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Integrated studies on *in vitro* propagation, phytochemical profiling, antioxidant activity, and antimicrobial potential of *Morus alba* L. leaves

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ABSTRACT

Morus alba L. (white mulberry) is an economically and medicinally important plant species belonging to the family Moraceae. The present investigation was undertaken to establish an efficient *in vitro* propagation protocol and to evaluate the phytochemical composition, antioxidant activity, and antimicrobial potential of methanolic leaf extracts of *M. alba* L. Leaf and stem explants were cultured on Murashige and Skoog (MS) medium supplemented with suitable concentrations of plant growth regulators. Callus induction was successfully obtained from both leaf and stem explants after four weeks of incubation. The calli initially appeared creamy white to light green and gradually increased in size. Shoot initiation was observed from stem explants through apical bud formation, whereas root formation was not recorded during the study period. Qualitative phytochemical analysis revealed the presence of phenols, terpenoids, steroids, alkaloids, tannins, saponins, anthocyanins, proteins, flavonoids, reducing sugars, phlobatannins, and glycosides. The total flavonoid content, total phenolic content, and total alkaloid content were determined to be 20.419 µg QE/mL, 4.111 µg GAE/mL, and 9.000 µg AE/mL, respectively. Antioxidant activity, evaluated by the DPPH assay, exhibited concentration-dependent free radical scavenging activity, with an IC_{50} value of 64.07 µg/µL. Antimicrobial activity was assessed against *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Candida albicans*. The highest inhibitory effect was observed against *P.aeruginosa*, with a maximum zone of inhibition of 12.5 mm. Statistical analysis using one-way ANOVA revealed highly significant differences among treatment groups ($p < 0.05$). The findings demonstrate that *M. alba* possesses considerable medicinal value and may serve as a promising source of natural bioactive compounds for pharmaceutical and therapeutic applications.

Key Words - *Morus alba*, micropropagation, phytochemical screening, antioxidant activity, antimicrobial activity, medicinal plants.

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INTRODUCTION

Medicinal plants have been used for centuries as an indispensable source of therapeutic compounds. Among these plants, *Morus alba* L. (white mulberry) occupies an important position because of its nutritional, economic, and medicinal significance. The species belongs to the family Moraceae and is widely distributed throughout tropical and subtropical regions of the world.

The leaves of *M. alba* have been extensively used in traditional systems of medicine for the treatment

of diabetes, inflammation, hypertension, microbial infections, and various metabolic disorders. Numerous investigations have demonstrated that the plant contains biologically active constituents such as flavonoids, alkaloids, tannins, phenolic acids, terpenoids, anthocyanins, glycosides, and steroids.

Increasing demand for medicinal plants has generated considerable interest in the development of efficient propagation techniques. Conventional methods of propagation often suffer from low multiplication rates, seasonal dependence, and susceptibility to diseases. Tissue culture techniques provide an alternative strategy for the rapid production of disease-free and genetically uniform plant materials.

The present investigation was therefore undertaken to establish a reliable *in vitro* propagation protocol and to evaluate the phytochemical composition, antioxidant activity, and antimicrobial potential of methanolic leaf extracts of *M.alba*.

MATERIALS & METHODS

Collection of plant materials

Fresh leaves and stem explants of *M. alba* were collected from healthy plants growing under natural conditions (Figure 1). The samples were transported to the laboratory, thoroughly washed with running water, and shade-dried before further analysis.

Surface sterilization

The explants were washed with distilled water and treated with 70% ethanol followed by sterilization with 0.1% mercuric chloride solution. Sterilized explants were subsequently rinsed several times with sterile distilled water.

Preparation of culture medium

Murashige and Skoog (MS) medium was used as the basal culture medium. The pH of the medium was adjusted to 5.8 before sterilization by autoclaving at 121°C under 15 psi pressure.

Callus induction and shoot initiation

Leaf and stem explants were inoculated onto culture media supplemented with different concentrations of plant growth regulators. Cultures were maintained at 25 ± 2°C under controlled photoperiodic conditions. Observations regarding callus induction, shoot initiation, and growth responses were recorded periodically.

Preparation of methanolic extracts

Fresh leaves were washed, shade-dried, powdered, and extracted using methanol. The extracts were filtered and concentrated before further phytochemical analyses.

Qualitative phytochemical analysis

Standard methods were employed for the detection of major phytochemicals, including alkaloids, flavonoids, tannins, phenols, terpenoids, steroids, anthocyanins, saponins, proteins, reducing sugars, and glycosides.

Quantitative estimation of phytochemicals

The total flavonoid content (TFC), total phenolic content (TPC), and total alkaloid content (TAC) were determined using standard spectrophotometric techniques.

Antioxidant activity

The antioxidant potential of the methanolic extract was evaluated by the DPPH radical scavenging assay.

Antimicrobial activity

The antimicrobial activity of the methanolic leaf extract was evaluated using the agar well diffusion technique.

RESULTS

In vitro Propagation of *M. alba* L.

The present study successfully established an efficient *in vitro* propagation protocol for *M. alba* using leaf and stem explants cultured on Murashige and Skoog (MS) medium supplemented with suitable plant growth regulators. Callus induction was observed after approximately four weeks of incubation in both leaf and stem explants. The callus initially appeared as a creamy-white to light-green mass and gradually increased in size with continuous incubation. The development of healthy callus indicated the viability of the explants and their positive response to the culture medium.

Subsequent observations revealed the development of apical buds from stem explants, which gradually differentiated into shoots exhibiting normal green growth. In contrast, leaf explants produced only callus tissue and failed to regenerate shoots. Root initiation was not observed during the experimental period. These results indicate that stem explants are more suitable than leaf explants for the *in vitro* propagation of *M. alba*.

Qualitative Phytochemical Screening

The methanolic leaf extract of *M. alba* was subjected to qualitative phytochemical analysis to determine the presence of biologically active compounds.

Table 1. Qualitative phytochemical profile of methanolic leaf extracts of *M.alba*

Phytochemical constituent	Test employed	Result
Phenols	Ferric chloride test	+
Terpenoids	Salkowski test	+
Steroids	Salkowski test	+
Alkaloids	Dragendorff's test	+
Tannins	Braymer's test	+
Saponins	Foam test	+
Anthocyanins	Hydrochloric acid test	+
Proteins	Millon's test	+
Flavonoids	Lead acetate test	+
Reducing sugars	Benedict's test	+
Phlobatannins	Hydrochloric acid test	+
Glycosides	Keller–Killani test	+

(+ indicates presence)

The analysis revealed the presence of all tested phytochemicals, demonstrating the rich phytochemical composition of *M. alba* leaves.

Quantitative Phytochemical Analysis

Total flavonoid content (TFC)

The absorbance value of the methanolic extract was recorded as 0.206, corresponding to a total flavonoid content of 20.419 µg QE/mL. The findings indicate the abundance of flavonoids in the leaf extract.

Table 2. Total flavonoid content of *M. alba* leaves

Sample	Absorbance	Flavonoid content
Methanolic extract	0.206	20.419 µg QE/mL

Total phenolic content (TPC)

The total phenolic content was estimated using gallic acid as the standard compound. The absorbance of the sample extract was recorded as 0.156, corresponding to a total phenolic content of 4.111 µg GAE/mL.

Table 3. Total phenolic content of *M. alba* leaves

Sample	Absorbance	Phenolic content
Methanolic extract	0.156	4.111 µg GAE/mL

Total alkaloid content (TAC)

The absorbance of the methanolic extract was recorded as 0.081, corresponding to a total alkaloid content of 9.000 µg AE/mL.

Table 4. Total alkaloid content of *M. alba* leaves

Sample	Absorbance	Alkaloid content
Methanolic extract	0.081	9.000 µg AE/mL

Antioxidant Activity

The antioxidant activity of the methanolic extract was evaluated using the DPPH free radical scavenging assay. The percentage of radical scavenging activity increased progressively with increasing extract concentration.

Table 5: Determination of IC_{50} Methanolic extract of *M. alba* L. using DPPH Radical Scavenging Assay.

Morus alba L.			Methanol	
Concentration (µg/µl)	Control	Samples	%RSA	IC_{50}
20	938	558	40.51172708	64.07441
40	938	501	46.58848614	
60	938	483	48.50746269	
80	938	432	53.9445629	
100	938	390	58.42217484	
200	938	270	71.21535181	

IC_{50} = 64.07 µg/µL

These findings indicate a strong antioxidant potential of the methanolic leaf extract.

Antimicrobial Activity

The methanolic extract exhibited significant antimicrobial activity against the selected microorganisms.

Table 6. Mean inhibition zone (mm)

Microorganism	10 µL	20 µL	30 µL	40 µL	Positive control
Staphylococcus aureus	0	8	10.5	10.5	31
Pseudomonas aeruginosa	8	10	10	12.5	33.5
Candida albicans	7	7	8	8.5	32.5

P. aeruginosa exhibited the highest sensitivity to the extract.

Statistical Analysis

One-way ANOVA revealed highly significant differences among the treatment groups.

Table 7.

Parameter	Value
F value	78.07
P value	9.92×10^{-9}
F critical	3.11

These findings confirmed the concentration-dependent antimicrobial activity of the extract.

Fig. 1. Collection site of *M. alba*. L.

Fig. 2. Washed healthy leaves of *M. alba* L.

Fig. 3. Stem explants

Fig. 4. MS culture medium.

Fig. 5. Inoculation of explants

Fig. 6. Autoclave used for sterilization.

Fig. 7. Callus and shoot initiation from leaf and stem explants.

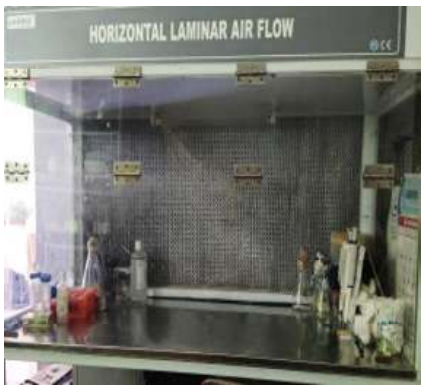


Fig. 8. Laminar airflow chamber.

Fig. 9. UV–visible spectrophotometer.

Fig. 10. Digital weighing balance.

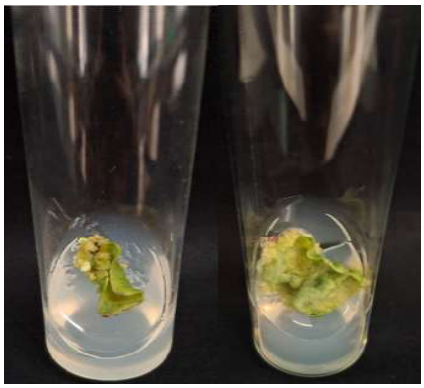


Fig. 11: Callus Formation from explants of *M. alba* L. leaf (4 weeks)

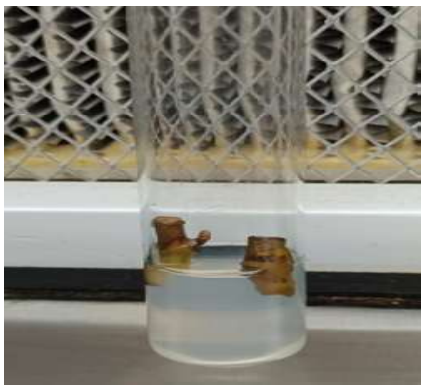


Fig. 12: Apical bud formation from stem explants of *M. alba* L. leaf (4-5 weeks)

Fig. 13. Filtration of plant extracts.

Fig. 14. Preparation of culture medium.

Fig. 15. Quantitative phytochemical estimation.

Fig. 16. Experimental procedures employed during the study.

Fig. 17. Antimicrobial activity against *P. aeruginosa*.

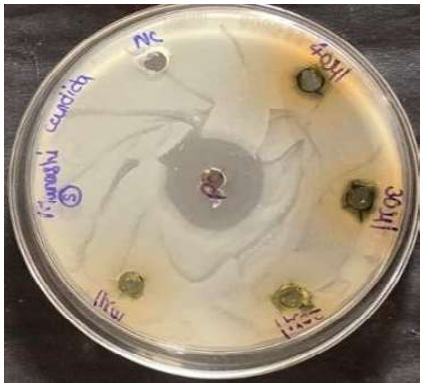


Fig. 18. Antimicrobial activity against *C. albicans*.

Fig. 19. Antimicrobial activity against *S. aureus*.

Table 8: Quantitative phytochemical analysis of Total Flavonoid content of *M. alba* leaf extracts.

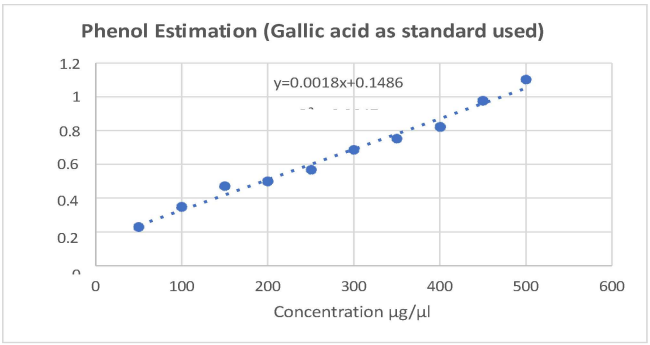
Flavonoid Estimation (Quercetin as standard used)	
Conc. µg/µl	**Absorbance at 415nm**
10	0.101
20	0.16
30	0.281
40	0.322
50	0.44
60	0.573
70	0.625
80	0.71
90	0.815
100	0.95
***Morus alba* L. Leaf**	**0.206**

Table 9: Quantitative phytochemical analysis of Total Phenolic content of *M. alba* leaf extracts

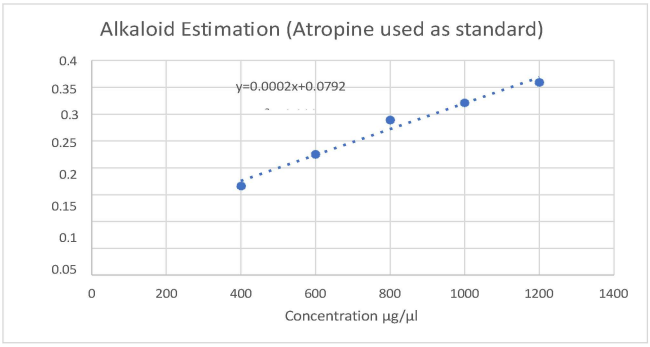
Phenol Estimation (Gallic acid as standard used)	
Conc. µg/µl	**Absorbance at 750 nm**
50	0.228
100	0.348
150	0.471
200	0.499
250	0.567
300	0.687
350	0.752
400	0.821
450	0.977
500	1.102
***Morus alba* L. leaf**	**0.156**

Table 10 : Quantitative phytochemical analysis of Total Alkaloid content of *M.alba* leaf extracts.

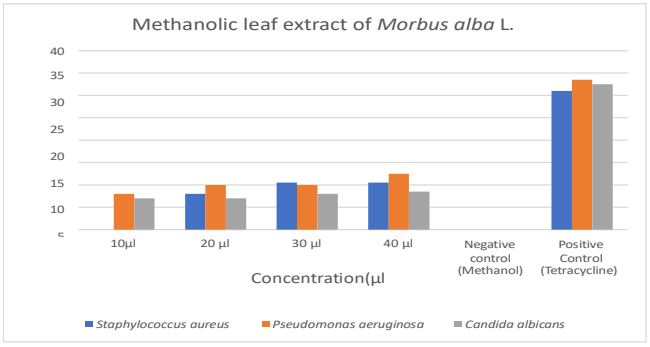
Alkaloid Estimation (Atropine used as standard)	
Conc. µg/µl	**Absorbance at 470 nm**
400	0.166
600	0.225
800	0.289
1000	0.321
1200	0.359
***Morus alba* L. Leaf**	**0.081**



Graph 1: Estimation of Total Phenol content



Graph 2: Estimation of Total Alkaloid content



Graph 3. Antimicrobial activity of the methanolic leaf extract of *M. alba* L. against selected microbial strains.

DISCUSSION

The present investigation demonstrated the successful establishment of an *in vitro* propagation system for *M. alba* using stem and leaf explants. The successful induction of callus tissues indicates the suitability of Murashige and Skoog medium supplemented with appropriate growth regulators for cellular proliferation.

The inability of leaf-derived callus tissues to regenerate shoots suggests that the hormonal balance employed during the experiment may not have been sufficient to induce organogenesis. Similar observations have been reported in previous investigations, which demonstrated the superiority of nodal and stem explants over leaf tissues for shoot regeneration.

Qualitative phytochemical analyses confirmed the presence of a wide range of biologically active compounds. The presence of flavonoids, alkaloids, phenols, tannins, and terpenoids demonstrates the considerable pharmacological importance of *M. alba*. Flavonoids and phenolic compounds are recognized as potent antioxidants because of their ability to neutralize reactive oxygen species and reduce oxidative stress.

The results of the DPPH assay demonstrated a concentration-dependent increase in free radical scavenging activity. The IC… € value obtained in the present investigation indicates a considerable antioxidant capacity, which may be attributed to the presence of phenolic compounds and flavonoids.

The methanolic extract also exhibited remarkable antimicrobial activity against bacterial and fungal species. The greatest inhibitory activity was observed against *P. aeruginosa*, followed by *S. aureus* and *C. albicans*. The observed antimicrobial activity may be associated with the presence of alkaloids, tannins, flavonoids, terpenoids, and glycosides.

The highly significant ANOVA results confirmed the reliability of the experimental findings and demonstrated that increasing extract concentration significantly enhanced antimicrobial activity.

CONCLUSION

The present investigation established an efficient protocol for the *in vitro* propagation of *M.alba* using stem explants. Qualitative and quantitative analyses revealed that the methanolic leaf extract contained substantial concentrations of flavonoids, phenols, alkaloids, tannins, terpenoids, glycosides, and anthocyanins.

The antioxidant assay demonstrated strong free radical scavenging activity, while antimicrobial assays confirmed inhibitory activity against both bacterial and fungal pathogens. These findings suggest that *M. alba* may serve as an important source of natural bioactive compounds for pharmaceutical, nutraceutical, and therapeutic applications.

Further investigations involving the isolation and characterization of active compounds are recommended.

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