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Evaluation of antioxidant activity of aqueous extract of Mulberry fruit powder

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Abstract

In the present work, we have examined the antioxidant activity of the aqueous extract of *Morinda citrifolia* fruit using phosphomolybdenum test and reducing power assay methods. With the help of a soxhlet apparatus, distilled water was used as the extraction solvent to get the fruit powder. The total phenolic content in the leaf extract was measured analytically using Folin-Ciocalteu reagent method. The IC50 of extract in blocking phosphomolybdenum radial was found to be 140.45 µg/mL. The data for reducing power show that the extract was able to reduce the ferricyanide within 20 minutes of interaction and hence is powerful antioxidant. From this the study, the ability of the extract to inhibit phosphomolybdenum and also to cause effective decrease of free radicals has been proven

Keywords

Mulberry, extract, antioxidant, reducing power, phosphomolybdenum

Introduction

Morinda citrifolia Linn. (family: Rubiaceae) is also known as noni, Indian mulberry, hog apple, and cheese fruit. The fruit, flower, leaves, bark, and root of M. citrifolia have been used for diverse medicinal purposes as traditional plant-based medicine. In fact, the fruits of this plant have benefited the Polynesians over 2000 years as an alternative medicine for antibacterial, antiviral, antifungal, antitumor, anthelmintic, analgesic, hypotensive, anti-inflammatory, and immune-enhancing effects (Nayak&Shettigar, 2010). The main issue right now is mulberry seeds, which have great healing qualities and are important in the field of pharmacology. Due to their high carbohydrate content, mulberries are eaten in many ways, including juice, wine, tea, jams, jellies, and other foods that are good for you (Kumaresan et al., 2008; Singhal et al., 2010). Many people use mulberries as medicine because they can help with liver and kidney problems, as well as weakness, tiredness, and anemia. People with heavy urine incontinence, tinnitus, dizziness, constipation, and anemia can also take it. It is also used to treat fever, sore throat, sadness, and anemia. Also, a few studies show that mulberry fruit juice can improve health by calming nerves, speeding up the metabolism of booze, and making the immune system stronger.

Phytochemicals like anthocyanins, phloridzin, quercetin, chlorogenic acid, resveratrol, and rutin have also been found to be present in mulberries (Gundogdu et al., 2011; Shrikanta et al., 2015; Tomas et al., 2015; Yang et al., 2016). It is the phytochemicals and nutraceuticals in mulberries that give them their many health benefits, including fighting cancer (Aggarwal et al., 2004), lowering cholesterol (Jiang et al., 2013), lowering blood sugar (Swathi et al., 2017), protecting nerves and the liver (Rebai et al., 2017), reducing inflammation (Chen et al., 2016), killing bacteria (Budiman et al., 2017), reducing fat storage (Peng et al., 2011), and lowering blood sugar levels (Yang et al.

There are many bioactive compounds in the fruit extract, and only a few radical scavenging assays have been done on it. Because of this, we tried to test the antioxidant activity of the water-based extract of *Morinda citrifolia* fruit using the phosphomolybdenum assay and the reducing power assay.

Material and Methods

Collection of fruit powder

The powder of mulberry fruit was purchased from online platform Indian jadi booty. The powder was used as obtained.

Extraction of fruit powder

The fruit powder was extracted using distilled water as the extraction solvent with the aid of soxhlet apparatus. Briefly, 35 g of the fruit powder was evenly packed in the extractor of the soxhlet apparatus and extracted with 250 mL distilled water. The extraction process was carried out for about 5 h and the hot extract was filtered through Whatman filter paper. The volume of solvent was reduced under evaporation and finally the extract was dried on water-bath to obtain a resinous extract (Khanna & Chauhan, 2021).

Phytochemical Screening of extract

To find out what kinds of chemicals were in the extract, scientists looked for alkaloids, glycosides, saponins, phenolics, tannins, flavonoids, and sterols.

Total Phenolic content Assay

We used the Folin-Ciocalteu reagent method and gallic acid as a standard to find out how much total phenolic content was in the leaf extract (Singh &Narwaria, 2021). To find the total phenolic content, 200 εL of the sample was mixed with 1.4 mL of clean water and 100 µL of Folin-Ciocalteu solution. It was left to sit at room temperature for 15 minutes before 300 µL of a 20% Na2CO3 water solution was added. It was then left to sit at room temperature for another 2 hours. A UV-Vis spectrophotometer was used to test the solution's absorbance at 760 nm. The control solution was made with 200 µL of methanol and the right chemicals. It was heated and prepared the same way as the other samples. The results were given as milligrams of gallic acid equivalent (GAE) per 100 grams of dry sample.

Antioxidant Assay

Phosphomolybdenum assay

0.588 mL of sulfuric acid, 0.049g of ammonium molybdate, and 0.036g of sodium phosphate were mixed together to make the test solution. The end amount was 10ml, which was made with pure water. One milliliter of DMSO was mixed with 10 mg of plant extract for the test. One milliliter of the chemical solution was added to 100µl of the material. It was put in a bath of hot water at 95°C for 90 minutes. The solution's absorption was measured at 695 nm after 90 minutes. As a reference, 10 mg/mL of ascorbic acid in DMSO was used (Khan et al., 2012). The Phosphomolybdenum reduction potential (PRP) of the samples that were tested was given as a percentage using the following formula:

% of inhibition = (control OD - sample OD/ Control OD) x 100.

Reducing power assay

To get different amounts of the extract (50–250 µg/mL), 2.5 mL of 0.2 M phosphate buffer (pH 6.6) was added to 1.0 mL of methanol, and the whole thing was mixed with 2.5 mL of 1% potassium ferricyanide. It was kept at 50°C for 20 minutes, and then 2.5 mL of 10% TCA was added. The reaction mixture was weakened by adding the same amount of distilled water to 2.5 mL. The absorption was recorded at 700 nm after 0.5 mL of 0.1% FeCl3 was added. A rise in the absorption of the reaction mixture means that it can reduce more (Bhalodia et al., 2013).

Results and Discussion

Extraction, phytochemical screening and total phenolic content

The dry powder extract was a dark brown color, and the yield of the extraction was 11.3%. It showed that alkaloids, phenolics, glycosides, tannins, flavonoids, and sterols were present. (Table 1).

Table 1. Phytochemical analysis of *Morinda citrifolia* fruit aqueous extract

Phytochemical	Test	Observation	Inference
Alkaloid	*Mayer's reagent*	cream colour precipitate	Alkaloid Present
	Hager's reagent	yellow colour precipitate	
	Wagner's reagent	reddish brown precipitate	
Flavonoid	*Shinoda test*	Red color	Flavonoid Present
	Alkaline reagent test	Yellow color that turns red on acidification	
	Zinc HCl reduction test	red color	
Glycoside	*Froth Test*	No Frothing	Glycoside absent
	Bontrager's Test	No coloration	
Phenolic and Tannins	*Ferric chloride*	Blue green color	Phenolics and Tannins present
	Gelatin Solution	No precipitate	
	Alkaline reagent test	Yellow to red precipitate	
	Ninhydrin Test	No coloration	
Sterols	*Salkwoski Test*	Crimson red color	Sterols present

The Folin-Ciocalteau method was used to find the total phenolic content, which was 39.46 GAE/g of the extract.

Antioxidant action

Phosphomolybdenum assay

This method is based on the reduction of phosphomolybdic acid to phosphomolybdenum blue complex by sodium sulfide. The obtained phosphomolybdenum blue complex is oxidized by the addition of nitrite and this causes a reduction in intensity of the blue colour. The percent reduction of phosphomolybednum is reported as percentage (Table 2).

Table 2 % PRP of aqueous extract of *Morindacitrifolia* fruit

Conc (µg/mL)	PRP (%)	
	Extract	Ascorbic acid
50	17.2±0.25	92.1±0.39
100	29.6±0.42	-
150	53.4±0.63	-
200	71.3±0.13	-
250	83.5±0.64	-

Values are mean ± SEM of six determinations

The IC_{50} value was calculated and found to be 140.45 µg/mL.

Reducing power assay

As shown in Table 3, the extracts' ability to lower potassium ferrocyanide (standard) at different times is shown. Assaying reducing power is based on the idea that substances with a reduction potential react with potassium ferricyanide (Fe3+) to make potassium ferrocyanide (Fe2+). This then reacts with ferric chloride to make a ferric–ferrous complex that is most absorbent at 700 nm.

Table 3 Reducing Power of aqueous extract

Conc (µg/mL)	Absorbance at 700 nm		
	10 min	20 min	30 min
50	0.056	0.061	0.072
100	0.097	0.099	0.108
150	0.139	0.147	0.161
200	0.176	0.188	0.193
250	0.221	0.231	0.245

Values are mean ± SEM of six determinations

The test for reducing power showed that the extract could lower the ferricyanide within 20 minutes of contact, which means it is a strong antioxidant.

Conclusion

Morinda citrifolia has been used as a medicine to keep people healthy and to stop some diseases, like those that affect the skin, brain, GI tract, heart, liver, and cancer. The DPPH test and the FRAP analysis both showed that the fruit juice could be used as an antioxidant. Based on this study, it was shown that the extract can both stop phosphomolybdenum from working and effectively get rid of free radicals.

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