Evaluation of Antioxidant Activity of the Selected frequently used Traditional Wild Medicinal Plants from Lakhimpur, Assam (India)

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Abstract

North Eastern India is a very diverse area because of its unique types of flora as well as from different tradition and community. *Terminalia arjuna*, *Cheilocostus speciosus* and *Clerodendrum glandulosum* are three important wild medicinal plants of family Combretaceae, Costaceae and Lamiaceae respectively, used by most of the people of Assam as medicine to cure certain illness like cardiovascular diseases, jaundice, high blood pressure etc. The aim of the present study is to evaluate the antioxidant activity of these three plant samples. Cold maceration method in methanol was applied in the preparation of extract and antioxidant activity was determined using DPPH free radical scavenging method. IC₅₀ (inhibition concentration) value was calculated for all sample. For comparison of total antioxidant activity pure ascorbic acid was taken as standard. The study shows the highest antioxidant activity by *T. arjuna* with IC₅₀ value at 12.508 µg/ml, while *C. speciosus* and *C. glandulosum* shows considerably lower antioxidant activity with IC₅₀ value at 300.532 µg/ml and 205.086 µg/ml.

Key words: Traditional medicinal plant, Antioxidant activity, DPPH, IC₅₀ value, *Clerodendrum glandulosum*, *Cheilocostus speciosus*, *Terminalia arjuna*

INTRODUCTION

Medicinal plants form the backbone of several indigenous traditional systems of medicine (Ramya *et al.* 2008). North-east India is known as the home of different people of different tradition and community and also home of diverse flora, from evergreen tropical forest to alpine forest. Assam is a part of the northeastern region of India with a high number of endemic flora and fauna (e.g. *Messua assamica* i.e. *Sia nahar* in Assamese). Annually different climatic condition is available in Assam; therefore, various types of plant species are found in this region. Though some region of Assam is comparatively developed yet natives in many other places were under poverty with weak purchasing power and depends everything on forests for their livelihood such as foods, medicines, fire-woods, etc. Various wild plants are used as medicines from ancient time. People of Assam specifically hold this ancient tradition of using wild plants as medicine till today. Various plants such as *Acorus calamus*, *Centella asiatica*, *Pogostemon benghalensis*, *Hibiscus acetosella*, *Terminalia arjuna*, *Cheilocostus speciosus*, *Clerodendrum glandulosum* etc. used traditionally as medicines against various diseases. Different plant parts produce pharmacologically active compounds like flavonoids, alkaloids, tannin, steroids, glycosides, phenols, fixed oils etc. (Sharma *et al.* 2008). Medicinal plants, a source of remedies, were widely used as alternative therapeutic tools for the prevention or treatment of many diseases

(Kaur & Mondal 2014). Antioxidants are considered as one of the important bioactive compounds on account of many health benefits along with their pivotal role in delaying oxidative rancidity of numerous food (Moharram & Youssef 2014). Oxidation is a chain reaction that releases free radicals like reactive oxygen species (ROS) and reactive nitrogen species (RNS), some of which are very dangerous and toxic to human health. So, antioxidants are considered an essential element that should provide in our daily diet. Antioxidants are available in both natural and synthetic condition. Natural antioxidants are obtained from mainly various medicinal plants, vegetables etc. Medicinal plants are therefore investigated for their antioxidant properties, and the demand for natural antioxidants are increasing day by day (Peschel *et al.* 2006). Leaves of *Clerodendrum glandulosum* (*Nefafu* in Assamese), boiled vegetable of which is believed to control blood pressure (Bordoloi & Borthakur 1997). *Cheilocostus speciosus*, a shady and moist growing wild plant the rhizome decoction of which is believed to cure jaundice (Lim, 2014), similarly believed by the local people of Assam. The water decoction of dried and powdered bark of *Terminalia arjuna* is also widely used by ethnic people of Assam for curing various heart-related diseases, and its bark-juice is used as cardi tonic by some people (Khandekar & Pansare 2018). Since plants with medicinal properties were bound to show antioxidant activity, these three frequently used medicinal plants (Table 1) of the area have been subjected to antioxidant activity [PLATE - I].

MATERIALS AND METHODS

Collection of plant material: The samples (i.e. *Clerodendrum glandulosum*, *Cheilocostus speciosus* and *Terminalia arjuna*) were collected during August – October of 2017 from Lakhimpur area of Assam, India. Samples were then brought to the Department of Botany, Rajiv Gandhi University, Arunachal Pradesh for further analysis.

Table 1. Names of the selected plant species and their traditional medicinal uses.

Assamese name	Botanical name	Family	Traditional uses as medicine
<i>Nefafu</i>	<i>Clerodendrum glandulosum</i> Lindl.	Lamiaceae	Boiled leaves consumed to control high blood pressure
<i>Jomlakhuti</i>	<i>Cheilocostus speciosus</i> (J.Koenig) C.D.Specht	Costaceae	Rhizome extract is taken to cure jaundice
<i>Arjun</i>	<i>Terminalia arjuna</i> (Roxb. ex DC.) Wight & Arn.	Combretaceae	Aqueous extract (decoction) from dried powdered bark to control blood sugar, juice from fresh bark as cardio-tonic, and to cure cough.

Preparation of extract: Extract for the detection of antioxidant activity was prepared in 100% methanol as solvent. Collected samples were washed properly and shade dried at room temperature. Dried samples were powdered and stored. Cold maceration method is used in extract preparation by allowing sample powder to mix with methanol. Methanol and ethanol have been proven as the effective solvent to extract phenolic compounds. After five to seven days, mixtures were filtered and filtrates were concentrated using rotary evaporator. And, now the extract is ready for further quantitative tests.

Quantitative test for determining antioxidant activity: For quantitative part DPPH (2,2-Diphenyl-1-picrylhydrazyl) free radical scavenging activity method was used. The process for determining free radical scavenging activity of plant extracts was measured

PLATE - I. Selected ethnomedicinal plants for antioxidant study: **A.** *Terminalia arjuna*; **B.** *Cheilocostus speciosus*; **C.** *Clerodendrum glandulosum*

by steps describing by Singh & Asha (2017). DPPH scavenging method is based on the reduction of purple color of stable free radical DPPH into yellow color. Decreases in the absorbance of DPPH indicate an increase in the DPPH radical scavenging activity (Hazarika *et al*, 2012). Briefly, 1 mg of extract was measured for each sample and seven different concentration of each sample extract was made mixing with the same solvent i.e. methanol, such as 1000, 500, 250, 125, 62.5, 31.25, 15.625 $\mu\text{g/ml}$. 100 μl of the sample from each concentration was pipette out in 96 well plates and add 200 μl of 0.1 mM DPPH solution (0.0039 gm DPPH powder mix with 100 ml of methanol). Incubated for 30 minutes and absorbance was taken at 517 nm using Thermo-Scientific Multiskan Spectrophotometer. Methanol was used as the blank. Ascorbic acid was taken as standard. Four replicates were taken against each concentration to see the accuracy of the test. Instead of methanol distilled water was used as the solvent for ascorbic acid. A control solution (solvent + DPPH, without sample) was also used for the calculation of free radical scavenging activity of each replica of all three sample.

Statistical analysis: All statistical analysis was done by using excel. For free radical scavenging activity of the samples, calculations were done by following formula:

$$\text{DPPH scavenging activity (\% of free radical inhibition)} = (A' - A)/A' * 100$$

Where,

A' = absorbance of control

A = absorbance of plant samples

Graphs were drawn between concentration (X-coordinate) and inhibition % (Y-coordinate) by logarithmic plotting for all and IC_{50} was calculated.

RESULTS

DPPH free radical scavenging activity- Among all three samples *T. arjuna* shows high scavenging activity against DPPH and found to have lower IC_{50} value (12.508 $\mu\text{g/ml}$) compared to *Cheilocostus speciosus* and *Clerodendrum glandulosum* (Tables 2 & 3), which shows IC_{50} value at 300.532 $\mu\text{g/ml}$ and 205.086 $\mu\text{g/ml}$ respectively. IC_{50} (inhibition concentration) is that concentration at which 50% of the free radicals will be inhibited by any source of antioxidant. Lower the IC_{50} value, higher will be the antioxidant activity of any sample. Being a pure antioxidant ascorbic acid (standard) shows IC_{50} at 7.038 $\mu\text{g/ml}$ concentration. Thus, present investigation shows that *Terminalia arjuna* have the high amount of antioxidant activity among all tested samples, because it has close IC_{50} value with the standard ascorbic acid (Table 4). Like standard ascorbic acid, *T. arjuna* also shows its scavenging power at a very low concentration (Figure 2).

Figure 2. DPPH scavenging activity of samples v/s standard in terms of IC_{50} values.

Table 2. DPPH free radical scavenging activity of methanol extract of *T. arjuna* (bark) and of standard ascorbic acid at different concentrations

Concentration ($\mu\text{g/ml}$)	Inhibition % of ascorbic acid	Inhibition % of <i>T. arjuna</i>
1.562	10.981 $\pm$ 3.008	5.348 $\pm$ 1.837
3.125	21.538 $\pm$ 0.829	13.371 $\pm$ 2.710
6.250	43.289 $\pm$ 1.285	22.699 $\pm$ 1.481
12.50	82.918 $\pm$ 1.141	46.517 $\pm$ 0.655
25.00	93.952 $\pm$ 1.154	72.699 $\pm$ 2.077
50.00	95.172 $\pm$ 0.659	93.657 $\pm$ 0.469
100.00	95.928 $\pm$ 0.871	95.584 $\pm$ 0.215

Table 3. DPPH free radical scavenging activity of methanol extracts of leaves of *C. glandulosum* and *C. speciosus*

Concentration (µg/ml)	Inhibition % of <i>C. glandulosum</i>	Inhibition % of <i>C. speciosus</i>
15.62	4.262 ± 1.045	7.453 ± 0.968
31.25	9.207 ± 0.754	10.290 ± 1.001
62.50	16.368 ± 0.426	11.900 ± 0.961
125	28.474 ± 0.378	19.607 ± 1.263
250	49.702 ± 0.546	36.928 ± 1.677
500	80.008 ± 0.295	69.198 ± 3.113
1000	86.615 ± 0.403	80.294 ± 0.570

Table 4. IC₅₀ values of tested samples with the standard

Samples	IC ₅₀ value (µg/ml)
<i>T. arjuna</i>	12.508
<i>C. glandulosum</i>	205.086
<i>C. speciosus</i>	300.532
Ascorbic acid (standard)	7.038

DISCUSSION

Plants have always been a rich source of phytochemicals, because of the presence of several biologically active compounds. The medicinal property of a plant depends on the presence of various secondary metabolites such as phenolics, terpenoids or alkaloids (Swargiary *et al*, 2016). Flavonoids, for instance, are a secondary metabolite that exhibits tremendous medicinal property including antioxidant, anticancer, antibacterial etc. (Kumar & Pandey 2013). The present study revealed that the tested plant samples have scavenging power against stable DPPH radicals and proves that they have antioxidant potential suggesting the possible presence of important phytochemicals. The *T. arjuna* shows the highest antioxidant activity with the least IC₅₀ (12.508 µg/ml), which is similarly, recorded by M. Shahriar *et al*, 2012 from the bark of *T. arjuna* using various solvent extracts. The IC₅₀ of *C. glandulosum* is comparatively higher (205 µg/ml), a closely similar value of the IC₅₀ 168.89 µg/ml was reported by Doley Pradip *et al*, 2016 and the antioxidant potential of the plant was also reported by Sanjay Jagtap *et al*, 2014. The IC₅₀ of *C. speciosus* is comparatively highest (300 µg/ml) which is similarly reported as medium – high by Jha *et al*. (2010).

Conclusion

From the present study, it can be concluded that *T. arjuna*, *C. glandulosum*, and *C. speciosus* show the antioxidant activity in descending order of *T. arjuna* >> *C. glandulosum* > *C. speciosus*. This study justifies the use of these wild plants by ethnic people in their health benefits. The presence of antioxidant activity of these plants shows the potency of these tested plants to prepare medicines and to use as a source of competent natural antioxidant.

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