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REGULAR ARTICLE

DETERMINING THE ANTIOXIDANT ACTIVITY OF CERTAIN MEDICINAL PLANTS OF SONITPUR, (ASSAM), INDIA USING DPPH ASSAY

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SUMMARY

As part of a screening program for biologically active compounds in medicinal plants of northeast India, twelve plants were investigated for their phytochemical screening and anti-oxidant activity. The antioxidant activity was estimated by using 2, 2- diphenyl-picryl-hydrazyl (DPPH) free radical assay. *Oroxylum indicum, Ipomoea aquatica* and *Moringa oleifera* exhibited strong antioxidant activity as compared to other plants. *Oroxylum indicum* showed the highest antioxidant activity. The present study indicated that these plants are of therapeutic potential due to their high free-radical scavenging activity. The role of phytochemical constituents of these important medicinal plants in traditional medicine treatment is discussed.

Keywords: DPPH assay, antioxidant activity, Phytochemical constituents.

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1. Introduction

Free radicals (superoxide, hydroxyl radicals and nitric oxide) and other reactive species (hydrogen peroxide, hypochloric acid and proxynitrite) produced during aerobic metabolism in the body, can cause oxidative damage of amino acids, lipids, proteins and DNA [1, 2]. By their ability to react with and damage many structures in the body, free radicals are involved in various related physiological processes and diseases such as ageing, cancer and atherosclerosis [3-5]. Nature, however, has endowed each cell with adequate protective mechanisms against any harmful effects of free radicals. Antioxidants are one such substances which have the capability to neutralize free radicals or their actions. Recently, there has been growing interest in natural antioxidants of plant origin because they have greater application in the food industry for increasing the stability and shelf life of food products. Moreover, they also

find use as nutraceuticals and phytoceuticals as they have significant impact on the status of human health and disease prevention [6].

North eastern region of India is the rich source of biodiversity which includes high potential of naturally occurring medicinal plants. It is one of the 'biodiversity hotspots' of the world supporting about 50% of India's biodiversity [7]. Seven states of the North eastern region, excepting Assam, are hilly states with varying climatic and forest vegetation zones which contain varying types of medicinal plants which either do not occur in other parts of the country have less potential [8]. Several researchers have reported the medicinal properties of plants based on the traditional knowledge system of this region [9-11].

Despite being age-old and time tested practices, issues are often raised about the efficacy, safety and quality of such medicines. There are concerns for the consumers as well as regulatory authorities across the countries. In spite of well-practised knowledge of herbal medicine and occurrence of a large number of medicinal plants, the share of India in the global market is not up to the mark [12].

Therefore, it is worthwhile to use modern science and technology tools for verifying therapeutic potential of medicinal plants as antioxidant as per international standards. Such information may be of potential value in the design of further studies to unravel novel treatment strategies for disorders associated with free radicals-induced tissue damage [13]. In view of the above, the current study was carried out to evaluate antioxidant activity of certain medicinal plants of Sonitpur district of Assam, India.

2. Materials and methods

Plant Material and Extraction Procedure: The plants were collected from around the Tezpur area (Sonitpur district) in Assam and were identified with the help of relevant literature. The voucher specimen of the collected plants were deposited at Defence Research Laboratory Tezpur. The Baseline information on the medicinal utility of these plants was also collected by carrying out literature survey (Table 1). Different plant parts were shade-dried at room temperature and ground in a mortar. 50g of the powdered plant material was extracted with 500 ml of methanol for 3 consecutive days. The coloured solution obtained from each of the plant material was concentrated by Rotary evaporator [14]. The concentrate (extract) obtained was preserved at -20 0C for further experimentation.

Phytochemical analysis: All the plant extracts were qualitatively tested for the presence of chemical constituents (Phenols, Tannins, Alkaloids, Flavonoids and Saponins) Methodologies used for the phytochemical examination are given in Table 2.

Free radical scavenging activity: The antioxidant activities were determined using DPPH, (Sigma-Aldrich, Germany; M.W. 394.32) as a free radical. 1 mg/ml solution of plant extract in methanol (PEIM) was prepared. 6 x 10-5 mol/L DPPH in methanol (DPPHIM) was prepared. 0.1 ml of PEIM was added to 3.9 ml of DPPHIM. The decrease in absorbance at 515nm was recorded at 1 min interval upto 15 min or until the reaction reached a plateau. Initially, absorption of blank sample containing the same amount of methanol and DPPH solution was prepared and measured as a control [15]. Ascorbic acid (Merck; M.W. 176.13) was used as standard. The experiment was carried out in triplicate. Free radical

scavenging activity was calculated by the following formula [16] -

% Inhibition = [(AB – AA) / AB] X 100

Where, AB = Absorbance of blank DPPH solution,

AA = Absorbance of tested extract (t=15min)

3. Results

The baseline information (botanical name, family, hindi and local name, medicinal utility and part used) of the selected plants has been presented in Table 1. Overall, the plants under study are reported to have a wide range of medicinal utilities. Phytochemical constituents of the plants under study have been depicted in Table 3. In general, phenols and flavonoids were present in all the tested plant extracts. Tannins were also present in majority of the samples except *C. filiformis* and *H. cordata*. Similarly, alkaloid were also found in almost all the samples except *E. alba*. Saponins were absent in *E. alba*, *H. cordata* and *O. indicum*. Scavenging activity of different plant extracts on DPPH radical has been shown in Fig. 1. As evident from the Fig. 1, there was noticeable variability in the antioxidant activity of plant extracts. Maximum scavenging activity was registered for *O. indicum* bark. Out of twelve plants screened, *O. indicum*, *I. aquatica* and *M. oleifera* were found to be good radical scavengers with the percent inhibition of 79.29±0.805, 70.29±0.869 and 63.05 ± 2.135 respectively whereas the per cent inhibition for ascorbic acid was 96.540 ± 0.652 which is used as a standard. There was no considerable difference in antioxidant activity of A. aspera, *A. scholaris* and *C. filiformis*.

The presence of flavonoids, tannins, alkaloids and saponins in A. aspera has been reported [17]. Results pertaining to phytochemical constituents of E. alba are parallel with earlier report [18]. Similarly, Vasu et al. [19] while studying the biomolecule and phytochemical properties of some aquatic angiosperms have also reported phenol, alkaloid, tannin and saponin and flavonoids in *I. aquatica*, which corroborates to the current results. Krishnaiah et al. [20] reported presence of tannin, saponin, flavonoids and alkaloid in *M. oleifera* and *C. asiatica*. Tannins play important role in promoting wound healing [21, 22]. This could be the reason why the bark paste of *M. oleifera* is applied on wounded portion of the body to give relief from pain (Table 1). Stem bark of *O. indicum* contains alkaloid, tannin and flavonoids [23]. In the present investigation, DPPH free radical effect of extracts of *O. indicum, I. aquatica* and *M. oleifera* was quite noticeable. It is well documented that phenols [24] flavonoids [25] are strong antioxidants. In addition, the presence of flavonoids and tannins (Table 3) suggests the reason why the leaves of O. indicum are used for the treatment of diarrhoea (Table 1). Flavonoids can inhibit the development of fluids that result in diarrhoea by targeting the intestinal cystic fibrosis transmembrane conductance regulator Cl^- transport inhibiting cAMP-stimulated Cl^- secretion in the intestine [26]. Also, tannins are very much astringent in nature and have high potential treating intestinal disorders such as diarrhoea and dysentery [27]. Apart from being useful for the treatment of intestinal disorders, *O. indicum*, has been considered as a potential anticancer medicinal plant [28]. Recently, the extract of *O. indicum* showed the toxicity on tumor cell lines [29].

Table 1: Plant species and their medicinal utility

S. No.	Botanical name & Family	Local name	Hindi name	Part used	Medicinal utility	Mode of preparation	Ref.
1	*Achyranthes aspera Linn.* (Amaranthaceae)	Apong	Latjira	Whole plant	Dysentery, piles , skin eruption, ulcer	Crushed juice taken orally	34
2	*Alstonia scholaris* R.Br. (Apocynaceae)	Chatiyana	Chitaban	Bark,Leaves, Milky juice	Periodic fevers, dysentery, tonic, stimulalnt	Decoction of bark 60-100ml b.i.d., A decoction of the leaves is used inberiberi	35
3	*Cassytha filiformis* L. (Lauraceae)	Honbori aloti	Amarbeli	Whole plant	Tonic, Ulcer, Inflamed eyes	**Paste** – applied on any inflammation, wounds, skin ailments and pain. **Powder** –helps in purifying blood and improves digestion, relieving from pain and arthritis conditions	44
4	*Centella asiatica* L. (Umbellifereae)	Manimuni	Manuki	Whole plant, Leaves	Mental disorder, Tonic, Leprosy	Sedative, plant paste is applied on boils and tumors; leaf decoction is taken in cough.	35
5	*Eclipta alba* Hassk. (Asteraceae)	Keheraj	Bhangara	Leaves	Viral hepatitis, hair hygiene, memory disorder ,Asthma, Conjunctivitis	Fresh leaf juice, 6-12ml b.i.d., external application also.	35
6	*Hibiscus sabdariffa Linn.* (Malvaceae)	Tenga morapat	Lalambari	Flower, Leaves, Fruit	The fruit possesses anti ascorbic properties, leaves emollient. referegent, tonic. Hypertention,	James, Jellies, Bevarges. Juice.	36, 37
7	*Houttuynia cordata* Thunb. (Saururaceae)	Mesondary	-------	Leaves	Diarrhoea and Culinary to food items, Constipation, Indigetion	Boiled leaves juice	35
8	*Ipomoea aquatica* Forsk. (Convolulaceae)	Kalmaushak	Kalmisag	Young shoots Leaves	Skin diseases, Liver diseases	The plant used as vegetable	38
9	*Moringa oleifera* Forsk. (Moringaceae)	Sajina goch	Saijan	Root , Leaves Bark, Fruits	Painkiller, Dental cure	Bark paste is applied on wounded portion f the body to give relief from pain. Root bark dried and powdered to make a paste and applied at the injured teeth	35
10	*Oroxylum indicum* Vent. (Bignoniaceae)	Bhatgila	Soyanka	Root bark, Stem bark, Seed	Tonic , Dibates, Diarrhoea , Dysentery, Purgative	The powder and infusion of the bark are diaphoretic and useful is acute rheumatism	38, 39
11	*Paederia foetida* L.(Rubiaceae)	Bhedailota	Gandh bhaduli	Leaves , Twigs	Dysentry, Stomach problem	The leaf juice is useful in treatment; leaves are used in preparation of curry.	35
12	*Sida acuta* Burm. (Malvaceae)	Sonborial	bala	Root, Whole plant	Root astringent, Tonic and useful in various nervous and Urinary diseases	Prescribed in infusion and in conjuction with ginger in case of fever.	36

Baicalein, a flavonoid extracted from a methanolic extract of *O. indicum* inhibited proliferation of a cancer cell line in vitro via induction of apoptosis [30]. Kumar et al. [18] also observed significant free radical scavenging effect on DPPH by M. oleifera. The suppressive effect of *I. aquatica* [31] and O. indicum [32] extracts on free radicals has also been reported.

Table 2: Standard methods used for phytochemical analysis

S. No.	Chemical constituent	Test	Reference
1	Phenols	Ferric chloride test	[40]
2	Tannins	Lead acetate test	[41]
3	Alkaloids	Wagner's test	[42]
4	Flavonoids	Alkaline reagent test	[41]
5	Saponins	Foam test	[43]

Fig. 1: Scavenging activity of plant extracts on DPPH radical. Error bars are standard error of mean.

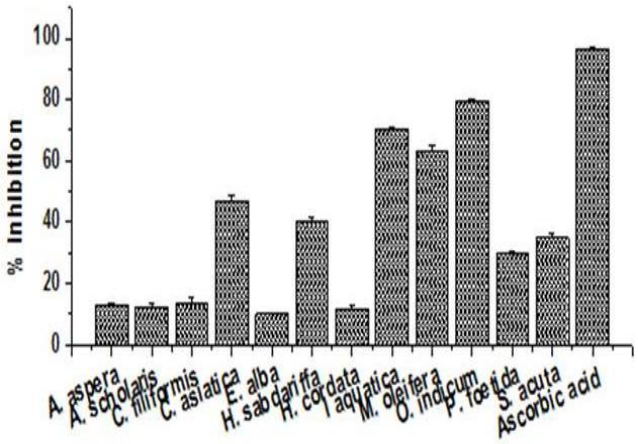

Table 3: Chemical groups identified in the plant extracts

S. No.	Plant extracts	Part used	Phenols	Tannins	Alkaloids	Flavonoids	Saponins
1	*Achyranthes aspera*	Seed	+	+	+	+	+
2	*Alstonia scholaris*	Bark	+	+	+	+	+
3	*Cassytha filiformis*	Whole plant	+	-	+	+	+
4	*Centella asiatica*	Leaves	+	+	+	+	+
5	*Eclipta alba*	Leaves	+	+	-	+	-
6	*Hibiscus sabdariffa*	Fruit	+	+	+	+	+
7	*Houttuynia cordata*	Whole plant	+	-	+	+	-
8	*Ipomoea aquatica*	Leaves	+	+	+	+	+
9	*Moringa oleifera*	Bark	+	+	+	+	+
10	*Oroxylum indicum*	Bark	+	+	+	+	-
11	*Paederia foetida*	Whole plant	+	+	+	+	+
12	*Sida acuta*	Leaves	+	+	+	+	+

+ Present; - Absents

Conclusion

In the present study, the antioxidant activity of twelve traditionally used medicinal plants grown around the Tezpur (Sonitpur) of the North Eastern region of India was evaluated. *Oroxylum indicum*, *Ipomoea aquatica* and *Moringa oleifera* exhibited strong antioxidant activity as compared to other plants. *Oroxylum indicum* showed the highest antioxidant activity. *Oroxylum indicum* is used in folk medicine as a cure of various diseases [33]. In the traditional Indian medicine, the root bark, stem and leaf are prescribed for snake bite, diarrhea and dysenteries [33]. Recently, inhibitory effect of *O. indicum* extract on proliferation of cancer cell lines has also been reported [30]. Considering the worthwhile significance of *O. indicum* as potential medicinal plant, bio-assay guided studies and structural elucidations of active isolates are under progress in our laboratory, which would further enhance the possible utility of the compounds in medical sciences.

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