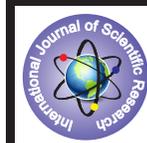


Phytochemical Examination and Evaluation of *In-Vitro* Antioxidant Potential Activity of *Celastrus Paniculatus* Seeds Extract



Chemistry

KEYWORDS : *Celastrus paniculatus*-wild seeds , Phytochemical Screening, Antioxidant activity, DPPH,

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ABSTRACT

Celastrus paniculata wild. (Family-Celastraceae) commonly known as Malkangni, Jyotishmati, Intellect Tree, Bitter sweet, as an important Indian medicinal plant. The present study is directed to phytochemical investigations and evaluation for their antioxidant potential against (DPPH) 2, 2 diphenyl-1-picrylhydrazyl free radical and compared with Ascorbic acid. The percentage of inhibition was found to be the IC50 value was 343.65 µg/ml, the result showing that *Celastrus paniculata* exhibits potential free radical scavenging. The preliminary phytochemical studies show the presence of terpenoids, steroids, saponins, flavonoid, carbohydrate, glycoside etc.

INTRODUCTION:-

Phytochemical investigation of natural products are always an interesting target for scientists in nineteenth century, especially on historically medicinal plants (Seeds, Fruits, Roots, Rhizomes, Leaves, Vegetables, Medicinal herbs etc.) have provide a good source of a wide variety of phytoconstituents. Nature have been a source of medicinal agent for thousand of years, and an impressive number of modern drugs have been isolated from natural resources, many based on their use in traditional medicine. Compounds and extracts obtained from the Mother Nature's diversity have found uses in medicines i.e. allopathic, homeopathic, and ayurveda agriculture and health products in ancient and modern societies around the world. Medicinal plants are now more focused then ever because they have the capability of production many potential benefits to human beings in line of medicine.

The medicinal power of herbal plants lie in phytochemical constituents that cause actual pharmacological action on human body.¹ The most power full bioactive phytoconstituents are terpenes, steroids, alkaloids, flavanoids, tannins, carbohydrate, amino acid, glycosides, phenoles compounds etc.² The value of phytomedicine and traditional pharmacology is nowadays gaining increasing recognition in modern medicine because the search for new potential medicinal plants is frequently based on an phytomedicine basis. In the phyto-pharmacological approach, local knowledge about the potential use of the plants are very useful as compared to the random approach where indigenous knowledge is not taken into consideration. *Celastrus Paniculata* is one of the most important medicinal plant of Celastraceae family locally is known as "Jyotishmati" Malkangni. It is used as a brain tonic to promote intelligence and to sharpen the memory³. The green plants are the storage house of many compounds which may act as a role of secondary metabolites "compounds which conduct metabolic activities"⁴. The seed oil is intellect promoting & used for curing epilepsy seeds yield as much as 52% oil by weight which is also useful in abdominal disorders headache, joint pain, leucoderma, paralysis, ulcer etc⁵. Oil stomachic tonic good for cough, asthmas and also used in leprosy. It is found that *C. paniculata* oil has a beneficial effect on the learning and memory process in mentally retarded children⁶. It is brain clearer and believed to promote intelligence⁷. In our country, a vast section of population still resides and inhabits rural and remote forest areas where there no facility. And the people, especially the tribal's, totally depend on herbal resources for the alleviation of their ailments.

BOTANICAL DESCRIPTION OF C. PENICULATA-

C. Paniculata is wild woody liane belongs to the Family celastaceae. The plant is Commonly known as Malkangni, Black oil tree, Intellect tree, "jyotishmathi" in the Ayurvedic system of medicine^{8,9}. It grows throughout India at the height of almost 1800-2000 meters. Because of its high medicinal values & destruction of habitat, this species has faced the stage threat and its abundance is very less in tropical forests of India and it has reached the stage of vulnerable¹⁰.

SCIENTIFIC CLASSIFICATION



Order	Celastrales
Kingdom	Plantae
Family	Celastraceae
Species	Celastru
Genus	Paniculatus

MATERIAL AND METHODS

Collection of plant materials-

The fresh specimen and seeds of selected plant *C. paniculatus* having medicinal value were collected from the forest of Rahatgarh hills, Sagar district in Madhya Pradesh India. The plant materials and specimen were authenticated by Dr. Madhuri Modak Professor Department of Botany Govt. M.V.M. Bhopal , the voucher specimen (Herbarium No.1123.49-125) .

1 Extraction of seeds of *C. Paniculata* :-

The seeds were dried in shade with occasional shifting and then powdered with a mechanical grinder, passing through sieve 40 mesh, and stored in a air tight container. The powdered material was filled in the thimble of Soxhlet apparatus and exhaustively extracted with petroleum ether (40°C) for about 48 cycles^{11,12}. The solvent was distilled off at low temperature under vacuum and concentrated on water-bath to get concentrated extract, after extraction with Petroleum ether the material was refluxed with other solvents like Benzene, Chloroform, alcohol and finally with water¹³.

2 Phytochemical Examination :-

Plant contain several constituents and some of them are present at very low concentration. In spite of the modern chemical analytical procedure available only rarely phytochemical screening succeed in isolating and characterization all secondary metabolites present in the plant extract. The preliminary phytochemical screening of the plant is carried out by testing of different class of compounds using standard methods to identify the compound showing in Table No. 01. The seeds of plant

for qualitative identification of phytochemical constituents using standard conventional protocol. The chemical constituents of the extract were identified by qualitative analysis of chemical test, which indicates the presence of terpenoids, steroids, flavanoids, carbohydrates, reducing sugar, tannins, saponins, phenolic compounds and glycosides.

ANTIOXIDANT ACTIVITY

DPPH Radical Scavenging Assay:-

The free radical scavenging activity of extract and the standard ascorbic acid were measured in term of hydrogen donating or radical scavenging ability using the stable radical 2,2-diphenyl-1-picrylhydrazyl radical(DPPH).To evaluate the antioxidant activity of specific constituents, antioxidants were allowed to react with a stable radical DPPH in methanol solution. Extent of DPPH radical scavenging was determined by the decrease in intensity of violet colour in the form of IC₅₀ values.

The procedure involved UV-spectrophotometric determination. The three solutions i.e. Standard, Test and Control were prepared.

Preparation of Standard Ascorbic acid solutions:

Different solutions (50 - 1000µg/ml) of the ascorbic acid were prepared in methanol. 1.5 ml of each solution of ascorbic acid were mixed with 1.5 ml of 200µM DPPH solution and incubated for 30 min at room temperature in dark. Absorbance of each solution was taken after 30 min against methanol (as blank) at 517 nm.

Preparation of Test solutions:

Different solutions of the *Celastrus paniculata* seed extract were prepared in methanol (50 - 1000µg/ml), 1.5 ml of each solution of extract were mixed with 200 µM DPPH solution and incubated for 30 min at room temperature in dark. Absorbance of each solution of extract were taken after 30 min against methanol (as blank) at 517 nm.

Preparation of Control solution:

For control, 1.5 ml of methanol was mixed with 200 µM DPPH solution and incubated for 30 min at room temperature in dark. Absorbance of the control was taken after 30 min against methanol (as blank) at 517 nm.

Percentage of antioxidant activity of plant extract and Ascorbic acid was calculated by using formula:

$$I\% = \frac{Ac - (At - Ab)}{Ac} \times 100$$

Where

I% = Percentage inhibition

Ac = Absorbance of control (methanol and 200 µM DPPH solution)

At = Absorbance of ascorbic acid / plant extract with 200 µM DPPH solution after 30 min.

Ab = Absorbance of ascorbic acid / plant extract without 200 µM DPPH solution.

Calculation of IC₅₀ value:-

IC₅₀ values denote the concentration of sample, which is required to scavenge 50% of DPPH free radical concentration. IC₅₀ value was determined from the plotted graph of scavenging activity against the different concentration of extracts.^{15,16}

RESULTS AND DISCUSSION

Celastrus paniculata wild, is a traditional medicinal plant use for ancient times in therapeutically curing memory enhancing, anti-inflammatory, sedative, anti-epileptics. Phytochemical screening reveal that the presence of various phytoconstituents such as terpenoids, steroids, alkaloids, carbohydrate, flavonoids, glycosides, tannins, saponin and antioxidant activity de-

termined in selected medicinal plant *Celastrus paniculata* wild. The antioxidant potential of petroleum ether extract of *Celastrus paniculata* wild was studied by the DPPH assay, at different concentration, ranging from 50 to 1000 µg/ml. The antioxidant activity of these plant can be determined precisely, conveniently and quickly using DPPH testing. The DPPH antioxidant assay is based on the ability of DPPH a stable free radical, to decolorize in the presence of antioxidants. The percentage of inhibition was found to be the IC₅₀ value 343.65 µg/ml . DPPH is a stable free radical system which is applies an essential model by *in vitro* antioxidant evaluation. Further investigation on the isolation, characterizations and identification of antioxidant constituents in the plant may lead to chemical entities with potential for chemical use. The petroleum ether extracts obtained. Seeds of *C. paniculata* have been raw material for the synthesis of many drugs and thus remain an important source of new therapeutic agent. It is found that *C. paniculata* oil has a beneficial effect on the learning and memory process in mentally retarded children¹⁶

Table No.01 = Phytochemical profile of *C. paniculata*

S.No.	Experiment	Petroleum ether extract
1 Test for Triterpeneoids & Stereroids		
1.1	Libberman and Burchard's test	+++ve
1.2	Solkowski's test	+++ve
2 Test for Flavanoids		
2.1	Alkaline reagent test	+ve
2.2	Shinoda test	+ve
3. Test for Alkaloids		
3.1	Mayer 's reagent test	-ve
3.2	wagner's reagent test	-ve
3.3	Hager's reagent test	-ve
4. Test for Carbohydrates		
4.1	Molish's test	+ve
4.2	Barfoed' test	+ve
5. Test for Reducing sugar's		
5.1	Fehling's test	+ve
5.2	Benedict's	+ve
6. Test for proteins and amino acid		
6.1	Ninhydrin test	-ve
6.2	Biurest test	-ve
7. Test for Tannin and Phenolic compound		
7.1	Ferric chloride test	+ve
7.2	Lead Acetate test	+ve
8. Test for Saponin		
8.1	Faom Test	+ve
9. Test for Glycoside		
9.1	Bortrager's test	+ve
9.2	Keller- killiani test	+ve

Note:- +++ve = Higher percent of Phytocostituents ,
 +ve = Lower percent of Phytocostituents
 -ve =absence of Phytocostituents.

1. Free radical scavenging assay (DPPH assay)

Table 1 : % Inhibition of DPPH by Ascorbic acid

S. No.	Conc. (μ g/ml)	Absorbance (Control), A_c	Absorbance (Test), A_t	Inhibition	IC ₅₀ (μ g/ml)
1.	50	0.796	0.361	54.65	30.27
2.	100		0.329	58.67	
3.	250		0.27	67.71	
4.	500		0.217	72.74	
5.	1000		0.124	84.42	

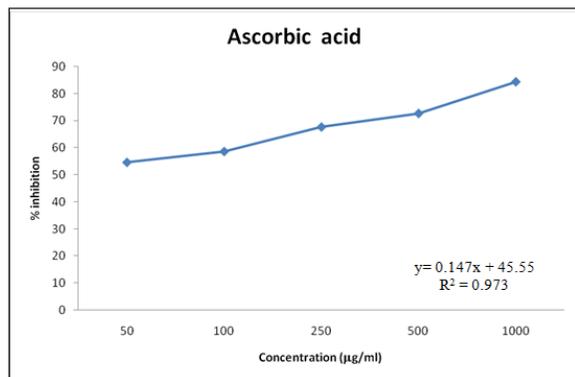


Fig.01: Standard curve of ascorbic acid. Graph represent regression curve of ascorbic acid by DPPH assay method

Table 2: % Inhibition of DPPH by Petroleum Ether Extract

S. No.	Conc. (μ g/ml)	Absorbance (Control), A_c	Absorbance (Test), A_t	Inhibition	IC ₅₀ (μ g/ml)
1.	50	0.796	0.735	7.66	343.65
2.	100		0.7014	11.93	
3.	250		0.653	17.96	
4.	500		0.577	27.51	
5.	1000		0.494	37.94	

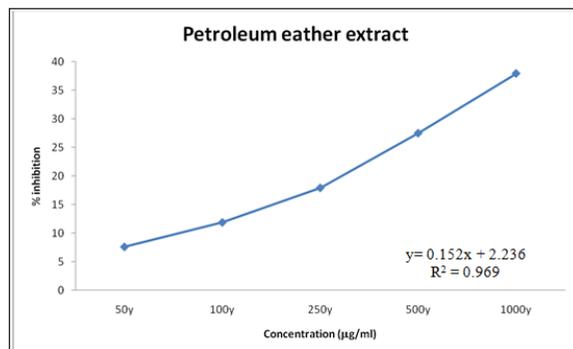


Fig.02: Graph represent regression curve of petroleum ether extract by DPPH assay method

CONCLUSION

Phytochemical screening as shown table no.1 reveal that petroleum ether extracts contain terpenoids, steroids, alkaloids, flavanoids, tannins, carbohydrate, amino acid, glycosides, phenoles compounds etc. which have been reported to be responsible for potential bioactive constituents. The plant extract of *Celastrus paniculata* has shown statistically significant result in their estimation of *in-vitro* antioxidant activity. The result obtained in the present study, indicates that the petroleum ether extract of *Celastrus paniculata* exhibits free radical scavenging. The plant extract of *Celastrus paniculata* has shown statistically significant result in their estimation of *in-vitro* antioxidant activity. The result obtained in the present study, indicate the petroleum ether extract of *Celastrus paniculata* extract exhibits free radical scavenging. The petroleum ether extract obtained from *C. paniculata* though successive solvent extraction in order to prove that the ethno pharmacological applications of the plant in Indian folk medicines. Phytochemical screening of *C. paniculata* is preliminary and important aspect. It is concluded from the data that petroleum ether extracts of *Celastrus paniculata* seeds exhibited significant role in medicinal chemistry for formulation of life saving drugs.

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