

Investigation of Antioxidant Potentials of *Acacia nilotica*, *Ocimum sanctum* and *Alpinia nigra*

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Abstract

Acacia nilotica, *Ocimum sanctum*, and *Alpinia nigra* are used traditionally in different ailments in rural settings of Bangladesh. These medicinal plants were studied for their total phenolic and total flavonoid contents as a partial approach to rationalizing the use of them. Antioxidant activity was also determined to measure ferric reducing power assay and 1,1'-diphenyl-1-picrylhydrazyl radical (DPPH) scavenging activity. *A. nilotica* showed the highest total phenolic content while *O. sanctum* showed highest flavonoid contents among the studied three plants. Similarly, IC50 values of the extracts of *A. nilotica*, *O. sanctum* and *A. nigra* against DPPH were 39.62, 48.81, 70.85 µg/ml, respectively. The reducing power of the extract was found to be concentration dependent and *O. sanctum* showed the highest reducing power followed by *A. nilotica*. The reducing power of these two plants was fairly close to the positive control of ascorbic acid. Further studies are suggested such as *in-vivo* testing and elucidation of the mechanism of action of inherited bioactive compounds to support its folkloric use in the treatment of diseases.

Keywords: Antioxidant potential; *Acacia nilotica*; *Ocimum sanctum*; *Alpinia nigra*; DPPH assay; Reducing power; Total phenolic; Total flavonoid

Introduction

The inequity between the manifestation of Reactive Oxygen Species (ROS) inside a biological system and detoxification capability of injurious intermediates is known as Oxidative stress [1]. The chain reaction initiated by a ROS can severely harm an individual cell and its surroundings. Research shows that a chain reaction evolves in the presence of different free radicals like superoxide, Peroxide, etc. [2].

In these recent years, concern on oxidative stress has raised to a higher level after numerous fundamental research were found to be finalizing significant relations between oxidative stress and aging or different chronic diseases including cancer

[3]. Antioxidants have been playing a role against oxidative stress to ensure the safety of biomolecules and prevent oxidative deterioration. Many studies have shown that polyphenolic compounds are effective as antioxidants and act against lipid oxidation in phospholipids' bilayers [4].

However a growing number of synthetic antioxidants have become another issue, scientists needed to consider carefully. At this situation, it is widely suggested that the use of natural antioxidants can be a very good alternative solution instead of using synthetic butylated hydroxyanisole, butylated hydroxytoluene, propyl gallate etc. where a few of these agents are believed to induce cancer and liver dysfunction [5,6]. On the contrary, it is very likely to assume that the natural antioxidants would be safer than most other synthetic materials serving as oxidative stress fighter.

A. nilotica is traditionally used as astringent, anti-oxidant, natriuretic, antispasmodial, diuretic, intestinal pains and diarrhea, coughs, leucorrhea and sclerosis [7]. *O. sanctum* Linn, a small herb seen throughout Bangladesh, have been recommended for the treatment of bronchitis, bronchial asthma, diarrhea, dysentery, skin diseases, chronic fever, insect bite, etc. [8]. The ethnopharmacological uses of *A. nigra* include treatment of jaundice, gastric ulcers, parasitic infections, inflammation, etc. [9,10].

As our continuous approaches [11-13] to justify traditional claims of different medicinal plants of Bangladesh, the antioxidant potentials of *A. nilotica*, *O. sanctum*, *A. nigra* were investigated.

Materials and Method

Plant material

Roots *A. nilotica*, leaves of *O. sanctum* and rhizomes of *A. nigra* from were collected from Noakhali district of Bangladesh and identified by officials of Bangladesh National Herbarium, Dhaka where voucher specimens (No. 38212, 38234, 38358, respectively) have also been deposited. Materials dried at room temperature and coarsely ground before extraction. Samples

(250 g each) were extracted separately at room temperature by percolation method using 750 ml of 80% methanol for each sample. The resulting extract was concentrated over a rotary vacuum until a crude solid extract was obtained, which was then subject to solvent fractionation by n-hexane and little amount of water. The obtained n-hexane fraction was separately concentrated under room temperature and used directly for the study.

Chemicals

Methanol, n-hexane, Folin-Ciocalteu reagent, sodium carbonate, gallic acid, crystalline aluminum chloride, crystalline sodium acetate, quercetin, 1'-1' diphenyl picryl-hydrazyl (DPPH), potassium ferricyanide, trichloroacetic acid, ferric chloride, butylated hydroxy toluene (BHT), ascorbic acid. All reagents were of analytical grade and purest quality available in a university lab.

Total phenolic content determination

Total phenolic compound contents were determined by the Folin-Ciocalteu method [14]. Extract solution of 250 µg/ml for each extract were prepared in water. To 0.5 ml of extract solution, 2.5 ml of Folin-Ciocalteu reagent (1:10 v/v diluted with distilled water) and 2.0 ml of Na₂CO₃ (7.5% w/v) solution were added. The mixture was incubated for 20 minutes at room temperature and then the absorbance was measured at 760 nm by UV-spectrophotometer and using the standard curve prepared from Gallic acid solution with different concentration (0.78125 µg/ml to 100 µg/ml), the total phenolic contents of the sample was measured and it was expressed as µg of GAE (Gallic acid equivalent)/mg of the extract [15].

Total flavonoid content determination

The flavonoids content of the n-hexane extracts of *A. nilotica*, *O. sanctum*, *A. nigra* were determined following Aluminium chloride colorimetric method [16]. Extracts were taken in methanol in different beakers to make the concentration of 500 µg/ml for each plant. Then 5 ml of an analyzed solution of three different extracts were taken in different test tubes and 2.5 ml of AlCl₃ reagent (133 mg crystalline aluminum chloride and 400 mg crystalline sodium acetate were dissolved in 100 ml of methanol) was added individually in all taken test tubes and absorbance recorded at 430 nm. Various dilutions of quercetin (125 µg/ml to 6.25 µg/ml) were prepared in methanol and a standard curve was plotted. The amount of flavonoids was calculated as quercetin equivalent from the calibration curve of quercetin.

DPPH radical-scavenging activity

The antioxidant activities of the plants extracts on the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) were estimated by the method of Brand-Williams [17]. Briefly, 3 mL of DPPH solution (20 µg/ml) was added to 2 ml each of the test solutions

(500-0.977 µg/ml, total 10 different conc.), and was incubated in the dark at room temperature for 30 min. The absorbance values were read at 517 nm, and converted into percentage antioxidant activity, using the formula below. This procedure was the same for all three different plants extracts.

$\% \text{ inhibition} = [1 - (\text{Absorbance of Sample} / \text{Absorbance of control})] \times 100$

Reducing power determination

The reducing power of three selected plants' extracts were determined according to the method previously described [18]. 1 ml of test samples of different concentrations (500 µg/ml to 3.906 µg/mL) was mixed with 2.5 ml phosphate buffer (pH 6.6) and 2.5 ml of (1% w/v) potassium ferricyanide. The mixture was incubated at 50°C for 20 min. Trichloroacetic acid of 2.5 ml was added to the mixture and it was then centrifuged at 3000 rpm for 10 min. The supernatant solution of 2.5 ml was taken in another test tube and 2.5 ml of distilled water, 0.5 ml of ferric chloride (0.1% w/v) were added to the tube and mixed. The absorbance was measured against a blank at 700 nm. Increased absorbance of the reaction mixture indicated the increased reducing power. This procedure was the same for all three different plants extracts. Butylated hydroxytoluene (BHT) was used as a positive control in DPPH radical scavenging assay and ascorbic acid (AA) as a positive control in ferric reducing power assay.

Results and Discussion

Antioxidants are used in the food industry, to stabilize foods that by their composition would in the presence of oxygen and other reactive oxygen species undergo significant loss in quality such as the development of rancidity from the oxidation of unsaturated fats resulting in off-odors, off-flavors, and discoloration by decomposition of components of the food.

In this study, we examined total phenolics, total flavonoids and evaluated the antioxidant effects of n-hexane extracts of roots of *A. nilotica*, leaves of *O. sanctum* and rhizome of *A. nigra*.

Total phenolic content and total flavonoid content

Recent years have seen an exponential increase in research to identify and characterize of phenolic and flavonoid contents from medicinal plants. Phenols show antioxidant activity by destroying lipid free radicals or preventing the conversion of hydroperoxides into free radicals, that is why it is responsible for varying the antioxidant capacity of plants [19].

Total phenol compounds, as determined by Folin Ciocalteu method, was reported as µg gallic acid equivalents/mg of extract powder, by reference to a standard curve ($y=0.018x+0.043$, $R^2=0.999$). The total flavonoid contents were reported as µg quercetin equivalent/mg of extract powder, by reference to a standard curve ($y=0.009x+0.005$, $R^2=0.988$) (Table 1).

Table 1: Total phenol and flavonoids contents of tested plants extracts.

n-hexane extract of	Total phenol contents gallic acid equivalent ($\mu\text{g}/\text{mg}$)	Flavonoid contents Quercetin Equivalent ($\mu\text{g}/\text{mg}$)
<i>A. nilotica</i>	74.89	46.67
<i>O. sanctum</i>	13.55	258.66
<i>A. nigra</i>	7.77	69

A. nilotica n-hexane root extracts showed the highest amount of phenolic content and *O. sanctum* leaves extract showed the highest amount of flavonoid content among three investigated plants' parts. Whereas the lowest amount of phenolic and flavonoid content were found in *A. nigra* and *A. nilotica*, respectively. The order of magnitude of total phenolic content among these plants is *A. nilotica*>*O. sanctum*>*A. nigra* and for total flavonoid is *O. sanctum*>*A. nigra*>*A. nilotica*. Phenolic acids are found as antioxidants in nature, e.g. caffeic acid, ferulic acid, vanillic acid, and rosmarinic acid are widely distributed in the plant kingdom [20] where the last one was found as potent active substance against HIV [21].

DPPH scavenging assay

The DPPH assay constitutes a quick and low cost sensitive method where the decolorization of the radical solution from purple to light yellow is evaluated in the presence of the plant extract at 517 nm using UV spectroscopy [22]. The IC₅₀ value is defined as the concentration of plant extract that causes 50% loss of the DPPH activity and was calculated by linear regression plots of the percentage of antiradical activity against the concentration of the tested extract. IC₅₀ value found from this study for *A. nilotica*, *O. sanctum*, *A. nigra* is 39.62 $\mu\text{g}/\text{ml}$, 48.81 $\mu\text{g}/\text{ml}$, 70.85 $\mu\text{g}/\text{ml}$, respectively.

In DPPH scavenging assay, *A. nilotica* extract exerts far better antioxidant activity than the others. This antioxidant test establishes the potency of *A. nilotica* as an oxidative stress fighter where *O. sanctum* and *A. nigra* shows relatively less potentiality (Table 2).

This result also determines an approximately proportional relationship between phenolic content and free radical scavenging power that is similar to other studies [14,23]. The order of magnitude of antioxidant activity among these three plants is *A. nilotica*>*O. sanctum*>*A. nigra*.

Ferric reducing power assay

The reduction of ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) is measured by the intensity of the resultant Perl's Prussian blue solution from yellow which absorbs at 700 nm. Greater the intensity of the colour, greater will be the absorption; consequently, greater will be the antioxidant activity [24]. Many studies reported that reducing power was associated with the antioxidant activity and this relationship of phenolic compound has been well established in several plant sources [25,26].

The reducing power of selected plants' extracts and positive reference standards is shown in Table 3.

Table 2: DPPH free radical scavenging capacity (% inhibition) of selected plants extracts and reference standard.

Concentration ($\mu\text{g}/\text{ml}$)	% inhibition			
	<i>A. nilotica</i>	<i>O. sanctum</i>	<i>A. nigra</i>	BHT
500	82.82	76.8	67.66	94.1
250	80.36	69.61	65.26	93.67
125	74.53	62.43	58.68	90.37
62.5	69.01	58.56	50.89	79.81
31.25	46.01	49.17	43.71	57.22
15.62	26.99	39.23	32.93	38.85
7.81	11.96	19.34	24.55	26.5
3.9	10.12	11.05	14.37	16.56
1.95	5.52	8.29	5.38	15.96
0.97	3.37	3.87	1.79	14.45
IC ₅₀ ($\mu\text{g}/\text{ml}$)	39.62	48.81	70.85	18.56

Table 3: Reducing the potential of plants extracts and ascorbic acid.

Conc. ($\mu\text{g}/\text{ml}$)	Absorption at 700 nm			
	<i>A. nilotica</i>	<i>O. sanctum</i>	<i>A. nigra</i>	AA
500	2.245	2.589	0.429	3.035
250	1.493	1.338	0.322	1.995
125	0.88	0.719	0.292	1.234
62.5	0.463	0.402	0.26	0.762
31.25	0.32	0.339	0.244	0.516
15.62	0.29	0.28	0.235	0.369
7.81	0.211	0.273	0.224	0.297

3.9	0.197	0.229	0.218	0.232
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The highest absorption (2.589) was found for *O. sanctum* and lowest (0.429) for *A. nigra* among the three extracts. *A. nilotica* and *O. sanctum* showed fairly good antioxidant activity close to positive standard ascorbic acid. The reducing power of extracts increased steadily with increasing concentrations (Figure 1) and varied significantly with different concentrations. The found order of magnitude of ferric reducing power in this study is ascorbic acid > *O. sanctum* > *A. nilotica* > *A. nigra*.

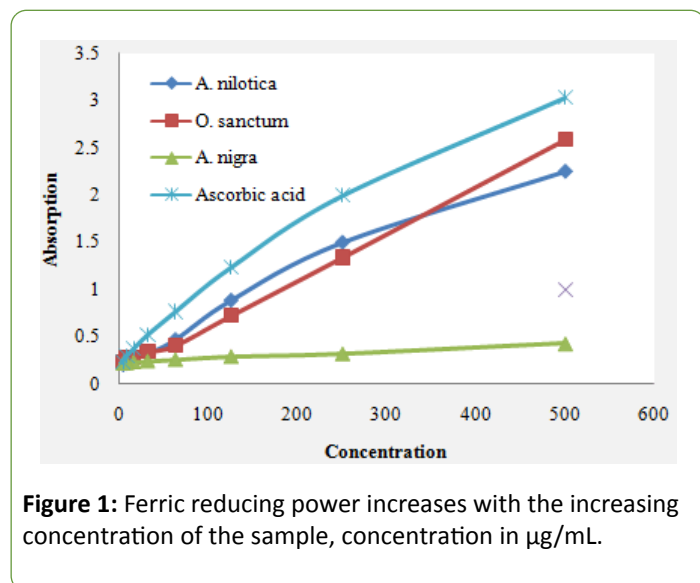


Figure 1: Ferric reducing power increases with the increasing concentration of the sample, concentration in µg/mL.

Conclusion

A. nilotica and *O. sanctum* showed good antioxidant properties and *A. nigra* showed comparatively less antioxidant properties. It is however, worthwhile to further investigate the in vivo potentials of this plant and also isolate the active components which could ultimately lead to their application in the food industry as an antioxidant flavour or in pharmaceutical formulations.

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