linear regression analysis of dose-response curve plotting between % inhibition and concentrations (Iranshahi *et al*., 2009). The optical density was recorded and % inhibition was calculated using the formula given below Percent (%) inhibition of DPPH activity = {(A-B)/A}x100, where, A = optical density of the blank and B = optical density of the sample.

Anthelmintic activity

The anthelmintic assay was carried out as per the Ayaiyeoba *et al*., 2001. Adult earthworms (*Pheretima posthuma*), were used to evaluate anthelmintic activity *in vitro*. Earthworms of 3-5 cm in length and 0.1-0.2 cm in width (same type) were collected from Kerala Agricultural University, Mannuthy. Test samples of hot water extract was prepared at the concentrations, 25,50,100 mg/ml in distilled water and three worms i.e. *Pheretima posthuma*, of approximately equal size were used for all the experimental protocol were placed in each nine cm petri dish containing 25 ml of above test solution of extracts. Albendazole (25 mg/ml and 50mg/ml) was used as reference standard as advocated earlier. All the test solution and standard drug solution were prepared freshly before starting the experiments. Observations were made for the time taken for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms was recorded after ascertaining that worms neither moved when shaken vigorously.

RESULT

Preliminary phytochemical analysis of hot water extract of Brassica nigra

Plants have always been recognized for its wealthy source of phytochemicals. The medicinal property of a plant depends on these secondary metabolites such as phenolics, terpenoids, or alkaloids. In the present study the aqueous extract of *Brassica nigra* the phytochemical such as carbohydrate, amino acid, protein, alkaloid, saponin, steroid, phenol and tannin were reported (Table 1).

Total phenolic contents of the extracts were determined spectrophotometricallyby Folin-Ciocalteu colorimetric method. Total phenolic content of hot water extract of *Brassica nigra* were expressed as microgram (µg) catechin equivalents (CE) / µg of extract (Fig 1).

Table 1 Preliminary Phytochemical screening of aqueous extract of *Brassica nigra*

Sl No	Plant Constituents	Test / Reagent	Observation	Presence/ absence
1	Carbohydrate	Molisch Test	Violet Ring	++
		Iodine Test	Blue Colouration	–
		Benedict's Test	Appearance of green, yellow or red colouration	++
2	Proteins	Biuret Test	Violet to pink colouration	+++
3	Amino Acids	Ninhydrin Test	Blue to violet colouration	+++
4	Alkaloids	Mayer's Test	White precipitate	++
5	Steroid	Salkowski reaction	Chloroform layer appears red and acid layer shows greenish yellow fluorescence	++
6	Saponins	Foam test	Persistent Foam	++
7	Phenols and Tannins	Folin Test	Blue Colouration	++
		Bromine water	Decolouration of Bromine water	++
		Acetic acid	Red Colouration	–

+ low, ++ Average, +++ High, ' –' Nil

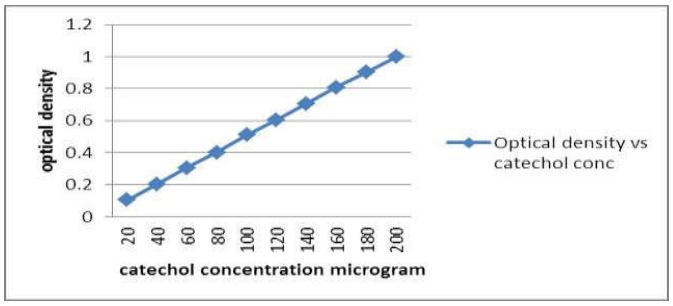

Fig 1 Catechol calibration curve

The phenolic content in *Brassica nigra* was 42 µg CE/ 5 µg of extract, 30µg CE/ 2.5µg of extract and 28 µg CE/ 1µg of extract, using the standard curve of catechin (Table 2).IC50 value of Brassica nigra was found to be 2.65 µg.

Table 2 Total Phenol Quantification of hot water extract of *Brassica nigra*

Sl. No.	Plant Extract	Concentration (µg/ µl)	Phenol concentration (µg/µl CE)
1	*Brassica nigra*	5	42
		2.5	30
		1	28

DPPH free radical scavenging activityof hot water extract of Brassica nigra

DPPH assay is routinely practiced for measurement of free radical scavenging potential of an antioxidant molecule and an easy method for the evaluation of antioxidant properties of compounds. When compared to the control, Table 3 showed the decrease in absorbance of DPPH free radical due to the scavenging ability of the different concentrations of plant extract. 1 µg, 2.5 µg and 5 µg of *Brassica nigra* showed percentage of inhibition of DPPH was 44.7%, 60% and 70% respectively (Fig 2). IC50 value (µg/ml) of *Brassica nigra* was found to be 2.64µg/ml. These results demonstrated that the extracts of *Brassica nigra* seeds have effective activity by reducing the free radical scavenging activity.

Table 3 DPPH free radical scavenging activityof hot water extract of *Brassica nigra*

Sl No	Plant Extract	Concentration (µg)	Optical density (OD)		Percentage of inhibition	IC50 (µg)
			Initial	Final		
1	Control		0.340			
2	*Brassica nigra*	1	0.157	0.188	44.7	2.64
		2.5	0.172	0.133	60	
		5	0.340	0.101	70	

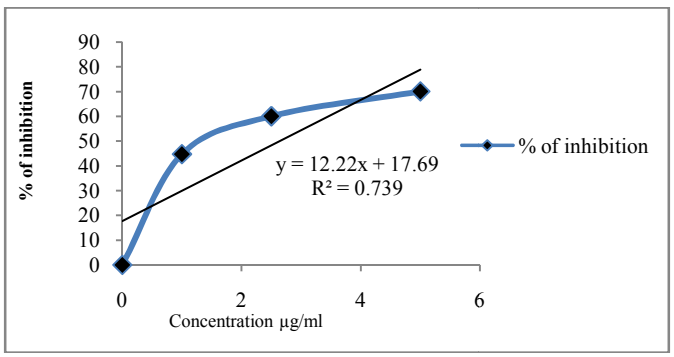

Fig 2 DPPH free radical scavenging activity of hot water extract of *Brassica nigra*

Anthelmintic activity of hot water extract of Brassica nigra

The anthelmintic activity of *Brassica nigra* hot water extract and standard drug Albendazole were studied by observing the time taken for paralysis and death of earthworms. In 50 mg/ml of standard drug Albendazole, the earthworm get paralysed and died after 2 min and 6 min respectively. In 25 mg/ml of Albendazole the time for paralysis and death of *Pheretima posthuma* was found to be 4 min and 18 min respectively. Earthworms get paralysed in 100 mg/ml of *Brassica nigra* at 4 min and died after 7 min. In 50 mg/ml of *Brassica nigra*, earthworms get paralysed after 8 min and died after 27 min. In 25 mg/ml aqueous extract of *Brassica nigra,* the paralysis and death of the earthworms occurred after 33 min and 45 min respectively (Fig 3). When compared to standard drug Albendazole, it was found that the anthelmintic effect of hot water extract of *Brassica nigra* was found to be dose-dependent manner.

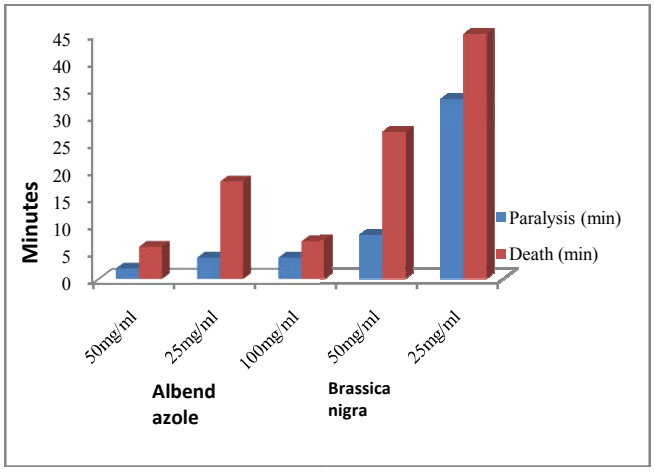

Fig 3 Anthelmintic activity of hot water extract of *Brassica nigra*

DISCUSSION

Secondary metabolites present in medicinal plants plays an important role in maintaining the normal functions of the human body. Phytonutrients play a positive role by maintaining and modulating immune function to prevent specific diseases (Gupta and Prakash, 2014). The phytochemical analysis of hot water extract of *Brassica nigra* indicated the presence of carbohydrate, amino acid, protein, alkaloid, saponin, steroid, phenol and tannin. The phytochemical analysis on ethanolic extract of *Brassica nigra* have been reported the presence of phytochemicals like tannin, volatile oils, anthraquinone glycosides, saponins and flavonoids, alkaloid, reducing sugar and terpenes (Hossein *et al*., 2011).

Several of such compounds are known to possess potent antioxidant activity (PrashithKekuda *et al*., 2009)

The wormicidal activity of hot water extract of *Brassica nigra* suggests that it is effective against parasitic infections. The anthelmintic activity of alcoholic extract of seeds of *Brassica nigra* showed against *Pheretima posthuma* and *Ascardia galli* (Upwar *et al.,* 2011). According to Obi *et al.* (2009) glycosides, alkaloids, proteins and tannins are responsible for the anthelmintic activity of *Brassica nigra*.

Tannins, the polyphenolic compounds, are shown to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation or, binds to the glycoprotein on the

cuticle of parasite, and cause death (Vidhyadhar *et al*., 2010). Further, in future it is necessary to identify and isolate the possible active phytoconstituents responsible for the anthelmintic activity and study its pharmacological actions.

CONCLUSION

Phytochemical analysis showed the presence of primary and secondary metabolites in the hot water extract of *Brassica nigra*. The extract also possesses antioxidant and anthelmintic activity. Further investigations are needed to elucidate the mechanisms of action of active components of the extract.

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