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Qualitative determination and Quantitative estimation of phytochemicals in *Madhuca latifolia* Roxb.

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Abstract

In the present study secondary phytoconstituents of *Madhuca latifolia* Roxb were qualitatively determined and quantitatively estimated. Qualitative determination was carried out in leaves, stem bark and root bark. The chemical extraction was done using different solvents likes acetone, ethanol, chloroform and water. These plant parts showed the presence of secondary phytochemicals such as saponins, alkaloids, glycosides, steroids, phenols, terpenoids, flavonoids and tannin. The estimated quantity of total alkaloid was highest in leaves 238 mg/g and lowest in stem barks 026 mg/gm. The quantity of total flavonoids and total saponins estimated was more in leaves 297 mg/gm and 221mg/g as compare to stem barks and root barks. The result of the present investigation supports the traditional as well as pharmacological uses of this plant in preparing drug, chemicals and therapeutic agent for various ailments.

Plants are the natural resources of drug and medicine and are being practices since ancient time for the treatment of different type of ailments[5]. Medicines obtained from plant source are known as an herbal medicine, and the herbal medicine are one which make human healthy without causing any of adverse effect. According to World Health Organization report approximately total 80% people of the world fulfill their primarily treatment by phytopathy[14]. Medicinal properties of plants are due to the presence of secondary metabolites and generally secondary metabolites are biosynthetically derived from primary metabolites and they serve as medicinal drug and protestant against various pathogenic microbes. Different secondary phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases. For example, alkaloids protect against chronic diseases. Saponins protect against hypercholesterolemia and

antibiotic properties. Steroids and triterpenoids show the analgesic properties[13].

Madhuca latifolia Roxb. commonly known as Mahua is frost resistant tree from the dry tropics and sub tropics belongs to family sapotaceae[1] common in deciduous forest and dry soil forests. The tree is usually found in scattered in pastures and cultivated field in central India and distributed mainly in Andhra Pradesh, Bihar Chhattisgarh, Madhya Pradesh, Odisha, and Uttar Pradesh. *M. latifolia* (Mahua) is commonly known for its commercial properties such as for food fodder and fuels. The crude extracts of plant also has high ethanomedicinal uses such as in Anti-diabetic, Snake-bite poisoning[9] Antibacterial[17] Rheumatisms, Anti- Ulcer, Bleeding, Skin diseases, Anti-inflammatory[15]. The present study deals with the qualitative and quantitative estimation of secondary in present *M. latifolia* Roxb. This study will provide information regarding quality and quantity of secondary metabolites present in the various plant parts of *M. latifolia* (Mahua).

For the preparation of the manuscript relevant literature[1-18] has been consulted.

Collection and identification of plant material :

The fresh plant parts such as leaves, root bark and stem bark of *Madhuca latifolia,* were collected from nursery of forest department Baktara near Madir hassaud Raipur Chhattisgarh, India during the winter season in the month of January- February in 2016. The plant was identified with help of flora[6].

Processing :

Leaf, stem bark and root bark of *Madhuca latifolia* were shade dried and grinded finely through mechanical grinder into powder form. These were then used for various extract preparations.

Preparation of extract :

The extract of leaf, stem bark and root bark of *Madhuca latifolia* was done in acetone, ethanol, chloroform and water separately with the help of soxhlet apparatus. For this 20 g of plant part powder was taken for 200ml of acetone, ethanol, chloroform and water was taken separately for extraction in different solvents. After extraction, the extracts were filtered through Whatman No. 41 filter paper. The concentrated extracts were subjected to qualitative test for the determination of various phytochemical constituents as per standard procedures[7,8,18].

Preparation of reagent :

All the reagent including Dragendorff's, Wagner's, Mayer's were prepared according to available literature [Flinn scientific, 2011]

Qualitative determination of secondary phytoconstituents :

Test for alkaloids :

i). Dragendorff's test: The extracts were treated with few drops of Dragendorff's reagent orange brown precipitate indicates the presence of alkaloids.

ii). Wagner's test: The extracts were treated with few drops of Wagner's reagent reddish brown precipitate indicates the presence of alkaloids.

iii). Mayer's test: The extracts were treated

with few drops of Mayer's reagent white or pale precipitate indicates the presence of alkaloids.

iv). Hager's test: The extracts were treated with Hager's reagent, appearance of yellow colour precipitate indicates the presence of alkaloids.

Test for Flavonoids :

i). Shinoda test: Ethanolic extracts of sample taken in test tube and added four pieces of magnesium ribbon and followed by concentrated HCl was added drop wise. Appearance of colour after few minutes orange to red indicated flavones, red to crimson indicated Flavonoids, crimson to magenta indicated flavonones.

ii). Sodium hydroxide test: 2 ml of concentrated extracts were added with 2 ml of the 10% aqueous sodium hydroxide solution produce yellow colouration. Change in colour from yellow to colourless on addition of dilute hydrochloric acid which indicates the presence of flavonoids.

iii). Zn test: 2 ml extracts were treated with Zn dust and conc. HCl development of red colour indicates presence of Flavonoid.

Test for Phenols :

i). Ferric chloride test: Concentrated extracts were treated with 2ml of water and 10% aqueous ferric chloride solution. An appearance of blue colour indicates presence of phenol.

ii). Lead acetate test: Concentrated extracts were treated with about 1% solution of gelatin and 10% NaCl. Formation of white precipitate indicates presence of phenols.

Test for Saponins :

i). Foam test: 5ml of filtrate was diluted with 20ml of water and was vigorously shaken. The test tube was observed for the presence of stable foam upon standing.

Test for Steroids :

Liebermann Burchardest test: Concentrated extracts were added with 2ml of acetic anhydride and 1 ml of concentrated sulphuric acid development of colour bluish to green indicated the presence of steroids.

Test for Terpenoids :

Salkowski test: 2 ml of each extracts was mixed with 2ml of chloroform and concentrated H_2SO_4 (3ml) was carefully added to form a layer. An appearance of red brown colour indicated the presence of terpenoids.

Test for Tannins :

i). Alkaline reagent test: Extracts was treated with 10% NaOH solution formation of intense yellow colour indicated the presence of tannins.

ii). Ferric chloride test: Extracts was treated with 2ml of water and 5% aqueous ferric chloride solution formation of green colour indicated presence of tannins.

iii). Gelation test: Extract was treated with aqueous solution of gelatin and added sodium chloride, white buff colour precipitate indicated the presence of tannins.

Test for Cardiac glycosides :

i). Keller-Killani test: Extracted was treated with 1ml of $FeCl_3$ reagent (mixture of 5% of $FeCl_3$ and 99 volume of glacial acetic acid). To this solution few drop of cons. H_2So_4 was added appearance of greenish blue color within few minutes indicated the presence of cardiac glycosides.

ii). Legal test: Extract is treated with few ml of pyridine add 2 drop of nitroprusside and 1 drop of 20% sodium hydroxide solution deep red colour indicated the presence of cardiac

glycosides.

Quantitative estimation of secondary Phytoconstituents :

Alkaloid estimation by using Harborn method[7]

5g of each plant parts sample was weighed separately into a 250 ml capacity beaker and added 200ml of 10% solvent of acetic acid in ethanol then the beaker was covered to check evaporations of solvent and allowed to stand for 4 hour. This was filtered and extracts was concentrated on water bath to ¼ of original volume. After this cons. ammonium hydroxide was added drop wise into concentrated extracts until the precipitation was completed. The solution was allowed to settle the precipitate and filtered. Filtered precipitate washed with dil. ammonium hydroxide and then again filtered. This precipitate residue was alkaloid which was further dried and weighed.

Weight of total alkaloids: $\frac{W2 - W1}{W3}$ g

Where, W1 = weight of crucible, W2 = weight of crucible with alkaloids, W3 = initial weight of plant sample taken for estimation.

Flavonoids estimation by using Boham and Kocipai *Method*[4]

10g of each plant parts samples were weighed separately into a 250ml capacity beaker, to this 100ml of 80% aqueous methanol was added for extraction at room temperature. The extract was filtered through Whatman No. 42 (125mm) filter paper and was collected separately in 500ml capacity beaker. Extraction procedure was repeated four times for the same sample. The extracts were transferred every time in same 500ml capacity beaker. Collected filtrate was then transferred to crucible and evaporated till dryness on water bath and then the dried extract was weighed.

Weight of total flavonoids: $\frac{W2 - W1}{W3}$ g

Where, W1 = weight of crucible, W2 = weight of crucible with flavonoids, W3 = initial weight of plant sample taken for estimation.

Saponin estimation by using Nahapetian and Bassiri *Method*[12]

Suspension was prepared of 10g of each plant parts sample in 200ml of 20% ethanol separately. These sample suspensions were heated over water bath for 4 hour at 55ºC with continuous stirring. These samples were filtered and each extracts collected separately in 500ml capacity of beaker. Obtained residues re- extracted with 100ml of 20% ethanol. Combine extracts heated over water bath at about 90 till volumes were reduced to 40ml. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 30 ml of n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the sample were dried in

the oven and weighted.

Weight of total saponins: $\frac{W2 - W1}{W3}$ g

Where, W1 = weight of crucible, W2 = weight of crucible with saponins, W3 = initial weight of plant sample taken for estimation.

In screening process alkaloids, tannins, saponins, flavonoids and terpenoids, glycosides, phenols showed variation in different solvents such as ethanol, acetone, chloroform and water (table-1) in various plant parts viz. leaves, stem barks and root barks. Water extract of plant parts revealed the presence of Alkaloid, Phenol, Saponins, Tannin, Terpenoids and sodium hydroxide test of Flavonoids was slightly positive for leaf extract. Acetone extracts showed the presence of Alkaloid, Flavonoid, Phenol, Tannin, Terpenoids and cardiac glycosides. In ethanol extracts of plant parts showed the presence of highest number of secondary phytochemicals such as Alkaloid, flavonoids, Phenol, Saponins, Tannin, Terpenoids and cardiac glycosides. Very few phytochemicals were reported in chloroform extracts such as Terpenoids, steroids but killer killani test of cardiac glycosides and gelation test of tannins was slightly positive for chloroform extract of root bark. Quantitative estimation of secondary phytoconstituents were carried out for shade dried powdered materials of various plant parts such as leaves, stem barks and root barks through various standard methods. It was found that the quantity of estimated alkaloid was 238 mg/g, 026 mg/g and 166 mg/g, flavonoids 297mg/g, 141 mg/g and 162 mg/g, saponins 221 mg/g, 139 mg/g and 049mg/g in leaves, stem barks and root barks respectively. Estimated total alkaloid yield percentage was found to be highest in leaves 23.8 % followed by root barks and then stem barks percentage of flavonoids also more in leaves 29.7 % and followed by stem barks. Highest percentage of saponins was reported 22.1% in leaves as compare to stem barks and root barks (table 2).

These secondary metabolites of plant have wide range of pharmacological value and are used to prepare drug and cosmetics. Alkaloids are used to reduce blood pressure, as analgesic and show bactericidal effects. Flavonoids were also found to be present in all the extracts of the plant parts. They are known to show anti-cancerous effect basically gastric carcinoma and aerodigestive tract cancer, detoxifying Agent and as inhibitors of tumourogenesis. Saponins were found to present in the extracts of all the plant parts. Saponins are known to show anti-inflammatory, hypercholesterolemia[11] and anti-obesity properties. The extracts of the plant parts of *Madhuca latifolia* Roxb showed presence of terpenoids, phenol, tannins and cardiac glycosides. Most of terpenoids has potent biological activities in anti-aging and overall beauty enhancement[10]. Phenol and Tannins both has antimicrobial properties. Despite of this phenol is also used as emulsifying agent in medicine[2]. It also has antioxidant[16], anti-inflammatory and cytotoxic property. Tannins are known to be used as anti-irritants. Cardiac glycosides were also determined in *Madhuca latifolia* Roxb. which has therapeutic uses in cardiac disease. Secondary bioactive chemical compound have potential activity against various microbes such as fungus, bacteria, protozoa, mycoplasmas etc. this bioactive chemical play vital role as protectant[3].

Table-1. Table showing qualitative determination of secondary metabolites in leaves, stem bark and root bark of *Madhuca latifolia* Roxb.

S. No.	Secondary Metabolites	Test	Plant leaves, stem bark, and root barks with various chemicals extract											
			Aqua. Extracts			Acetone extracts			Ethanol extracts			Chloroform extracts		
			A	B	C	A	B	C	A	B	C	A	B	C
1.	Alkaloids	Wagner's	-	-	-	-	+	+	+	+	+	-	-	-
		Mayer's	+	-	+	+	+	+	+	+	+	-	-	-
		Hager's	+	+	+	+	+	+	+	+	+	-	-	
2.	Flavonoids	Shinoda	-	-	-	+	+	+	+	+	+	-	-	-
		NaOH	+	-	-	-	-	-	+	+	+	-	-	-
		Zn test	-	-	-	+	+	+	+	+	+	-	-	-
3.	Phenols	Ferric chloride	+	+	+	+	+	+	+	+	+	-	-	-
		Lead acetate	+	+	+	+	+	+	+	+	+	-	-	-
4.	Saponins	Foam	+	+	+	-	-	-	+	+	+	-	-	-
5.	Steroids	Liebarmann's	-	-	-	-	-	-	-	-	-	+	+	+
6.	Tannins	Ferric chloride	+	+	+	+	+	+	+	+	+	-	-	-
		Alkaline	-	-	+	+	-	-	+	+	+	-	-	-
		Gelation	+	+	-	-	+	+	+	+	+	-	-	+
7.	Terpenoids	Salkowski	+	+	+	+	+	+	+	+	+	+	+	+
8.	Cardiac glycosides	Keller-Killani	-	+	-	-	-	-	+	+	+	+	+	+
		Legal test	-	+	+	+	+	+	+	+	+	-	-	-

KEY: A = Indication for leaves, B = Indication for stem bark, C = Indication for roots bark
(+)Ve Present and (-) Ve Absent

Table-2. Quantitative estimation of leaves, stem barks and root barks of Madhuca latifolia Roxb.

Plant parts	Fresh weight in gm	Dry weight in gm	% of dried material	extracted Alkaloid in each 5 gm	% Yield of Total Alkaloid	extracted Flavonoid in each 10 gm	% Yield of Total Flavonoid	extracted Saponin in each 10 gm	% Yield of Total Saponin
Leaves	100	55.81	55.81	1.19	23.8	2.97	29.7	2.21	22.1
Stem Barks	100	45.42	45.42	0.13	2.6	1.41	14.1	1.39	13.9
Root barks	200	65.36	32.68	0.83	16.6	0.81	8.1	0.49	4.9

Each value of extracted secondary metabolites is presented as mean (n = 3).

$$\% \text{ Yield calculated by formula} = \frac{\text{Extracted weight of particular secondary metabolites}}{\text{total wieght used for extraction}} \times 100$$

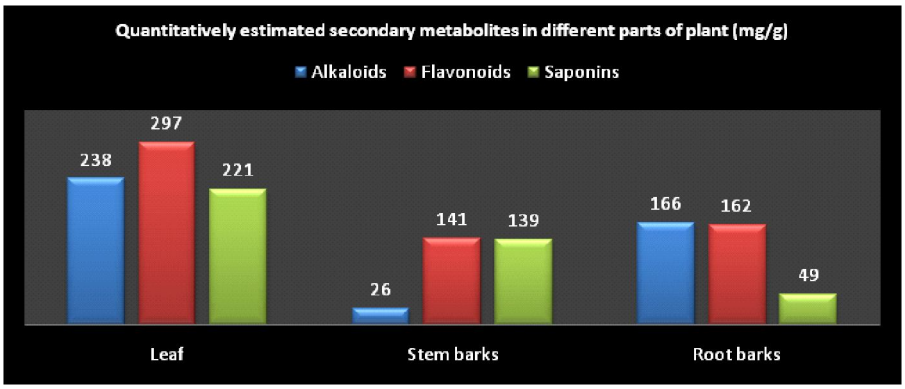

Fig.1: Comparative bar of qualitatively estimated Secondary metabolites in different plant parts (mg/g).

(a) *Madhuca latifolia* Roxb.

(b) Leaf

(c) Stem bark

(d) Root bark

Fig. 2: *Madhuca latifolia* Roxb. and their selected plant parts for qualitative and quantitative estimation.

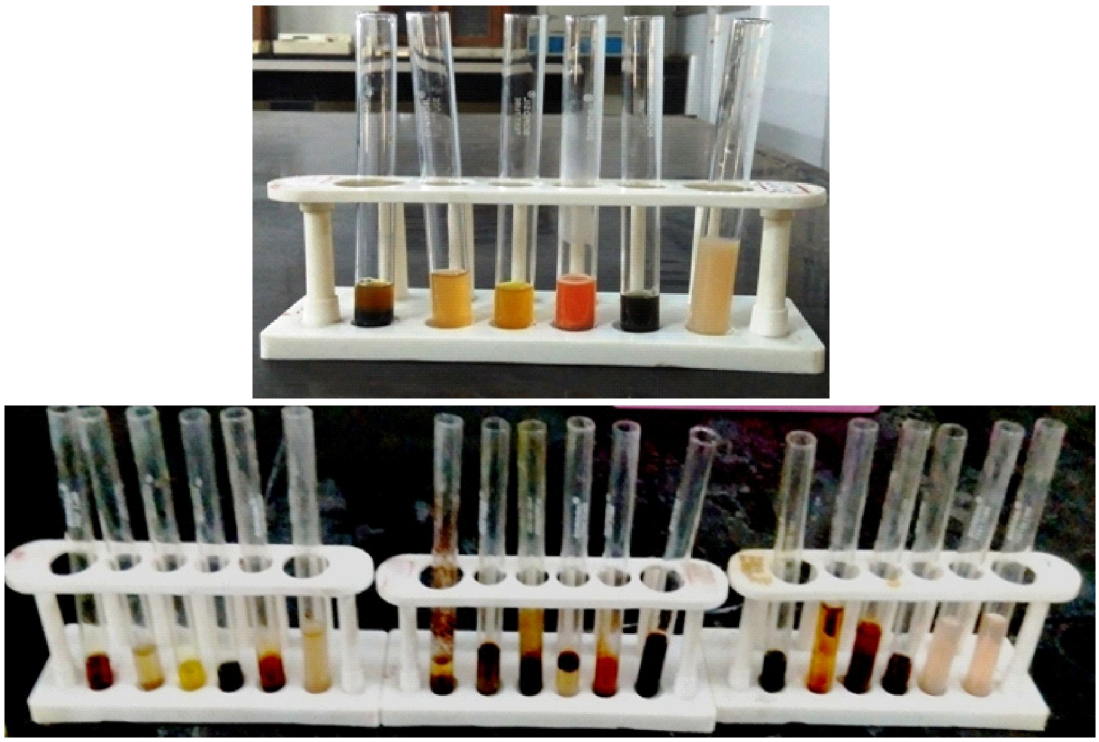

Fig.3: Qualitative phytochemical determination in different plant parts of *Madhuca latifolia*

The results of qualitative determination and quantitative estimation of *Madhuca latifolia* Roxb. showed the presence of wide varieties of secondary metabolites including Saponins, Alkaloid, Steroids, Phenolic compound, Tannins, Glycosides, Terpenoids, Flavanoids. Thus it can be concluded that *Madhuca latifolia* Roxb. seemed to have the potential to act as a source of useful drugs due to the presence of various compounds present in different plant parts that are vital for good health.

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