Phytochemical Analysis and TLC Profile of *Madhuca indica* Inner Bark Plant Extract

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Abstact – *Madhuca indica* is a plant of Indian origin having tremendous therapeutic potential but is not fully utilized. The present study, primarily aims to carry out a preliminary phytochemical screening so as to detect the major class of compounds present in M. indica inner bark and to perform thin layer chromatography (TLC) profiling of all sequential extracts. Phytochemical analysis was performed by various qualitative methods and TLC profiling was carried out using various extracts of hexane, chloroform, ethyl acetate, acetone and methanol. Thesolvent system of varying polarity, hexane, ethyl acetate, and acetic acid respectively. Qualitative phytochemical analysis reflects the presence of alkaloids, glycosides, saponins, phenolic compounds, amino acids, and carbohydrates in the plant extract. TLC profiling of the M. indica was constituted different coloured phytochemical compounds with different Rf values. The ethyl acetate and acetone extracts in the drug is carried out to establish the biomarker compound. The present study provides evidence that solvent extract of *M. indica* contains medicinally important bioactive compounds and this justifies the use of plant species as traditional medicine for treatment of various diseases.

Index Terms – *Madhuca indica*, inner bark, phytochemical, TLC, Rf value.

1 INTRODUCTION

The substantial proportions of the population of India have been using traditional medicines since many centuries (Amit Pandey and Parul Singh 2011). The World Health Organization (WHO) estimated that 80% of the populations of developing countries rely on traditional medicines, mostly plant drugs, for their primary health care needs (Prabakaran *et al.*, 2011). Medicinal plants are believed to be an important source of new chemical substances with potentialtherapeutic effects. Herbalism is a traditional medicinal or folk medicine practice based on use of plantsand plant extracts (Acharya and shrivastava, 2008). Plants are being an effective source of both traditional and modern medicines are genuinely useful for primary healthcare. The medicinal plants produce wide range array of bioactive molecules and rich source of medicines. (Agharkar, 1991).

Madhuca Indica is a large, shady deciduous tree both wild and cultivated, found indifferent parts of India (Kirtikar and Basu, 1987). The medicinal properties attributed to this plant are stimulant, demulcent, emollient, heating and astringent (Awashthi and Mitra, 1967). The bark is good remedy for itch, swelling, fracture and snake bite poisoning, internally employed in diabetes mellitus (Khaleque *et al*., 1969). Its bark is used to cure leprosy and wounds (Shruthi *et al*., 2010).

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Madhuca indica Plant inner bark was selected for this study is based on its traditional medicinal use (Rajendra Prasad Gujjeti and Estari Mamidala, 2012). The purpose of the present study isto investigates the phytochemical analysis and TLC profile of different extracts of *Madhuca indica* inner bark. The obtained results provide a support for the use ofthis plant in traditional medicine and its further investigation and isolation of Phytoconstituents.

2 MATERIAL AND METHODS

2.1 Plant collection

Plant was selected for this study is based on its traditional medicinal use. Fresh inner bark was Collected from the Chintoor mandal, Khammam district of Andhra pradesh, India, in the month of September 2012. The plant voucher specimens identification was done with the help of Prof.Vastsavaya.S.Raju Department of Botany Kakatiya University, Warangal and the same was deposited at Infectious Diseases & Metabolic Disorders Research Lab, Department of Zoology, Kakatiya University, Warangal.

2.2 Preparation of plant extract

After collection of plant material the inner bark sample were dried at room temperature until they were free from moisture. The bark subjected to size reduction to get coarse powder was then stored in a clean dry air tight container.The air dried bark (200gr) of *M. Indica* powder was subjected to maceration using different solvents(hexane, chloroform, ethyl acetate, acetone, and methanol etc) for seven days. The extract was filtered reddish brown syrupy mass was obtained and it was finally dried at low room temperature under pressure in a rotary vaccum evaporator (Thermotech, buchi type model th-012).

2.3 Preliminary Phytochemical Tests

The extracts obtained were subjected to qualitative tests the identification of various plant constituents. Tests for alkaloids, carbohydrates, glycosides, standard procedures to identify the constituents as described by standard methods (Horbone,1983).

2.4 TLC Profile

For TLC analysis plate with Silica gel 60 F254 TLC (Merck, Germany), 7X6 cm was cut with ordinary household scissors. Plate markings were made with soft pencil. Glass capillaries were used to spot the sample for TLC applied sample volume 1-µl of sample by using capillary at distance of 1 cm at 5 track, by the different solvent system Hexane : Acetic acid (9:1) solvent system I, In solvent system II-Hexane : Ethyl acetate: Acetic acid (5:4:1), In solvent system III- Hexane : Ethyl acetate : Acetic acid (4:4:2), In solvent system IV-Hexane : Ethyl acetate : Acetic acid (3:6:1), In solvent system V-Hexane:Ethyleacetate:Aceticacid (2:7:1) used. After presaturation with mobile phase for 20 min for development were used. Freshly prepared iodine spray reagents were used to detect the bands on the TLC plates. The movement of the analyte was expressed by its retention factor (R_f), values were calculated for different samples.

$$R_f = \frac{\text{Distance traveled by the solute}}{\text{Distance traveled by the solvent front TLC plates}}$$

3 RESULTS

3.1 Percentage of yield extract

The yield of sequential extracts (g) is shown in [Table 1]. The amount obtained from hexane, chloroform, ethyl acetate, acetone and methanol extracts are 7.100 gm, 6.380 gm, 6.050 gm, 4.930 gm, and 6.480 gms respectively.

3.2 Phytochemical analysis

Phytochemical screening of the sequential extract of *M. indica* revealed the presence of various bioactive components of which phenolics, saponins, alkaloids, tannin, Glycosides, Proteins, Carbohydrates, and Amino acids are the most prominent components and the result of phytochemical test is presented in [Table 2].

Among these phytochemical tests, Alkaloids, Glycosides, Saponins were present in all solvent extracts where as phenolic compounds and tannins were present in Hexane and Methanol extracts, carbohydrates were found in hexane, acetone, and methanol extracts, amino acids were present in ethyl acetate, acetone, and methanol extracts. Phytosterols are absent in all solvent extracts.

3.3 TLC Profile

TLC of all sequential extracts of *M. indica* obtained by sequential extraction methods was carried out to confirm its nature by analysing TLC chromatograms and to isolate active ingredients from the extracts. TLC of hexane extract of *M.indica* revealed the presence of 4 spots having R_f values of 0.14, 0.25, 0.40 and 0.75 respectively when a solvent phase of Hexane: Acetic acid (9:1) solvent system I was used. In solvent system II-Hexane: Ethyl acetate: Acetic acid (5:4:1), two spots were obtained having R_f of 0.69 and 0.84 respectively. In solvent system III Hexane: Ethyl acetate: Acetic acid (4:4:2), 3 spots were obtained having R_f of 0.23, 0.56 and 0.98 respectively. In solvent system IV-Hexane : Ethylacetate : Acetic acid (3:6:1), 1 spot is obtained having R_f of 0.86, and In solvent system V Hexane : Ethyleacetate : Acetic acid (2:7:1), 1 spot is obtained having R_f of 0.83.

TLC of chlorofom extract of *M.indica* revealed the presence of 6 spots having R_f values of 0.20, 0.26, 0.33,0.43, 0.60 and 0.71 respectively when a solvent phase of Hexane:Acetic acid (9:1) solvent system I was used. In solvent system II, three spots were obtained having R_f of 0.60, 0.61 and 0.90 respectively. In solvent system III, 4 spots were obtained having R_f of 0.10, 0.30, 0.75 and 0.96 respectively. In solvent system IV, 3 spots were obtained having R_f of 0.25,0.73 and 0.93 In solvent system V, 1 spot is obtained having R_f of 0.90.

TLC of Ethyl acetate extract of *M.indica* revealed the presence of 6 spots having R_f values of 0.26, 0.33, 0.40,0.53, 0.83 and 0.88 respectively when a solvent phase of Hexane:Acetic acid (9:1) solvent system I was used. In solvent system II, three spots were obtained having R_f of 0.16,0.66 and 0.91 respectively. In solvent system III, 4 spots were obtained having

Table 1 :- Extractive values of different extracts of *MadhucaIndica* inner bark

S.No	Solvent	Colour of extract	Yield of the extract (in gm)	Percentage yield(%w/w)
1	Hexane	Dark red	7.100	3.55%
2	Cholroform	Dark red	6.380	3.19%
3	Ethyle acetate	Brown	6.050	3.025%
4	Acetone	Dark red	4.930	2.465%
5	Methanol	Dark red	6.480	3.240%

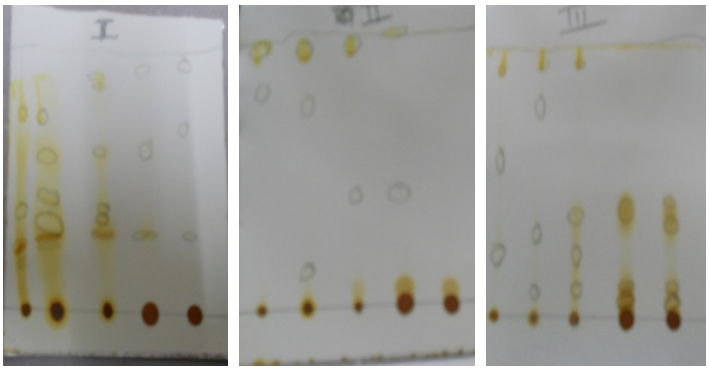
Table 2: Qualitative Phytochemical analysis of the different extracts of *Madhuca indica* inner bark

S.No	Phytoconstituents	Tests	Hexane	Chloroform	Ethyle acetae	Acetone	Methanol
1	Alkaloids	Hager's test	+ve	+ve	+ve	+ve	+ve
2	Corbohydrates	Fehilings test	+ve	-ve	-ve	+ve	+ve
3	Glycosides	Borntrager's test	+ve	+ve	+ve	+ve	+ve
4	Saponins	Froth forming test	-ve	+ve	+ve	+ve	+ve
5	Phenolic compounds	Lead acetate test	+ve	-ve	-ve	-ve	-ve
6	Tanins	$Fecl_{3\ test}$	-ve	-ve	-ve	-ve	+ve
7	Phytosterols	Libermann-Buchard test	-ve	-ve	-ve	-ve	-ve
8	Protiens	Biuret test	+ve	-ve	-ve	-ve	-ve
9	Aminoacids	Ninhydrin test	-ve	-ve	+ve	+ve	+ve

0.08, 0.23, 0.30 and 0.91 respectively. In solvent system IV, 4 spots were obtained having R_f of 0.03, 0.25, 0.63 and 0.91 In solvent system V, 2 spots are obtained having R_f of 0.66 and 0.93 (Figure 1 & 2).

TLC of Acetone extract of *M.indica* revealed the presence of 3 spots having R_f values of 0.30, 0.60, and 0.91 respectively when a solvent phase of Hexane: Acetic acid (9:1) solvent system I was used. In solvent system II, three spots were obtained having R_f of 0.08, 0.41 and 0.95 respectively. In solvent system III, 3 spots were obtained having R_f of 0.05, 0.16 and 0.40 respectively. In solvent system IV, 5 spots were obtained having R_f of 0.03, 0.25, 0.30, 0.33 and 0.63 In solvent system V, 4 spots are obtained having R_f of 0.38, 0.50, 0.75 and 0.96 (Figure 1 & 2).

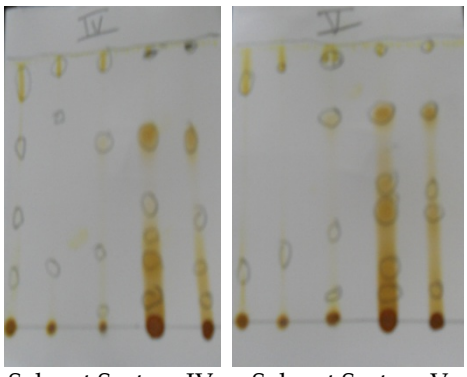
Fig:1 TLC profile of different extracts of *M. indica* inner bark



Solvent System-I Solvent System-II Sovent System-III

TLC of Methanol extract of *M. indica* revealed the presence of 3 spots having R_f values of 0.33, 0.71, and 0.96 respectively when a solvent phase of Hexane:Acetic acid (9:1) solvent system I was used. In solvent system II, 1 spot is obtained having R_f of 0.08. In solvent system III, 4 spots were obtained having R_f of 0.05, 0.16, 0.33 and 0.40 respectively. In solvent system IV, 4 spots were obtained having R_f of 0.30, 0.41, 0.58, and 0.66 In solvent system V, 3 spots are obtained having R_f of 0.36, 0.73, and 0.96 (Figure 1 & 2).

Fig:2 TLC profile of different extracts of *M. indica* inner bark



Solvent System-IV Solvent System-V

4. DISCUSSION

For the pharmacological as well as pathological discovery of novel drugs, the essential information regarding the chemical constituents are generally provided by the qualitative phytochemical screening of plant extracts (Talukdar *et al*., 2010).

In the present study, qualitative tests for all five extracts showed significant indication about the presence of metabolites. Alkaloids, Glycosides, and Saponins, were found to be present in the all the sequential extracts of *M. indica* while Phenolic compounds and Tannins were present in very low amounts in the extracts of hexane and methanol. These findings of phytochemicals were good enough to reflect its importance.

TLC profiling of all 5 extracts gives an impressive result that directing towards the presence of number of phytochemicals. Various phytochemicals gives different R_f values in different solvent system. This variation in R_f values of the phytochemicals provides a very important clue in understanding of their polarity and also helps in selection of appropriate solvent system for separation of pure compounds by column chromatography. Mixture of solvents with variable polarity in different ratio can be used for separation of pure compound from plant extract. The selection of appropriate solvent system for a particular plant extracts can only be achieved by analysing the R_f values of compounds in different solvent system. Different R_f values of the compound also reflect an idea about their polariy. This information will help in selection of appropriate solvent system for further separation of compound from these plant extracts.

5. CONCLUSION

The results obtained in the present investigation indicated *M. indica* inner bark as a rich source of secondary metabolites. These findings suggested that *M.indica* could be a potential source of natural antioxidant having great importance as therapeutic agent and preventing oxidative stress related degenerative diseases. The inner bark of *M. indica* can provide lead molecules which could be useful substrate for the synthesis of new broad spectrum antibiotics for the treatment of infections caused by the organisms. Further purification, identification and characterization of the active compounds would be our priority in future studies.

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