

Phytochemical Screening, Total Phenolic and Antioxidant Capacity of Root Extracts of *Morus indica* L.

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ABSTRACT

In the present study phytochemical properties of root of Morus indica L. was evaluated. The ethanolic and acetone extract of root of Morus indica L. (Moraceae) is used in the present study. The root of the plant was studied for the presence of phyto-chemicals and total phenolics, flavonoids, reducing power assay and total antioxidant activity was evaluated by folin-ciocalteu, aluminum chloride, Chang-Wei Hsieh et al. and by phospho-molybdate assay respectively. The acetone extract showed the presence of phenols, flavonoids, tannins, resins, terpenoids, glycosides, cardiac glycosides, steroid, anthraquinone, reducing sugar and saponins except alkaloids. While the ethanolic extract showed absence of reducing sugar only. Total phenolic content was found to be 214.71 ± 2.21 mg and 190.61 ± 2.88 mg GAE/g, total flavonoid content showed 123.39 ± 2.04 mg and 113.09 ± 7.25 mg QE/g, total reducing power assay showed 231.07 ± 11.18 mg BHA/g and 107.41 ± 4.98 mg BHA/g and total antioxidant activity showed 584.98 ± 7.01 mg and 287.3 ± 17.3 mg AAE/g dried extract in ethanolic and acetone extract respectively. The ethanolic extract of RoMi showed higher amount of phenolics, flavonoids, reducing power activity and total antioxidant activity than the acetone extract. The data obtained in the present study has enhanced phenolic and flavonoid concentration that are very good source of antioxidant activity.

Key words: Phytochemical, *Morus indica* L., Folin-Ciocalteu, Aluminum Chloride, Quercetin, Phosphomolybdate.

INTRODUCTION

Medicinal plants are a source for a wide variety of natural products, such as the phenolic acids and flavonoids which are very interesting for their antioxidant properties¹⁷. Polyphenols has an important role in the prevention and restriction of free radicals. In

addition to their ability to act as an efficient free radical scavengers, their natural origin represents an advantage to consumer in contrast to synthetic antioxidants which their use is being restricted due to their carcinogenicity¹⁶.

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Photochemicals present in such medicinal plants have significant antioxidant potentials that are associated with lower occurrence of several human diseases². Phytochemicals are the chemicals produced by various parts of the plants. These bioactive constituents of plants are steroids, terpenoids, phenols, resins, reducing sugar, flavanoids, alkaloids, tannins, anthraquinone and glycosides. *Morus indica* L., the fast growing flowering plant under the family Moraceae, commonly known as mulberry, is found widely in India and South East Asia. The Bodo tribe of BTAD (Bodoland Territorial Area District), Assam, India, is observed to use the root of this plant in curing Liver disorder. Kabesh⁸, investigated the phytochemical analysis and anti-microbial activity of aqueous and methanolic extracts of *Catharanthus roseus*. Qualitative analysis of phytochemical screening reveals the presence of alkaloids, phenol, saponin and proteins. Khyade⁹, told that 80 per cent methanolic extract of mulberry leaves (*Morus alba* L.) is found to have high amount of total phenolic compound in fresh weight. The same extract showed cytotoxicity activity on MCF-7 (Breast cancer cell line), HT-29 (colorectal cancer cell line), WRL-68 (Normal liver cell line). Methanolic extract showed good antioxidant effect, which may suggest its use as food supplement for cancer patients. Delouee and Urooj⁴, examined antioxidant properties and total phenolic contents of various solvent leaf extracts of *Morus indica* L. Experimental models including iron (III) reducing capacity, total antioxidant capacity, DPPH radical scavenging activity and *in vitro* inhibition of ferrous sulphate-induced oxidation of lipid system were used for characterization of antioxidant activity of extracts. Methanolic extracts with the highest level of phenolic contents was the most potent antioxidant in all assay.

Mulberry (*Morus alba* L.) leaf is traditionally used as herbal medicine in China to treat diabetes, protect the liver and lower blood pressure. Kim¹⁰, investigated antioxidant activity, total polyphenol content and main flavonoid contents in Mulberry leaf. Rutin,

isoquercitrin and astragal in are the main flavonol compounds. The main objective of the present study was Screening of phytochemical constituents of root of *Morus indica* L. by different solvents *viz.*, ethanol, acetone, chloroform, ethyl acetate and petroleum ether, and to quantify the total phenolic, flavonoid, reducing power assay and total antioxidant capacity in the root of *Morus indica* L.

MATERIALS AND METHODS

Collection and identification:

Fresh plant root was collected from Bismuri Bashbari area (Lat: 26°33'42.6"N and Lon: 90°16'17.2"E) under the Kokrajhar District, BTAD, Assam, India in between April and May of 2016. The biological identification of the plant was carried out by Dr. A.A. Mao (Scientist-F) at B.S.I. Shillong, Meghalaya, India prior to initiation of the said work.

Chemicals:

Chemicals used for the study were of analytical grade and were purchased from HiMedia.

Extract preparation of Sample:

The sample was washed properly with clean water and kept in room temperature in dark place for 20-30 days to be well dried. After that, they were crushed up and ground to get homogeneous fine powder by a grinder. Extracts were prepared by soaking 200 g of dried powdered plant material in 1L of all the five solvents and kept in dark at room temperature for 48 hrs. After 48 hrs, the extracts were filtered twice with a Whatmann filter paper No. 42 (125mm). The extracts were concentrated at oven set at 40°C and then kept in a dark place at 4°C till their use in the different studies.

Phytochemical screening:

The preliminary phytochemical screening of various active compounds from the root of *Morus indica* L. by five solvents *viz.*: ethanol, ethyl acetate, acetone, chloroform and petroleum ether were accomplished.

1. Detection of phenols⁷

In each test tube, previous filtered extracts of 5ml volume were taken, following which 1ml

of FeCl_3 (1 %) and 1ml $\text{K}_3(\text{Fe}(\text{CN})_6)$ (1 %) were added. The appearance of fresh radish blue color indicated the presence of polyphenols.

2. Detection of flavonoids⁷

Two solutions “A” and “B” were prepared from the plant extracts. The solution “A” contains 5ml of extract previously prepared. The solution B consists of 5ml of solvent added to 5ml of KOH (50%). Then the two solutions “A” and “B” were mixed together in equal volume. The presence of flavonoids is indicated by the appearance of yellow color.

3. Detection of tannins⁵

In a 2 ml of the extracts, a few drops of 1% lead acetate were added. A yellow precipitate formation indicates the presence of tannins.

4. Detection of resins⁷

In a 5ml of each previous filtered extracts, 10ml of 4% HCl were added. The appearance of turbidity indicates the presence of resins in the extracts.

5. Detection of terpenoids³

To 0.5g each of the extract, 2ml of Chloroform and 3ml of concentrated H_2SO_4 was carefully added to form a layer. The appearance of reddish brown on the interface indicated the presence of terpenoids.

6. Detection of alkaloids⁷

0.2g of the powder extract of root of the plant was dissolved in 10ml of 1% HCL. Then, they were transferred to a water bath for few minutes. After that, 1ml of the filtrated extract was treated with 2-4 drops of Dragen dorff's reagent. The presence of alkaloid is indicated by the appearance of an orange reddish precipitation.

7. Detection of Glycosides⁵

The extract was hydrolysed with HCl for few hours on a water bath. To this, 1ml of pyridine and a few drops of Na-Nitroprusside solution was added and then it was made alkaline with NaOH solution. Appearance of pink to red colour shows the presence of glycosides.

8. Detection of Cardiac Glycosides⁵

To 0.5 g of extract were diluted to 5ml in water followed by addition of 2ml of Glacial acetic acid containing one drop of ferric chloride solution. This was under layered with

1ml of conc. H_2SO_4 . Appearance of brown ring at the interface indicated the presence of a deoxy-sugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring.

9. Detection of reducing sugar⁸

To 1ml of extract equal volume of Fehling's A and Fehling's-B solution were added. Formation of red colour indicates the presence of sugar.

10. Detection of steroids⁸

In 1ml of extract, equal volume of chloroform and concentrated H_2SO_4 were added sidewise. A red colour appearance at the lower chloroform layer indicates presence of steroids.

11. Detection of Anthraquinones⁵

Borntrager's test

About five ml of each extract solution was hydrolysed with dilute H_2SO_4 treated with benzene. And 1 ml of dilute ammonia was added to it. Presence of rose pink colour is the positive response for anthraquinones.

12. Detection of saponins¹⁴

About 0.5 mg of the extract was shaken with five ml of distilled water. Formation of frothing (appearance of creamy miss of small bubbles) shows the presence of saponins.

Total phenolic content (TPC)

Determination of total phenolic content was done by using the folin-ciocalteau method¹¹ which was adopted from Swain and Hillis (1959). 150 μl of the extract was taken in a test tube following which 2400 μl of doubled distilled water was added and again 150 μl of 0.25 N folin-ciocalteureagent were combined and then mixed properly. The above mixture was allowed to react for 3 minutes. After that, 300 μl of 1N Na_2CO_3 solution was added and mixed well. The reaction mixture was incubated at room temperature in the dark for 2hrs. The absorbance was taken at 725 nm using a PC based double beam spectrophotometer (Systronics) by taking Gallic acid as standard and the result were expressed in milligram (mg)of gallic acid equivalent (GAE)/gm of dried extract.

Total flavonoid content (TFC)

Determination of total flavonoid content was determined by aluminum chloride method⁶. From both the extracts, 0.5 ml of extracts were prepared at various concentrations (0.1, 0.25 and 0.5 mg/ml) were reacted with 1.5 ml of 95% ethanol, 0.1 ml of 10 per cent aluminum chloride hexahydrate, 0.1 ml of 1 M potassium acetate (CH₃COOK), and 2.8 ml of doubled distilled water for 40 min at room temperature. Finally, the absorbance of the mixture was measured at 415 nm using PC based double beam spectrophotometer (Systronics). Quercetin was used as the standard.

Total reducing power assay

Total reducing power assay was determined by the method of Chang-Wei Hsieh¹³ et al. An aliquot of 5ml of sample at different concentrations was reacted with 2.5 mL of phosphate buffer (0.2 M) and 2.5 ml of potassium ferricyanide (1 %). The above mixture was then incubated at 50°C for 20 min. After cooling, 2.5 mL of trichloroacetic acid (10%) was added. After that, 5 mL of the above reaction liquid was mixed with 5 mL of distilled water and 1 mL of iron (III) chloride (0.1%). Finally, the absorbance was measured at 700 nm using PC based double beam spectrophotometer (Systronics). BHA was used as a positive control.

Total antioxidant capacity

Total antioxidant capacity was studied by phospho molybdate method¹⁵. An aliquot of 0.3 ml extract of different concentrations (50, 100 and 200 µg/ml) was added to 3 ml of reagent (0.6 M H₂SO₄, 0.028 M sodium phosphate, 0.004 M ammonium molybdate). The above reaction mixture was incubated for 90 min at 95°C in a water bath. After the sample was cooled to room temperature, the absorbance was measured at 765 nm. Ascorbic acid served as standard. Results were expressed in milligram (mg) ascorbic acid equivalent (AAE)/gm dried extract. All experiments were performed in triplicate.

RESULTS AND DISCUSSION**Phytochemical screening**

Phytochemical screening of RoMi revealed significant differences in the constituents of the five different extracts. Except reducing

sugar, ethanolic (70%) extract tested positive for all the major phytochemicals mentioned above; Acetone extract showed negative for alkaloids only; Ethyl acetate extract tested positive for all phytochemicals except tannins. Chloroform extract tested positive only for phenols, flavonoids, glycosides, terpenoids, alkaloids and negative for tannins, resins, cardiac glycosides, reducing sugar, steroid, anthraquinone where as petroleum ether extract showed presence of only phenols, terpenoids, alkaloids and very high steroids. The presence of phenolic compounds, flavonoids and tannins was found to be very high in ethanol (70%) and acetone extract (Table 1). Flavonoids and tannins and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers¹².

Total phenolic and flavonoids content

Estimation of total phenolic, flavonoid, reducing power assay and antioxidant capacity in the 70% ethanol and acetone extract of RoMi was shown in table 2. The phenolic content was determined from the (graph 1) linear curve of standard gallic acid ($y = 0.0166x + 0.0913$; $r^2 = 0.9959$). Higher content of total phenol (214.71 ± 2.21 mg GAE/g) was found in 70% ethanol than acetone extract (190.61 ± 2.88 mg GAE/g). Whereas the flavonoid content was determined from the (graph 2) linear curve of standard quercetin ($y = 0.0123x - 0.0214$; $r^2 = 0.9986$). Ethanolic extract showed the higher content of total flavonoid (123.39 ± 2.04 mg QE/g) than acetone extract (113.09 ± 7.25 mg QE/g dry extract).

Total reducing power assay in the 70 per cent ethanol and acetone extract of RoMi was determined from the (graph 3) linear curve of standard butylated hydroxyl-anisole ($y = 0.0175x + 0.0896$; $r^2 = 0.9982$). 70 per cent ethanol showed the higher content of total BHAEE (231.07 ± 11.18 mg BHAEE/g) than acetone extract (107.413 ± 4.98 mg BHAEE/g). Total antioxidant capacity was determined from the (graph 4) linear curve of standard ascorbic acid ($y = 0.0143x + 0.0907$; $r^2 = 0.9993$). Ethanolic extract showed the higher antioxidant activity (584.98 ± 7.01 mg AAE/g) than acetone extract (287.24 ± 17.3 mg

AAE/g). In plants phenolic contents are widely distributed. Phenolic compounds are important plant antioxidants, which exhibited considerable scavenging activity against radicals. It has been reported that the antioxidant activity of phenol is mainly due to their redox properties, hydrogen donors and

singlet oxygen quenchers¹³. Higher amount of phenolic and flavonoid content corresponds to their stronger antioxidant activity. Therefore, phenolics and flavonoids play vital role in reducing the risk of various human diseases. Thus, antioxidant capacity of a sample can be attributed mainly to its phenolic compounds¹.

Table 1: Phytochemical screening of root of *Morus indica*

Screening test	Ethanol (70%)	Ethyl acetate	Acetone	Choloroform	Petroleum ether
1. Phenols	+++++	+++	+++++	++	++
2. Flavonoids	+++	++	+++	+	--
3. Tannins	++++	--	+++	--	--
4. Resins	++++	+	+++	--	--
5. Terpenoids	++++	+++	+++	+	++
6. Alkaloids	+++	+	--	++	+
7. Glycosides	+++	++	++	+++	--
8. Cardiac Glycosides	++	+	+	--	--
9. Reducing sugar	--	+	+++	--	--
10. Steroid	++	+	+	--	++++
11. Anthraquinone	++	+	+	--	--
12. Saponins	+++	+	+	-	-

Table 2: Phytochemical Contents of RoMi

Plant extract	Total phenolic content (mg/g GAE of dry extract)	Total Flavonoid content (mg/g QE of dry extract)	Total reducing power assay (mg/g BHAE of dry extract)	Total antioxidant capacity (mg/g AAE of dry extract)
RoMi-Ethanol 70%	214.71 ± 2.21	123.39 ± 2.04	231.07 ± 11.18	584.98 ± 7.01
RoMi-Acetone	190.61 ± 2.88	113.09 ± 7.25	107.41 ± 4.98	287.24 ± 17.3

Values are the average of triplicate experiments and values are expressed as mean ± sd

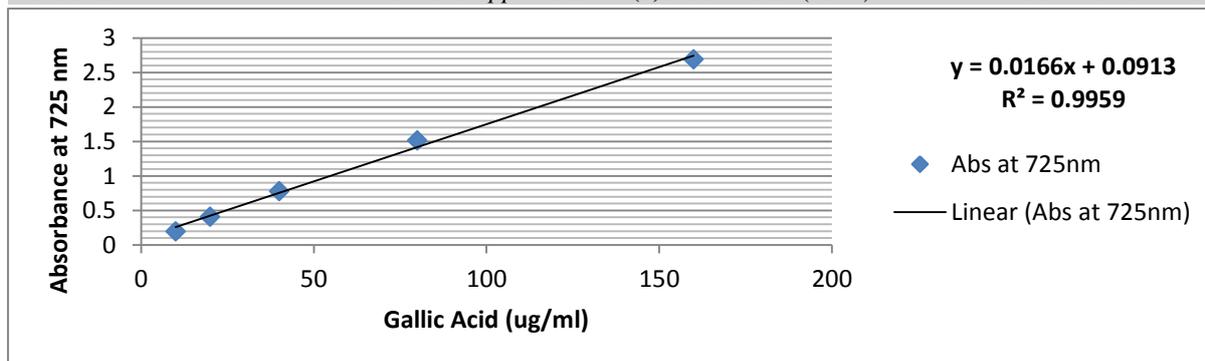


Fig. 1: Linear curve of standard Gallic acid (at a concentration of 10, 20, 40, 80 and 160 ug/ml) for determination of total phenolic content

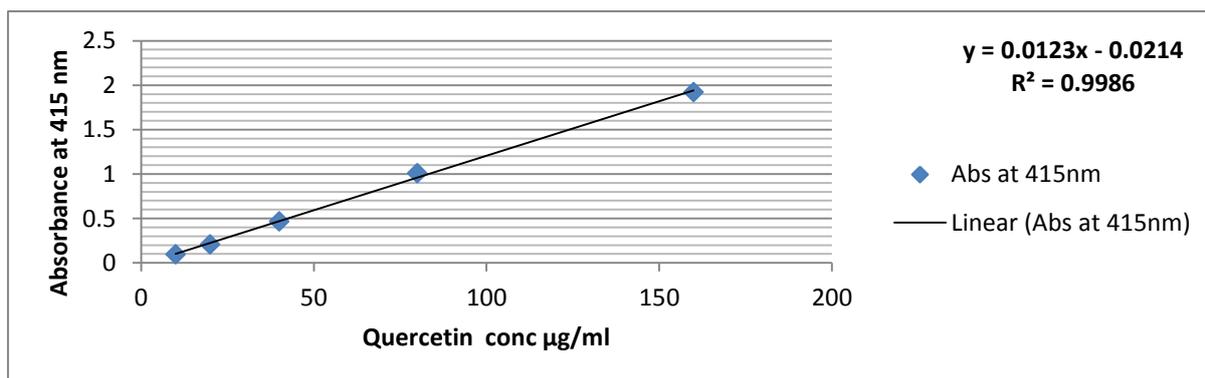


Fig. 2: Linear curve of standard Quercetin (at a concentration of 10, 20, 40, 80 and 160 ug/ml) for determination of total flavonoid content

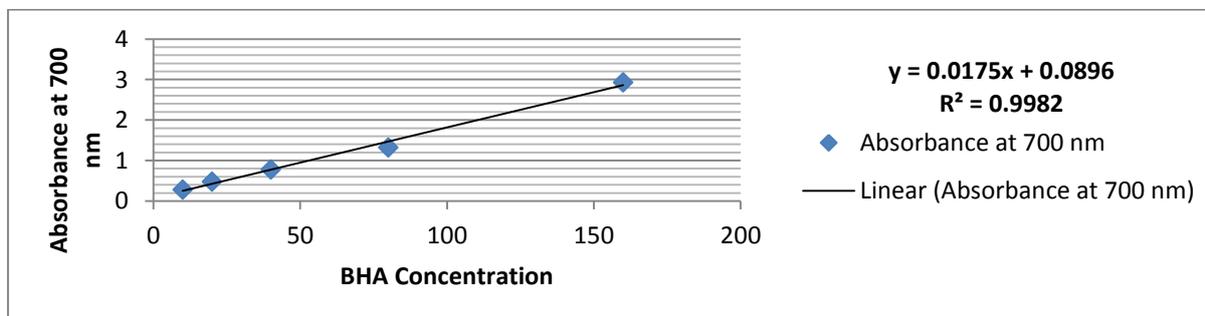


Fig. 3: Linear curve of standard BHA (at a concentration of 10, 20, 40, 80 and 160 ug/ml) for determination of total reducing power assay

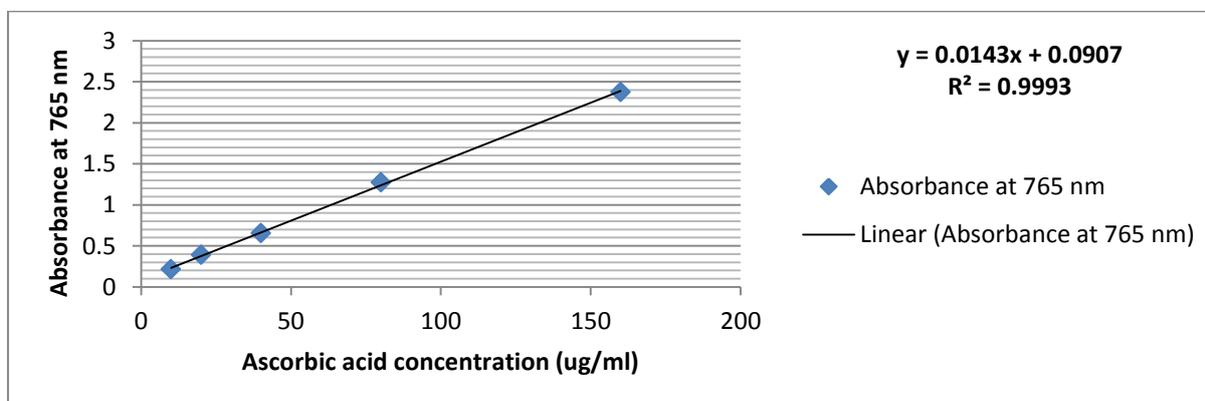


Fig. 4: Linear curve of standard Ascorbic acid (at a concentration of 10, 20, 40, 80 and 160 ug/ml) for determination of total antioxidant activity

CONCLUSION

The ethanolic (70 %) extract showed better result than acetone extract in all the assays of present study. Phenolic content (214.71 ± 2.21 mg GAE/g) and flavonoid content (123.39 ± 2.04 mg QE/g) of 70% ethanol was slightly better than that of acetone extract (190.61 ± 2.88 mg GAE/g) and (113.09 ± 7.25 mg QE/g) dry extract respectively. How the result obtained from the total reducing power assay and total antioxidant capacity showed big differences. The total reducing power assay and antioxidant capacity of ethanolic (70%) extract showed (231.07 ± 11.18 mg BHAE/g) and (584.98 ± 7.01 mg AAE/g) against the acetone extract (107.413 ± 4.98 mg BHAE/g) and (287.24 ± 17.3 mg AAE/g) dry extract respectively. The present work promises huge potential in identifying and establishing efficacy of bioactive components thereby making them establish their candidature for future therapeutic treatment.

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