SCREENING OF IN *VITRO* ANTI OXIDANT ACTIVITY OF METHANOL EXTRACT OF STEM BARK OF *OROXYLUM INDICUM* (L.) KURZ

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ABSTRACTS
This plant has history of many health benefits local uses in Nagaland. Therefore, the main objectives of the present work are to explore the invitro antioxidant potentiality of methanol stem bark extract from the plant Oroxylum indicum indigenous to Nagaland. The stem bark was collected and air dried at room temperature and the extraction was done by successively Soxhlet apparatus. Total phenolic, total flavonoid were analyzed. Antioxidant capacity and free radical scavenging potentialities of the plant extract were done by performing several in vitro methods, viz; 1,1-diphnyl-2-picrylhydazyl (DPPH), reducing power assay and hydrogen peroxide scavenging activity. The results of phenolic and flavonoids were found to be highest in methanolic extract of the bark of Oroxylum indicum. The study shows that the plant has potential source of antioxidant and could be used for mitigating the detrimental effect of oxidative stress and reactive oxygen species-mediated disease and thus justifies its used in traditional medicine.

KEYWORDS: Oroxylum indicum, extract, antioxidant, DPPH assay, Reducing power, Nitric oxide

INTRODUCTION

The knowledge of traditional healing system is as old as the human civilization [17]. WHO states that 80% of the world's population depends on plants for their medicinal needs [1][2][10]. Herbal plants play a vital role in healthcare programs specially in the developing countries, medicinal plants and plant extract represent the oldest and the most widespread form of medication [13][19]. They are the resource of the new drugs and many of the modern medicines are produced directly from the plant, therefore the study deals with the standardization of both qualitative and quantitative analysis of antioxidant activity [2][7]. Herbal plants have been used for many healing properties and they remain the primary sources of novel biological active components [14]. Medicinal plants and other natural products possessing pharmacological models have been considered as alternative therapy for the treatment of various disease [17]. These chemical compounds are classified into primary and secondary metabolites, and the secondary metabolites and other chemical constituents contribute to their medicinal value [8].

The used of medicinal plants all over the world predates the introduction of antibiotics and other modern drugs. Products of higher plants have been shown to be effective sources of chemotherapeutic effect, and other chronic disorders such as coronary heart disease, Alzheimer's disease without the underlying side effect [5][8][11]. Herbal plants have been the main productive source of leads for the development of drugs. According to WHO (World Health Organization), world's population relies on traditional medicine for their health needs due to better cultural acceptability, fewer sides effects and better compatibility with the human body [7].

An antioxidant helps in inhibiting the oxidation of any other molecules. As antioxidant have been reported to prevent oxidative damage caused by free radicals, it can interfere with the oxidation process by reacting with the free radicals, chelating catalytic metals and by acting as oxygen scavenger [8][11]. Antioxidants from plant materials terminate the action of the radicals thereby protecting the body from various disease [15]. Free radical is toxic by-product of aerobic respiration, free radicals tends to cause all types of cell damages and antioxidants neutralize free radicals that are defined as atoms or groups of

atoms having unpaired electrons [3][9]. They are significant regarding the fact that they reduce oxidative stress which could affect and damage biological molecules [4][11].

Plant description: *Oroxylum indicum*
Size: medium-sized, soft-wooded tree
Height: attaining height of 10–16 m
Color: dull brown
Leaves: leaves are broad, leaflets are ovate, wavy, and acuminate. Leaf fall occurs during winter season (January) each year.
Flower: flowering season from June to July, flowers is generally situated at the apics of branches and its length is about 30 cm or more.
Fruits: fruits are follicle, 30-90 cm long and 5-10 cm broad
Seeds: seeds are round with papery wings

MATERIALS AND METHODS:

Collection of a plant:

The stem bark of *Oroxylum indicum* was collected during the month of June and July 2019 from the forest of Jaboka village, Nagaland, India. The dried stem bark was used for the study. The stem was collected accordingly and were shade dried. The Herbarium sheet of the plant was prepared, and the plant was identified as *Oroxylum indicum* (L.) Kurz (Bignoniaceae) by Dr. Chaya Deori office of the Scientist-E, Botanical Survey of India, Eastern Regional centre, Shillong. The identification number was No: BSI/ERC/Tech/2019-2020/721.

Figure:3 *Oroxylum indicum* plant

Figure:2 Dried Bark of the plant

Figure:3 Coarse powder of the plant

Chemical and requirements:

Petroleum ether, Chloroform, Methanol, Folin-Ciocalteu's, sodium carbonate, gallic acid, aluminum chloride ($Alcl_3$), Sodium nitrite, sodium chloride, quercetin, DPPH, Griess reagent.

Preparation of extract:

The collected fresh stem bark of the plant were cleaned, by handpicking the foreign material, washed and dried under shade for three weeks. The air-dried stem bark was crushed and the powdered stem bark (3500gm) were extracted at room temperature successively by Soxhlet apparatus using petroleum ether followed by chloroform and methanol as solvent respectively for 50 hours or till the solvent in the siphon tube become colorless. The solvents were recovered by distillation under reduced pressure using rotary vacuum evaporator to obtain the extracts. The extracts were stored in refrigerator and the methanol extract was used for evaluation of different biological activities.

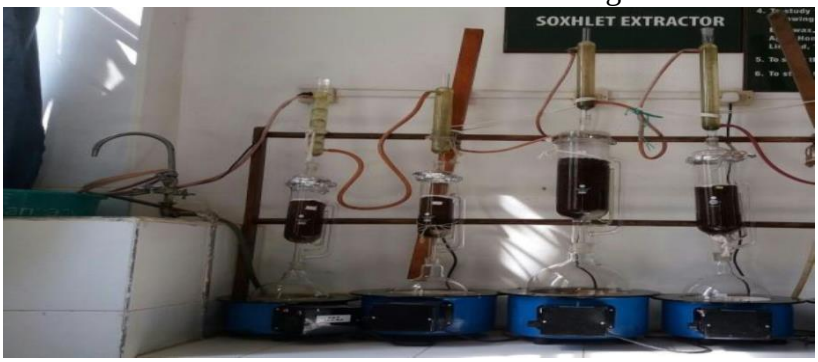

Fig2: Extraction of plants by using Soxhlet's apparatus

EVALUATION OF INVITRO ANTIOXIDANT ACTIVITY

Total phenolic content:

Total phenolic content of the extract was performed spectrophotometrically according to the Folin-Ciocalteu method. 1ml (5oµg/ml) of the extract treated with 5ml of Folin's reagent (10-fold dilution) after 4 minutes add 4ml of (20% sodium carbonate) keep in dark room at room temperature for 1 hour and absorbance were observed and measured at 750nm using Uv- visible spectrophotometer. Gallic acid was used as a standard. The total phenolic content was qualified by a calibration curved obtained from measuring the absorbance of the known concentration of Gallic acid [6][19].

Total flavonoid content:

The total flavonoid content was determined by using Aluminum chloride ($AlCl_3$) colorimetry assay method. 1ml of the methanolic stem bark extract was taken and add 2ml of methanol, 3ml of 5% sodium nitrite, 0.3ml of 10% aluminum chloride and kept for 6minutes and 2ml sodium hydroxide (1M) was added, and make up the volume up to 10ml with methanol and kept for 1 hour at room temperature and the absorbance was measured at 510nm using spectrophotometry, the total flavonoid contend was express in terms of milligram quercetin equivalent per gram of stem bark extract of *Oroxylum indicum* [9][19].

Determination of 2,2 diphenyl-1-picrylhydrazyl (DPPH) Radical scavenging activity:

DPPH radical scavenging activity was performed by taking 1ml of different concentration of stem bark extract and mixed with freshly prepared 0.1mM DPPH. The reaction mixture was shake and incubate in the dark room for 50 minutes at room temperature. The absorbance was measured at 517nm using spectrophotometry. The radical scavenging activity of DPPH was calculated as follows [4][6][7][8].

$$\text{Scavenging effect\%} = (Abs_{control} - Abs_{sample})/Abs_{control} \times 100$$

Determination of reducing power:

The methanolic extract of stem bark was diluted at different concentration (20,40,60,80,100µg/ml) and 1ml of each dilution was mixed with 2.5ml phosphate buffer (ph-6.6) and 2.5ml of 1% potassium ferricyanide and the mixture was incubated at 50ºc for 30 minutes. After cooling 2.5ml of 10% TCA was added and centrifuge for 10 minutes at 3000rpm. 2.5ml of supernatant was dilute with 2.5ml of distilled water and add 0.5ml of freshly prepared 0.1%ferric chloride solution and the absorbance of the mixture was measured at 700nm. Ascorbic acid was used as a standard and the higher the absorbance indicates the increased in reducing power [8][9].

Determination of nitric oxide scavenging activity:

The methanolic extract stock solution was prepared (100µg/ml) and the concentration was serial diluted with methanol to get concentration 20-100 (g/ml) and was stored at 4ºc for later use. Griess reagent was prepared by mixing an equal amount of 1% sulfanilamide in 2.2% phosphoric acid, 0.1 naphthyl ethylenediamine dihydrochloride in 2.5 phosphoric acid immediately before use. 0.5ml of 10mM sodium nitroprusside in phosphate buffer saline and to this 1ml of different concentration of methanolic was added and incubated at 25ºc for 180 minutes. After incubation the extract was mixed with equal volume of freshly prepared Griess reagent, control was prepared by mixing all the reagents except the extract. The absorbance was observed and measured at 546nm using Uv-visible spectrophotometry [7].

RESULTS:

Determination of total phenolic content:

The total phenolic content of the methanolic extract of stem bark of *oroxylum indicum* was expressed as mg of gallic acid equivalent /gm and was found to be 203.52 mg GAE/g as showed in Fig.4.

Determination of Total flavonoid content:

The total flavonoid content of the extract of *Oroxylum indicum* stem bark was determined by aluminium chloride method. The total flavonoid was expressed as mg of quercetin (y=0.0015x +0.036,

R^2 =0.9889) respectively. The flavonoid content in the extract of Oroxylum indicum was found to be 14.66 mg QE/g of the extract as showed in Fig.5.

Determination of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity:

The IC_{50} of the *Oroxylum indicum* stem bark was found to be 37.39 µg /ml as compared to standard (Ascorbic acid) which exhibited 45.055µg /ml. The result indicates that *Oroxylum indicum* exhibit antiradical activity, however the standard Ascorbic acid showed significantly higher DPPH activity than the sample *Oroxylum indicum* as showed in (Table.1, fig.6).

Reducing power assay:

The reducing power of the standard ascorbic acid was significantly higher compared to the sample *Oroxylum indicum*. However, the antioxidant present in the sample cause the reduction of Fe^{3+} to Fe^{2+} and thus proving its reducing power as showed in (Table.2, Fig.7)

Nitric oxide scavenging assay:

The methanol extract of *Oroxylum indicum* stem bark shows Nitric oxide scavenging activity with increasing concentration of the extract. The mean IC_{50} values for and standard extract were found to be 24.74 (µg/ml) and 37.87 (µg/ml) respectively. The readings were indicated in (Table.3, Fig.8) below.

DISCUSSION:

The plants studied was chosen due to tradition-based medicine which has many local uses and also due to growing trend in the evidence-based validation of traditional herbs. In the present study we investigate the antioxidant activity of the stem bark extract of *oroxylum indicum.*

The total phenolic component exhibit antioxidant activity through adsorption and neutralization of the free radicals as showed in (Fig.4). whereas flavonoid and flavanol show antioxidant activity through scavenging or chelating process [19]. The high content of the flavonoid and phenolic compounds in *Oroxylum indicum* stem bark can explain their high radical scavenging activity as showed in (Fig.5).

DPPH is a free radical which is stable at room temperature and this method is often employed to determine the antioxidant activity of many plant extracts. The concentration in g/ml of the sample to scavenge 50% of the DPPH radical is called IC_{50} and lower IC_{50} values indicate higher antiradical activity. IC_{50} was calculated from the graph by plotting the % scavenging against concentration [16]. In the table shows a steady increase in the percentage inhibition of DPPH radicals as concentration increases which is commonly observed with plant extracts. The percentage DPPH scavenging activities is a basis for evaluating the free radical scavenging potentials of the extracts. From the above result of DPPH, the IC_{50} showed the percentage inhibition was decreasing with the decreased in concentration of test as well as standard sample [4][5] (Table.1, Fig.6). The reducing power assay is often used to evaluate the ability of an antioxidant to donate an electron. In this assay, the presence of antioxidant in the sample resulted into reduction of the ferric cyanide complex (Fe^{3+}) to the ferrous cyanide form (Fe^{2+}) [8][9]. Higher the absorbance of the reaction mixture, higher would be the reducing power, suggesting that the sample was able to terminate the radical chain reaction as showed in (Table.2, Fig.7). Nitric oxide scavenging ability of the stem bark extract of oroxylum indicum shows significant elevation in the Nitric oxide scavenging activity at different concentration dependent manner. Fig, table3 [7] (Table.3, Fig.8).

Table 1: Inhibition of methanolic extract of stem bark of *Oroxylum indicum* and ascorbic acid.

Concentration (µg) /ml)	% inhibition of extract	% inhibition of Ascorbic acid
20	55.28±0.053	46.26±0.087
40	71.62±0.107	82.22±0.305
60	78.26±0.235	90.40±0.159
80	83.33±0.161	95.032±0.0843
100	95.73±0.2351	96.299±0.024

Table 2: Rates of reducing power between the extract and standard

Concentration (µg/ml)	Absorbance	
	Extract (mean ± SEM)	Ascorbic acid (mean ± SEM)
20	0.2626±0.00088	0029±0.00115
40	0.237±0.00086	0.187±0.0033
60	0.3386±0.00084	0.258±0.0057
80	0.347±0.00033	0.431±0.0057
100	0.355±0.0017	0.597±0.00057

Table 3: Nitric oxide scavenging rate of extract and standard Ascorbic acid

Concentration (µg/ml)	% Inhibition Extract (mean ± SEM)	% Inhibition Ascorbic acid (mean ± SEM)
20	57.183±0.1479	40.9282±2.494
40	68.077±0.0977	60.2320±0.2110
60	78.718±0.074	75.105±0.2791
80	84.52±0.0488	78.27±0.1055
100	90.3189±0.1230	79.64±0.550

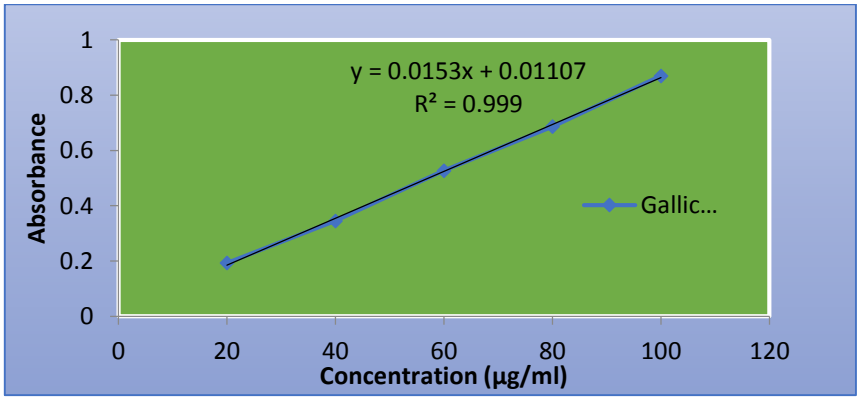

FIG:3 Standard calibration curved for total phenolic content

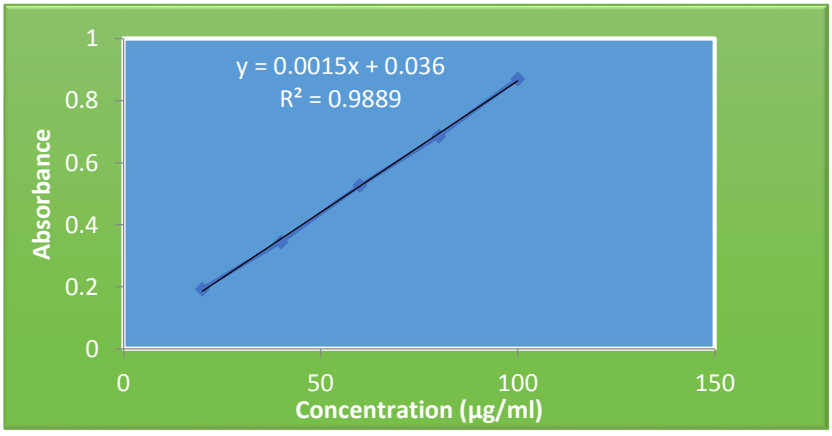

FIG:4 Standard calibration curve for total flavonoid content

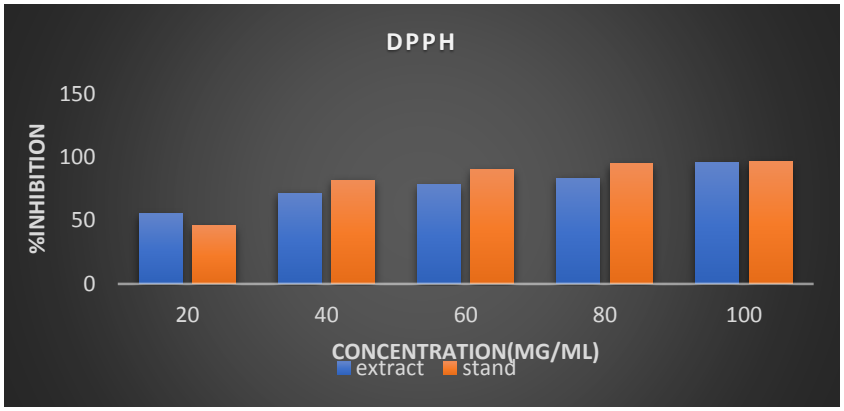

FIG:5 DPPH scavenging activity of stem bark methanolic extract of *Oroxylum indicum* and standard Ascorbic acid

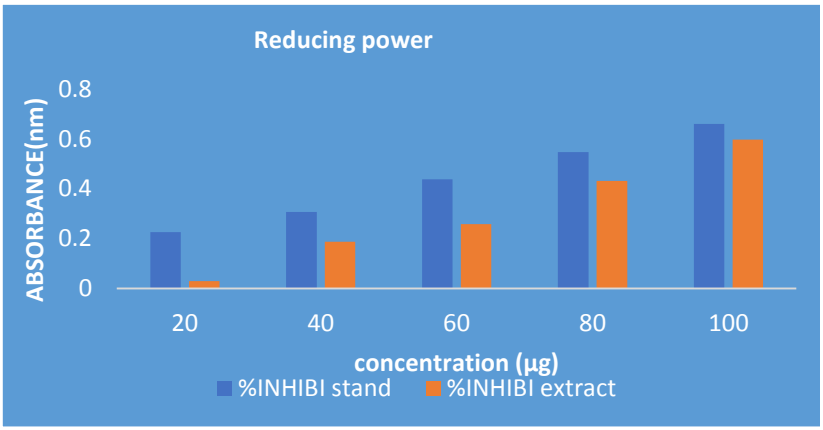

FIG:6 Reducing power assay of stem bark methanolic extract of Oroxylum indicum and standard Ascorbic acid

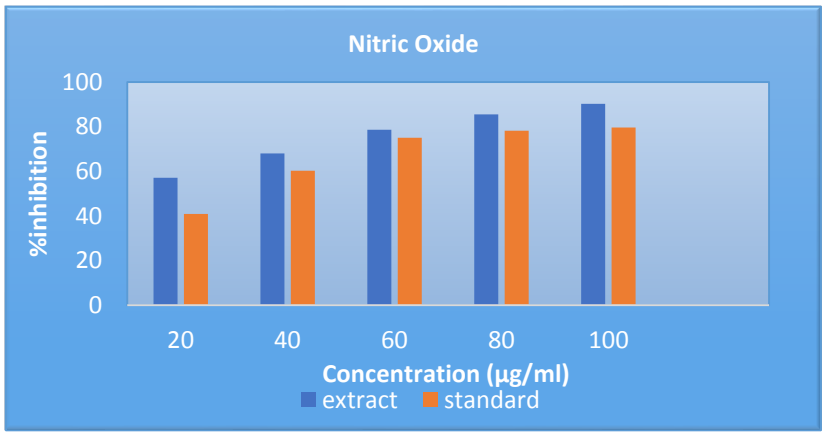

FIG:7 Nitric oxide scavenging rate of stem bark extract and standard Ascorbic acid

CONCLUSION:

In this present study the methanolic extract of the stem bark *of oroxylum indicum* shows potential antioxidant activity and free radical scavenging which leads to possible prevention of various possible oxidative stress related disease, therefore it is essential for identifying the phytocompounds to identify their pharmacological properties, further investigation is being under the progress for isolation and characterization of the novel phytoconstituents.

AKNOWLEDEMENT:

I would sincerely thankful, Head of department pharmacy Dr. H. Lalhlenmawia (Regional Institute of Paramedical and Nursing science Aizwal) for providing all the necessary facilities for the work.

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