

## Evaluation of free radical scavenging activity of *Neiebuhria apetala* DUNN leaf extracts

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**Abstract-** The present investigation is to assess the antioxidant properties of leaf extracts of *Neiebuhria apetala* using different in vitro methods. The antioxidant activity was studied by DPPH radical scavenging method, hydroxyl radical scavenging activity, superoxide radical scavenging activity, ABTS radical scavenging activity and reducing power methods. Ethanol extract of leaf showed very good antioxidant properties. The present study exposed that ethanol extract of *N. apetala* include effective potential source of natural antioxidant, which might be useful in preventing the progress of various oxidative stresses.

**Keywords-** Medicinal plants, ROS, Antioxidant activity, DPPH, Superoxide assay

### I. INTRODUCTION

Free radicals such as oxygen, superoxide and hydroxyl are biologically important substances which naturally release from human tissues. The highly reactive radicals can cause oxidative damage to DNA, lipids and proteins [1]. Therefore, free radicals result in many disorders like cancer, cardiovascular diseases and diabetes mellitus [2]. Many compounds carried out free radical scavenging are substances having antioxidant activity such as flavonoid and phenolic compounds or phenolic rich plant extracts. Ascorbic acid, the standard antioxidant in this study, acts as a chain breaking scavenging agent that impairs the formation of free radicals in the process of intracellular substances formation throughout the body, including collagen bone matrix and tooth [3]. Several methods have been developed to estimate the antioxidant capacity of different plant materials [4]. A single assay is not sufficient to evaluate the total antioxidant activity [5].

*Capparis* species is also known as Caper plants. These are familiar as a potential cause of valuable nutrients and biochemical compounds with physiological function. The multiple biological activities includes antibacterial, antifungal, hepatoprotective, anthelmintic, antidiabetic, anti-inflammatory, anti-cancer, and antihyperlipidemic. These activities as well as folk medicinal uses of Caper plants have been attributed to the presence of functional bioactives, such as phenolic acids, flavonoids, alkaloids, phytosterols, natural sugars, vitamins, and organic acids [6]. Hence the present

study, different solvent extracts of *N. apetala* leaf was investigated for their antioxidant activity using DPPH radical scavenging activity, hydroxyl radical scavenging activity, superoxide scavenging activity, ABTS radical cation scavenging activity and reducing power assay.

### II. RELATED WORK

Presently, more interest has been focused on the use of natural remedies to protect the human body especially brain tissues from the oxidative damage caused by free radicals. For the past two decades, several medicinal plants have shown such effectiveness through the traditional methods of ethnopharmacology. Keeping this in view, the present study has been conducted to evaluate the comparative antioxidant activity of folklore medicinal plant (*N. apetala*) which are traditionally used to treat various diseases.

### III. MATERIALS AND METHODS

#### Collection of plants

The fresh plant parts of *N. apetala* (leaf) were collected from Petchiparai, Western Ghats, Kanyakumari District, Tamil Nadu, India. The gathered samples were cut into small pieces and shade dried until the fracture is identical and even. The dried plant material was crushed or grinded by using a blender and separated to get uniform particles by using sieve No. 60. The final uniform powder was used for the extraction of active constituents of the plant material.

### Preparation of extract

100 g of the coarse powder of *N. petala* leaf was extracted successively with 250 ml of alcoholic and organic solvents (Peroleum ether, Benzene, Ethyl acetate, Methanol and Ethanol) in a Soxhlet apparatus for 24 hrs. All the extracts were filtered through Whatman No. 41 filter paper separately and all the extracts were concentrated in a rotary evaporator. All the concentrated extracts were subjected for *in vitro* antioxidant activity.

### Antioxidant activity

#### DPPH radical scavenging activity

The DPPH is a constant free radical and is extensively used to measure the radical scavenging activity of antioxidant component. This process is based on the reduction of DPPH in methanol solution in the company of a hydrogen donating antioxidant due to the arrangement of the non-radical form DPPH-H [7]. Using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) the free radical scavenging action of all the extracts was assessed as per the previously reported process [7]. DPPH of 0.1 mM solution in methanol was prepared. 1 ml of this solution was poured into 3 ml of the solution at different concentrations (50, 100, 200, 400 and 800 µg/ml). The mixtures were shaken dynamically and allowed to stand at room temperature for 30 minutes. After that the absorbance was measured at 517 nm using a UV-VIS spectrophotometer (Genesys 10s UV: Thermo electron corporation). Ascorbic acid was employed as the reference. The lesser absorbance values of reaction mixture identify higher free radical scavenging action. Using the subsequent formula the ability to scavenge the DPPH radical was computed.

$$\text{DPPH scavenging effect (\% inhibition)} = (A_0 - A_1) / A_0 \times 100$$

Where, A<sub>0</sub> is the absorbance of the control and A<sub>1</sub> is the absorbance of the test samples and reference. All the tests were carried out in triplicates and the outcomes were averaged.

#### Hydroxyl radical scavenging activity

According to the modified method of Halliwell *et al.* [8] the scavenging ability for hydroxyl radical was projected. Stock answers of FeCl<sub>3</sub> (10 mM), Ascorbic Acid (1 mM), EDTA (1 mM), H<sub>2</sub>O<sub>2</sub> (10 mM) and Deoxyribose (10 mM) were put in distilled deionized water. The assay was executed by adding 0.1 ml EDTA, 0.1 ml H<sub>2</sub>O<sub>2</sub>, 0.01 ml of FeCl<sub>3</sub>, 0.36 ml of deoxyribose, 1.0 ml of the extract of diverse concentration (50, 100, 200, 400 & 800 µg/ml) dissolved in distilled water, 0.33 ml of phosphate buffer (50 mM, pH 7.9), 0.1 ml of ascorbic acid in sequence. The mixture was then incubated at 37°C for 1 hour. 1.0 ml of the incubated mixture was mixed with 1.0 ml of 10% TCA and 1.0 ml of 0.5% TBA (in 0.025 M NaOH containing 0.025% BHA) to develop the pink chromogen measured at 532 nm. The hydroxyl radical scavenging achievement of the extract is accounted as % inhibition of deoxyribose. The degradation is figured by using the succeeding equation

Hydroxyl radical scavenging activity = (A<sub>0</sub> - A<sub>1</sub>) / A<sub>0</sub> X 100 where, A<sub>0</sub> is the absorbance of the control and A<sub>1</sub> is the absorbance of the test samples and reference. All the tests were carried out in triplicates and the results were averaged.

#### Superoxide radical scavenging activity

The superoxide anion scavenging action was calculated as elucidated by Srinivasan *et al.* [9]. The superoxide anion radicals were made in 3.0 ml of Tris - HCl buffer (16 mM, pH 8.0) containing 0.5 ml of NBT (0.3 mM), 0.5 ml NADH (0.936 mM) solution, 1.0 ml extract of different concentrations (50, 100, 200, 400 & 800 µg/ml) and 0.5 ml Tris - HCl buffer (16 mM, pH 8.0). The reaction was started by adding 0.5 ml PMS solution (0.12 mM) to the mixture, incubated at 25°C for 5 min and the absorbance was estimated at 560nm against a blank sample, ascorbic acid. The percentage inhibition was determined by using the following equation

$$\text{Superoxide radical scavenging activity} = (A_0 - A_1) / A_0 \times 100$$

where, A<sub>0</sub> is the absorbance of the control and A<sub>1</sub> is the absorbance of the test samples and reference. All the tests were achieved in triplicates and the results were averaged.

#### Antioxidant Activity by Radical Cation (ABTS+)

ABTS assay was supported on the slightly modified technique of Huang *et al.* [10]. By reacting 7 mM ABTS solution with 2.45 mM potassium persulphate, ABTS radical cation (ABTS<sup>+</sup>) was prepared. This mixture is permitted to be in the dark at room temperature for 12-16 hrs previous to use. With ethanol to an absorbance of 0.70 + 0.02 at 734 nm the ABTS<sup>+</sup> solution was added. Following this trolox standard to 3.9 ml of diluted ABTS<sup>+</sup> solution or addition of 100 µL of sample, absorbance was calculated at 734 nm by Genesys 10S UV-VIS (Thermo scientific) accurately after 6 minutes. Results were expressed as trolox equivalent antioxidant capacity (TEAC).

ABTS radical cation activity = (A<sub>0</sub> - A<sub>1</sub>) / A<sub>0</sub> X 100 where, A<sub>0</sub> is the absorbance of the control and A<sub>1</sub> is the absorbance of the test samples and reference. All the tests were repeated thrice and the end results were averaged.

#### Reducing Power

The reducing power of the extract was established by the method of Kumar and Hemalatha [11]. 1.0 ml of solution containing 50, 100, 200, 400 & 800 µg/ml of extract was mixed up with sodium phosphate buffer (5.0 ml, 0.2 M, pH 6.6) and potassium ferricyanide (5.0 ml, 1.0%). The mixture was incubated at 50° C for 20 minutes. Then 5ml of 10% trichloroacetic acid was added and centrifuged at 980 g (10 minutes at 5°C) in a refrigerated centrifuge. The upper layer of the solution (5.0 ml) was diluted with 5.0 ml of distilled water and ferric chloride and absorbance read at 700 nm. The experiment was performed thrice and results were averaged.

#### IV. RESULTS AND DISCUSSION

##### DPPH radical scavenging activity

The antioxidant activity of crude leaf extracts of *N. apetala* were tested using the constant free radical, DPPH. Unlike DPPH radical scavenging is considered to be good *in vitro* model widely used to assess antioxidant efficacy within a very short time. In its radical form, DPPH disappears on reduction by an antioxidant compound or a radical species to become a stable diamagnetic molecule resulting in the color change from purple to yellow, due to the formation of diphenyl picryl hydrazine, which could be taken as an indication of the hydrogen donating ability of the tested samples [12]. DPPH radical scavenging activity of petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of leaf of *N. apetala* are shown in figure 1. The scavenging effect of ascorbic acid, the standard and various solvent extracts studied increases with the increase in the concentration. Among the solvent tested, ethanol extract leaf of *N. apetala* exhibited the highest DPPH radical scavenging activity. At 800µg/ml concentration, ethanol extract of leaf of *N. apetala* possessed 128.22 % . The concentration of *N. apetala* leaf ethanol extract needed for 50 % inhibition (IC<sub>50</sub>) was 32.43 mg/ml, while ascorbic acid needed 29.16 mg/ml (Table 1).

##### Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity of petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of leaf of *N. apetala* are shown in figure 2. The methanol extract of *N. apetala* leaf showed a very potent hydroxyl radical scavenging activity. At 800µg/ml concentration, *N. apetala* leaf possessed 138.16 % hydroxyl radical scavenging activity. The concentration of *N. apetala* leaf methanol extract needed for 50 % inhibition (IC<sub>50</sub>) was found to be 37.16 mg/ml, whereas 28.17 mg/ml (Table 1) needed for ascorbic acid. The hydroxyl radical scavenging ability of the extracts was determined by its ability to compete with deoxyribose for hydroxyl radical. The crude extracts (methanol) of *N. apetala* extract compete with deoxyribose and diminish chromogen formation in a dose dependant manner. In this assay, 2-deoxy-2-ribose was oxidized when exposed to hydroxyl radicals generated by Fenton-type reaction. Scavenging of OH<sup>+</sup> is an important antioxidant activity, because of its very high reactivity, which can easily cross the cell membranes at specific sites and reacts with most of the biomolecules and furthermore cause tissue damage and cell death. Thus, removing OH<sup>+</sup> is very important task for the protection of living systems [13].

##### Superoxide anion radical scavenging activity

All the extracts of *N. apetala* leaf were subjected to be superoxide radical scavenging activity and the results are shown in figure 3. The ethanol extract of leaf of *N. apetala* exhibited the maximum superoxide radical scavenging activity 138.22% at 800µg/ml concentration. This scavenging

activity was higher than that of ascorbic acid, the standard which had 119.61% scavenging activity. The IC<sub>50</sub> value of ethanol extract of *N. apetala* leaf on superoxide radical was found to be 36.93 mg/ml and 30.16 mg/ml for ascorbic acid, respectively (Table 1). Superoxide anions are the most common free radicals in *in vivo* and are generated in a variety of biological systems and the concentration of superoxide anions increases under conditions of oxidative stress. Superoxide anion is a reduced form of molecular oxygen created by receiving one electron. Superoxide anion is an initial free radical from mitochondrial electron transport systems. Mitochondria generate energy using a 4-electron chain reaction, reducing oxygen to water. Some of the electrons escaping from the chain reaction of mitochondria directly react with oxygen and form superoxide anion. It plays an important role in the formation of other reactive oxygen species, such as hydrogen peroxide, hydroxyl radical or singlet oxygen in living systems [14].

##### ABTS radical cation scavenging activity

The different solvent extracts of *N. apetala* leaf were subjected to be ABTS radical cation scavenging activity and the results are shown in figure 4. The methanol extract of *N. apetala* leaf exhibited potent ABTS radical cation scavenging activity in concentration dependent manner. At 800µg/ml concentration, the methanol extract of *N. apetala* leaf possessed 128.16%. The quantity of *N. apetala* methanol extract required to produce 50% inhibition of ABTS radical 32.07 mg/ml whereas 30.22 mg/ml (Table 1) needed for trolox. The ABTS chemistry involves direct generation of ABTS radical mono cation with no involvement of any intermediary radical. It is a decolorization assay, thus the radical cation is performed prior to addition of antioxidant test system, rather than the generation of the radical taking place continually in the presence of antioxidant. The results obtained imply the activity of the extract either by inhibiting or scavenging the ABTS radicals since both inhibition and scavenging properties of antioxidants towards ABTS radicals have been reported earlier [15].

##### Reducing Power

Table 5 showed the reducing abilities of different solvent extracts of *N. apetala* leaf were compared to the standard ascorbic acid. Absorbances of the extracts were increased with the concentration. A higher absorbance indicated a higher reducing power. Among the solvent tested, the methanol extracts of leaf of *N. apetala* exhibited higher reducing activity. This result indicates that the extracts may consist of polyphenolic compounds that usually show great reducing power. This has been justified by methanol extract being the most reducing agent with highest phenolic content. Reducing power assay measures the electron-donating capacity of an antioxidant [16]. In this assay, the yellow color of the test solution changes to various shades of green and blue, depending on the reducing power of each compound. Presence of reducers causes the conversion of the

Fe<sup>3+</sup>/ferricyanide complex to the ferrous form used in this method may serve as a significant indicator of its antioxidant capacity [17,18].

## V. CONCLUSION

The results of the present study revealed that *N. apetala* leaf extracts possessed potent free radical scavenging ability in methanol and ethanol extracts. The activity observed may be attributed to the presence of phenolic and flavanoid contents in the methanol and ethanol extracts and further we conclude that this plant is a potential candidate for natural antioxidant.

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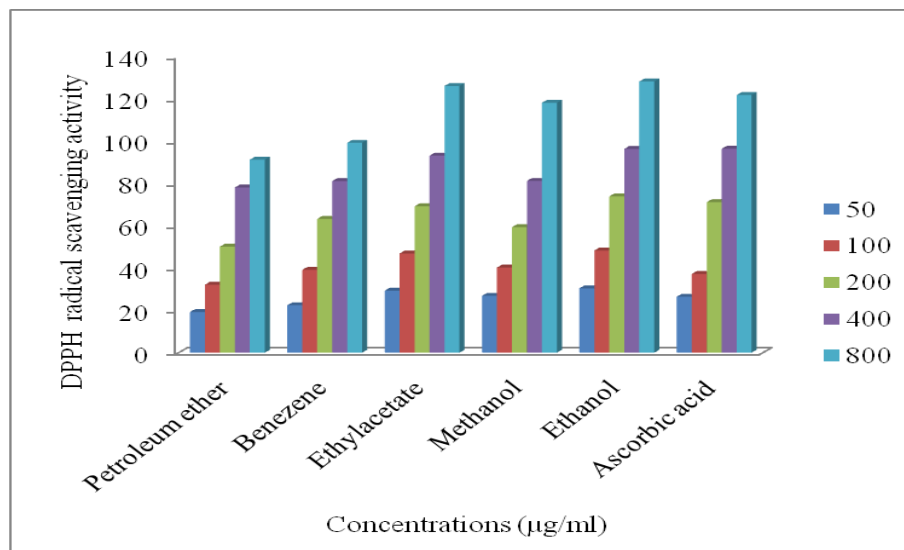


Figure 1: Effect of different solvent extract of *N. apetals* leaf on DPPH assay

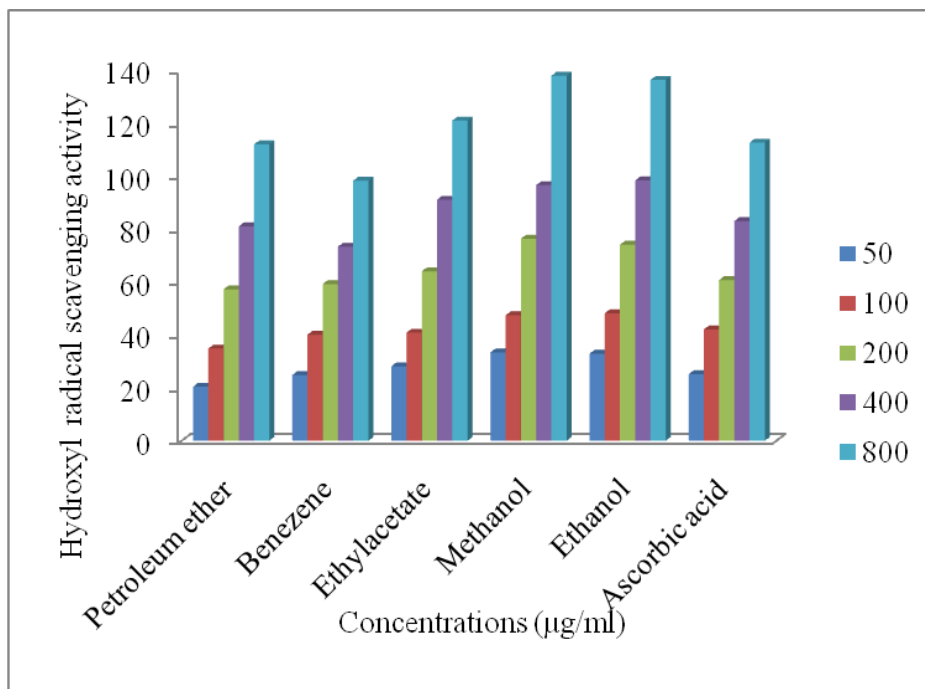


Figure 2: Effect of different solvent extract of *N. apetals* leaf on Hydroxyl assay

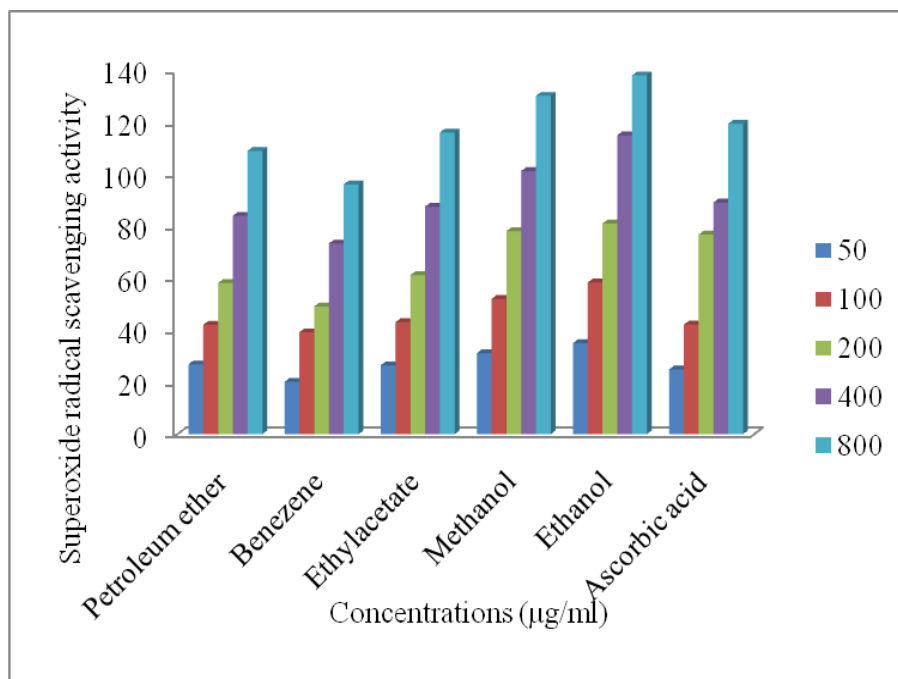


Figure 3: Effect of different solvent extract of *N. apetals* leaf on Superoxide anion assay

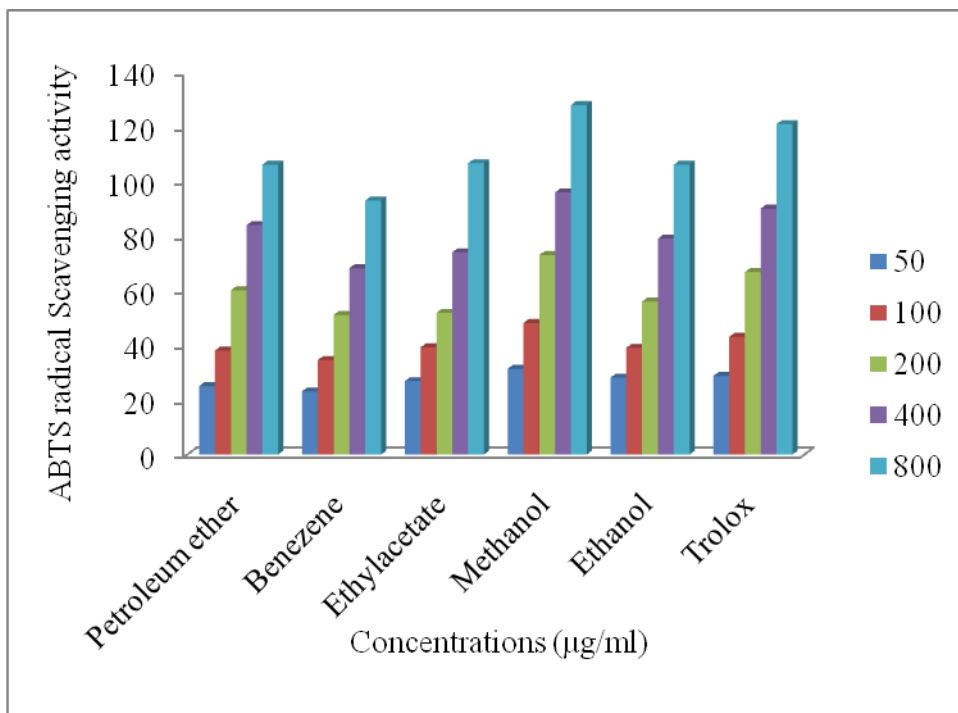


Figure 4: Effect of different solvent extract of *N. apetalis* leaf on ABTS assay

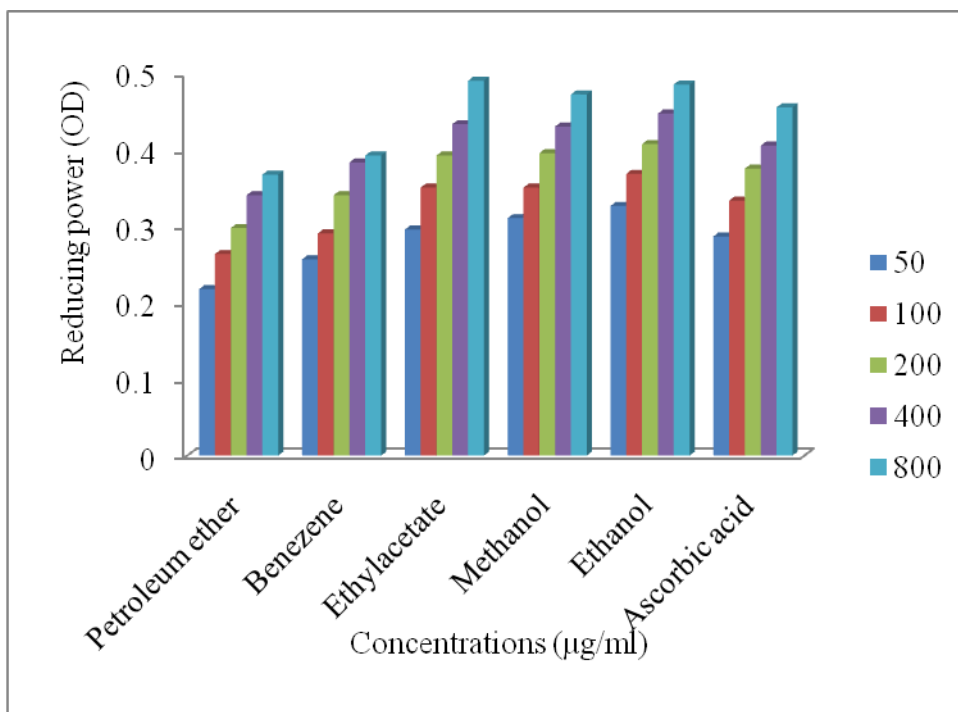


Figure 5: Effect of different solvent extract of *N. apetalis* leaf on reducing power assay

Table 1: IC<sub>50</sub> values of different solvent extracts of the leaf extracts of *N. apetals*

Solvents	IC <sub>50</sub> (µg/ml)			
	DPPH	Hydroxyl	ABTS	Superoxide anion
P.ether	24.11	27.91	26.97	28.93
Benzene	26.16	26.23	23.06	24.89
Ethyl acetate	31.84	31.45	27.07	28.95
Methanol	28.16	37.16	32.07	34.22
Ethanol	32.43	36.54	27.12	36.93
Ascorbic acid	29.16	28.17	-	30.16
Trolox	-	-	30.22	-