Pavithra T.* and Tamizh Mani T.

Department of Pharmacognosy, Bharathi College of Pharmacy, Bharathinagar, Maddur Taluk, Mandya District, Karnataka, India.

*Corresponding Author: Pavithra T.
Department of Pharmacognosy, Bharathi College of Pharmacy, Bharathinagar, Maddur Taluk, Mandya District, Karnataka, India.

ABSTRACT
Odina wodier Roxb (Anacardiaceae) is a tropical tree commonly called as Indian ash tree. Its various parts is said to possess medicinal property and used in Ayurveda and other ancient system of medicine for the treatment of various ailments. Our present study deals with the finding of the phytochemical constituents present in odina wodier leaf extracts. It was performed by generally accepted laboratory technique for qualitative determination. It was observed that alkaloids, flavonoids, tannins, steroids were present more in methanolic extract and were lesser amount in ethyl acetate extract, petroleum ether extract and chloroform extracts. To ascertain the medicinal properties of the plant and also to assess its anti-oxidant potential of methanolic leaf extract of odina wodier. The results revealed that methanolic extract is mainly composed of oxygenated hydrocarbons and predominantly phenolic hydrocarbons. These different active phytochemicals may be responsible for wide range of activities, which may help in the protection against incurable diseases. The future In-vivo investigation should be carried out to confirm the activity in animal models.

KEYWORDS: Odina wodier, phytochemical screening and In-vitro antioxidant.

INTRODUCTION
Based on the material of origin, Ayurvedic medicines are divided into three classes, namely herbal, mineral and animal. Among this, herbal formulation has gained great importance and rising global attention recently. Ayurveda has about 700 type of plants listed in its medicinal systems. The use of such herals is mentioned in the ancient Ayurvedic literature such as Chakara Samhita and Sushruta Samhita. The discovery of herals is further complemented with knowledge on the method of isolation, purification, characterization of active ingredients and type of preparation. The term “herbal drug” determines the part/parts of a plant (leaves, flowers, seeds roots, barks, stems etc.) used for preparing medicines.

Herbal medicines are employed in a wide variety of health related applications ranging from treatment of common colds to treatment of cancer. Antioxidant plays an important role in the alleviation of diabetes and obesity due to oxidation stress. Many plants often contain substantial amounts of antioxidants including VitaminC and E, Carotenoids, flavanoids and tannins and can be utilized to scavenge the excess free radicals from human body. Antioxidant-based drug formulation is used for the prevention and treatment of complex diseases and cancer. The plant Odina Wodier Roxb is a moderate-sized or large deciduous tree, with thick soft branchlets belonging to the family Anacardiaceae. This tree occurs in hotter parts of India. Its wood is a light brown, tough, moderately hard and heavy. It is used for furniture, flooring, and general construction. It has been used as a source of tannin and has been used as a medicinal plant. The bark is used for the treatment of rheumatism and bronchitis. The leaves are used for the treatment of skin diseases and are also used as a paste for leprosy ulcers. Gum of the tree made into an ointment with coconut milk or into liniment with brandy is a good application to sprains and bruises. Internally, gum is given in asthma and as a cordial to women during lactation. Leaves boiled in oil are also applied to sprains and bruises, to local swellings and pains of the body. For rheumatism a paste of the leaves mixed with black pepper is a useful application.

Antioxidant based drug formulation is used for the prevention and treatment of complex diseases and cancer. In the present study, our investigation aims to find out the constituents by preliminary phytochemical analysis and to investigate the antioxidant activity of petroleum ether, ethyl acetate, chloroform and methanolic extract via DPPH radical model, Hydrogen...
peroxide, nitric oxide, reducing assay on odina wodier leaves.

**MATERIALS AND METHODS**

Collection of plant material: The fresh plant leaves were collected in the month of October from the Chamundeswari hills, Mysore, India. It was duly identified by the forest revenue officer Mrs. Vinutha DRFO (Deputy Range forest officer) and authenticated by Botanist Dr. Gurukar Mathew, Head of the Department of Botany, Bharathi College. After collection the leaves was washed thoroughly with running tap water, cut into small pieces and shade dried. The dried material was then pulverized separately into coarse powder by a mechanical grinder. The voucher specimen was preserved in laboratory for further reference.

Preparation of extracts

The powdered leaves of *odina wodier* were extracted by using petroleum ether, ethyl acetate, chloroform, and methanol in soxhlet apparatus by using standard procedure. The distillates were collected and distilled separately to yield the concentrated extracts. These extracts dried using rotary vacuum evaporator. It was kept in a desiccator until used.

Preliminary Phytochemical Screening of Extracts

Extracts prepared by using petroleum ether, ethyl acetate, chloroform and methanol successively were subjected to qualitative chemical tests for identification of various phytoconstituents. Phytochemical examinations were carried out as per the standard methods set by World health organization (WHO).

1.0 Detection of alkaloids

The Extract was dissolved in dilute Hydrochloric acid and filtered. The acidic layer was used for testing the alkaloids.

Dragendorff’s test: (solution of Potassium Bismuth Iodide)

Filtrates were treated with Dragendorff’s reagent gives of reddish brown precipitate indicates the presence of alkaloids.

Wagner’s test: (Iodine in Potassium Iodide)

Filtrates were treated with Wagner’s reagent gives reddish brown precipitate indicates the presence of alkaloids.

Mayer’s test: (Potassium Mercuric Iodide)

Filtrates were treated with Mayer’s reagent. Formation of a yellow coloured precipitate indicates the presence of alkaloids.

Hager’s test: (saturated picric acid solution)

Filtrates were treated with Hager’s reagent. Presence of alkaloids confirmed by the formation of yellow colored precipitate.

1.1 Detection of Glycosides

Extracts were hydrolysed with dil. Hydrochloric acid and then subjected to test for glycosides.

Legal’s test: Extracts were treated with sodium nitroprusside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

Modified Borntrager’s test: Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammoniacal layer indicates the presence of anthranol glycosides.

1.2 Detection of phytosterols and triterpenes

Salkowski’s test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Concentrated Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

Liebermann Burchard’s test: Extracts were treated with Chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Concentrated Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

1.3 Detection of Diterpenes

Copper acetate test: Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

1.4 Detection of saponins

Froth test: Extracts were diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.
1.5. Detection of phenolic compounds flavonoids and Tannins

**Ferric Chloride test:** Extracts were treated with 3-4 drops of 5% ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

**Gelatin test:** To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

**Lead-acetate test:** Test solution was treated with 10% lead acetate solution. Formation of yellow precipitate indicates the presence of flavonoids.

**Shinoda test:** To the alcoholic solution of test sample, a few fragments of magnesium ribbon and conc. HCl were added. Appearance of magenta colour after few minutes indicates presence of flavonoids.

1.6. Detection of carbohydrates

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

**Molisch’s test:** Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube and 2 ml of Conc. Sulphuric acid was added carefully along the sides of the test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.

**Benedict’s test:** Filtrates were treated with Benedict’s reagent and heated gently on water bath. Formation of orange red precipitate indicates the presence of reducing sugars.

**Barfoed’s test:** Filtrate was treated with Barfoed’s reagent and heated on water bath. Orange red precipitate indicated presence of mono and disaccharides.

**Fehling’s test:** Filtrates were hydrolysed with dilute Hcl, neutralized with alkali and heated with Fehling’s A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

1.7. Detection of proteins and amino acids

**Xanthoproteic test:** The extract was treated with few drops of concentrated. Nitric acid. Formation of yellow colour indicates the presence of proteins.

**Ninhydrin test:** To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of purple colour indicates the presence of amino acid.

**Millon’s test:** Test solution was treated with millon’s reagent and boiled on a water bath. Formation of white precipitate, which turns red on heating, indicates the presence of amino acids.

**Biuret test:** The extract was treated with 0.7% copper sulphate solution followed by few drops of sodium hydroxide. Formation of purplish violet colour indicates the presence of proteins.

1.8. Detection of Fixed oils and Fats

**Spot test:** Extracts were dissolved in a suitable solvent and applied as a spot on filter paper. Appearance of a clear-transparent spot indicates the presence of fixed oils.

**Saponification test:** The test solution was mixed with 3ml of 0.1 N NaOH, along with a drop of Phenolphthalein. Heated over water bath for 20 minutes until the colour decolorizes.

**Antioxidant Studies**

**DPPH free radical scavenging activity**

2.36g of the DPPH was dissolved in 100ml of methanol to get \( 6 \times 10^{-5} \) M methanolic solution of DPPH. Different concentration of Standard and plant extract like 20, 40, 60, 80 and 100 μg/ml were prepared by diluting with methanol. 1ml of each diluted Standard and test samples were mixed with 3ml of DPPH solution in each test tube. Control was prepared by adding 1ml of methanol and 3ml of DPPH. The test tubes were covered with aluminum foil to protect from light and kept in dark place for 15 min. Methanol was used as blank. Absorbance of standard, control and test extract was at 517nm using UV- Visible spectrophotometer. The % inhibition was calculated by using following formula and compared with the values of standard Ascorbic acid.

\[
\text{% inhibition of DPPH activity} = \left( \frac{A_0 - A_t}{A_0} \right) \times 100
\]

Where \( A_0 \) is the absorbance of control and \( A_t \) is the absorbance of the extract/standard.

**Hydrogen peroxide Scavenging Assay**

The Hydrogen peroxide-scavenging activity, The extract (20, 40, 60, 80 and 100 μg/ml) / standard (ascorbic acid-20-100μg/ml) different concentrations were dissolved in 3.4 ml of 0.1M phosphate buffer (pH 7.4) and mixed with 0.6ml of 40 mM solution of hydrogen peroxide. The reaction mixture was incubated for 19 minutes and absorbance was measured at 230 nm for each concentration, a separate blank sample was used for background subtractions.

\[
\text{Percentage inhibition} = \left( \frac{A_{\text{control}} - A_{\text{test/standard}}}{A_{\text{control}}} \right) \times 100.
\]

**Determination of total phenolic content**

0.5ml of a methanol solution of the crude extract of concentration of 1mg/ml was mixed with 5ml Folin ciocalteu reagent (1:10 v/v distilled water) and 4 ml (75g/L) of Sodium carbonate. The mixture was vortexed for 15 second and allowed to stand for 30min at room temperature in dark place for colour development and the absorbance was measured at 760 nm against methanol as blank by using a UV- visible spectrophotometer. The
total phenolic was expressed as gm of GAE (Gallic acid equivalent)/100gm of the dried extract using the following equation obtained from a standard Gallic acid calibration curve.

**Total Flavonoids Concentration**
0.5ml of a methanol solution of the extract of concentration of 10 mg/ml was mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. Sample blank was prepared in similar way by replacing aluminium chloride with distilled water. Both sample and blank solution were filtered through double rings filter paper before measuring the absorbance. Both sample and blank solution were allowed to stand for 30 min at room temperature. After 30 minute incubation period, the absorbance of the reaction mixture was measured at 415 nm against a blank by using a UV-visible spectrophotometer. All the tests were carried out in triplicate and average absorption was noted for each time. The total flavonoids was expressed as mg of Quercetin equivalent per gram of dried Extract by using the equation obtained from a standard Quercetin calibration curve.

**Nitric Oxide Radical Scavenging Assay**
In nitric oxide radical inhibition assay, ascorbic acid (1mg/ml) and different concentrations (20- 100μg/ml) was used as the standard. Test Sample solution were prepared from extract in phosphate Buffer. The concentrations of sample solution were 20,40,60,80,100 μg / ml. The reaction mixture (3ml) contained sodium nitroprusside (10mM,2ml), phosphate buffer saline (0.5ml) and standard solution/the extract(0.5ml). It was incubated at 25oC for 150 minutes. After incubation 0.5ml of reaction mixture containing nitric oxide was taken and mixed with 1ml of sulphanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for completion of the reaction process of diazotisation. Then 1ml of 1- naphthylamaine was added, mixed and was allowed to stand for 30min at 250 C. The concentration of nitrite was assayed at 540nm and was calculated with the reference to the absorbance of the standard nitrite solution. Ascorbic acid was taken as standard.

The percentage inhibition was calculated using the formula.

**Percentage inhibition =[(Acontrol - Atest /standard) / Acontrol] 100.**

Where Acontrol is the absorbance of control, Atest/standard is the absorbance of the extract/standard.

**Determination of reducing power assay**
1 ml of the methanol solution of the crude extract of different concentrations (20, 40, 60, 80, 100μg/mL) was mixed with 2.5 ml phosphate buffer and 2.5 ml potassium ferricyanide [K3Fe(CN)6] (1%). The mixture was incubated at 50°C for 20min. 2.5mL of trichloroacetic acid (TCA) was added to the mixture, which was then centrifuged at 3000rpm for 10min. The upper layer of the solution was separated and mixed with 2.5 ml distilled water and 0.5ml FeCl3. The absorbance was measured against a blank at 700nm. All the tests were carried out in triplicate and average absorption was noted for each time. L-Ascorbic acid was used as positive control. Percentage (%) increase in reducing power was calculated as follows:

% Increase in reducing power = (ATest / ABlank) -1 X 100

Where ABlank is absorbance of blank (containing all reagents except the test material)
ATest is absorbance of test solution.

**RESULTS AND DISCUSSION**
The extracts were subjected for qualitative chemical analysis for the identification of various phytoconstituents, revealed the presence of Flavonoids, phenolic compounds etc. Results of the chemical tests for each extract was recorded and tabulated in the table 1.

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Chemical test</th>
<th>SOLVENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>PE</td>
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<tr>
<td>1.</td>
<td>Alkaloids</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dragendroffs</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Wagner’s</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Mayer’s</td>
<td>-</td>
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<td></td>
<td>Hager’s</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>Glycosides</td>
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</tr>
<tr>
<td></td>
<td>Legal’s</td>
<td>-</td>
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<td></td>
<td>Modified Borntrager’s</td>
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</tr>
<tr>
<td>3.</td>
<td>Phytosterols and Triterpenoids</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Salkowski</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Libermann burchard</td>
<td>+</td>
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Pavithra et al.

European Journal of Pharmaceutical and Medical Research

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Conc µg/ml</th>
<th>Ascorbic Acid</th>
<th>Methanolic extract*</th>
<th>Ethyl acetate extract*</th>
<th>Pet ether extract*</th>
<th>Chloroform extract*</th>
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<td>66.88</td>
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<td>52.13</td>
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<td>77.5</td>
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<td>62.36</td>
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<td>83.55</td>
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<td>60.06</td>
<td>72.31</td>
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<tr>
<td>5</td>
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<td>75.08</td>
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<td><strong>IC₅₀</strong></td>
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<td><strong>36.23</strong></td>
<td><strong>38.30</strong></td>
<td><strong>56.0</strong></td>
<td><strong>3.4</strong></td>
<td></td>
</tr>
</tbody>
</table>

Table No. 2: Percentage inhibition for Antioxidant activity by DPPH radical scavenging method.

Graph 1: Percentage inhibition for Antioxidant activity by DPPH radical scavenging method.

Determination of Total phenolic content

The crude extract of Odina wodier roxb leaves by using different solvent like pet ether, chloroform, ethyl acetate and methanol, were subjected to total phenolic content determination. Based on the absorbance values of extract solution, reacted with Folin-ciocalteu reagent and compared with the standard solution of gallic acid equivalents, result of colorimetric analysis of the total phenolics are given in table no3. Total phenolic content of the samples are expressed as mg of GAE(gallic acid equivalent) per gm of dried extract.

Graph 2: Standard cure of Gallic acid.

Expansions: P.E-Petroleum ether extract, C.E-Chloroform extract, M.E-Methanolic extract, E.A-ethyl acetate extract

Percentage inhibition for Antioxidant activity by DPPH radical scavenging method

Medicinal plants are an important source of antioxidants. Typical phenolic that possess antioxidant activity are known to be mainly phenolic acids and flavonoids. It is reported that the phenolic are responsible for the variation in the antioxidant activity of the plant. Flavonoids are phenolic acids, which serve as an important source of antioxidants found in different medicinal plants and related phytomedicines. The antioxidant activity is due to their ability to reduce free radical formation and to scavenge free radicals. The stable radical DPPH had been used widely for the determination of primary antioxidant activity. The DPPH antioxidant assay is based on the ability of a stable free radical to decolorize in the presence of antioxidants.

Among successive solvent extracts of highest percentage inhibition by DPPH radical scavenging assay exhibited in methanolic extract 90.33±3.03 at 100µg/ml and lowest percentage inhibition in ethyl acetate extract 45.55±0.62 at 20µg/ml. The results are represented in Table No.2.
Table 3: Total phenolic content in different sample extracts.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Conc((µg/ml))</th>
<th>Absorbance</th>
<th>mg of gallic acid equivalent per gm of dried extract.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pet ether</td>
<td>1(µg/ml)</td>
<td>0.608</td>
<td>109.2</td>
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<tr>
<td>Chloroform</td>
<td>1(µg/ml)</td>
<td>0.598</td>
<td>126.9</td>
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<tr>
<td>Ethyl acetate</td>
<td>1(µg/ml)</td>
<td>0.521</td>
<td>262.8</td>
</tr>
<tr>
<td>Methanol</td>
<td>1(µg/ml)</td>
<td>0.506</td>
<td>289.2</td>
</tr>
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</table>

Determination of Total Flavonoid content
To determine the total flavonoid content of crude extract of Odina wodier roxb leaves using Chand et al method. The total flavonoids content of the sample is expressed as mg of Quercetin per gm of dried extract. By using the standard curve equation of Quercetin ($Y=11.924X-0.0307, R^2=0.9993$).

Table 4: Total flavonoid content assay.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Conc(µg/ml)</th>
<th>Absorbance</th>
<th>mg of Quercetin equivalent per gm of dried extract.</th>
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<tbody>
<tr>
<td>Pet ether</td>
<td>10 (µg/ml)</td>
<td>0.349</td>
<td>95.4</td>
</tr>
<tr>
<td>Chloroform</td>
<td>10 (µg/ml)</td>
<td>0.430</td>
<td>115.8</td>
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<tr>
<td>Ethyl acetate</td>
<td>10 (µg/ml)</td>
<td>0.761</td>
<td>198.9</td>
</tr>
<tr>
<td>Methanol</td>
<td>10 (µg/ml)</td>
<td>0.842</td>
<td>219.3</td>
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</table>

Determination of antioxidant activity of selected plant extracts by Hydrogen peroxide scavenging method
The scavenging ability of petroleum ether, chloroform and methanol extracts of *Odina wodier roxb* Hydrogen peroxide scavenging assay is shown in table no.5. The percentages of inhibitions were increased with increasing concentrations of the extracts.

Table no 5.

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Conc (µg/ml)</th>
<th>Ascorbic acid</th>
<th>Methanolic extract*</th>
<th>Chloroform extract*</th>
<th>Pet ether extract*</th>
<th>Ethyl acetate extract*</th>
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<td>5.</td>
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<td>85.11</td>
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<td>55.06</td>
<td>73.11</td>
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Nitric Oxide Radical Scavenging Assay
Nitric Oxide (NO) is a forceful pleiotropic negotiator in numerous physiological process like smooth muscle relaxation, inhibition of platelet aggregation, neuronal signalling and cell mediated toxicity parameter. This free radical is a diffusible one, which plays a lot of roles as an effect or molecule in many parts biological systems together with neuronal messenger, anti-microbial, anti-inflammatory and anti-tumour activities. Furthermore Nitric Oxide plays a key position in repairs of wound by influence angiogenesis and inflammation. Hence this study will assist us to considerate the role of our extract in various physiological scheme such as diminution of...
inflammation, smooth muscle relaxation and platelet aggregation.

The scavenging activity of the extract in opposition of nitric oxide released by sodium nitroprusside was inspected. Nitric oxide (NO) scavenging assay is based on the scavenging capability of the extracts as well as Ascorbic acid, which is used as standard. The scavenging of NO was found to amplify in dose dependent manner. In the present study the plant extracts competes with oxygen to react with nitric oxide and thus inhibits the generation of anions. The results are presented in Table 6.

Table 6: Nitric Oxide Radical Scavenging Assay

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Conc (µg/ml)</th>
<th>Ascorbic acid</th>
<th>Methanolic extract*</th>
<th>Chloroform extract*</th>
<th>Pet ether extract*</th>
<th>Ethyl acetate extract*</th>
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<td>78.44</td>
<td>74.38</td>
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<td>72.11</td>
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</table>

CONCLUSION

In preliminary phyto chemical studies of *odina wodier* Roxb indicates the presence of alkaloids, Phytosterols, triterpenoids, Saponins, phenolic compounds and flavonoids were found more in methanolic extract compare to other extracts. The overall antioxidant activity of these extracts might be attributed to its flavonoids, phenolic and other phytochemical constituents. These could be a source of natural antioxidant that could have greater importance or slowing oxidative stress related to degenerative diseases. Further research work to be carried out to isolate bioactive molecules responsible for their activity and to

Determination of reducing power assay

Reducing power is connected with antioxidant activity which might serve as a considerable reflection of the antioxidant activity. Reducing power effect of the components was considered as a sign of that, they are electron donors and can trim down the oxidized intermediates of lipid peroxidation processes, as a result, they can act as primary and secondary antioxidants. In this assay, the colour of the test solution was yellow and it vary to a variety of shades of blue and green depending on the ability for the reducing power of each compound. Existence of reducers causes the translation of the Fe3+/ferricyanide complex used in this method. Among each and every one of the extracts methanolic leaf extract of *odina wodier* exhibited respectable activity with utmost inhibition of about 75.8% at a concentration of 100 µg/ml when paralleled to standard Ascorbic acid at the same concentration for which the percentage inhibition is 85.4%. The results are presented in Table 7.

Table 7: Determination of reducing power assay

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Conc (µg/ml)</th>
<th>Ascorbic acid</th>
<th>Methanolic extract*</th>
<th>Chloroform extract*</th>
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CONCLUSION

In preliminary phyto chemical studies of *odina wodier* Roxb indicates the presence of alkaloids, Phytosterols, triterpenoids, Saponins, phenolic compounds and flavonoids were found more in methanolic extract compare to other extracts. The overall antioxidant activity of these extracts might be attributed to its flavonoids, phenolic and other phytochemical constituents. These could be a source of natural antioxidant that could have greater importance or slowing oxidative stress related to degenerative diseases. Further research work to be carried out to isolate bioactive molecules responsible for their activity and to
investigate and screen the compounds to evaluate other biological activities.

REFERENCES