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## PHYTOCHEMICAL SCREENING, ANTIMICROBIAL ACTIVITY OF GEMMOTHERAPEUTICALLY WHITE MULBERRY (*MORUS ALBA*) LEAVES

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### ABSTRACT

The present study was designed to find out phytochemical analysis and antimicrobial activity of leaves of Mulberry plant. The phytochemical analysis was performed on quantitative and qualitative basis. The qualitative basis indicated the presence of alkaloids, glycosides, flavonoids, steroids, tannins, saponins and Anthraquinone. The quantitative analysis showed alkaloids (40%), glycosides (20.05%), flavonoids (14%), steroids (3.5%), tannins (11.9%) and saponins (11.5%) and anthraquinone (0.5%) presence. Antimicrobial activity against bacteria has been observed in the leaves of Mulberry (*Morus alba*) and showed zone of inhibition, *Escherichia coli* (7±3.3) mm, *Staphylococcus aureus* (8±3.1), *Bacillus subtilis* (9±2.5), *Pasturella multocida* (8±3.1) mm.

**Keywords:** Phytochemical analysis, antimicrobial activity, mulberry plant.

### INTRODUCTION

Plants are rich source of phytochemicals and are used to kill the micro-organism by inhibiting their growth (Hammer et al., 1999). The phytochemicals possess antimicrobial properties and are used from ancient time as western medicine (Akerle et al., 1993). Traditional practitioner still use plants to cure infectious diseases or healing of wound. Medicinal plants on scientific analysis showed antibiotics properties against pathogens (Levy et al., 2006). Among medicinal plants, the Moraceae family is widely used for their antimicrobial activities against pathogenic organisms (Dev, 2010). In family *Moraceae*, the genus *Morus* is worldwide in distribution due to fast growing nature, produces cheap source of fruit and possess antioxidant properties (Newman et al., 2007). The white mulberry (*Morus alba*) is native plant of South Asia and found abundantly in warmer habitat. In Asian countries, this plant is widely used as herbal tea, feeding of silk worm (sericulture), fodder, making baskets and grown in orchards for fruits (Datta, 2000). The current research was designed with

objective to check the phytochemical constituents and antimicrobial properties of fresh leaves of *M. alba*.

### MATERIAL AND METHODS

Fresh leaves of *M. Alba* were collected from different areas of Pothwar. These fresh leaves were washed thoroughly with distilled water and then weighed. These leaves were dried by evaporation in oven. This material was blended in alcohol and glycerin mixture in the ratio of (2:1). The mixture was placed in cool and shaded environment for one month followed by shaking at different intervals to help maceration process. Stock of this material was prepared by filtering it under constant pressure and kept for further 48 hours, followed by another filter. The alcohol was removed by evaporation in rotary and further kept at 65° C temperature in an incubator to evaporate extra alcohol.

#### *Phytochemical Screening*

The powdered leaves were subjected to phytochemical screening for the presence of the alkaloids, tannins, saponins, steroids using standard photochemical protocol used by Brain and Turner (1975)

## **Quantitative Estimation of Phytochemicals**

### **i. Extraction of Alkaloids**

The Paste of plant material (25 g) was mixed with 5 % Na<sub>2</sub>CO<sub>3</sub> solution and transferred to a 500 ml flask, by adding 50 ml of chloroform. The solution was refluxed for 20 minutes, cooled, filtered and transferred to the agitator for 5 minutes. The upper layer was removed and made volume up to 5 ml.

Further 1% H<sub>2</sub>SO<sub>4</sub> (25 ml) was added and extracted using 20 ml of CHCl<sub>3</sub>. The aqueous phase was separated, and ammonium hydroxide was added to alkaline it and then extracted with 10 ml portions of CHCl<sub>3</sub> successively. Then chloroform layers were washed with water (5 ml) followed by reducing volume (5 ml) by distillation. The absolute alcohol (2 ml) was added to the residues and evaporated at 100° C to dryness and solid residue obtained were crude alkaloids. The percentage yield of alkaloids was determined.

% yield of alkaloids = Weight of alkaloids obtained × 100 / Total weight of Sample

### **ii. Extraction of Flavonoids**

The paste plant material (25 g) was mixed with 100 ml ether in flask and refluxed for 1 hour at temperature 60°C, filtered and dried at 20° C. The filtrate was mixed with methanol of concentration 100 ml, refluxed (2 hour), filtered and evaporated. The crude flavonoids were determined by the following formula (Vongsak, 2013).

% yield of flavonoids = Weight of flavonoids obtained × 100 / Total weight of Sample

### **iii. Extraction of Tannic Acid**

The paste (25 g) of plant material was mixed with acetone and water (70% and 30%) and employed for extraction. Purification of this material was carried out in order to remove the pigments and phenolics by following Hagerman (1996).

% yield of Tannic acid = Weight of tannic acid obtained × 100 / Total weight of Sample

### **iv. Saponins Extraction**

The plant material (25 g) was placed in soxhlet extractor with solvent (200 ml) i-e ether for six hours at temperature 60°C to be defatted. The residue was kept in open air for overnight to evaporate the solvent. This plant material was then placed in the thimble of soxhlet extractor again alongwith methanol solvent (200 ml) till the colorless extraction. The solvent was evaporated and calculated percent yield of saponins by following Sharma et al., (1982).

% yield of saponins = Weight of saponins obtained × 100 / Total weight of Sample

### **v. Extraction of Steroids**

The powdered plant material (10 g) and Gemmo-therapeutically treated *M. alba* was then weighed and transferred to a round bottom flask with ethyl acetate (100 ml). The solution was refluxed for 20 minute at 40°C and then filtered. The filtrate was mixed with 5% KOH (2-50 ml). Two layers were separated: ethyl acetate layer and steroids layer (The ethyl acetate layer was extracted with of 5% HCl (2-50 ml) by evaporation) while steroid layer persist.

% yield of steroids = Weight of steroids obtained × 100 / Total weight of Sample

### **vi. Extraction of Glycosides**

The grounded plant material (25 gram) was boiled with 90 ml ethyl alcohol and filtered. The filtrate was mixed with lead sub acetate solution (30 ml) to remove chlorophyll and other pigments. The filtrate was treated with distilled water (45 ml saturated with H<sub>2</sub>S) to remove lead sub acetate.

The pure filtrate was then dried on an electric water bath and the percentage (%) yield of crude glycosides was calculated.

% Yield of Glycosides = Weight of glycosides obtained  $\times$  100 / Total weight of Sample

### **Antibacterial Activity**

Antibacterial activities of alcohol and gemmo extracts of leaves of *M. alba* were tested against bacterial species (*Staphylococcus aureus*, *Escherichia coli*, *Pasturella* and *Bacilli*) in vitro as reported by Coventry and Allen (2001). The antibacterial activity was checked against bacteria were *S. aureus*, and *E. coli*.

### **Preparation of Disc and Media**

Wicks paper disc of 10 mm were used. Agar media was prepared by following Cruickshank (1975).

The solution of peptone and agar-agar were prepared by distilled water and yeast extract and NaCl were added. The solution was autoclaved.

### **Dispersion of Medium**

15 ml of medium was poured in Petri plates (9 cm) for gel formation (2-3 mm).

### **Testing Antibacterial Activity**

The bacteria (*S. aureus*, and *E. coli*) incubated at (37 $\pm$  0.2 $^{\circ}$  C) on nutrient agar. Petri plates (9 cm) were incubated with 0.01ml of cultured media (110 -118 bacteria per mL). Agar media was distributed into each inoculated Petri dishes. Discs of Methanolic gemmo extracts (25  $\mu$ L, 50  $\mu$ L and 75  $\mu$ L) were placed on agar medium. Medium were placed at 37 $^{\circ}$  C for 24 hours. Commercially available antibiotics Saparaxin and Rocephin were used as standard reference.

### **Phytochemical Analysis**

Phytochemical analysis of *M. Alba* was carried out in order to determine the presence of different phytoconstituents quantitatively as well as qualitatively.

**Table 1: Broth Composition**

Chemicals	Amount	Chemicals	Amount
Peptone	2.5 g	pH	7.4
Yeast Extract	1.5 g		
Agar-agar	20 g		
NaCl	2.5 g		
Distilled water	500 ml		

### **Alkaloids Glycosides Flavonoids and Saponins**

Methanolic extract of *M. Alba* leaves showed brown precipitates when Mayers & Wagner's reagents were added. These results showed alkaloids were present in this plant. Quantitatively alkaloids isolated from *M. Alba* leaves were 40%. Methanolic extract of *M. alba* showed light brown precipitates with Fehling solution as well as with Benedict solution. These observations suggested the presence of glycosides in *M. alba* leaves. Quantitatively glycosides isolated from *M. Alba* leaves were 20.05%. Plant materiel was extracted Plant materiel was extracted with 30 ml absolute ethanol for 5 minutes. Mixture was filtered & in 5 ml of filtrate solution, 2 ml of AlCl<sub>3</sub> was added. Indication of yellow precipitates confirmed the presence of flavonoids in *M. Alba* leaves. Quantitatively 14% flavonoids are present in *M. Alba* leaves When small quantity of ground plant materiel of *M. Alba* was shaken with distill water, considerable froth was produced which lasts for several hours (Sofowora, 1982). Saponins are used for hypercholesterolemia, antioxidant, anti-inflammatory, anti-cancer & gentle blood cleanser. Quantitatively 11.5% saponins were present in *M. Alba* leaves.

### **Tanic Acid, Steroids and Anthraquinone**

Plant material was extracted with 20 ml of distilled water for 5 minutes. Then, 5 drops of FeCl<sub>3</sub> were added. Blue black precipitates indicated the presence of

tannins. Quantitative analysis showed that 11.9% tannins were present in *M. Alba* leave. In methanol extract of *M. Alba* 1-2 drop of Liebermann Bur chard reagent was added. Violet coloration appears which indicated the presence of steroids. Quantitative analysis showed 43.5% steroids were present in *M. Alba* leave. On extraction of plant material with absolute ethanol, then addition of n, n dimethyl aniline solution, No red coloration appeared. Anthraquinone was only 0.5% in *M. Alba* leaves.

## RESULTS

**Phytochemical Screening** The result of phytochemical screening has reported the

presence of alkaloids, saponins, flavonoids, glycosides, steroids, tannins and anthraquinone (Table 2).

### Antibacterial Activity

The results of antibacterial activity against *E.Coli* methanolic extract of *M. Alba* leaves had showed inhibition zone 7 mm. Against (*Staphylococcus. Aurous*) plant extract of *M. Alba* leaves showed 8 mm inhibition zone (Table 3). Methanolic extract of *M. Alba* leave showed inhibition zone 8mm against *B. subtilis*. It is a gram positive bacterium. Against *P.multocida* plant extract of *M. Alba* showed 9 mm inhibition zone.

**Table 2: Quantitative analysis of phytoconstituents.**

Constituent	Result	% age yield
Alkaloids	Positive	40%
Glycosides	Positive	20.05%
Flavonoids	Positive	14%
Saponins	Positive	11.5%
Tannic Acid	Positive	11.9%
Steroids	Positive	3.5%
Anthraquinone	Negative	0.5%

**Table 3: Antibacterial activity of plant leaves extract of *Morus Alba* showing inhibition zones for four different strains of Bacteria.**

Sr.No.	Tested Microorganism	Standard	Inhibition zone (mm)
1	<i>Escherichia Coli</i>	25±9.3	7±3.3
2	<i>Staphylococcus Aurous</i>	19±4.5	8±3.1
3	<i>Bacillus subtilis</i>	25.5±8.6	9±2.5



**Figure 1:** Inhibition zone produced by Plant extract against *E.Coli* , *Staphylococcus aureus* and *Bacillus subtilis*.

## DISCUSSION

The plants possess different kinds of metabolites and these serve as potential source of antimicrobial activities against pathogens and have medicinal importance due to minimum toxicity (Wang et al., 2008). The trend of phytochemicals uses as medical therapy is going to increase (Grayer and Harborne, 1994).

Our results reported the presence of phytochemical glycosides, alkaloids, flavonoids, steroids, tannins and the presence of these phytochemical also confirmed the findings of (Vikas et al., 2015)

Our results of phytochemical analysis shows alkaloids (40%), glycosides (20.05%), flavonoids (14%), steroids (3.5 %), tannins (11.9%) and saponins (11.5 %) and anthraquinone (0.5%) while similar analysis was conducted by Ayoola et al., (2011) at Nigeria .

The present finding reports saponins, alkaloids, flavonoids, tannins percentage concentration is greater than (Ayoola et al., 2011) reports. The flavonoids content ( $11.59 \pm 2.11$ ) mg/g in leaves of (*M. alba*) has been also reported by (Wang et al., 2011).

Our results of antimicrobial activity against bacteria *Staphylococcus aureus* show similar inhibition zone as reported by (Ayoola et al., 2011) inhibition zone ( $8 \pm 3.1$ ) mm. the present findings of inhibition zone by *Escherichia Coli*

( $7 \pm 3.3$ ) mm also confirm the findings of (Ayoola et al., 2011).

The present finding reports inhibition zone by leaves of (*M. alba*) for (*Bacillus subtilis*) ( $9 \pm 2.5$ ) while similar study was reported that present bacterial species is more sensitive (Wang et al., 2011). The antimicrobial activity by leaves of (*M. alba*) for (*Bacillus subtilis*) is also reported by (Roessler et al., 2009).

## CONCLUSION

The present findings revealed that natural plant extracts are potential source of antimicrobial activity which inhibit growth of microbial populations so these plants should be used in pharmaceutical industries for production of cheap medicine to conserve human health. These natural plants should be used as food supplements.

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