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Evaluation of antioxidant activity of flower and seed oil of *Azadirachta indica A. juss*

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Abstract: The present study on evaluation of antioxidant activity of the flowers and seed oil of Neem plant *Azardirachta indica A. Juss* revealed that the ethanolic extract of flowers and seed oil at 200 µg/ml producing the highest free radical scavenging activity i.e. 64.17±0.02% and 66.34±0.06% respectively. The Neem oil has the highest amount of total phenol content (132 µg/ml) which is responsible for highest percentage of inhibition of DPPH radical. In conclusion Neem flower and seed oil have potential for use in human health which is used as food by common people and in diabetes and Neem seed oil is widely used for variety of diseases and also antioxidant potential for use in different pharmaceutical industries.

Keywords: Neem oil, Flower, Antioxidant activity, DPPH scavenging assay

INTRODUCTION

The different parts of Neem *Azardirachta indica A. Juss* (seeds, leaves, flowers and bark) have a vast pharmacological activity and are used as raw materials for pesticide, medicine and other commodities.(Ghimeray *et al*., 2009; Wadher *et al*., 2009; Rashid *et al*., 2004; Olabinri *et al*., 2009; Aromdee and N. Sriubolmas, 2006). Essential oils may have antioxidant properties and their consumption can influence immune cell functions (Svoboda *et al*., 1992). The biological activity of the oils can be compared with the activity of synthetically produced pharmacological preparations and should be investigated in the same way (Colgate, 1993; Svoboda *et al*., 1998a; Svoboda and Deans, 1995; Baratta *et al*., 1998). The high antioxidant activities of plant phenolic compounds are attractive to the food industry, prompting their use as replacements for synthetic antioxidants and also as nutraceuticals have a significant role in preventing many diseases.

The Neem flowers are also useful in medicine, food and pharmaceutical fields (Aromdee and Sriubolmas, 2006).

The present study has been undertaken to evaluate the antioxidant activity as well as the compounds related to antioxidant such as phenol in Neem flower and seeds which are not documented although other parts such as leaf, bark and root has been studied.

MATERIALS AND METHODS

Collection of plant materials and oil extraction: The plant materials (flowers and seeds) were collected from Botanical garden of B.J.B. Autonomous College, Bhubaneswar, Orissa between the months of January and April during afternoon time only because they normally open in the afternoon, emanating a strong smell during night. Extraction of oil was done by Mechanical press method which is the most widely used method to extract Neem oil from Neem seed. However, the oil produced with this method usually has a low price, since it is turbid and contains a significant amount of water and metal contents (Liauw *et al*., 2008). In this method seeds were washed properly and dried for 1-2 days for moisture free. Then they were placed in a tub or container and a form of press or screw is used to squeeze the seeds until the oil is pressed out and collected.

The fresh Neem flowers were collected, washed by tap water and then extracted for 10 to 12 hours through soxhlet apparatus by using multi-solvents (water, methanol and ethanol). Then collected solutions were filtered through Whatman No-1 filer paper. The extracts were evaporated to dryness under reduced pressure at 90^0C by Rotary vacuum evaporator to obtain the crude extracts and stored in a freezer condition at 4^0C until used for further analysis.

Phenolic estimation: The total phenolic content of oil and flowers were determined by using Folin-Ciocalteu Spectrophotometric method according to the method described (Kim *et al*., 2007) using UV-vis Spectrophotometer at 650 nm. The results were expressed as catechol equivalents (µg/ml).

Antioxidative activity: The antioxidant activity of the Neem flower and oil on the basis of the scavenging activity of the stable 2, 2- diphenyl-2-picrylhydrazyl (DPPH) free radical was determined according to the method

described in (Brand-Williams *et al.,* 1995) with slight modification. The following concentrations of extracts were prepared 40 µg/mL, 80 µg/mL, 120 µg/mL, 160 µg/mL and 200 µg/mL. All the solutions were prepared with methanol. 5 ml of each prepared concentration was mixed with 0.5mL of 1mM DPPH solution in methanol. Experiment was done in triplicate. The test tubes were incubated for 30 min. at room temperature and the absorbance measured at 517 nm. Lower the absorbance of the reaction mixture indicates higher free radical scavenging activity. Ascorbic acid was used as a standard and the same concentrations were prepared as the test solutions. The difference in absorbance between the test and the control (DPPH in ethanol) was calculated and expressed as % scavenging of DPPH radical. The capability to scavenge the DPPH radical was calculated by using the following equation.

Scavenging effect (%) = (1-As/Ac) ×100

As is the absorbance of the sample at t =0 min.

Ac is the absorbance of the control at t=30 min.

RESULTS AND DISCUSSION

Phenol content and antioxidant activity: In our present study (Table 1), the ethanolic fraction of Neem flower exhibited the highest free radical scavenging activity i.e. 64.17±0.02% in comparison to methanol and water fractions i.e. 52.30±0.05% and 41.03±0.06% respectively. From above all the essential oil obtained from Neem seed exhibited the highest position in showing antioxidant activity i.e. 66.34±0.06% as well as phenolic content with 132µg/ml (Table 2). The total phenolic concentration of Neem flower and oil showed a positive correlation with antioxidant capacity. Dorman *et al*. (1995) screened *Pelargonium* Sp., *Monarda citriodora* var. *citriodora*, *Myristica fragrans*, *Origanum vulgare* Sp. *hirtum* and *Thymus vulgaris* for their antioxidative effect using a thiobarbituric acid (TBA) assay. Similar reports by (Viuda-Martos *et al*., 2010) on antioxidant activity of oils has been observed in clove oil i.e. 98.74%. Thyme essential oil produced the highest percentage inhibition of DPPH radical i.e. 89.84% and rosemary essential oil producing the highest effect with 76.06%. The oils showed active antioxidant capacities at extremely low level of dilution. Rosemary has long been recognized as having antioxidant molecules and these have been identified as carnosic acid, carnasol, carsolic acid, rosmaridiphenol and rosmarinic acid, found in ethanol-soluble fraction. Antioxidant properties are also found in the volatile oil fraction. These findings (Dorman *et al*., 1995) suggest that in certain cases, antioxidants can be pro-oxidant and can stimulate free radical reactions. In our results we found that the essential oil showed more effectiveness in free radical scavenging activity i.e. 66.34±0.06% than the flower extracts which supports the findings obtained from Dorman *et al*. (1995).

The presence of higher percentage of yield and higher values of phenol content in ethanolic extracts show that phenolic constituents must be responsible for such properties. The presence of higher content of phenolic compounds may serve as good source of antioxidantal activity and may be used in pharma/food industries. It is in agreement with the data of (Goncalves *et al.,* 2005). Antioxidant activity of Neem oil was probably due to presence of terpenes and phenolics present in essential oil as the evidence found in the paper (Oyedeji *et al*., 2005).

The study (Table 1 and 2) showed that there is a relationship between total phenolic content and antioxidant activity in both flower and oil of Neem plants. Several studies have demonstrated regarding correlation between phenolic content and antioxidant activity (Yang, *et al*., 2002). In addition to total phenol content antioxidant activity in the plant parts of *Azardirachta indica A. Juss* is possible owing to the presence of azadirachtin, nimbidin and nimbin in seed oil and nimbosterol, myricitin and kaempferol in flowers (Gupta, 2008).

IC_{50} value: IC_{50} value is defined as the concentration of substrate that causes 50% loss of the DPPH activity and was calculated by linear regression method of plots of the percentage of antiradical activity against the concentration of the tested compounds. As shown in Table-1, the essential oils and extracts of bud obtained from methanol and ethanol are able to reduce the stable free radical 2,22 -diphenyl-1-picrylhydrazyl (DPPH) to the yellow colored diphenyl picrylhydrazine. The studies on antioxidants suggested that free radical scavenging activity of essential oils was more than that of the other

Table 1. Antioxidant activity of neem oil and flower.

Conc. of sample in (µg/ml)	Antioxidant activity (%) Neem oil	Antioxidant activity (%) Neem flower		
		Water	Methanol	Ethanol
40	54.61±0.01	31.38±0.04	44.15±0.05	50.02±0.03
80	59.61±0.08	33.30±0.01	46.31±0.02	54.64±.0.05
120	61.00±0.04	36.19±0.08	49.06±0.01	56.32±0.04
160	64.42±0.02	39.11±0.04	51.56±0.07	63.82±0.08
200	66.34±0.06	41.03±0.06	52.30±0.05	64.17±0.02

Table 2. Phenolic content and IC_{50} values of neem oil and flower.

Name of the sample	Phenol content (µg/ml)	IC_{50} values (µg/ml)
Neem oil	132	39
Neem Flower (W)	15	< IC_{50} Values
Neem Flower (M)	34	140
Neem Flower (E)	86	102

two extracts i.e. ethanolic and methanolic. Among the given extracts of different solvents, the essential oils of seeds exhibited the strongest free radical scavenging activity with IC_{50} value of 39 µg/ml. The results obtained from these findings (Table 1 and 2) showed a linear relationship between the reciprocal of IC_{50} value and the total polyphenol content of *Azardirachta indica A. Juss* indicating that the polyphenol content strengthening the antioxidant activity (Katsube *et al*., 2004).

Conclusion

Multi solvent extract experiment of Neem flower revealed highest percentage of antioxidant activity and phenol content in ethanol solvent. The *in vitro* antioxidant study of Neem flower and oil demonstrate that the oil has more ability to scavenge DPPH. High antioxidant activity in seed oil is attributed to total phenol content. Antioxidant potential expressed as IC_{50} were 39 µg/ml for oil and 102µg/ml for flower in comparison to ascorbic acid as standard. Neem flower is used as seasonal food of common people as well as tribals of our country of its therapeutic value. All parts of Neem plant have advantages in medicinal treatments and industrial products including flower and seed oil. Still researchers are exploiting the therapeutic usefulness of the plants through various research projects. Certain new pharmacological activities may open new vistas of drug discovery and development enriching the therapeutic wealth of *Azardirachta indica A. Juss*.

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