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Research Article

PRELIMINARY PHYTOCHEMCIAL SCREENING OF LEAF EXTRACT OF MULBERRY (*MORUS INDICA*) FROM CHHATTISGARH

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ABSTRACT: Mulberry **(***Morus Indica)* belongs to family *Moraceae* is a perennial tree, it is used as a traditional medicine and modern drug preparation, mainly constitutes diet for the silk worm. The preliminary phytochemcial screening of three extracts (methanolic, ethanolic, and aqueous) of Mulberry leaves revealed that it contains Alkaloids, Steroids, Reducing sugars, Tannins, Phenols, Flavonoids, Proteins and Amino acids, Terpenoids, Cardiac glycosides which give the medicines several healing properties. The separation of the bioactive compounds like flavonoids was carried out using thin layer chromatography (TLC).Total Flavonoid content (TFC), Total Phenol Content (TPC), and Total tannin content (TTC) were calculated by standard graph of Lambert -Beer law using Double Beam Spectrophotometer (Systronics).

Key words: *Morus indica*, Phytochemcials, Spectrophotometer, Thin Layer Chromatography

INTRODUCTION

Phytochemicals are bioactive chemicals of plant origin. They are regarded as secondary metabolites because the plants that manufacture themselves may have little need for them. They are naturally synthesized in all parts of the plant body; bark, stem, root, flower, fruits, seeds, leaves, etc. (Tiwari, Kumar and Kaur, 2011). The quantity and quality of photochemical present in plant parts may differ from one part to another. (M. Lahlou, 2004).

Phytochemicals are organic substances and could be obtained in both primary and secondary metabolic process; they also provide a source of medicine since the earliest time. The plant kingdom has proven to be the most useful in the treatment of diseases and they provide an important source of all the world's pharmaceuticals. The most important bioactive constituents of plant are steroids, flavonoids, alkaloids, tannins, terpenoids, glycosides, etc. Antibiotics or antibacterial substances like saponins, glycosides, flavonoids, and alkaloids etc, are found to be distributed in plants. (Edeoga & Okwu, 2005) and (Hafiza, Parveen, Ahmed & Hamid, 2002). From these phytoconstituents, saponins have been reported to exhibit hemolytic and foaming activity, antifungal, anti-inflammatory, fungistatic, molluscidal (Feroz &Nagata, 1985), (Takagi & Zehavi, 1986). The presence of a phytochemical of interest may leads to its further isolation, purification, and characterization. Then it can be used as the basis for a new pharmaceutical product. (Das, Shrivastava and Tiwari, 2010). The mulberry (Morus spp.) is an important tree in the sericultural industry because it's leaves constitute the sole source of food for the Mori silkworm (Bombyx mori). Qualitative and quantitative improvements in mulberry varieties play a vital role in industrial advances. Mulberry belongs to the Moraceae family and to the genus Morus. It is a perennial tree or shrub as an economically important plant. The economic importance of mulberry is due to it's leaf, it has been estimated that nearly 60% of the product cost of silkworm cocoon is incurred by mulberry leaf production (Das & Krishnaswami, 1965). Mulberry leaves containing more water, total sugar and soluble carbohydrate and less mineral are best relished by silkworms. Nutritive requirement of silkworm larvae vary with the maturity of leaves fed. Silkworms required leaves of high moisture content as it is easy to digest and late age silkworms required mature leaves with less moisture content as late age silkworms have the strength to digest mature leaves. The fruits of mulberry has a tonic effect on kidney energy and thus, it is used as an antiphlogistic, a diuretic and an expectorant, (Koyuncu,F., 2004). Morus fruit has good source of several phytonutrients and contain high amounts of total phenolics, total flavonoids and ascorbic acid (Ercisli & orhan, 2007) and (Koyuncu, 2004).

On the other hand too much mature leaves do not contain sufficient biochemical contents. Keeping in view, the importance of medicinal value of mulberry leaves, present study aims to identify and evaluate bioactive content present in Mulberry leaves with three (Methanol, Ethanol, and Water) different solvent systems. Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. (Tiwari, Kumar and Kaur, 2011). The present work is to carry out a preliminary phytochemical screening of leaves extracts of *Morus Indica*. Mulberry of Chattisgarh region in order to know the presence of secondary metabolites and better understand the pharmaco dynamic properties of this extracts.

MATERIALS AND METHODS

Collection of Mulberry Leaf Samples

Healthy mulberry leaves were collected from the plants present in campus of Devleela Biotech, Raipur, Chhattisgarh. Since tender, medium and coarse leaves at various developmental stages were selected for qualitative and quantitative screening of phytochemicals.

Chemicals Required

All the chemicals and solvent systems were used are analytical grade and purchased from Hi Media Pvt. Ltd. Mumbai, India.

Preparation of samples

Leaf samples collected were washed thoroughly with tap water followed by distilled water, then wiped and dried under shade followed by oven drying at 60° C-65° C till constant weight was attained. Completely dried leaf samples were ground using an electric blender to obtain a fine powder. The powder was further passed through successive cycles using Soxhlet's apparatus using all the three solvents 200 ml each with 20 gms of dried plant material. The resulting extract is filtered and concentrated in vacuum evaporator (Roteva: Medica Instrument Mfg. Co.). The concentrated extract is then used to determine the presence of phytoconstituents.

Preliminary Screening of Phytochemicals

Test for Alkaloids

About 0.5gm of the plant extract was mixed in 8ml of 1% HCl, warmed and filtered. 2ml of the filtrate was treated separately with both reagents (Maeyer's and Dragendorff's), after which it was kept for overnight incubation at room temperature. The appearance of pale yellow with reddish precipitate indicates the presence of alkaloids. (1)

Test for Flavonoids (Shinado's Test)

To the extract, a few magnesium turnings and a few drops of concentrated hydrochloric acid were added and boiled for five minutes. Red coloration identifies the presence of flavonoids.(1)

Test for Terpenoids (Salkowki's test)

1ml of chloroform was added to 2ml of each extract followed by a few drops of concentrated sulphuric acid. A reddish brown precipitate produced immediately indicated the presence of terpenoids. (17)

Test of Tannins

The 0.5 gm of sample was boiled in 20ml of distilled water in a test tube and then filtered. The filtration procedure was simple using filter paper, then 0.1% $FeCl_3$ was added to the filtrate and observed for blue black coloration, which showed the presence of tannins.(1)

Test for Phenols (Ferric chloride test)

A fraction of the extract was treated with aqueous 5% ferric chloride and observed for the formation of deep blue or black colour. (17)

Test for Saponins

2 ml of extract was added to 6ml of distill water in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam for 15 minutes that confirm the presence of saponins. (17)

Test for Quinones

A small amount of extract was treated with concentrated hydrochloric acid and observed for the formation of yellow precipitation (or coloration). (17)

Test for cardiac Glycosides (Keller Kelliani's test)

5ml of extract was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1ml of concentrated sulphuric acid. A brown ring at the interface indicated the deoxysugar characteristics of cardenolides. A violet ring may appear below the ring while in the acetic acid layer, a greenish ring may form. (3).

Test for Carotenoids

1gm of sample was extracted with 10ml of chloroform in a test tube with vigorous shaking. The resulting mixture was filtered and 85% sulphuric acid was added. A blue color at the interface showed the presence of carotenoids. (3)

Test for Reducing Compounds

1gm of sample was dissolved 10 ml distill water and was boiled for 5minutes. The mixture was filtered while hot and the cooled filtrate was made alkaline to litmus paper with 20% sodium hydroxide solution. The resulting solution was boiled with an equal volume of Benedict qualitative solution on a water bath. The formation of a brick red precipitate depicted the presence of reducing compound. (3).

Test for Carbohydrate (Fehling's test)

The minimum amount of extract was dissolved in 5ml of distill water and filtered. The filtrate was treated with 1ml of Fehling's reagent A and B and heated in a boiling water bath for 5-10 minutes. Appearance of reddish orange precipitate shows the presence of carbohydrate. (16)

Test for Protein and Amino acids

2ml of filtrate was treated with 2-5 drops of 1% ninhydrin in acetone solution and placed in water bath for 1-2 minutes. Observe the formation of purple color. (16)

Thin Layer Chromatography

Thin layer chromatography (TLC) was performed on a silica gel plate (Silica Gel GF254, Merck), Glass plates of dimensions 20×20 cm were coated with Silica gel to get a thickness of 0.25 mm. Plates were allowed to dry. Thus Solvent system used was n-butanol-acetic acid-water (3:1:1) as eluents for separation of extracts. TLC tank was allowed to equilibrate for at least 30 min. 2µl of each extract and standard of flavonoids rutin 1% in methanol (Himedia) was spotted and allowed to run.

The spots were visualized by exposing the plates to iodine vapors and Rf value of standard amino acids as well as those in the given sample was calculated as follows-

$$Rf = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by the solvent system}}$$

Quantitative screening of phytochemicals

Determination of Total Flavonoid Contents (TFC)

Total flavonoid content was measured by the aluminum chloride colorimetric assay. Rutin (Himedia) is used as the standard for estimation of total flavonoids in the prepared extract. 10 mg of rutin was dissolved in 10mL of methanol to get 1 mg/ml solution.

The assay comprises of the following steps.

Preparation of Calibration Curve: Different dilutions of standard solution were added to 10ml test tubes containing 2ml of water. To the above mixture, 0.15 ml of 5% $NaNO_2$ was added. After 5 minutes, 0.15 ml of 10% $AlCl_3$ was added. After 6 min, 1ml of 1 M NaOH was added and the total volume was made up to 5ml with distilled water. Then the solution was mixed well and the absorbance was measured against a freshly prepared reagent blank at 510 nm. Readings were taken in a Double beam Spectrophotometer (Systronics).

Preparation of Test Solution: 10 gm of extract was dissolved in 10 ml of methanol to get 1 mg/ml solution. Required volume of the above solution was transferred into a 10 ml standard flask and color development was carried out as that for standard.

Determination of Total tannin content (TTC)

Total tannin content was determined by spectrophotometric method. Tannic acid (Himedia) is used as the standard for estimation of total phenol in the prepared extract. 10 mg of tannic acid was dissolved in 100mL of methanol to get 0.1 mg/ml solution. (Modified from Toth and Pavia, 2001; Makaar, 2007).

The assay comprises of the following steps:

Tannin precipitation and analysis

Add 0.5ml methanol to 2ml tubes containing 10mg PVP and place on ice. For each sample, transfer 0.5ml of each extract to a tube with PVP and methanol, vortex for ten seconds, and incubate on ice for 30 minutes. Prepare two test blanks with 1ml methanol and 10mg PVP. Centrifuge samples and test blanks for 2mons and keep them on ice. Transfer 0.75ml of supernatant into a new tube with 10mg PVP only for a second precipitation step. Repeat the same steps for blanks. Vortex both samples and blanks for 10 seconds and incubate on ice cubes for 30 mins. Then finally centrifuge the samples and transfer supernatants in different concentration in 10ml test tubes for " Folin-ciocalteau reagent assay".(as described below for phenol estimation).

Determination of Total phenol content (TPC)

Total phenol content was determined by spectrophotometric method. Tannic acid (Himedia) is used as the standard for estimation of total phenol in the prepared extract. 10 mg of tannic acid was dissolved in 100mL of methanol to get 0.1 mg/ml solution (modified from Ainsworth and Gillespie, 2007).

The assay comprises of the following steps.

Preparation of Calibration Curve

Different dilutions of standard solution were added to 10ml test tubes containing 1ml of water. To the above mixture, 0.5ml of Folin-ciocalteu reagent was added. Followed by 1.0 ml of 7.5 % Na_2CO_3 was added and the total volume was made up to 5ml with distilled water.

Then the solution was mixed well and the absorbance was measured against a freshly prepared reagent blank at 760 nm, after an incubation of 90 minutes at room temperature. The final tannic acid concentration will be 1-5µg/ml. Readings were taken in a Double beam Spectrophotometer (Systronics).

Preparation of Test Solution:

10 mg of extract was dissolved in 100 ml of methanol to get 0.1 mg/ml solution. Required volume of the above solution was transferred into a 10 ml standard flask and color development was carried out as that for standard.

RESULT AND DISCUSSION

The Methanolic, Ethanolic, and aqueous extracts were subjected to preliminary phytochemical screening to identify the various phyto-constituents present in the plant. The preliminary phytochemical screening revealed that carbohydrates, saponins, alkaloids, flavonoids, proteins, terpenoids and cardiac glycosides were present (Table-2). Flavonoids are important active components in mulberry leaves, which can effectively inhibit the oxidative modification of human lipoprotein. The amount of total flavonoids content, total phenol content and total tannin content of methanolic, ethanolic, and aqueous extracts were expressed in milligram of rutin (Himedia) and milligram of tannic acid equivalent respectively, using the standard curve equation (Table- 3).

Table 1: Determination of percentage yield of plant extracts in different solvent system

Plant name	Solvent		
	Methanol	Ethanol	Aqueous
Morus indica (mulberry)	3.5%	2.8%	3.0%

Table 2: Determination of presence of phytochemicals in different solvent system

Phytochemicals	Methanol	Ethanol	Aqueous
Alkaloid	+	+	+
Flavonoid	+	+	-
Terpenoid	+	+	+
Tannin	+	+	-
Phenol	+	+	+
Saponin	+	+	+
Quinone	-	-	-
Cardiac glycosides	+	+	-
Carotenoids	-	-	-
Reducing compound	+	+	+
Carbohydrate	+	+	+
Protein & amino acids	+	+	+
Steroids	-	-	-

Thin Layer Chromatography

After exposure of plates to iodine, brown spots were observed. R.f. value of the reference spot (rutin) was found to be 1.79.

Total Flavonoid Content

The Total Flavonoid contents was expressed as mg rutin equivalents (RE) per gram plant sample. TFC estimated in methanolic, ethanolic and aqueous extract of mulberry is described in table 3.

Total phenol content

The total phenol content was expressed as milligrams of tannic acid equivalents per gram of sample or extract (mg TAE/gm). TPC estimated in methanolic, ethanolic and aqueous extract of mulberry is described in table 3.

Total Tannin content

The total tannin content was expressed as

Tannins = Total phenol - phenol conc. in the solution analyzed after PVP precipitation

Table 3: Quantitative estimation of total flavonoid content, total phenol and total tannin content in leaf extract of Mulberry (*Morus indica*).

Solvent system	Total Flavonoid content (mg/ml	Total Tannin content (mg/ml)	Total phenol content (mg/ml)
Methanol	0.96	0.23	0.42
Ethanol	0.48	0.35	0.42
Distilled water	0.38	0.16	0.22

CONCLUSION

Phytochemicals found in leaf extracts of *Morus* species indicates their potential as a source of principles that may supply novel medicines and also to improve the health status of its users as a result of the presence of various compounds that are vital for good health. In our study Flavonoid content was found to be maximum as compared to phenol and tannin content. It has been recognized that flavonoids show antioxidant activity and their effects on human nutrition and health are considerable. Modern medical research has proven that mulberry leaf flavonoids enhance cerebrovascular and coronary blood flows, regulate arrhythmia, soften angiosclerosis, as well as decrease sugar and fat. Antibiotics or antibacterial substances like saponins, glycosides, flavonoids, and alkaloids etc, are found to be distributed in plants. (Edeoga & Okwu, 2005) and (Hafiza, Parveen, Ahmed & Hamid, 2002). Further, isolation, purification and characterization of the phytochemicals found in moraceae family tree should be studied. Quantiative analysis of other phytochemicals of this plant and also the anti-fungal and antioxidant activities should be investigated.

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REFERENCES

Ana. F. Vinha, Castro. A, Santos. A, Machado. M. and Marta. O. Soares. (2012). Phytochemical Characterization And Radical Scavenging Activity Of Aqueous Extracts Of Medicinal Plants From Portugal. European J. Med. Plant*:* Vol. 2, 4, 335-347.

Ayitey-Smith. E. and Addae-Mensah. I. (1997). A preliminary pharmacological study of Winnine: A piperine type of alkaloid from the roots of *Piper guineense.* W. Afr. J. Pharmacol. Drug Res. Vol. 4, 7-8.

Ajayi. I. A, Ajibade. O. and Oderinde. R.A. (2011). Preliminary Phytochemical Analysis of Some Plant Seeds. Research Journal of chemical sciences. ISSN 2231-606X.

Das. B. C. and Krishnaswami. S. (1965). Some observations on inter-specific hybridization in mulberry. Indian J. Sericulture. Vol.4, 1-8.

Das. K, Tiwari. R. K. S. and Shrivastava. D. K. (2010). Techniques for evaluation of medicinal plant products as antimicrobial agents: Current methods and future trends. J. Med. Plant Res. Vol. 4, 2, 104-111.

Edeoga. H.O, Okwu. D. E. and Mbaebie. B.O. (2005). Phytochemical constituents of some Nigerian medicinal plants. Afr. J. Biotechnol. Vol. 4, 685-688.

Ercisli. S. and Orhan. E. (2007). Chemical composition of white (*Morus alba*), red (*Morus rubra*) and black (*Morus nigra*) mulberry fruits. Food Chem. Vol. 103, 1380-1384.

Feroz. M, Ahmed. R, Sindhu. S. T. A. K. and Shahbaz. A.M. (1985). Preliminary Phytochemical Analysis of Some Plant Seeds. Pak. Vet. J. Vol. 13, 4.

Hafiza. M.A, Parveen. B, Ahmed. R. and Hamid. K. (2002). Phyto-chemical And Antifungal screening of *Medicago sativa* and *Zinnia elegans.* Online J. Biol. Sci. Vol. 2, 130-132.

Koyuncu. F. (1992). Micropropagation of *M. laevigata* black mulberry. Chemistry of Natural Compounds. Vol. 40, 368-369.

Koyuncu. F.(2004). Morphological and agronomical characterization of native black mulberry (*Morus nigra* L.) in Sutculer, Turkey. Plant Genet. Res. Vol. 138, 32-35.

Kubmarawa. D, Ajoku. G. A, Enworem. N. M. and Okorie. D. A. (2007). Preliminary phytochemical and antimicrobial screening of 50 medicinal plants from Nigeria. Afr. J. Biotechnol. Vol.6, 1690-1696.

Lahlou. M. (2004). Methods to study the phytochemistry and bioactivity of essential oils. Phytother. Res. Vol. 18, 435-445.

Nagata. K, Tajiro. T, Estsuji. H, Noboyasu. E, Shunuchi. M, and Chikao. N. (1985). Comellindin antifungal saponins isolated from *Camellia japonica* .Agric.Biol.Chem. Vol. 49, 1181-1186.

Sanchez. M.D. (2000). World Distribution and Utilization of Mulberry, Potential for Animal Feeding. FAO Electronic conference on mulberry for animal production (*Morus*-L).*Available online http://www.fao.org/ DOCREP/005/ X9895E/x9895e02.htm*.

Singh. R, and Sawhney. S.K. (2009). Introductory Practical Biochemistry. 7th Edn., Narosa Publishing House, New Delhi.pp: 19-20, 52-53.

Takagi. K, Hee. P.E. and Histoshi. k. (1980). Anti-inflammatory activities of hedergenin and crude saponin from *Saecindus mukorassi.* Chem.Bull. Vol. 28, 1183.

Tiwari. P, Kumar. B, Kaur. M, Kaur. G. and Kaur. H. (2011). Phytochemical screening and Extraction. Int. Pharma. Sciencia. Vol.1, 98-106.

Ugochukwu. S.C, Arukwe. U.I. and Ifeanyi. O. (2013). Preliminary Phytochemical Screening Of Different Solvent Extracts Of Stem Bark And Roots Of *Dennetia tripetala* G. Baker. Asian J. Plant Sci. and Research. Vol. 3, 3, 10-13.

Zaki. M, Kaloo. Z.A. and Sofi. M.S. (2011). Micropropagation of *Morus nigra* L. from Nodal Segments with Axillary Buds. World J.Agri.Sci. Vol. 7, 4, 496-503.