Research Article

Phytochemical Evaluation and Biological Studies on the Bark of *Odina wodier,* roxb.,

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ABSTRACT

In the present study, an attempt was made to investigate phytochemical evaluation and *in vitro* Antioxidant studies on the bark of *Odina wodier,* roxb. The crude drug powder of hydro-ethanolic extract of the above plant was taken for the study. In the Phytochemical Screening, Phenolic compounds, tannins, and carbohydrates were present. In nitric oxide radical inhibition assay and Hydrogen peroxide-scavenging activity, ascorbic acid (1mg/ml) was used as the standard in different concentrations (50-250µg/ml). Calculate the IC_{50} value (50% inhibitory concentration) from the graph for both *in vitro* Antioxidant studies.

Keywords: Phytochemical screening, *Odina wodier,* and Antioxidant.

INTRODUCTION

The plant *Odina wodier***,** Roxb., (Syn. *Lannea grandis*, Englr.) 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[1].

Decoction of bark is used as astringent, in cases of atonic dyspepsia and general debility. It is also used as a gargle in aphthous conditions of the mouth and also for tooth ache and as a lotion for skin eruptions. Fresh juice of the bark is used to sore of eyes and obstinate ulcer. Powdered bark mixed with neem oil is an application for chronic ulcers and skin diseases and also used as a paste for leprous 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[2].

Free radical reaction is an important pathway in a wide range of unrelated biological systems. A vast amount of circumstantial evidence implicates free radicals as the mediators of wide range of diseases[3]. These reactions cause several diseases like atherosclerosis, heart failure, hemorrhagic shock, neuro degenerative disorders[4], lung diseases, cancer, kidney damage, inflammation, diabetes mellitus, aging, etc[5,6].

As there is no reports of *Odina wodier* bark's , Here in the present study plant was taken for Phytochemical screening and Antioxidant studies on hydro-ethanolic plant extract of crude dried powdered drug was taken and evaluated. The phytochemical constituents were studied by qualitative analysis for performing various chemical tests and in antioxidant studies, nitric oxide radical inhibition assay and Hydrogen peroxide-scavenging activity was carried out.

MATERIALS AND METHODS

Collection of plant material

The stem bark *Odina wodier* used for the present studies was collected by incision and peeling from the mature plants in Dharmapuri district of TamilNadu. The plant was identified and authenticated Botanist Dr. TR Shanta from NADRI, Bangalore (Specimen no: 1043). Just after collection the bark 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 resulting powder was then used for extraction.

Preparation of Extract: The powdered drug was dried and packed well in Soxhlet apparatus and extracted with 1500 ml of hydro-ethanol (1:1) for 72 hrs. The extract was concentrated and dried using Rotary vaccum evaporator. It was kept in a desiccator until used.

Qualitative Phytochemical Screening

The crude drug powder of hydro-ethanolic extract of the stem bark *Odina wodier* was subjected to qualitative analysis for presence of chemical constituents.

The different qualitative chemical tests were performed for establishing phytochemical profile of extract obtained from soxhlet extractions. The following tests were performed on the extract to detect various phytoconstituents present in it [7'8.]

Detection of Alkaloids
The dried extracts were dissolved in few ml of 0.1N HCl and used for all the following tests.

Mayer's test
One ml of sample was taken and 1-2 drops of Mayer's reagent was added from the sides of the test tubes. The appearance of white precipitate indicates presence of alkaloids in the sample.

Wagner's test
One ml of sample was taken and 2-3 drops of Wagner's reagent was added from the sides of the test tubes. The appearance of reddish-brown precipitate indicates presence of alkaloids in the sample.

Hager's test
One ml of sample was taken and 1-2 ml of Hager's reagent was added into the test tubes. The appearance of yellow precipitate indicates presence of alkaloids in the sample.

Dragendroff's test
One ml of sample was taken and 1-2 ml of Dragendroff's reagent was added into the test tubes. The appearance of yellow precipitate indicates presence of alkaloids in the sample.

Detection of Saponins by foam test
50mg of extract was diluted with distilled water and volume was made up to 20 ml in a measuring cylinder. It was shaken well for 15 minutes. The appearances of layer of foam of about 2 cm indicate presence of saponins.

Detection of phytosterols by Liebermann-Burchards method
Sample was dissolved in 2ml of acetic anhydride and 1-2 drops of concentrated H_2SO_4 was added from the sides of the test tube. The array of change in colors from purple to green indicates presence of phytosterols.

Detection of Phenolic compounds
Ferric chloride
The extract was dissolved in 5ml of distilled water and few drops of neutral 5% $FeCl_3$ solution were added. The appearance of dark green color indicates the presence of tannins.

Gelatin
The extract was dissolved in 5ml of distilled water and 2 ml of 1% gelatin was added. Appearance of white precipitate indicates presence of phenols.

Folin-Ciocalteau reagent
The extract was dissolved in 3ml of water, 0.5ml of Folin-Ciocalteau reagent was added and incubated for 3minutes, and 2ml of 20% Na_2CO_3 solution was added. Appearance of blue coloured complex indicates the presence of phenols.

Carbohydrates
Take 100µl of sample and heat to evaporate the solvent. This dried extract is dissolved in 1ml of distilled water and used for all the following tests.

a) Molish's test
To 2 ml of sample, add 2 drops of alcoholic solution of α-naphthol. Shake the tube well. Add 1ml of concentrated H_2SO_4 slowly from the sides of the test tube. Let it stand. Appearance of violet ring at the interface indicates the presence of carbohydrates in the sample.

b) Fehling's test
1 ml sample is boiled with 1ml each of Fehling's A and Fehling's B. Red precipitate indicates the presence of carbohydrates in the sample indicates the presence of carbohydrates in the sample.

c) Barfoed's test
1ml sample is boiled along with 1ml of Barfoed's reagent on boiling water bath for about 2 minutes. The appearance of red precipitate indicates the presence of carbohydrates in the sample.

d) Benedict's test
0.5 ml of sample along with 0.5 ml Benedict's reagent is heated on boiling water bath for 2 minutes. Appearance of characteristic colours like yellow/orange/green/red indicates presence of carbohydrates in the sample.

Proteins & Amino acid
Take 100 µl of sample and heat to evaporate the solvent. This dried extract is dissolved in 1 ml of distilled water and used for all the following tests.

e) Million's test
To 2ml of sample, add few drops of million's reagent. White precipitate is a positive test for proteins.

f) Biuret test

To 2ml of sample, add 1 drop of 1% $CuSO_4$ solution and 1ml of 95% ethanol. Add excess of alcoholic KOH pellets to it. The appearance of pink colour in ethanol layer is indicative of presence of proteins.

g) Ninhydrin test

To 2 drops of Ninhydrin reagent add 2 ml of sample. The appearance of purple colour indicates the presence of amino acids.

Gum and mucilage

Take 100µl of sample and heat to evaporate the solvent. Add 10ml of distilled water. Add 25 ml of absolute alcohol. The appearance of white precipitate indicates the presence of gum.

ANTIOXIDANT STUDIES

A) Nitric Oxide Radical Scavenging Assay[9]

In nitric oxide radical inhibition assay, ascorbic acid (1mg/ml) and different concentrations (50-250µg/ml) was used as the standard. Test Sample solution were prepared from extract in phosphate Buffer. The concentrations of sample solution were 50,100,200,300 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 25^{0}C 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 25^0 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 =

$$[(A_{control} - A_{test\ /stanard)} / A_{control}\ ]\ 100\ .$$

Where $A_{control}$ is the absorbance of control, $A_{test\ /standard}$ is the absorbance of the extract/standard.

B. Hydrogen peroxide Scavenging Assay[10]

The Hydrogen peroxide-scavenging activity, The extract (50,100,200 and 300µg/ml) / standard (ascorbic acid- 50-250µg/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.

Percentage inhibition =

$$[(A_{control} - A_{test\ /stanard)} / A_{control}\ ]\ 100\ .$$

Statistics

The decolorization was plotted against the sample extract concentration,and a linear regression curve was established in order to calculate the IC50 (µg/ml) being the amount of sample necessary to decrease by 50% the absorbance of radicals.

RESULTS AND DISCUSSION

The extracts were subjected for qualitative chemical analysis for the identification of various phytoconstituents, revealed the presence of carbohydrates, tannins and phenolic compounds. Results of the chemical tests for each extract was recorded and tabulated in the table.1

In nitric oxide radical inhibition assay and hydrogen peroxide scavenging assay, the absorbance of the reaction mixture for different concentrations of standard and samples and control were recorded in triplicate. From this the percentage of inhibition were calculated by using the formula and tabulated in the table.2. The percentage inhibition obtained in the different concentration of sample extracts were compared to the percentage inhibition obtained with the standard.

From the table.3, it is clear that while studying the nitric oxide scavenging activity and hydrogen peroxide scavenging assay, IC50 value of hydro-ethanolic extract was 137.5µg/ml and 35µg/ ml respectively. Whereas the standard require100 µg/m42.5µg/ml for 50% inhibition respectively. Compared with standard, hydro-ethanolic extract was found to have significant antioxidant activity.

CONCLUSION

The screening of the phytochemical constituents of plant *Odina wodier., indicated* the presence of carbohydrates, phenolic compounds and tannins. The plants contains more metabolites there is need for further investigations using fractionated extracts and purified chemical components.

As a rule, presence of the compounds such as flavonoids, phenolics, and carotenoids exhibit antioxidant property. Hence, it is proved that the shown antioxidant activity is due to the presence phenolics and

tannins in the extract whose presence is detected by the phytochemical screening. As the chemical compounds are not isolated and identified, actually which compound is responsible for the above said activity. Hence, further study is essential and it is underway.

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Table 1: Showing results for qualitative phytochemical; screening of the hydro-ethanolic extract of *Odina wodier*

Phytochemical Screening of Hydro-ethanolic extract of *Odina wodier*.	
Alkaloids	
Mayer's	-
Wagners	-
Hager's	-
Dragendroff's	-
Carbohydrates	
Molisch's	+
Fehling's	+
Barfoed's	+
Benedict's	+
Saponin	
Foam test	-
Proteins & Amino acid	
Million's	-
Biuret	-
Ninhydrin	-
Phytosterols	
Liebermann-Burchards	-
Phenolic compounds and tannins	
Ferric chloride	+++
Gelatin	++
Lead acetate	++
Alkaline reagent	+
FC reagent	+++
Gum and mucilage	-

Table 2: showing Effect of Hydro-ethanolic extract of *Odina wodier* on nitric oxide radical and Hydrogen Peroxide Scavenging Assays

		Nitric oxide Free radical Scavenging Assay		**Hydrogen Peroxide Scavenging Assay**	
Sample	**Concentrations (µg/ml)**	**Absorbance**	**% inhibition**	**Absorbance**	**% inhibition**
Control	-	0.14	-	3.25	-
Standard	50 100 150 200	0.08 0.07 0.05 0.04	42.86 % 50.00 % 64.29 % 71.43 %	1.126 0.822 0.624 0.463	65.35 % 74.70 % 80.8 % 85.75 %
Hydro-ethanolic extract	50 100 200 300	0.12 0.08 0.05 0.04	14.29 % 42.86 % 64.29 % 71.42 %	0.892 0.869 0.716 0.534	72.55 % 73.26 % 77.99 % 83.57 %

Table 3: showing 50% Inhibition
Hydro-ethanolic extract of *Odina wodier*

Antioxidant Activity	Sample	IC50 value (µg/ml)
Nitric oxide Free radical Scavenging Assay	Standard (Ascorbic acid)	100 µg/ml
	Hydro-ethanolic extract	137.5 µg/ml
Hydrogen Peroxide Scavenging Assay	Standard (Ascorbic acid)	40 µg/ml
	Hydro-ethanolic extract	35 µg/ml

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