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Study of Antimicrobial Activity of Crude Extract of *Morus alba* Linn Stem Bark and its Topical Preparations

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ABSTRACT

The basic aim of this research is to study the antimicrobial activity of methanol extract of *Morus alba* stem bark extract. The result of the antimicrobial activities of the extract showed potent activity at the increasing concentration against *E. coli, S. aureus, MRSA, P. aeruginosa MDR, K. pneumoniae* and *K. pneumoniae MDR, Candida species*. From the result of antimicrobial activities of extract, topical preparations (gel and ointment) were designed in 10% concentration and evaluated using physiological parameters. The antimicrobial activities of formulated gel and ointment were compared with the plant extract. It was inferred from the result that formulated gels and ointment were good in appearance and showed potent inhibition against the tested micro-organisms which is almost same with that of plant extract. Thus, this study helps to explore medicinal potency of the plant being focused on biochemical compounds as a lead for the discovery of new antimicrobial drugs in future.

Keywords: Antimicrobial; Formulatiom Gel; Oinment; *Morus alba*

INTRODUCTION

Today, many drugs commonly used are of plant origin. Plants have so far played important role as a powerful healing ingredients all over the world especially in developing countries. Moreover in the developing world, 70-80% of population relies on traditional medicine based on plants for primary health care [Luitel et al., 2014]. A few number of new antibiotics in the last three decades was developed despite antibiotic resistance. Thus search for newer drugs which can fight against the infections is warranted [Cohen ML, 1992]. Herbal formulations are gaining popularity throughout the world. Delivery of drugs to the skin is an effective and targeted therapy for local skin disorders. This route of drug delivery has gained popularity because it avoids first-pass effects, gastrointestinal irritation, and metabolic degradation associated with oral administration [Tas et al., 2003]. *Morus alba* is a popular plant belonging to a family Moraceae that has long been used in Ayurvedic and many of traditional systems of medicine. Genus *Morus* consists of over 150 species; among these *M. alba* is dominant [Grajek et al., 2015]. It is a rich source of polyphenolic compound especially flavonoids and other compounds that showed antimicrobial, antidiabetic and antioxidant potential [Srivastava et al., 2006]. Traditionally, the

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bark of the plant is used as astringent, carminative and antiseptic. Decoction of bark is also useful in asthma, lung infections, chronic Bronchitis and dysentery [Aditya et al., 2012]. We aimed at (i) performing antimicrobial tests with extracts of *M. alba* and (ii) to formulate and evaluate physical parameter of formulated gel and ointment of *M. alba*

MATERIAL AND METHODS

Chemicals: Carbopol 934, Triethanolamine, Propylene glycol, Polyethylene glycol (PEG 4000 and PEG 400) were obtained from Lomus Pharmaceuticals, Gothatar, Bhaktapur, Nepal.

Plant Collection: The barks of *M. alba* was collected from Kathmandu district in the month of August (2017) and was identified at the National Herbarium and Plant Laboratory, Godavari, Lalitpur, Nepal (Voucher specimen No. 133).

Preparation of plant extract: The barks of *M. alba* was cut into pieces and dried at room temperature. Dried sample was then crushed into powder by grinder. The powder was passed through sieve No. 30 and stored in airtight container for further use. Extraction was done by cold maceration process and dried by evaporation under reduced pressure.

Antibacterial activity: Antibacterial assay of the extract was performed by agar well diffusion method in Muller Hinton Agar (MHA). In this study, screening and evaluation of antibacterial activity were performed. MHA plates of approximately 4 mm thickness was prepared. Sterile cotton swab was dipped into the prepared inoculums and carpet culture was done. Wells were made in the inoculated media plates and labeled properly. The diameter of well was 6 mm, 50 µl of the working solution (25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, and 3.125 mg/ml) of the plant extracts were loaded into the respective wells with the help of micropipette. DMSO was used as negative control. The plates were incubated at 37 °C for 12-18 hours. After overnight incubation, the inhibition zones were observed and the diameter of ≥12 mm was considered as having antibacterial activity [Kusuma et al., 2017].

Antifungal activity: Antifungal activity of the extract was performed by agar well diffusion method in Potato Dextrose Agar (PDA). In this study, screening and evaluation of antifungal activity was performed. PDA was prepared. Before using plates, they were dried in hot air oven at 36 °C for 15 minutes to remove the excess moisture from the surface of the media. A sterile cotton swab was dipped into the prepared inoculums and carpet culture was done. Wells were made in the inoculated media plates and labeled properly. The diameter of the well was 6 mm, 50 µl of the working solution (12.5 mg/ml, 25 mg/ml, 50 mg/ml, and 75 mg/ml) of the plant extracts were loaded into the respective wells with the help of micropipette. DMSO was used as negative control. Then, the plates were incubated at 25 degree Celsius for 48 hours. Zone of inhibition was observed and noted [Sato et al., 2000].

Formulation of gel: 1 g of Carbopol 934 was weighed and was dispersed in distilled water and mixed by stirring continuously in a magnetic stirrer. The mixture was neutralized by dropwise addition of triethanolamine. Mixing was continued until a transparent gel was formed. The extract of *M. alba* was incorporated into the gel base and mixed continuously for uniformity (Table 1) [Kikwai et al., 2005].

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Table 1: Formulation of Gel from methanol extract of *M. alba* L.

Formulation	10% Concentration	Control
Plant extract	5 g	_
Carbopol 934	1 g	1 g
Propylene glycol	5 ml	5 ml
Triethanolamine	Quantity sufficient	Quantity sufficient
Distilled water	Quantity sufficient to 50 ml	Quantity sufficient to 50 ml

*Formulation of ointment***:** The ointment base was prepared by fusion method. In this method, the constituents of the base were placed together in the basin and allowed to melt together at 37 °C. After melting, the ingredients were stirred gently maintaining a temperature of 37°C for a certain period of time and then cooled with continuous stirring. Then, plant extract dissolved in propylene glycol was mixed and stirred properly to form homogeneous ointment (Table 2) [Chhetri et al., 2010].

Table 2**:** Formulation of ointment from methanol extract of *M. alba* L.

Formulation	10% concentration	Control
Plant extract	5 g	_
PEG 400	16 g	16 g
PEG 4000	16 g	16 g
Propylene glycol	3.75 ml	3.75 ml
Distilled water	Quantity sufficient to 50 ml	Quantity sufficient to 50 ml

Evaluation of the product: The formulated product was subjected to evaluation (i) Physical evaluation: the color, appearance and the feel on application of the prepared herbal gel formulation were observed by visual inspection. (ii) Odor: it was done by mixing the gel in water and taking the smell. (iii) Determination of pH: the pH of the gel and ointment was determined by using a digital dissolved in 50ml water and the pH was determined by dipping the glass electrode completely into the solution system so as to cover the electrode. (iv) Spreadability: it indicates the extent of area to which gel readily spreads on application to the skin or affected part. The therapeutic potency of the drug also depends upon its spreading value. Spreadability is expressed in terms of time in seconds taken by two slides to slip off from the gel and ointment ,which is

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placed in between the slides under the direction of certain load. Lesser the time taken for the separation of two slides better the spreadability.

*Antimicrobial activity of the product***:** Antimicrobial activity of formulated gel and ointment was done similarly to the procedure of plant extract (agar well diffusion method). The formulated gel and ointment were weighed (equivalent to the concentration of plant extract) and were tested against the gram positive and gram negative organisms and *Candida s*pecies. Antimicrobial activity was recorded by measurement of the zone of inhibition around each disc in the plate using zone reader.

Statistical analysis: All the three experiments were taken. Results were subjected to Microsoft Excel 2010. The means and standard deviations were calculated. The data were expressed as the average value of three replicates and standard error (±).

RESULTS

The percentage yield of the plant extract of *M. alba* stem bark was found to be 6.05%. Antibacterial activity was tested against gram positive organism; *Staphylococcus aureus* (ATCC 25923), gram negative organism; *Escherichia coli* (ATCC 25922), *Klebsella pneumoniae* (ATCC 700603), Methicillin Resistant *Staphylococcus aureus* (MRSA), *Klebsella pneumoniae* MDR*, Pseudomonas aeruginosa* MDR (Table 3-5). Antifungal activity was tested against two organisms: *Candida albicans* and *Candida krusei.* (Table 6-8) (Figure 1,2). Gel was dark brownish in color with translucent appearance which showed excellent gelling property and ointment was light brown in color with good appearance having the pH 6.9 and 6.8 respectively which lies in the normal range of skin. From the result, it is concluded that formulated gel and ointment were stable.
Evaluation of gel and ointment is given Table 9.

Table 3: Antibacterial activity of plant extract

Microorganism	Methanol Extract (mg/ml) Zone of inhibition, mm (Mean± S.E)				Control*
	3.125 (0.15mg/well)	6.25 (0.3mg/well)	12.5 (0.62mg/ well)	25 (1.25mg/w ell)	20µg/ml (0.001mg/ well)
S. aureus	15.3±0.2	16.6±0.18	17.6±0.3	19.6±0.18	19.3±0.10
E.coli	14.6±0.1	16.3±0.18	17.6±0.15	19.3±0.10	19.6±0.15
K. pneumoniae	16.6±0.18	18.6±0.15	20.5±0.05	22.6±0.10	20.6±0.10
MRSA	15.6±0.18	17.3±0.3	18.6±0.1	20.3±0.1	15.3±0.18
P. aeruginosa (MDR)	17.6± 0.18	18.6±0.10	20.6±0.10	22.3±0.15	15.3±0.10
K.pneumoniae (MDR)	17.5±0.15	19±0.010	20±0.02	21.6±0.1	11.1±0.2

*Control = Neomycin, Diameter of well = 6mm

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Table 4: Antibacterial activity of gel from methanol extract of *M. alba* L.

Microorganisms	Gel of Methanol extract (mg/ml) Zone of Inhibition, mm (Mean ± S.E)				Control*
	3.125 (0.15mg/well	6.25 (0.31mg/well)	12.5 (0.62mg/well)	25 (1.2mg/well)	20µg/ml (0.001mg/well)
S. aureus	15.3±0.15	16.6±0.15	17.3±0.15	19.6±0.15	18.6±0.15
MRSA	15.3±0.15	16.6±0.18	17.5±0.15	18.3±0.15	14.6±0.18
P.aeuroginosa (MDR)	17.3±0.18	18.1±0.18	19.3±0.15	21.3±0.15	14.6±0.18

*Control= Neomycin, Diameter of well = 6 mm

Table 5: Antibacterial activity of ointment from methanol extract of *M. alba* L.

Microorganisms	Ointment of Methanol Extract (mg/ml) Zone of Inhibition, mm (Mean ± S.E)				Control*
	3.125 (0.1562mg/well)	6.25 (0.3125mg/well)	12.5 (0.625mg/well)	25 (1.25mg/well)	20µg/ml (0.001mg/well)
S. aureus	15.3±0.18	16.5±0.01	17.3±0.24	18.5±0.18	18.5±0.18
MRSA	15.1±0.15	16.5±0.18	17.2±0.18	18.1±0.18	15.6±0.18
P.aeruginosa (MDR)	17.1±0.18	17.9±0.01	19.1±0.18	21.2±0.18	15.5±0.15

*Control = Neomycin, Diameter of well = 6mm

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Table 6: Antifungal activity of Plant extracts

Microorganism	Methanol extract (mg/ml) Zone of inhibition, mm (Mean ± S.E)				Control*
	12.5 (0.62mg/well)	25 (1.25mg/well)	50 (2.5mg/well)	75 (3.75mg/well)	25mg/ml (1.25mg/well)
C. krusei	16.5±0.15	17±0.18	18.6±0.18	20.1±0.15	18±0.18
C. albicans	16.3±0.18	18.3±0.02	19±0.15	21.3±0.18	17.3±0.01

*Control = Cycloheximide, Diameter of well = 6mm

Table 7: Antifungal activity of formulation of gel

MicroorganisMm	Gel of Methanol extract (mg/ml) Zone of inhibition, mm (Mean ± S.E)				Control*
	12.5 (0.62mg/well	25 (1.25mg/well)	50 (2.5mg/well)	75 (3.75mg/well)	25mg/ml (1.25mg/well)
C. krusei	15±0.05	16.6±0.18	17.5±0.18	19±0.01	17.3±0.18
C. albicans	16.6±0.05	17.3±0.18	18.5±0.15	20.3±0.18	18.1±0.18

*Control = Cycloheximide, Diameter of well = 6mm

Table 8: Antifungal activity of formulation of ointment

Microorganism	Ointment of Methanol extract (mg/ml) Zone of inhibition, mm (Mean ± S.E)				Control*
	12.5 (0.625mg/well	25 (1.25mg/well	50 (2.5mg/well	75 (3.75mg/well)	25mg/ml (1.25mg/well)
C. krusei	14.5±0.18	16.3±0.1	17.6±0.24	18.1±0.20	18±0.18
C. albicans	15.3±0.05	17.6±0.10	18.3±0.15	19±0.15	17±0.10

*Control = Cycloheximide, Diameter of well = 6mm

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Figure 1: Antibacterial activity of plant extract, gel and ointment

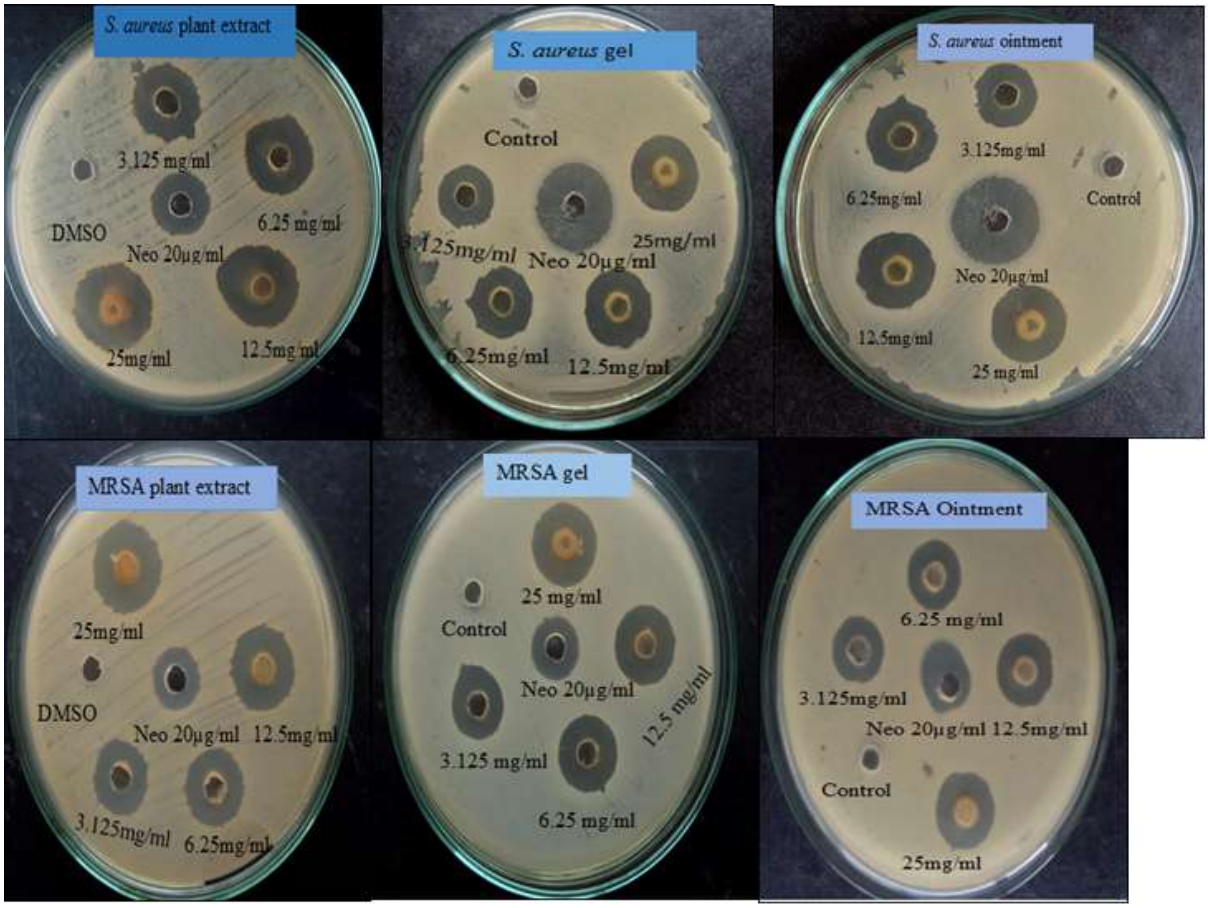

Figure 2: Antifungal activity of plant extract, gel and ointment against *Candida albicans*

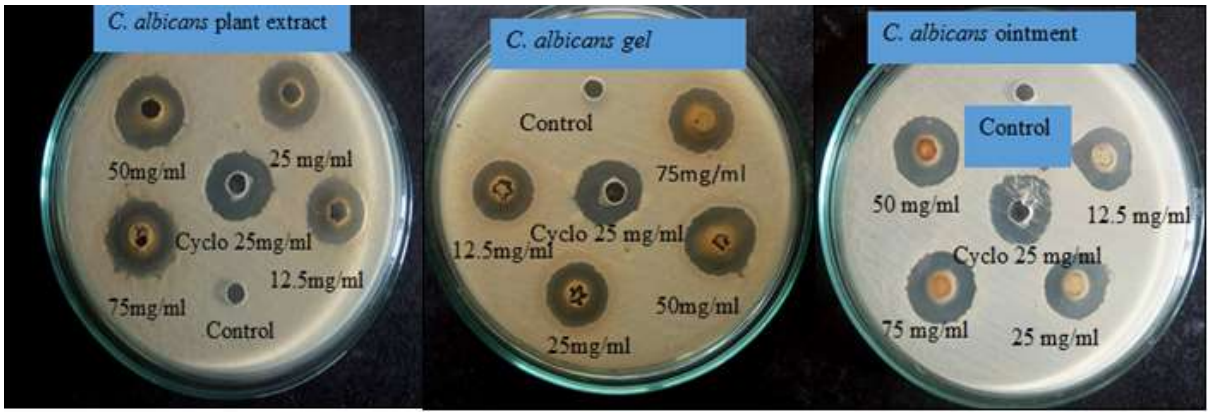

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Table 9**:** Evaluation of Gel and Ointment:

Physical Parameters	Gel	Ointment
Color	Dark Brownish	Light Brownish
Odor	Characteristics	Characteristics
Consistency	Smooth	Smooth
pH	6.9	6.8
Spread ability (gm.cm/s)	17.75	12.48
Solubility	DMSO, Propylene glycol, Boiling water, tween 80	DMSO, Propylene glycol, Boiling water, tween 80

DISCUSSION

In the present study, the antibacterial efficacy of *M. alba* extract was studied quantitatively by measuring the diameter of the zone of inhibition as an indicative of antimicrobial properties of the extracts which was found to be less potent when compared to standard. The antibacterial screening of methanol extracts of *M. alba* showed activity against both tested gram positive (*Staphylococcus aureus*) and gram negative bacteria *(Escherichia coli, Klebsella pneumoniae*). Moreover, it also showed a potent activity against the multi-drug resistance (*Pseudomonas aeruginosa* MDR*,* MRSA*, Klebsiella pneumoniae* MDR).The antibacterial potential of the plant extracts were studied with four different concentrations (3.125 mg/ml, 6.25 mg/ml, 12.5 mg/ml, 25 mg/ml). From the results, it was found that plant extract showed dose dependent response having strong activity against almost all tested organism. The methanol extract against *K. pneumoniae* and *P. aeruginosa* shows excellent activity ranging from 17- 23 mm as compared to other organisms. This shows that plant extracts had a greater zone of inhibition towards gram negative when compared to gram positive organisms. It indicates that gram negative organisms are more susceptible to the plant extract especially against multi-drug resistant. The plant extract was also tested against two yeasts (*Candida albicans* and *Candida krusei*). The results showed that plants extract showed a potent activity in dose dependent manner against tested organisms and the result was almost comparable to the cycloheximide. The maximum zone of inhibition was against *C. albicans*. From the results of antimicrobial activity of *M. alba* extract, gel and ointment were formulated at 10% concentration. The physiological properties of the prepared gel and ointment was evaluated for physical appearance, solubility, pH, spread ability, antimicrobial activity which showed satisfactory results. Also, the antimicrobial study of formulated gel and ointment was studied and compared to that of the plant extract. The formulation under examination (gel and ointment) showed broad spectrum of action against tested microorganisms. The antimicrobial activity of both gel and ointment formulation on agar plates varied according to tested organisms. This shows that incorporation of plant extracts into gel and ointment slightly decreases its activity which may be

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due to excipients present in it. The observed antimicrobial activity of the gel and ointment was due to the presence of active constituents of the extract and the activity also well maintained when it was converted to both gel and ointment. Thus, it can be used as battle against microorganisms in the form of topical formulations.

CONCLUSION

From this study, it was found that *M. alba* extract along with formulated gel and ointment have a potent antimicrobial activity which supports the traditional use of this plant as broad spectrum activity. Moreover, the results of different chemical and physical tests of gel and ointment showed that the formulation could be used topically in order to protect skin against damage caused by *S. aureus,* among which MRSA*,* the major causative agent. There is an increasing demand for herbal formulation in the world market. Both ointment and gel could become a media as a simple dosage form against infections caused by pathogenic microorganisms. Furthermore, this study demonstrates the great industrial value in harnessing the plant extract as an ingredient for formulation of antibacterial drugs.

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DECLARATION OF CONFLICT OF INTEREST

No conflict of interest is associated with this research work.

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