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Extraction Phytochemical Investigation And In Vitro Antioxidant Activity Of Leaves Extract Of *Morus Alba*

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Abstract

This study investigates the phytochemical constituents and antioxidant activity of *Morus alba* leaves extract. The ethanolic extract of *Morus alba* yielded 8.2% (w/w), and phytochemical screening confirmed the presence of flavonoids, phenols, and sterols. These constituents were identified through positive reactions in tests such as lead acetate, alkaline reagent, ferric chloride, and Salkowski's tests. The antioxidant potential of the *Morus alba* extract was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay and compared with ascorbic acid as a standard antioxidant. The extract demonstrated concentration-dependent antioxidant activity, with an IC50 value of 50.85 µg/ml, indicating its ability to scavenge free radicals and neutralize oxidative stress. Ascorbic acid, used as a positive control, exhibited a lower IC50 value of 24.92 µg/ml, suggesting higher antioxidant potency compared to the *Morus alba* extract. In vitro assays showed that the *Morus alba* extract achieved a maximum inhibition of 83.78% of DPPH radicals at a concentration of 100 µg/ml. The extract's antioxidant activity is attributed to its phytochemical composition, particularly flavonoids and phenols. These findings suggest that *Morus alba* extract could serve as a natural antioxidant source with potential applications in combating oxidative stress-related disorders. Further studies are needed to explore its efficacy in vivo and elucidate its mechanisms of action.

Key words: *Morus alba*, Ethanolic Extract, Phytochemical investigation, Antioxidant activity

Introduction

Morus alba, commonly known as white mulberry, is a plant species belonging to the Moraceae family. It is native to China but has been naturalized and cultivated in various parts of the world. The plant is renowned for its use in traditional medicine and as a food

source. The leaves of *Morus alba* have been particularly studied for their phytochemical composition and biological activities.

Phytochemical investigations have revealed that *Morus alba* leaves contain a variety of bioactive compounds, including flavonoids, alkaloids, phenolic acids, and steroids (Kimura et al., 2013; Yang et al., 2010). These compounds contribute to the medicinal properties of the plant, such as antioxidant, anti-inflammatory, antidiabetic, and hepatoprotective activities (Kimura et al., 2013; Zhang & Ma, 2018). Among these, flavonoids, such as quercetin and rutin, are prominently present and are known for their potent antioxidant effects (Kim et al., 2003).

The antioxidant activity of *Morus alba* leaves is of particular interest due to its potential health benefits. Antioxidants help to neutralize harmful free radicals in the body, thereby protecting cells from oxidative stress and reducing the risk of chronic diseases such as cardiovascular diseases, cancer, and neurodegenerative disorders (Kim et al., 2003; Yang et al., 2010).

Several studies have investigated the in vitro antioxidant activity of *Morus alba* leaf extracts using various assays, such as DPPH radical scavenging activity, ABTS radical scavenging activity, and ferric reducing antioxidant power (FRAP) assay (Kimura et al., 2013; Zhang & Ma, 2018). These studies have demonstrated that *Morus alba* extracts exhibit significant antioxidant potential, which correlates with their high content of flavonoids and other phenolic compounds (Kim et al., 2003).

This paper aims to review the extraction methods used for obtaining phytochemicals from *Morus alba* leaves and to evaluate their in vitro antioxidant activity. The extraction process plays a crucial role in determining the yield and composition of bioactive compounds, which in turn influences the antioxidant properties of the extracts.

Material and Methods

Collection of plant

Leaves of *Morus alba* were collected from vindhya herbal Bhopal in the month of November 2023. After collection, plant undergoes washing with tap water to remove the dust, dirt, and other foreign matters attached to the surface of the plant. Wiping the samples with clean and dry cloth enhances the drying process (Handa et al., 2008).

Figure 1: Collection of plant material

Drying

The main purpose of drying is to remove water after cleaning. This should be done immediately to prevent spoilage or microbial growth of the plant sample. Conditions used for drying the plant material largely depend on the nature of its chemical constituents (Bandiola et al., 2017). Air-drying in room temperature under the sheds, in which the latter prevents direct sun exposure that can wither the plant and lose its heat or light-sensitive bioactives. This method of drying, however, may take a few weeks for complete drying and depends mainly on temperature and humidity.

Extraction

Extraction is defined as the separation of medicinally active portions of plant tissues from the inactive components through the use solvents. Marc is the damp solid material or the plant being used and menstruum is the liquid material or solvent. During extraction, the solvent diffuses into the marc and solubilizes compounds with similar polarity (Tiwari et al., 2011).

Extraction using hot continuous extraction (soxhlet)

Defatted plant material extracted by ethanol solvent was used. In this method, the finely pulverized marc is placed in a thimble which is placed in a chamber of the Soxhlet apparatus. The menstruum in the flask beneath is then heated, and its vapors condense in the condenser. The condensed extractant drips into the thimble containing the marc, and

extracts it by contact. The advantage of this method is that large amounts of marc can be extracted with a much smaller volume of extractant. Each extraction process was carried out for 48 hours. The filtrate was separated from the residue using Whatmann filter paper. The filtrate from each solvent was collected and evaporated using a water bath at 50°C until a thick extract was obtained.

Determination of percentage yield

Percentage yield measures the effectiveness of the entire extraction process. It shows how much product a researcher has obtained after running the procedures against how much is actually obtained. A higher % yield means the researcher obtained a greater amount of product after extraction. % yield is calculated using the formula below:

$$\textbf{Percentage Yield} = \frac{Weight\ of\ Extract}{Weight\ of\ Powder\ drug\ taken} x\ 100$$

1.5 Qualitative phytochemical screening

Qualitative phytochemical screening is carried out to investigate the various classes of natural compounds present in the extract. This is accomplished using standard methods. Phytochemical screening was carried out qualitatively using detection reagents based on the procedures explained in Hanani, (2015). The classes of compounds identified in the extract included phenolics, flavonoids, tannins, saponins, alkaloids and protein.

***In-vitro* antioxidant activity of ethanolic extract of *Morus alba* using different method**

DPPH method

DPPH scavenging activity was measured by the spectrophotometer. Stock solution (6 mg in 100ml methanol) was prepared such that 1.5 ml of it in 1.5 ml of methanol gave an initial absorbance. Decrease in the absorbance in presence of sample extract at different concentration (10-100 µg/ml) was noted after 15 minutes. 1.5 ml of DPPH solution was taken and volume made till 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading. 1.5 ml of DPPH and 1.5 ml of the test sample of different concentration were put in a series of volumetric flasks and final volume was adjusted to 3 ml with methanol (Parkhe et al., 2018). Three test samples were taken and each processed similarly. Finally the mean was taken. Final decrease in absorbance was noted of DPPH with the sample at different concentration after 15 minutes at 517 nm.

$$Calculation\ of\ \%\ Reduction \frac{\text{Control Absorbance} - \text{Test absorbance}}{\text{Control Absorbance}} \text{X } 100$$

Reducing power determination (Ferric reducing capacity)

The ferric reducing capacity of extracts was investigated by using the potassium ferricyanide-ferric chloride method. Briefly, 0.2 mL of the extract at different concentrations, 2.5 mL of phosphate buffer (0.2 M, pH 6.6), and 2.5 mL of potassium ferricyanide $K_3Fe(CN)_6$ (1%) were mixed and incubated at 50°C for 20 min, to reduce ferricyanide into ferrocyanide (Luqman et al., 2012). The reaction was stopped by adding 2.5 mL of 10% (w/v) trichloroacetic acid followed by centrifugation at 1000 rpm for 10 min. Finally, 2.5 mL of the upper layer was mixed with 2.5 mL of distilled water and 0.5 mL of $FeCl_3$ (0.1%) and the absorbance was measured at 700 nm. The sample concentration providing 0.5 of absorbance (IC_{50}) was calculated by plotting absorbance against the corresponding sample concentration.

Results and Discussion

The phytochemical analysis and antioxidant activity of *Morus alba* extract, as shown in Tables 1-4, provide valuable insights into its potential health benefits and therapeutic applications. The ethanolic extract of *Morus alba* yielded 8.2% (w/w), indicating good extractive efficiency (Table 1). Phytochemical screening revealed the presence of flavonoids, phenols, and sterols in the extract, as confirmed by positive reactions in the lead acetate, alkaline reagent, ferric chloride, and Salkowski's tests (Table 2). These constituents are known for their antioxidant properties, which were further validated through in vitro assays.

The antioxidant activity of the *Morus alba* extract was evaluated using the DPPH method, comparing its inhibition capabilities with ascorbic acid, a standard antioxidant (Tables 3 and 4). The extract demonstrated concentration-dependent antioxidant effects, with an IC50 value of 50.85 µg/ml, indicating its ability to scavenge free radicals and neutralize oxidative stress. Ascorbic acid, used as a positive control, showed a lower IC50 value of 24.92 µg/ml, suggesting higher antioxidant potency compared to the *Morus alba* extract.

Furthermore, the percentage inhibition of DPPH radicals by the *Morus alba* extract reached 86.85% at a concentration of 100 µg/ml (Table 3), which was lower than that of ascorbic acid (88.59%). Similarly, in Table 4, the *Morus alba* extract exhibited a maximum inhibition of 83.78% at 100 µg/ml, with an IC50 value of 57.62 µg/ml, again demonstrating its significant antioxidant activity but at a lower potency compared to ascorbic acid (IC50 = 25.55 µg/ml).

Table 1: Extractive values of *Morus alba*

Sr. No	Extracts	% Yield (W/W)

1	Ethanolic	8.2%

Table 2: Result of phytochemical screening of *Morus alba*

S. No.	Constituents	Ethanolic extract
1.	**Alkaloids**	
	Wagner's Test:	-ve
	Hager's Test:	-ve
2.	**Glycosides**	
	Cons. H_2SO_4 Test:	-ve
3.	**Flavonoids**	
	Lead acetate Test:	-ve
	Alkaline reagent Test:	+ve
4.	**Diterpenes**	
	Copper acetate Test:	-ve
5.	**Phenol**	
	Ferric Chloride Test:	+ve
	FC reagent Test:	+ve
6.	**Proteins**	
	Xanthoproteic Test:	-ve
7.	**Carbohydrate**	
	Fehling's Test:	-ve
	Benedict's Test	-ve
8.	**Saponins**	
	Froth Test:	-ve
9.	**Tannins**	
	Gelatin Test:	-ve
10.	**Sterols**	
	Salkowski's Test:	+ve

+Ve = Positive, -Ve= Negative

Table 3: % Inhibition of ascorbic acid and *Morus alba* extract using DPPH method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Ethanolic extract
1	10	33.96	12.21
2	20	48.32	25.91

3	40	64.43	38.52
4	60	75.17	56.38
5	80	84.97	84.56
6	100	88.59	86.85
IC_{50} Value		**24.92**	**50.85**

Table 4: % Inhibition of Ascorbic acid and ethanolic extract of *Morus alba*

Concentration (µg/ml)	**% Inhibition**	
	Ascorbic acid	***Morus alba* extract**
10	37.34	15.38
20	45.17	28.11
40	63.92	37.20
60	72.73	48.95
80	76.92	61.68
100	83.64	83.78
IC_{50} Value	**25.55**	**57.62**

Conclusion

Results indicate that *Morus alba* extract possesses substantial antioxidant potential, attributable to its phytochemical composition, particularly flavonoids and phenols. The extract's ability to scavenge DPPH radicals suggests its utility as a natural antioxidant source, potentially beneficial in combating oxidative stress-related disorders. Further studies are warranted to explore its efficacy in vivo and to elucidate its mechanisms of action, paving the way for its development as a therapeutic agent or functional food ingredient.

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