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PHYTOCHEMICAL, ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY OF VARIOUS SOLVENT EXTRACTS FROM FRUITS OF *MORUS INDICA.*

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ABSTRACT

In this study, fruits of an indigenous varieties of Mulberry namely *Morus indica* was investigated for their phytochemical content, antioxidant and antibacterial potential. The antioxidant activities were carried out on three different solvent extracts of fruits of *M.indica* by free radical scavenging assay, ABTS radical cation scavenging assay, Hydroxyl radical scavenging assay, Fe^{3+} reducing power assay and phosphomolybdenum reduction assay methods. It was found that the methonolic extract had the highest antioxidant activity. Further,qualitative and quantitative analysis of phytochemicals found that the phenolic content (8.8 µg/GAE) was comparitively lower whereas the flavanoid and reducing sugar content (33.77 µg/QE and1.6317 µg/ml of glucose) was higher than the other sps. of *Morus*. The antibacterial activity was carried out by well diffusion method and the methanolic extract was found to be effective against six different bacterial strains-*Staphylococcus aureus, Micrococcus luteus, Echerichia coli, Proteus vulgaris, Klebsiella pneumoniae and Bacillus subtilis.*

KEYWORDS: *Morus indica*, antioxidant, radical scavenging, phytochemical, antibacterial activity.

INTRODUCTION

Morus Indica belongs to the Kingdom: *Plantae*(Angiosperms, Eudicots, Rosids), Order: *Rosales*, Family: *Moraceae* and Tribe: *Moreae*. The taxonomy of *Morus* is complex and disputed amongst the published 150 species names which are differing in sources. Only 10–16 are generally cited as being accepted by the vast majority of botanical authorities. *Morus* classification is found in India, South East Asia, North America and even further complicated by widespread hybridisation, wherein the hybrids are fertile. The fruit of the white mulberry – an East Asian species extensively naturalized in urban regions has a different flavor, sometimes characterized as refreshing and a little tart, with a bit of gumminess to it and a hint of vanilla. The white mulberry is considered an invasive exotic and has taken over extensive tracts from native plant species, including the red mulberry.

The ripe fruit is edible and is widely used in pies, tarts, wines, cordials, and herbal teas. The fruit of the black mulberry (native to southwest Asia) and the red mulberry (native to eastern North America) have the strongest flavor, which has been likened to 'fireworks in the mouth'. The fruit and leaves are sold in various forms as nutritional supplements. The mature plant contains significant amounts of resveratrol, particularly in stem bark. Mulberry fruit color derives from anthocyanins, which are under basic research for mechanisms of various diseases.

Anthocyanins are responsible for the attractive colors of fresh plant foods, including orange, red, purple, black, and blue. These colors are water-soluble and easily extractable, yielding natural food colorants. Due to a growing demand for natural food colorants, their significance in the food industry is increasing.A cheap and industrially feasible method has been developed to extract anthocyanins from mulberry fruit which could be used as a fabric tanning agent or food colorant of high color value (above 100). Scientists found that, of 31 Chinese mulberry cultivars tested, the total anthocyanin yield varied from 148 to 2725 mg per liter of fruit juice. All the sugars, acids, and vitamins of the fruit remained intact in the residual juice after removal of the anthocyanins, so the juice could be used to produce products such as juice, wine, and sauce.

Figure no. 1: The Collected Sample of Mulberry Fruit.

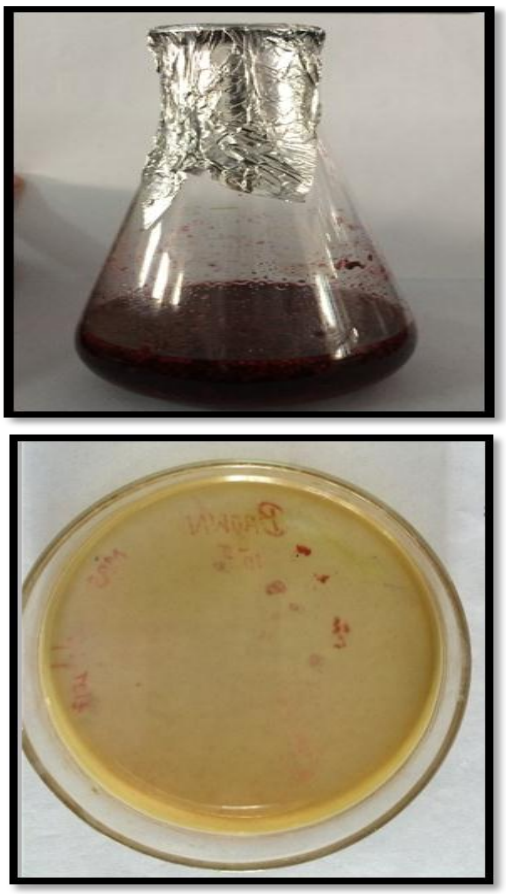


Figure no. 2 Extraction and Condensation process.

Table no. 1: Free Radical Scavenging Property-DPPH Method.

S.no	Conc µg/ml	% inhibition		
		Hexane	Ethanol	Methanol
1	20	32	31.7	34.7
2	40	34	40.7	40.2
3	60	34.2	41.6	55.6
4	80	43.3	47.3	63
5	100	45.3	53.1	63.7
6	120	52.7	61.1	66.1

Table 2: Antioxidant Property- ABTS Assay.

S.no	Conc. µg/ml	% Inhibition		
		Hexane	Ethanol	Methanol
1	5	42.2	4.2	18.4
2	10	43.7	11.3	25.4
3	15	44.7	14.1	30.6
4	20	45	30	38.6
5	25	45.9	45.8	63.5
6	30	47.5	57.9	64.1

Table 3: Free Radical Scavenging Property-Hydroxyl Assay.

S.no	Conc µg/ml	% Inhibition		
		Hexane	Ethanol	Methanol
1	20	35	57.2	52.8
2	40	35.5	57.7	58.6
3	60	35.9	58.5	59.4
4	80	36.3	59.7	61.3
5	100	39.3	60.5	62
6	120	40.3	60.8	65

Table 4: Reducing Property- FRAP.

S.no	Conc. µg/ml	Absorbance		
		Hexane	Ethanol	Methanol
1	20	0.008	0.216	0.065
2	40	0.067	0.241	0.083
3	60	0.078	0.257	0.273
4	80	0.088	0.316	0.376
5	100	0.12	0.319	0.392
6	120	0.221	0.435	0.504

Table 5: Total Antioxidant Property-Phosphomolybdenum Assay.

S.no	Conc. µg/ml	Absorbance		
		Hexane	Ethanol	Methanol
1	20	0.075	0.004	0.122
2	40	0.23	0.005	0.15
3	60	0.294	0.04	0.306
4	80	0.311	0.098	0.447
5	100	0.373	0.36	0.556
6	120	0.397	0.377	0.601

Table 6: Qualitative phytochemical analysis.

S.no	Phytochemical	Results
1	Terpenoid	+
2	Flavanoids	+
3	Alkaloid	_
4	Phenols	+
5	Glycoside	+
6	Steroid	+
7	Saponins	_
8	Tannins	_
9	Reducing Sugars	+

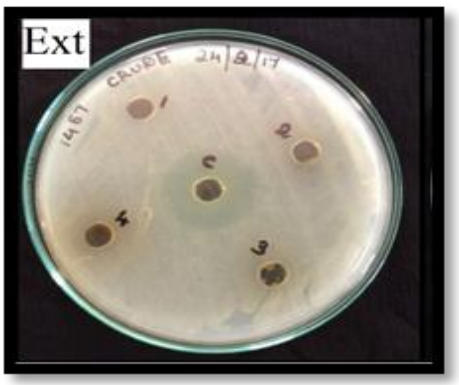
Table 7: Quantitative Phytochemical Analysis -Total phenol content.

S.no	Phytochemical	Quantitative Amount
1	Phenols	8.80 µg/GAE
2	Flavanoids	33.77 µg/QE
3	Reducing Sugars	1.6317µg/ml

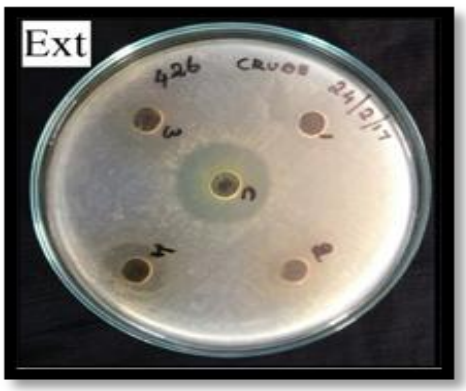
Table 10: Antibacterial Activity.

Zone of Inhibition at Various Concentrations (in mm)					
Strain Name	**Control (Tetracycline)**	**250 µg/ml crude**	**500 µg/ml crude**	**750 µg/ml crude**	**1000 µg/ml crude**
Staphylococcus aureus	35	10	12	17	19
Micrococcus luteus	23	-	12	13	13.5
Echerichia coli	26	-	-	10	12
Proteus vulgaris	25	10	14	15	17
Schigella flexneri	21	-	-	-	-
Klebsiella pneumoniae	23	-	13	14	15
Bacillus subtilis	23	10	12	13	15

Staphylococcus aureus



Shigella flexneri

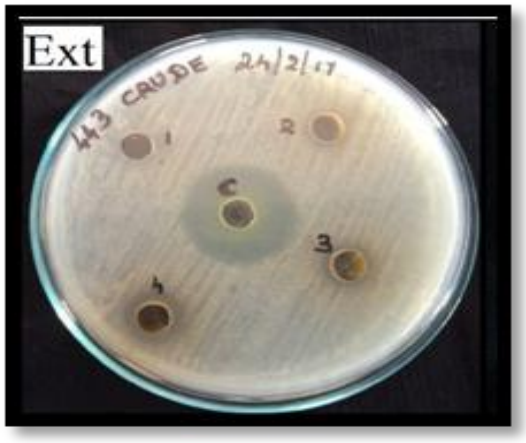


Proteus vulgaris

Micrococcus luteus

Klebsiella pneumoniae

Bacillus subtilis



Escherichia coli
Figure no. 3: Antibacterial Activity Exhibited by Methanolic Extract

MATERIALS AND METHODS

COLLECTION OF SAMPLE

In the present study, the *Morus indica* (mulberry) fruits were collected from the Central Silk Board and Research Institute, Bangalore, India. The samples were safely preserved and were brought to the laboratory for cleaning, removing the adhered debris and associated biota using fresh double distilled water. The plant samples were cut and were dried for 15 days.

EXTRACTION OF PLANT MATERIAL

The fruits were cut and were dried for up to 15 days. After sufficient drying one part of the dried sample was soaked in ten parts of methanol, Ethylacetate and hexane solvents. The conical flask containing the above was placed in a shaker overnight. The conical flask was removed the following day and the solvent was collected after filtration. The collected solution was then condensed (40-50^0 C) to get the crude extract which can be further fractionalized for experimentation.

ANTIOXIDANT ASSAYS

FREE RADICAL SCAVENGING ACTIVITY

The antioxidant activity was determined by DPPH scavenging assay according to Williams *et al*., 1995. Various concentrations of solvent extracts (methanol, hexane and ethyl acetate) of *Morus indica* was added to freshly prepared DPPH (1,1-Diphenyl-2-picryl hydrazyl) solution (2ml)t and incubated in dark at 37°C for 20 min and read at 517 nm. The data were expressed as the percent decrease in the absorbance compared to the control. Ascorbic acid was used as reference compound. The percentage inhibition of radical scavenging activity was calculated.

%Radical scavenging potential = [(Control OD-Sample OD)/Control OD] X100.

AZINO-BIS (3-ETHYL BENZO THIAZOLINE-6-SULPHONIC ACID) (ABTS) ANTIOXIDANT ASSAY

The crude extract was taken in various concentrations and this assay was performed per the method of Delgado-Andrade *et al*., 2005. Test sample of varying concentration were allowed to react with 500µL of the ABTS solution for 15minutes in dark condition. Then the absorbance was taken at 734 nm using the spectrophotometer.

% Radical scavenging potential = [(Control OD-Sample OD)/Control OD] X100.

HYDROXYL RADICAL SCAVENGING ACTIVITY

The scavenging activity of extract of *Morus indica* on hydroxyl radical was measured according to the method of Olabinri*et al*., 2010. The % hydroxyl radical scavenging activity is calculated by the following formula.

% Radical scavenging potential = [(Control OD-Sample OD)/Control OD] X100.

FERROUS REDUCING ANTIOXIDANT POWER ASSAY (FRAP)

The reducing power assay was determined by slightly modified method of Yen and Chen, 1995. The crude extract was taken in various concentrations and was mixed with 2.5ml of phosphate buffer (0.2 M, pH 6.6) and 2.5ml of potassium ferricyanide (1%), and incubated at 50°C for 30minutes. Then, 2.5ml of trichloro acetic acid (10% v/v) was added to the mixture and then centrifuged at 3000 rpm for 10 min. Finally, 2.5ml of upper layer solution was mixed with 2.5ml of distilled water and 0.5ml $FeCl_3$ (0.1%) and the absorbance was measured at 700 nm EDTA served as standard.

PHOSPHOMOLYBDENUM ASSAY

Total antioxidant capacity can be calculated by the method described by Prieto *et al*., 1999.

QUALITATIVE PHYTOCHEMICAL ANALYSIS

Preliminary screening of secondary metabolites such as alkaloids, flavanoids, saponins, coumarins, anthraquinones, terpenoids, steroid and sterols were carried out according to the common phytochemical methods described by Harborne., 1984. The different qualitative chemical tests were performed for

establishing the profile of given extract for its chemical composition.

QUANTITATIVE PHYTOCHEMICAL SCREENING

DETERMINATION OF TOTAL PHENOL CONTENT

The total phenolic content in methanolic extract of *Morus indica* determined with the Folin-Ciocalteau reagent. 0.1ml of extract was added to 46ml of distilled water followed by 1ml of Folin–Ciocalteau reagent and mixed thoroughly and kept at room temperature for 3min.To that 3ml of 2% sodium carbonate was added and periodically shake for 2hours and the absorbance was measured at 760nm.

DETERMINATION OF TOTAL FLAVANOIDS CONTENT

0.5 ml of plant extract was weighed and to the extract 1.5 ml of ethanol (95%), 0.1 ml of aluminum chloride (10%), and 0.1ml of potassium acetate (1 M), 2.8 ml of distilled water was added. The incubation period was carried out for 30 minutes and the OD was measured at 415nm.The amount of total flavanoids was expresses as µg QE/ml of sample.

DETERMINATION OF TOTAL REDUCING SUGAR CONTENT

Reducing sugar content was determined by preferring 1ml of plant extract and 1ml of dinitro salicylic acid, which was mixed and was kept in water bath for 15 minutes at 55-60^{o}c and then it was cooled and finally absorbance was measured at 540nm.

ANTIBACTERIAL ACTIVITY

The nutrient agar was prepared and sterilized in autoclave. Various gram negative and gram positive bacteria were swapped onto the solidified agar using cotton swaps. In the plates the wells were punched and the different concentrations of extract of *Morus indica* along with control tetracycline was diffused in the wells. The plates were incubated overnight and the results were observed. This was performed according to the method of Kori *et al*., 2014.

RESULTS

COLLECTION AND PREPARATION OF SAMPLE EXTRACT

The sample was collected and was brought safely to the laboratory. It was then washed and thoroughly rinsed with distilled water. The plant filtrate was completely filtered and was subjected to condensation process to obtain as a semi-paste in nature. The crude extract of the fruit was evaluated for various phytochemical activities.

ANTIOXIDANT ASSAY

The antioxidant assays were done for the various plant samples and the results were found as below.

FREE RADICAL SCAVENGING ASSAY

The DPPH antioxidant scavenging assay was done on the various solvent extract samples and the results are tabulated below in Table no. 1. From this research, it was found that the DPPH Free Radical Scavenging Assay that the methanol derived *Morus indica* fruit extract had the highest radical scavenging activity in that of the crude extract. The results obtained show that the crude methanol extract has the IC_{50} of 36.1 µg/ml and the ethyl acetate and hexane have comparatively lower IC_{50} of 29.5 µg/ml and 21.9 µg/ml respectively.

AZINO-BIS (3-ETHYL BENZO THIAZOLINE-6-SULPHONIC ACID) (ABTS) ASSAY

The ABTS antioxidant assay was done on the various samples and the results are tabulated below in Table no. 2. From this research, it was found that the ABTS radical Scavenging Assay that the methanol derived *Morus indica* fruit extract had the highest radical scavenging activity in that of the crude extract. The IC_{50} for the various extracts were 105 µg/ml for the methanol extract and this was the highest, this was followed by the Ethylacetate extract which has an IC_{50} of 91.6 µg/ml and then hexane extract with 79.1 µg/ml.

HYDROXYL RADICAL SCAVENGING ASSAY

The Hydroxyl radical scavenging assay was done on the various samples and the results are tabulated below in Table no..3 From this research, it was found that the Hydroxyl Radical Assay that the methanol derived *Morus indica* fruit extract had the highest radical scavenging activity in that of the crude extract. The IC_{50} for the various extracts were 143 µg/ml for the methanol extract and this was the highest, this was followed by the Ethylacetate extract which has an IC_{50} of 132 µg/ml.

FERROUS REDUCING ANTIOXIDANT POWER ASSAY (FRAP)

The Ferrous reducing power assay was done on the various samples and the results are tabulated below in table no. 4. From this research, it was found that the Ferrous reducing power Assay that the methanol derived *Morus indica* fruit extract had the highest reducing capability in that of the crude extract at a conc. of 120 µg/ml.

PHOSPHOMOLYBDENUM ASSAY

The Phosphomolybdenum assay was done on the various samples and the results are tabulated below in Table no. 5. From this research, it was found that the Phosphomolybdenum Assay that the methanol derived *Morus indica* fruit extract had the highest radical scavenging activity in that of the crude extract at a conc. of 120 µg/ml.

QUALITATIVE PHYTOCHEMICAL ANALYSIS

Phytochemical compounds were screened in the methanol extract of fruits of species *Morus indica* through qualitative method. The phytochemical analysis

showed the presence of terpenoids, flavanoids, phenols, glycoside, steroids and reducing sugars (Table no. 6). The presence of these phytochemicals might have a functional role in the antibacterial activity.

QUANTITATIVE PHYTOCHEMICAL ANALYSIS

The quantitative tests were performed and reported in the Table no.7. Its seen that flavanoids are high in content than phenols and reducing sugars.

ANTIBACTERIAL ACTIVITY

The antibacterial activity of methanolic fruit extract of *Morus indica* is seen in Figure no.3. In the present study, antibacterial activity was highest against the following three species*: Staphylococcus aureus*, *Bacillus subtilus*and *Proteus vulgaris.* Also, it shows no antibacterial activity against *Schigella flexneri.* Thus, it is show that the methanolic extract is effective against six bacterial species.

DISCUSSION

The antioxidant activities were carried out on various solvent extracts of *M.indica* by DPPH free radical, ABTS radical cation, Hydroxyl radical scavenging assays, Fe^{3+} reducing power and phosphomolybdenum reduction assay methods. It was found that the methonolic extract had the highest antioxidant activity. The qualitative and quantitative analysis of phytochemicals were carried out for the methonolic extract of *M.indica using* standard procedures and it was found that the phenolic content (8.8 µg/GAE) was comparitively lower whereas the flavanoid and reducing sugar content (33.77 µg/QE and1.6317 µg/ml of glucose) was higher than the other sps. of *Morus*. The antibacterial activity was carried out by well diffusion method and the methanolic extract was found to be effective against six different bacterial strains.

CONCLUSION

The replacement of synthetic with natural antioxidants may be advantageous. In the present study, methanolic extract of fruits of *M.indica*tested with respect to their total phenolic and flavonoid content, antioxidant capacity and antibacterial property. The existence of phenolic and flavonoid compounds was confirmed. The antioxidant capacity was measured by DPPH free radical scavenging method was proven to be high and it was also effective against six different bacterial strains. The *M.indica* fruits can successfully be used as a good therapeutic agent against human pathogens and also for the successful development of drug delivery in the near future.

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