

IN VITRO ANTIOXIDANT ACTIVITY OF BARK EXTRACTS OF *OROXYLUM INDICUM* (L) VENT**PRIYANKA SAHA, PRAKASH ROY CHOUDHURY, SUBRATA DAS, ANUPAM DAS TALUKDAR*,
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ABSTRACT**Objectives:** The aim of the present work is to screen the antioxidant potentiality of the bark extract of *Oroxylum indicum* (L) Vent, ethnomedicinally prescribed plant as hepatoprotective.**Methods:** Bark extracts were prepared using Soxhlet apparatus. Total phenol, flavonoid, and total alkaloid were analyzed using Folin-Ciocalteu assay, aluminum chloride colorimetric assay, atropine standard, respectively. Antioxidant capacity and free radical scavenging potentialities were done by several *in vitro* methods, viz.; 1,1-diphenyl-2-picrylhydrazyl (DPPH), hydrogen peroxide scavenging activity, reducing power assay, metal chelating activity, ferric reducing ability of plasma (FRAP) assay, and thiobarbituric acid (TBA) method.**Result:** Total phenol, flavonoid, and alkaloid were found to be highest in methanolic extract of *O. indicum* (L) Vent. Methanolic extract of the bark showed very low inhibitory concentration (IC₅₀) and effective concentration values for DPPH assay and reducing power assay compared to another extract. In hydrogen peroxide scavenging activity assay, the lowest IC₅₀ value was recorded in methanolic extract with 153.45±0.06 µg/ml. The FRAP assay for *O. indicum* methanolic extract showed the highest activity at 940.09±0.07 µg/mL of ascorbic acid equivalent. The ferric reducing capacity and TBA values of the plant extracts confirmed the presence antioxidant principles in the bark of the said plant.**Conclusion:** These underused plants may be used for mitigating the detrimental effect of oxidative stress and reactive oxygen species-mediated disease and thus justifies its use in folklore medicines.**Keywords:** Antioxidant, Free radical, Oxidative stress, Phenols, Flavonoids, Alkaloids, *Oroxylum indicum* (L) Vent.© 2017 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>) DOI: <http://dx.doi.org/10.22159/ajpcr.2017.v10i8.18977>**INTRODUCTION**

Antioxidants are composites that protect cells against free intermediated processes. Reactive oxygen intermediates or free radicals, products of oxidative stress, are responsible for tissue damage of living system, in aging and the pathogenesis of diseases such as arthritis, atherosclerosis, diabetes, and cancer [1-4]. Several studies explained plants as natural antioxidant sources due, mainly, to the presence of flavonoids, which act in decreasing free radical formation and also in foraging free radicals [5-7]. Phytochemicals, belonging to several chemical groups in various plants, are drawing the attention of scientist to find more effective antioxidants from plant species.

Oroxylum indicum (L) Vent. important medicinal plant is locally known as Shonapatha in Bengali language in Southern Assam of India. This plant mostly occurs in Japan, China, Bangladesh, and Malaysia. In India, it is found in Western, Southern, and North East region up to an altitude of 1200 m and found mainly in ravine and moist places in forests. The use of this plant for various ailments is part of traditional folklore of various communities in Manipur (India) such as Anal, Kuki, Mao, Maram, Tangkul, and Zeliangrong. The traditional knowledge of Maram Naga village of Senapati district, Manipur, reveals that the decoction of *O. indicum* bark can be used as a potent anticancer medicine, especially against nasopharyngeal cancer [8,9]. Stem bark is used to treat bile problems gastric and liver disorder including hepatic cancer [10]. The fruits of this plant are used among Mao tribe as an expectorant which improves the appetite [11-14]. Leaves of this plant are prescribed in snake bite and liver disorders such as jaundice [13,14]. Many phytochemical such as biochanin A possesses antifungal action and tumor necrosis factorα [12]. Ellagic acid is another important polyphenolic compound found in the leaves of this plant [15]. *O. indicum*

leaves also reported to have a beneficial effect on enlarged spleen, headaches, gastric, and hepatic ulcers [12]. A few apparent preliminary antioxidant studies of *O. indicum* have been undertaken [15,16], but a detailed report on antioxidant efficacy of the plant is still to be evaluated. The present work was conducted to understand and focus on evaluating the comparative antioxidant property of bark extracts.

METHODS**Collection of plant material**

The bark of plant was collected from Southern Assam of Cachar District (Northeast India). It was identified and submitted in Assam University Herbaria with voucher specimen no. 2578. After proper washing, the dried parts of the plant were pulverized separately into fine powder and were used for preparation of extracts.

Chemicals and equipment

All chemicals used were of analytical reagent grade. Ascorbic acid, atropine, gallic acid, t-butylhydroxyanisole, aluminum chloride, Folin-Ciocalteu reagent, ammonium thiocyanate, disodium phosphate, monosodium dihydrogen phosphate, trichloroacetic acid, hydrogen peroxide, iron (III) chloride, and iron(II) chloride were purchased from Merck Chemicals (Mumbai); and 2,2-diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid, glacial acetic acid and tripyridyl-striazine (TPTZ) were purchased from Himedia Laboratories (Mumbai). Absorbances were taken using ultraviolet visible spectrophotometer (Labomed, Inc., USA). Other chemicals and solvents were purchased from Merck Chemicals, Mumbai, India.

Preparation of extracts

About 50 g of powdered bark samples of both the plants were used for extraction by Soxhlet system using different solvents, viz.; hexane, ethyl

acetate, acetone, and methanol. The filtrates were concentrated under reduced pressure by a rotatory evaporator. The resulting residue was then filtered and stored at 4°C for further antioxidant assays.

Quantitative phytochemical analysis

Quantitative phytochemical assays were performed with focus on testing different chemical groups present in different solvent extracts of *O. indicum*.

Determination of phenols

Total phenolic constituent of the extracts was determined with standard protocol [17]. 1 ml of 2000 µg extract solution was added to a volumetric flask. 45 ml distilled water and 1 ml Folin-Ciocalteu reagents were then added and shaken vigorously. After 3 minutes, 3 ml of Na₂CO₃ (2%) solution was added, and the mixture was allowed to stand for 2 hrs by intermittent shaking. Absorbance was measured at 760 nm. Results were expressed in microgram gallic acid equivalent (GAE)/mg of plant extracts.

Determination of flavonoids

Total flavonoid content (TFC) was determined using the Dowd method [18]. 1 ml of 2% aluminium trichloride (AlCl₃) was mixed in methanol with the same volume of the various extracts (2000 µg). The absorbance was read at 415 nm after 10 minutes against blank consisting of 1 mL extract solution with 1 ml methanol without AlCl₃, and the flavonoid content was expressed as microgram quercetin equivalent/mg of plant extract.

Determination of alkaloids

A part of dried solvent extract was dissolved in 2N HCL and then filtered. 1 ml of filtrate was washed with 10 ml chloroform in a separatory funnel. The pH of this solution was then adjusted to neutral with 0.1 N NaOH. 5 ml of BCG solution, and 5 ml of phosphate buffer were added. The mixture was extracted with chloroform by shaking; the extracted mixture was then diluted with chloroform [19]. The absorbance was measured at spectrum of 470 nm, and the result was expressed as microgram atropine equivalent/mg of plant extract.

In vitro antioxidant assays

Determination of DPPH free-radical scavenging activity

The free radical scavenging activity of the plant extracts was measured using DPPH by the method of Kumarasamy *et al.* [20] 80 µg/ml DPPH was prepared with methanol. Serial dilutions were carried out with the 1 mg/ml stock solutions of the extracts. 2 ml of each solution was then mixed with 2 ml of DPPH and allowed to stand for 30 minutes; the absorbance was then read at 517 nm. Ascorbic acid was used as standard. IC₅₀ value was also calculated using a concentration-response curve. Inhibition of DPPH free radical in percentage was calculated by formula:

$$\text{DPPH radical scavenging activity (\%)} = (A \text{ control} - A \text{ test}) / A \text{ control} \times 100$$

Reducing power assay

The reducing power assay was conducted according to the method of Oyaizu [21]. To 2.5 mL (1 mg/mL) of a plant extract, 2.5 mL of 0.2 mol/L sodium phosphate buffer and 2.5 mL of 1% potassium ferricyanide were mixed. After incubating the mixture at 50°C for 20 minutes, 2.5 mL trichloroacetic acid solution was added, and the mixture was centrifuged at 650 rpm and 25°C for 10 minutes. The supernatant (5 ml) was mixed with 5 ml distilled water and 1 mL ferric chloride solution. The absorbance was measured at 700 nm. Ascorbic acid was used as standard.

Hydrogen peroxide scavenging assay

Hydrogen peroxide scavenging potential of the plant extract was determined using the method described by Jayaprakasha *et al.* [22]. A solution of hydrogen peroxide (20 mM) was prepared in (phosphate buffer saline [PBS], pH 7.4). Different concentrations of the extract (20-100 µg/ml) in ethanol (1 ml) were added to 2 ml of hydrogen

peroxide solution in PBS. After 10 minutes the absorbance was measured at 230 nm against blank solution that contained hydrogen peroxide solution without the extract. The percentage of H₂O₂ scavenging of the plant extract was calculated as follows:

$$\% \text{ Scavenged [H}_2\text{O}_2] = [(Abs \text{ control} - Abs \text{ sample}) / Abs \text{ control}] \times 100$$

Ferric reducing antioxidant power (FRAP) assay

The antioxidant activity analysis using FRAP was performed according to the method reported by Benzie and Strain [23]. The stock solutions included 300 mM acetate buffer pH 3.6, 10 mM TPTZ solution in 40 mM HCl, and 20 mM FeCl₃.6H₂O solution. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ, and 2.5 ml FeCl₃.6H₂O. The temperature of the solution was raised to 37°C before use. Various solvent extracts (200 µl) were allowed to react with 2800 µl of the FRAP solution for 30 minutes in the dark condition. Readings of the colored product (ferrous-tripyridyltriazine complex) were taken at 593 nm. The FRAP values of samples were expressed as µg/mL of ascorbic acid equivalent (AAE).

Measurement of ferrous ion chelating ability

The chelating ion by various plant extracts from the bark of *O. indicum* was measured by the method of Yan *et al.*, [24]. Various concentrations of the solvent extracts such as, 50, 100, 150, 200, 250, and 300 µg/mL of *O. indicum* were added with 1 mL of 2 mM FeCl₂ separately. The reaction was initiated by the addition of 5 mM ferrozine (1 mL). Absorbance was measured at 562 nm after 10 minutes ascorbic acid was used as standard.

$$\text{Chelating activity (\%)} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

Thiobarbituric acid (TBA) method

This assay was performed according to the method reported by Kikuzaki and Nakatani [25]. 2 ml of 20% trichloroacetic acid and 2 ml of 0.67% of TBA were added to 1 ml of the sample solution. The mixture was placed in a boiling water bath for 10 minutes and then centrifuged at 3000 rpm for 20 minutes. The absorbance of the supernatant was measured at 552 nm. The inhibition rate was calculated using the following equation:

$$[(Ac - As) / Ac] \times 100, \text{ where Ac is the absorbance of the control and As is the absorbance of the sample.}$$

RESULTS

Total phenolic (TPC), TFC, alkaloid content (TAC) of the extract

In this study, *O. indicum* was recorded to possess highest phenolic, alkaloid, and flavonoid content in the methanolic extract with 118.84±0.62 µg GAEs/mg of extract, 81.42±0.62 µg quercetin/mg of extract, 111.66±0.01 µg atropine/mg of extract, respectively. TPC was calculated using the standard curve of gallic acid (standard equation curve equation: Y=0.0033x+0.0378, R²=0.9761), TFC was calculated using the standard curve of quercetin (standard equation curve equation: Y=0.03576x + 0.211, R²=0.9922), and TAC was calculated using the standard curve of atropine (standard equation curve equation: Y=0.0003x+0.0052, R²=0.9862).

DPPH radical scavenging assay

The results of DPPH radical scavenging activity of *O. indicum* bark extract and the standard ascorbic acid (IC₅₀=12.25±0.01 µg/ml) are presented in Table 1. The 50% inhibitory activity has been widely studied as a parameter to measure antioxidant activity. In this study, both the plant extract and the standard significantly scavenged the DPPH radical with increasing concentrations. Fig. 1 showed the dose response curve of DPPH radical scavenging activity. IC₅₀ of the methanolic extract (9.42±0.04 µg/ml) was found to be lowest than all another sample extract. However, with the addition of a larger amount of bark extract of both plants to the DPPH assay mixture, the degree of inhibition decreased, indicating a prooxidant effect.

Table 1: TPC, TFC, and TAC estimation of various extracts of *O. indicum*

Sample extract	TPC ($\mu\text{g GAEs/mg of extract}$) total mg of extract)	TFC ($\mu\text{g Quercetin/mg of extract}$) total mg of extract)	TAC ($\mu\text{g atropine/mg of extract}$)
Hexane	16.72 \pm 0.03	0.098 \pm 0.07	34.33 \pm 0.08
Ethyl acetate	76.72 \pm 0.03	76.70 \pm 0.03	38.00 \pm 0.02
Acetone	02.18 \pm 0.01	54.00 \pm 0.01	93.32 \pm 0.06
Methanol	118.84 \pm 0.62	81.42 \pm 0.62	111.66 \pm 0.01

TPC: Total phenolic content, TFC: Total flavonoid content, TAC: Total alkaloid contents. Values are expressed as mean \pm SD, n=3 sets in each group. Mean values followed in a column are significantly different ($p < 0.05$), SD: Standard deviation, *O. indicum*: *Oroxylum indicum*

Reducing power assay and FRAP assay

As shown in Fig. 2, various extracts exhibited a dose-dependent increase in the ferric ion reducing potential. The lowest effective concentration (EC_{50}) was recorded in the methanolic extract with 292.31 \pm 0.06 $\mu\text{g/ml}$ which was much higher than the EC_{50} of the standard, ascorbic acid (910.37 $\mu\text{g/ml}$). A detailed EC_{50} of various extracts and FRAP value of both the plant is depicted in Fig. 2. The FRAP values (Fig. 1) ranged from 324.55 to 967.12 $\mu\text{g/mL AAE}$ for various fractions of the plant.

Hydrogen peroxide scavenging activity

As shown in Fig. 1, *O. indicum* also demonstrated hydrogen peroxide decomposition activity in a concentration dependent manner with lowest IC_{50} exhibited by methanolic extract (IC_{50} =153.45 $\mu\text{g/ml}$) which was much lower than standard ascorbic acid, IC_{50} =228.97 $\mu\text{g/ml}$.

Metal chelating activity and TBA assay

As observed in DPPH and hydroxyl radical scavenging assays, the percentage of metal chelating activity was determined to be sample concentration dependent and it was increasing with the increase in the concentration of extract from 50 to 300 $\mu\text{g/mL}$. The percentage of inhibition of the metal chelation was varying from 35.77% to 37.53% (in 50 $\mu\text{g/mL}$ of extract) to 80.19-87.41% (in 300 $\mu\text{g/mL}$ extract). The IC_{50} value of the methanolic bark extract was highest with 121.54 $\mu\text{g/ml}$. The percentage of inhibition of free radicals by various concentrations of methanol samples was more or less to that of the respective concentration of the standard drug, ascorbic acid.

Result of TBA assay depicted the radical scavenging activity of various extracts of the plant where the IC_{50} values of methanolic extract of *O. indicum* (18.9788 $\mu\text{g/ml}$) where significantly more than ascorbic acid (IC_{50} =35.77 $\mu\text{g/ml}$, taken as standard).

Inhibitory concentration (IC_{50})

IC_{50} values of various bark extracts of *O. indicum* and standard ascorbic acid, BHT, Quercetin for DPPH, H_2O_2 radical scavenging activity, ferrous chelating activity, TBA are depicted in Fig. 1.

DISCUSSION

Highest phenolic and flavonoid content were recorded in methanolic extract of *O. indicum*, while hexane extract showed least. To understand the noticeable free radical scavenging activity of assigned extracts, DPPH assay was performed. As the maximum radical scavenging activity was exhibited by the methanolic extract, thus it is implying a positive correlation of both the phenol and flavonoid content to the antioxidant activity of a plant [26-29]. This activity was cross-validated by performing reducing power assay, H_2O_2 scavenging activity, FRAP assay, metal chelating activity, and TBA assay with all the extracts. For each individual assay, methanolic extract exhibited similar antioxidant efficacy. Furthermore, methanolic extract of *O. indicum* exhibited the higher antioxidant activity in comparison to the positive control. This finding invariably authenticates the antioxidant potential of *O. indicum* in general and methanolic extract in particular. Compounds such as biochanin A, bhrysin, baicalein, oroxylin A, dihydrobaicalein, β -sitosterol, iso-flavone, and prunetin present in the bark may have contributed to this activity [29].

Highest alkaloid content was noted in methanolic extract than other extracts used. Interestingly above extracts also showed highest

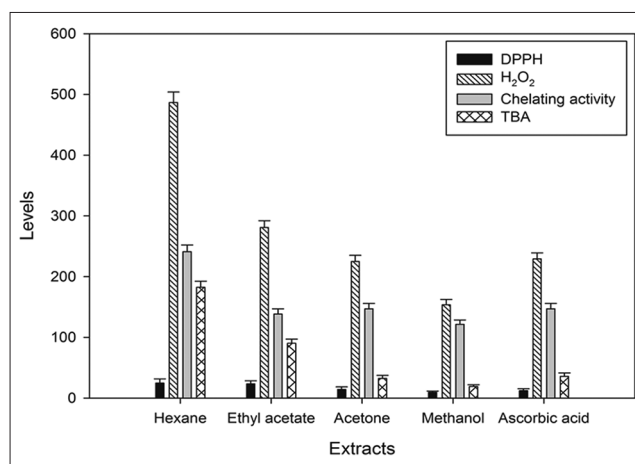


Fig. 1: DPPH radical scavenging activity, H_2O_2 , chelating activity and TBA activity of the various bark extracts of *O. indicum* in comparison with ascorbic acid

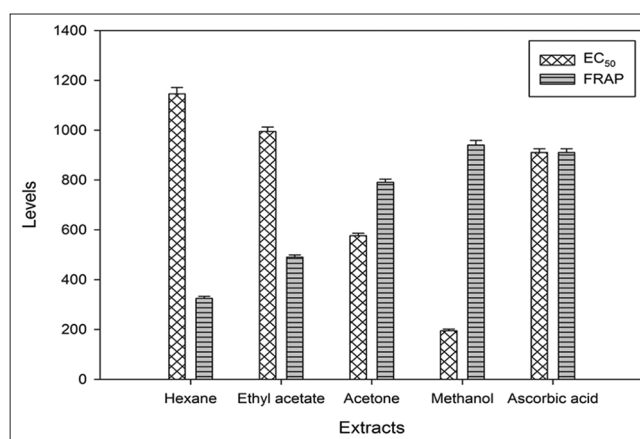


Fig. 2: Comparison of effective concentration and FRAP values of various fractions of bark parts of *O. indicum*

antioxidant activity than others. Although the general concept is that among all chemical groups, flavonoids and phenols are the major groups responsible for antioxidant-oxidant activity; our study proposes a direct correlation between alkaloid content and antioxidant activity of plants. While working with Turkish Tea polyphenols and alkaloid Erol *et al.* [30] proposed that alkaloid content is linked with antioxidant activity. Rackova *et al.* [31-33] suggested that probably high lipophilicity of the alkaloids is responsible for antiradical reactivity.

CONCLUSION

The study suggests that methanolic extract from *O. indicum* bark are a rich source of antioxidants. Experimental evidence pointing *O. indicum* a promising sources of active principles. Further detailed *in-vivo* experiments can establish its efficacy in this regard.

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