

Quantitative Determination of Total Phenolic Content in Stem Bark and Leaves Extracts of *Madhuca longifolia*

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Abstract: Total phenolic content (TPC) of *Madhuca longifolia* leaves and barks extracts were evaluated using the Folin- Ciocalteu method. This phenolics component is responsible for antioxidant activity. Gallic acid was used as a standard compound and the total phenols were expressed as mg / g gallic acid equivalents (standard curve equation: $y = 1.387x + 0.055$, $R^2 = 0.903$). The total phenol varied from 8.29 ± 0.52 to 32.54 ± 0.35 mg GAE /g. The highest concentration of phenols was measured in methanol and aqueous extracts. Total Phenol Content in plant extracts of the species of *Madhuca indica* stem bark and leaves depends upon the type of extracts and the polarity of solvent used in the extraction.

Keywords: *Madhuca longifolia*, total phenols, Folin- Ciocalteu reagent, Gallic acid.

Introduction

Madhuca indica is one of the most important Indian forest trees belonging to the family of Sapotaceae. It is found in abundance in the forests of Asian and Australian continents^{1,12,22,23} and it is a prominent tree in tropical mixed deciduous forests^{1,4} of West Bengal, Bihar, Orissa, Madhya Pradesh, Punjab and Uttar Pradesh^{15,5} and sub mountainous region of the Himalaya² in India. The flower have been traditionally used as cooling agent, tonic, aphrodisiac, astringent, demulcent and for the treatment of helminthes, acute and chronic tonsillitis, pharyngitis as well bronchitis³.

Madhuca longifolia leaves are expectorant and also used for chronic bronchitis and cushing's disease¹⁷. The distilled juice of the flower is considered a tonic, both nutritional and cooling and also in treatment of helminthes, acute and chronic tonsillitis, as well as bronchitis. The leaves are applied as a poultice to relieve eczema. The aerial parts are used for treatment of inflammation. The bark is a good remedy for itch, swelling, fractures and snake bite poisoning, internally employed in diabetes mellitus¹⁴. For bleeding gums stem bark is powdered and used as tooth powder for strengthening the gums. In diarrhoea a cup of infusion of bark is taken orally twice a day by the tribal. Besides the stem bark is used in chronic tonsillitis, fever, leprosy etc¹³.

Previous phytochemical studies on *madhuca indica* induced characterization of sapogenins, triterpenoids, steroids, saponins, flavonoids and glycosides¹⁴. The therapeutic value of the plant depends upon the active constituents present inside the different part of the plant which may be present in the small or large quantity¹⁸. The secondary metabolites are the important substance responsible for the main medicinal properties in the crude drugs²⁰. The leaves of Mahua tree contain saponin, an alkaloid and glucoside, sapogenin and other basic acid are found in the seeds. Thus it is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of the bacterial infection.

Besides this *M. indica* has antioxidants property, Antioxidants help the organisms in dealing with oxidative stress, caused by free radical damage. Free radicals are chemical species, which contains one or more unpaired electrons due to which they are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability. Reactive oxygen species (ROS) formed, such as superoxide anion, hydroxyl radical and hydrogen peroxide are highly reactive and potentially damaging transient chemical species. These are continuously produced in the human body, as they are essential for energy supply, detoxification.

Material and Methods

Aunthentication of Plant Material

The fresh plant parts stem bark and leaves of *Madhuca indica* were collected from the Mahatma Gandhi Chitrakut Gramodaya VishwaVidhyalay, U.P., India during the winter season in the month of October-November in 2012 and identified in the laboratory of the university.

Processing

Whole plant and leaves of *M.indica* were washed thoroughly with distilled water and then sun dried. After that sample was grounded finely to powder form, which was then used for extract preparation and analysis.

Extract Preparation

The extract of leaf and bark of *Madhuca indica* was prepared by Acetone, Methanol and Aqueous.

A. Aqueous Extract:

The Aqueous extract of leaf and bark was prepared by taken 100g of sample and 500ml of water in a beaker and left for 72 hrs. in a dark area.after that it was filtered by muslin cloth and it was kept on water bath untill the sample was concentrated 1/3rd.

B. Acetone Extract:

The Acetone extract of leaf and bark was prepared by taken 100g of sample and 500ml of Acetone in a beaker and left for 72 hrs. in a dark area.after that it was filtered by muslin cloth and it was kept on water bath untill the sample was concentrated 1/3rd.

C. Methanol Extract:

The Methanol extract of leaf and bark was prepared by taken 100g of sample and 500ml of Methanol in a beaker and left for 72 hrs. in a dark area.after that it was filtered by muslin cloth and it was kept on water bath untill the sample was concentrated 1/3rd.

Chemicals and Instruments

Folin- ciocalteu's phenol reagent, gallic acid, anhydrous sodium carbonate and methanol. UV spectrophotometer.

Preparation of Folin-Ciocalteu's Phenol Reagent

100 gm of sodium tungstate and 25gm of sodium molibedate were dissolved in 800 ml of water in a 1500 ml flask then 50ml of phosphoric acid and 100 ml HCl were added and refluxed for 10 hours. After cooling, 150 gm of lithium sulphate, 50 ml of water and 4 to 6 drops of bromine water were added and allowed to stand for 2 hours. The solution was boiled for 15 minutes and cooled before filtration. The reagent should have no greenish tint¹¹.

Procedure for determination of total phenolics contents

The amount of total phenolics in extracts was determined with the Folin- Ciocalteu reagent. Gallic acid was used as a standard and the total phenolics were expressed as mg/g gallic acid equivalents (GAE)⁴.A dilute extract of each plant extract (0.5ml of 1:10g/ml)or gallic acid is used as standard was mixed with Folin-Ciocalteu reagent (5ml,1:10 diluted with distilled water) and aqueous Na₂CO₃(4ml ,1M). The mixture was allowed to stand for 10min and absorbance was measured by calorimetrically at 765nm the standard curve was prepared using 0, 50, 100, 150, 200, 250mg of solutions of gallic acid in methanol water. All determination was performed in triplicate. The Folin-Ciocalteu reagent is sensitive to reducing compounds including polyphenols, thereby producing a blue colour upon reaction. This blue colour is measured spectrophotometrically. Thus total phenolic content can be determined^{19,14}.

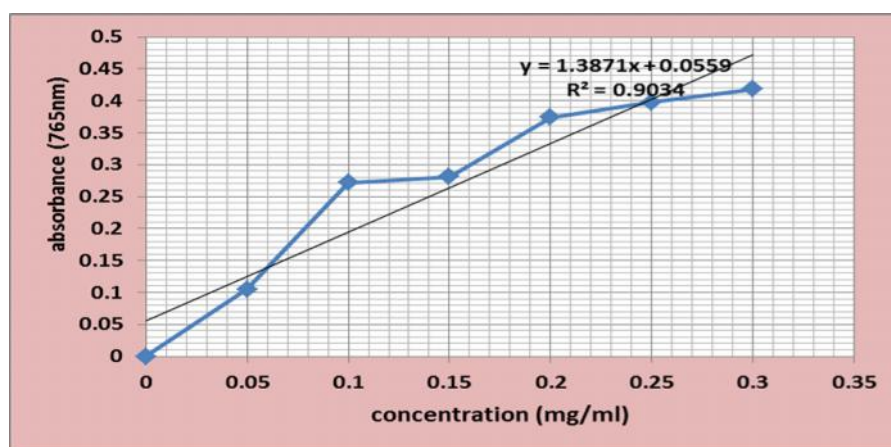
Table 1: Absorbance of Standard Compound (Gallic Acid)

X Concentration (mg/ml)	y-absorbance (Mean) $\lambda_{\max}=760$ nm
0	0
0.05	0.105
0.1	0.272
0.15	0.281
0.2	0.374
0.25	0.398
0.3	0.418

Table 2: Total Phenolic Content of *M indica* in Different Plant Extracts

Plant Parts	Sample extracts	Mean $\pm$ SD (mg GAE/g)
Bark	BAq	19.06 $\pm$ 0.15
	BM	32.54 $\pm$ 0.35
	BA	8.29 $\pm$ 0.52
Leaves	LAq	18.6 $\pm$ 0.20
	LM	32.5 $\pm$ 0.39
	LA	19.6 $\pm$ 0.24

Each value in the table was obtained by calculating the average of three experiments $\pm$ standard deviation

**FIG 1: Standard Gallic Acid**

Results and Discussion

80% of all the extracts of plant parts contained significantly highest amount of total phenol content (32.54 $\pm$ 0.35) and (32.5 $\pm$ 0.39) in stem bark and leaves methanol mg GAE/ g respectively. Generally total phenol content of extracts declined with decreasing polarity of the solvent used that is aqueous and acetone. The total phenol content examined in the plant extracts using the Folin- Ciocalteu's reagent is expressed in terms of gallic acid equivalent (the standard curve equation($y=1.3871x+0.0559$; $R^2 = 0.9034$). Where y is absorbance at 760 nm and x is total phenolic content in the different extracts of *Madhuca indica* expressed in mg/gm. Phenolic compounds are a class of antioxidant agents which acts as free radical terminators¹². Table.1 shows the variation of mean absorbance with concentration of Gallic acid. Table.2 shows the contents of total phenols that were measured by Folin Ciocalteu reagent in terms of gallic acid equivalent. The total phenol in the examined extracts ranged from 8.29 $\pm$ 0.52 to 32.54 $\pm$ 0.35 mg GAE /g. The highest concentration of phenols is measured in methanolic and aqueous extracts. Acetone extracts contains smaller concentration of phenols. The total phenol content in plant extracts of the species of *Madhuca indica* stem bark and leaves depends upon the type of extracts and the polarity of solvent used in extraction. High solubility of phenols in polar solvent provides high concentration of these compounds in the extracts obtained using polar solvents for the extraction.

Conclusion

The amount of total phenols was determined with the Folin-Ciocalteu reagent. Gallic acid was used as a standard compound and the total phenols were expressed as mg/g gallic acid equivalents. The maximum phenolic content was found in the aqueous extract (325.1 ± 0.39 mg/g) and (325.53 ± 0.39) in stem bark and leaves methanol GAE mg/ g respectively. The result of the present study showed that the extract of *Madhuca longifolia*, which contain highest amount of phenolic compounds which exhibited the greatest antioxidant activity. The high scavenging property of *Madhuca longifolia* may be due to hydroxyl groups existing in the phenolics compounds. Free radicals are often generated as by products of biological reactions or from exogenous factors. The involvements of free radicals in the pathogenesis of a large number of diseases^{9,6}.

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