Original Research article

In Vitro Antioxidant Activity of *Acacia nilotica* (L.) Delile from Lakhimpur District, Assam

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Received: May 30, 2019; revised; June 10, 2019; accepted: June 13, 2019

Abstract: Medicinal plants are the sources of almost all types of bioactive compounds that are needed for curing many human diseases. The plant *Acacia nilotica* (L.) Delile is a shrub grown in wild habitat with beautiful yellow coloured flowers. The plant is well known as "Tarua Kadam" in the Assamese community of Brahmaputra Valley. Bark of *Acacia nilotica* (L.) Delile plant is used by many people of Assam locally to treat stomach related disorders like diarrhea, abdominal pain and in the treatment of female reproductive problem such as menstrual pain etc. Traditionally, the patient is prescribed to take aqueous crude bark extract orally to cure stomach ailments. The objective of the present study was to evaluate the antioxidant activity of methanolic extract of the bark of *Acacia nilotica* plant. Antioxidants can inhibit the oxidation activity of free radicals that are responsible for various degenerative diseases. The antioxidant activity was determined by 2,2- diphenyl-1-picrylhyrazyl (DPPH) free radical scavenging activity method with L-ascorbic acid as a reference compound. Seven different fraction of sample extract were made in methanol such as 100, 50, 25, 12.5, 6.25, 3.125 and 1.562 µg/ml and absorbance were taken using Thermo multiskan spectrophotometer at 517 nm wave length. The antioxidant activity of the crude extract of *A. nilotica* was calculated as IC_{50} value against DPPH free radicals. Result shows a positive co-relation between the standard and crude sample with IC_{50} of 7.038 µg/ml and 32.116 µg/ml respectively. The result indicates the bark of *A. nilotica* to possess significantly high antioxidant activity.

Key words: *Acacia nilotica*, antioxidant activity, DPPH, free radical scavenging activity, IC_{50} value, methanolic extract.

Introduction

Antioxidant means “against oxidation” which defines that they can inhibit oxidation of other molecules. Oxidation of biological compounds inside the body of an organism produces free radicals some of them are proved as dangerous to human health. Free radicals like reactive oxygen species (ROS) which includes hydroxyl radicals, oxygen singlet, superoxide radicals and hydrogen peroxide and also some reactive nitrogen species (RNS) though play significant positive role such as cell growth regulation, intercellular signaling, phagocytosis etc. (Chang *et al.,* 2001). Yet overproduction of these free radicals can damage cellular organelles, DNA, RNA, lipid bilayer of cell membrane leading to cause many degenerative diseases like cancer, cardiovascular diseases, kidney damage, diabetes, mutation in DNA, RNA etc. (Halliwell, 1997). A number of medicinal plants and their crude extract have been shown to contain antioxidant activity (Jain *et al.,* 2011), in the form of phenolic compounds such as flavonoids, phenolic acids, tannins and polyphenols (Abhisekh *et al.,* 2013). Because of the presence of these phytochemicals plants have been used in treating many human diseases. There are so many evidences that reveal the use of fresh fruits and vegetables in diet which lowers the risk of certain cancer, diabetes, cardiovascular diseases (Zahin

et al., 2009). Therefore, antioxidants are necessary for our body as a dietary supplement to control and inhibit the free radical production (Chaturvedi, 2008).

A. nilotica is a significant shrubby plant of family Fabaceae. The bark juice of *A. nilotica* locally known as "Tarua Kadam" (Assamese) have been used by traditional healers as well as local people of Lakhimpur district of Assam for the treatment of stomach and abdominal pain related human disorders. Therefore, aim of the current study was to evaluate antioxidant activity of bark extract of this plant and to justify the use of the plant as medicine by the people.

Materials and methods

Chemicals and solvents: 100% methanol for maceration, pure ascorbic acid as standard reference, DPPH (2, 2-Diphenyl-1-picrylhydrazyl) for detecting the radical scavenging activity of the samples.

Collection of the plant material and extract preparation

Bark of *A. nilotica* (Fig. 1) was collected from the Lakhimpur district of Assam during august, 2017. For preparation of the extract, bark was washed properly and cut into pieces. Sample was then shade dried, grounded into powder and stored in an air tight amber colour bottle for future use. 20 g of bark powder was soaked in100% methanol for at least 5 days at room temperature and filtered the mixture using Whatman filter paper No. 1. The total filtrate was concentrated by rotary evaporator and extract was stored in 4^{0}C for further quantitative analysis.

Fig. 1. Photograph of *Acacia nilotica*

DPPH free radical scavenging activity test

Total antioxidant activity of the sample was carried out by DPPH free radical scavenging method described by Liyana-Pathiranan & Shahidi (2005) with little modification using Thermo Scientific Multiscan Spectrophotometer in place of cuvette UV-VIS spectrophotometer. DPPH is a very stable organic free radical with deep purple color. Upon receiving proton from any source, it loses its original purple colour. Disappearance of the purple colour of DPPH by any sample indicates the presence of antioxidant property of that sample. Working solution of the test sample, was prepared by homogenizing 1mg of the dry extract in 1ml of methanol (MeOH), from which a series of dilutions were prepared in the order 100, 50, 25, 6.25, 12.5, 3.125 and 1.562 µg/ml in MeOH. Pure Ascorbic acid was employed as standard. A working solution of ascorbic acid (1 mg/ml) was prepared in distilled water, from which dilutions were made similar to that of the sample. 100 µl from each serial dilutions with 200 µl of 0.1 mM DPPH were pipette out in well plate with four replicas of each concentration and incubated at room temperature for 30 minutes under darkness. Absorbance were taken at 517 nm wave length after 30 mins of incubation of both the crude and standard. Free radical scavenging activity was calculated using the formula:

DPPH scavenging activity (% of free radical inhibition) = (A-A)/A* 100

Where, A is the absorbance of the control and A* is the absorbance of the sample.

Statistical analysis

All statistical calculations were done using excel. Graphs were drawn between the concentration (X-coordinates) v/s

inhibition % (Y-coordinates) by the logarithmic plotting of points and calibrate the IC_{50} of both the sample and standard. The IC_{50} is calculated from the formula Y=a ln(x)±c. Where 'Y' is, the value representing 50% of the maximum inhibition; 'X', the concentration of the sample extract at which 50% inhibition of the DPPH radicles takes place.

Results

Table 1 shows the DPPH free radical scavenging activity of the bark extract of *A. nilotica* inhibition % (in quadruplets) as well as their average values at different concentrations. The table also shows the IC_{50} of antioxidant DPPH activity of bark extract of *A. nilotica* as 20.176 µg/ml.

Table 1. Table showing free radical Inhibition % of *A. nilotica* bark extract at different concentrations by DPPH method and its IC_{50} value.

Concentration (µg/ml)	Free radical inhibition % Per concentration in four replicates				Average inhibition % (± SD)	IC50 value
1.562	5.65	5.64	5.64	5.63	5.64 ± 0.008	
3.125	6.659	6.549	6.759	6.669	6.659 ± 0.086	
6.25	13.08	11.09	12.09	12.10	12.09 ± 0.812	20.176
12.5	19.67	20.672	19.500	22.846	20.672 ± 1.539	
25.0	46.896	48.513	45.381	53.262	48.513 ± 3.414	
50.0	59.381	59.674	59.519	60.122	59.674 ± 0.322	
100	90.218	89.709	88.278	84.907	88.278 ± 2.393	

Table 2. Table showing free radical Inhibition % of pure ascorbic acid at different concentrations by DPPH method and its IC50 value.

Concentration (µg/ml)	Free radical inhibition % Per concentration in four replicates				Average inhibition % (± SD)	IC50 value
1.562	8.692	9.398	9.573	7.905	8.892 ± 0.877	
3.125	20.371	21.645	22.281	21.971	21.567 ± 0.838	
6.25	43.501	42.016	42.653	46.622	43.698 ± 2.042	7.1985
12.5	73.456	80.759	79.759	74.830	77.201 ± 3.599	
25.0	80.854	92.308	84.005	84.201	85.342 ± 4.891	
50.0	95.279	94.218	95.703	89.02	93.555 ± 3.087	
100.0	96.180	94.642	96.552	98.314	96.422 ± 1.541	

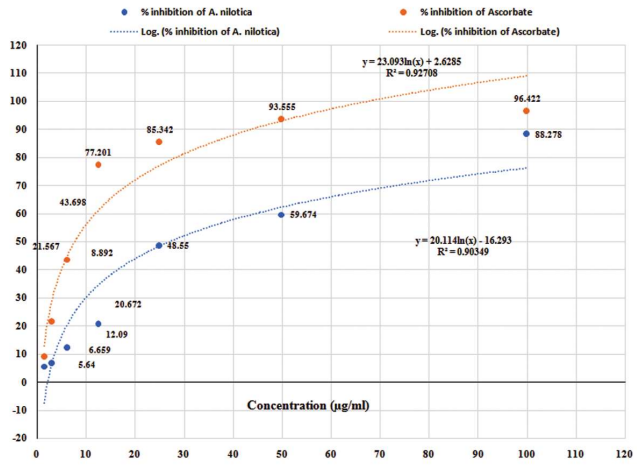

Fig. 2. Graph showing logarithmic curves between Average inhibition % vs Concentration (**µg/ml**) of Ascorbic acid and *A. nilotica*

Table 2 shows the DPPH free radical scavenging activity of the standard (pure ascorbic acid), inhibition % (in quadruplets) as well as their average values at different concentrations. The IC_{50} of antioxidant DPPH activity of ascorbate is also shown as 7.1985 µg/ml.

Discussions

The present study evaluates *in vitro* antioxidant activity of the crude extract sample of *A. nilotica* by DPPH free radical scavenging method. DPPH free radical assay is one of the most common and reliable method for determination of antioxidant potential. It is a highly stable free radical, when it reacts with a sample that has high antioxidant activity it gains electron from antioxidant and loses its original color (Ayam

and Asha, 2017). This method is based on decrease in deep purple to colorless of alcoholic DPPH solution (Wojdylo *et al.,* 2007). *A. nilotica* is a plant of multipurpose (Kaur *et al., 2005).* It is a tropical and subtropical genus with abundant species throughout Asia, Africa, America and Australia that occurs naturally in rural pastoral fields (Shittu, 2010; Ali *et al., 2012).* Present study showed that *A. nilotica* bark contains potent antioxidant activity. Similar work has been done on antioxidant activity of leaves and pods of *A. nilotica* by many researchers (Kalaivani and Mathew, 2010; Khan and Gohel, 2014; Gowri *et al., 2011*; Subhaswaraj *et al.,* 2017). Singh *et al.* (2009) have studied about antioxidant activity of ethyl acetate bark extract of *A. nilotica* and reported significant radical scavenging activity of the extract however, in this study it is the antioxidant study of the methanol extracts and shows different results. Moreover, there is no report from Assam on antioxidant activity of bark extract of *A. nilotica* plant. It was found during the study that bark of A. nilotica shows high antioxidant activity when extracted in MeOH with an IC_{50} value of 20.176 µg/ml (Table 1) calculated from the formula (y = 20.114ln(x) - 16.293) generated from the logarithmic curve between concentrations vs average inhibition % of oxidation by DPPH. The curve of concentrations vs average inhibition % of oxidation by DPPH drawn using excel was highly reliable which shows the goodness of fit value of R^2 = 0.90349.

The IC_{50} value of *A. nilotica* was found to be lesser than the well-known Pomegranate ethanolic seed extract (PESE) which was reported its IC_{50} ranging from 33.3 to 333 µg/ml (Karasu*et al*, 2012) also slightly lesser than the hexane extractand Diethylether fraction ($IC_{50,}$ 24.8±1.3) of *Chenopodium quinoa* (IC_{50}, 22.4±0.3), but higher than the Hexane extract of*Amaranthuscruentus* (IC_{50}, 15.9±0.3) (Nsimba *et al.*, 2008).

Conclusion

In conclusion, we might say that result of the present study support the use of this plant as herbal medicine by local people of Assam and also is a source of natural antioxidant. This study showed that *A. nilotica* bark extract possess a significant free radical scavenging ability comparable with pure ascorbic acid and other fruit like pomegranate and wild leafy vegetable like proved to have high antioxidant activity. It will be worthy to further investigate about effectiveness of *A. nilotica* in preventing degenerative diseases caused by free radicals.

Acknowledgements

The authors are grateful to the Centre with Potential for Excellence in Biodiversity (CPEBII), Rajiv Gandhi University, Arunachal Pradesh for providing the instrumentation help.

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