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Topical Preparation and Antimicrobial Study of Crude Extracts of Syzygium cumini (L) Skeel Leaves

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Abstract

The aim of the study is antimicrobial study of crude extracts of *S. cumini* leaves and further topical preparation from Methanolic extract. For this successive soxhlet extracted petroleum ether, diethyl ether, methanol and aqueous extracts of leaves of *S. cumini* were subjected to antimicrobial activity test (antibacterial and anti-fungal test). Antimicrobial activity testing was done by agar well diffusion method. The organism tested were gram positive organism: *Staphylococcus aureus (ATCC) and MRSA* and gram negative organism: *E. coli, Pseudomonas aeruginosa, Klebsellapneumonia for antibacterial* and *candida albican* for antifungal Excellent antimicrobial activity was shown by Methanolic extract in comparison to other extract. From the result of antibacterial test of plant extract further gel and ointment were formulated from Methanolic extract which too showed antibacterial activity comparable to plant extract.

Keywords: Successive Soxhlet; Antimicrobial Test; Gel and Ointment

Introduction

S. cumini, is one of the medicinally importance plant found in Nepal *S. cumini* commonly known as jamun is commonly distributed in tropical and sub-tropical region around the world [1]. The genus comprises about 1200-1800species and has a native range [2]. Traditionally, it was used for treatment of bacterial infection, diabetes, stomalgia, diarrhea [3]. Leaves of *S. cumini* also have hypoglycemic action and can be used for dermatopathy, stomatalgia. Its leaves works against multi resistance gram positive and gram negative bacteria.

Nepal occupying a central part of Himalayas is rich in flora and fauna. Nepal is rich in information about traditional plants and their use but it's hidden within some tribes due to their ritual believes or superstitions. But nowadays their attitude has changed and is open to provide information about traditionally important plants [4]. The study of traditional medicines and their manufacture has much to offer to sociocultural studies of many medical systems due to its acceptability, compatibility and lesser side effect [5]. Thus, it becomes rational to formulate product from *S. cumini* extract that supports the traditional use.

Materials and Methodology

Plant Collection

The leaves of *S. cumini* were collected from Kathmandu District which was duly identified as *Syzygiumcumini* (L.) Skeel in National herbarium and plant laboratory, Godawari, Lalitpur.

Extraction

Preparation of Plant Extract

The leaves of *S. cumini* was cut into pieces and dried in room temperature. Dried sample then crushed by grinder and was sieved through sieve number 30.

Method of Extraction

Extraction was done by successive soxhlet extraction process using Petroleum ether, diethyl ether, methanol and aqueous as solvent and the extracts was then dried by evaporation under reduced pressure.

Antibacterial Screening of Extracts

Plants have an amazing ability to produce a wide variety of secondary metabolites, like alkaloids, glycosides, terpenoids, saponis, steroids, flavonoids, coumarins and tannins. These biomlecules are the source of plant derived antimicrobial substances [6,7].

Procedure

Antimicrobial activity of Extract (Petroleum Ether, Diethyl ether, Methanol, Aqueous) was performed by agar well diffusion method by the use of Muller hinton agar. The organisms used were Gram positive organism: *Staphylococcus aureus (ATCC) and MRSA* and Gram negative organism: *E.coli, Pseudomonas aeruginosa, Klebsellapneumonia.* At first organism were inoculated in a plate and plant extract in a concentration of 25mg/ml, 50mg/ml, 100mg/ml and 200mg/ml were used for antibacterial test. DMSO was used as negative control and Neomycin as a positive control. The plates were then left for half an hour and were incubated at 37° c for 12-18 hour [8].

Antifungal Screening of Extract

Procedure

Antifungal activity of Extract (Petroleum Ether, Diethyl ether, Methanol, Aqueous) was performed by agar well diffusion method by the use of Potato dextrose agar. The organism used was *Candida albican.* At first organism were inoculated in a plate and plant extract in a concentration of 12.5mg/ml, 25mg/ml, 50mg/ml and 100mg/ml were used for antifungal test. DMSO was used as negative control and Cycloheximide as a positive control. The plates were then left for half an hour and were incubated at 37° c for 12-18 hours [9].

Formulation of Gel

Methodology

Carbopol was measured and was dispersed in distilled water and mixed by stirring continuously in a magnetic stirrer at 800r.p.m.The mixture was neutralized by drop wise addition of Triethanolamine (Table 1). Mixing was continued until transparent gel was formed. The extract of *S.cumini* skeels leaves Methanolic extract was incorporated into the gel base and mixed continuously for uniformity [10].

Materials	Control	With Plant Extract (20%)
Extract	-	5g
Carbopol	1g	1g
Propylene glycol	12.5ml	12.5ml
Triethanolamine	Q.s	Q.s
Distilled water	Q.s to 50ml	Q.s to50ml

Table 1: Formulation of gel (20%) from plant extract.

Formulation of Ointment

Methodology

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 at70°C (Table 2). After melting, the ingredients were stirred gently maintaining temperature of70 °C for certain periods and then cooled with continuous stirring. The prepared ointment was stored at room temperature [11].

Materials	Control	With plant Extract
Extract	-	5g
Polyethylene glycol 200	3g	3g
Polyethylene glycol 4000	3g	3g
Propylene glycol	10g	10g
Purified water	Q.s to 50ml	Q.s to 50ml

Table 2: Formulation of ointment (20%) from plant extract.

Evaluation of the Formulated Products

The formulated product was subjected to evaluation of physiological parameters.
Following parameters were followed:

- pH
- Color
- Odor
- Solubility
- Spreadability
- Antimicrobial activity

Physical Evaluation

The color, appearance and the feel on application of the prepared herbal gel formulation were observed by visual inspection (Tables 3-8).

Odor

It was done by mixing the gel in water and taking the smell.

Determination of pH

The pH of the gel was determined by using a digital dissolved in 50ml water and the pH was determined by dipping the glass electrode completely into the gel solution system so as to cover the electrode.

Spreadability

It indicates the extent of area to which gel readily spreads on application to 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 which is placed in between the slides under the direction of certain load (Table 8-12) Lesser the time taken for the separation two slides better the spreadability.
Spreadability=mass of weight*length/time taken to spread

Antimicrobial Activity

Antimicrobial activity of formulated gel and ointment was done similar to the procedure of extract

Result

Microorganism	Methanolic Extract (mg/ml) Zone of inhibition (Mean± S.E)				Control Neomycin (20µl)
	25	50	100	200	20µg/ml
	1.25mg/well	2.5mg/well	5mg/well	10mg/well	0.001mg/well
S.aureus	12.83±0.09	14±0.31	14.5±0.15	16±0.31	19.3±0.10
E.coli	19.66±0.18	19.83±0.24	20±0.31	21±0.18	19.6±0.15
K.pneumoniae					
	-	-	-	-	20.6±0.10
MRSA	13.83±0.2	14.83±0.24	15.5±0.15	16.6±0.18	15.3±0.18
P.aeruginosa(MDR)	19±0.18	11±0.18	12.1±0.09	15.66±0.18	15.3±0.10
K.pneumoniae(MDR)	15±0.18	17.5±0.158	18±0.0	20.6±0.18	11.1±0.2

Table 3: antimicrobial test of Methanolic extract of *S.cumini*.
Control= neomycin (20µg/ml)

Microorganism	Diethyl ether Extract (mg/ml) Zone of inhibition (Mean± S.E)				Control Neomycin (20µl)
	25	50	100	200	20µg/ml
	(1.25mg/well)	2.5mg/well	5mg/well	10mg/well	0.001mg/well
S.aureus	8.33±0.18	9.66±0.18	12.6±0.18	16.6±0.18	19.3±0.10
E.coli	-	8±0	9.33±0.18	10.6±0.18	19.6±0.15
MRSA	9.33±0.18	11.16±0.09	12.5±0.15	15.6±0.18	15.3±0.18

Table 4: Antimicrobial activity of Diethyl ether extract.
Control= neomycin 20µg/ml

Microorganism	Petroleum ether Extract (mg/ml) Zone of inhibition (Mean± S.E)				Control Neomycin (20µl)
	25	50	100	200	20µg/ml
	1.25mg/well	2.5mg/well	5mg/well	10mg/well	0.001mg/well
S.aureus	8.16±0.09	8.66±0.18	10.3±0.18	12.16±0.09	19.3±0.10
E.coli	-	-	-	11.6±0.18	19.6±0.15
MRSA	10.5±0.15	12.6±0.18	13.16±0.09	13.6±0.18	15.3±0.18

Table 5: Antimicrobial activity of Petroleum ether extract.
Control=neomycin 20µg/ml

Microorganism	Aqueous Extract (mg/ml) Zone of inhibition (Mean± S.E)				Control Neomycin (20µl)
	25	50	100	200	20µg/ml
	1.25mg/well	2.5mg/well	5mg/well	10mg/well	0.001mg/well
S.aureus	-	-	-	11	19.3±0.10
E.coli	-	11.6±0.18	12.5±0.15	14±0.3	19.6±0.15
MRSA	8.33±0.1	9.6±0.18	15.16±0.09	18.66±0.18	15.3±0.18

Table 6: Antimicrobial activity of aqueous extract.
Control=20µg/ml

Microorganism	GEL (mg/ml) Zone of inhibition (Mean± S.E)				Control Neomycin 20µl
	25	50	100	200	20µg/ml
	1.25mg/well	2.5mg/well	5mg/well	100mg/well	0.001mg/well
S.aureus	14.6±0.18	15.3±0.18	17.5±0.15	18.3±0.09	19.3±0.10
MRSA	11.66±0.18	13.33±0.18	14±0	14.6±0.18	15.3±0.18

Table 7: Antimicrobial activity of gel from Methanolic extract.
Control=20µg/ml

Microorganism	ointment (mg/ml) Zone of inhibition (Mean± S.E)			Control Neomycin (20µl)
	25	50	100	20µg/ml
	1.25mg/well	2.5mg/well	5mg/well	0.001mg/well
S.aureus	15.5±0.15	16.5±0.15	16±0.18	19.3±0.10
MRSA	10.66±0.18	13.33±0.18	13.66±0.18	15.3±0.18

Table 8: Antimicrobial activity of ointment.
Control=20µg/ml

Microorganism	Methanolic extract (mg/ml) Zone of inhibition (mm)			Control
	12.5	**25**	**50**	**25mg/ml**
	0.625mg/well	1.25mg/well	2.5mg/well	1.25mg/well
Candida albicans	12±0.15	13±0.18	14±0.18	18±0.10

Table 9: Antifungal activity of Plant extracts.
Control = Cyclohexamide

Microorganism	Gel of Methanolic extract (mg/ml) Zone of inhibition (mm)			Control
	12.5	**25**	**50**	**25mg/ml**
	0.625mg/well	1.25mg/well	2.5mg/well	0.001mg/well
Candida albicans	10±0.18	12.5±0.18	14±0.15	17±0.10

Table 10: Antifungal activity of formulation of gel.
Control = Cyclohexamide

Microorganism	Ointment of Methanolic extract (mg/ml) Zone of inhibition (mm)			Control
	12.5	**25**	**50**	**25mg/ml**
	0.625mg/well	1.25mg/well	2.5mg/well	0.001mg/well
Candida albicans	9±0.0	11±0.18	13±0.1	18±0.10

Table 11: Antifungal activity of formulation of Ointment.
Control = Cyclohexamide

Formulation	Colour	p^H	Solubility	Spreadibility (gm*cm/s)	Consistency
Gel 20%	Dark brown	6.9	Propylene glycol, DMSO, tween 80	12.22	Smooth
Ointment	Light brown	6.6	Propylene glycol, DMSO, tween 80	11	Smooth

Table 12: Evaluation of Gel and Ointment.

Discussion

The present study dealt with the biological studies, formulation and evaluation of various parameters of the topical preparation containing the Methanolic extracts of the leaves of *S. cumini.* The plant part was successively extracted with soxhlet extraction using petroleum ether, diethyl ether, methanol, and aqueous as a solvent with percentage yield 6.378%, 6.732%, 39.078% and 7.218% respectively. Maximum percentage is in methanol which may be due to difference in polarity of solvent as methanol being polar as per Haruna HM, et al.

The antibacterial screening of different extracts (petroleum ether, diethyl ether, methanol and aqueous) of leaves of Syzygium cumini was carried by agar well diffusion method. The concentration used was 25mg/ml, 50mg/ml, 100mg/ml and 200mg/ml where neomycin was

as standard .Neomycin being broad spectrum was used as standard for both gram positive and gram negative bacteria. The present study showed the maximum antibacterial activity by Methanolic extract of plant rather than petroleum ether, diethyl ether, aqueous.

The Methanolic extract showed activity against *S.aureus, MRSA, E.coli, P. aeruginosa, K. pneumonia* with excellent result whereas diethyl ether, petroleum ether and aqueous showed activity against *S.aureus, MRSA, E.coli* and no activity against *P. aeroginosa*. The activity of diethyl ether and aqueous extract was higher in comparison with petroleum ether extract. Mostly zone of inhibition shown was greater than 12mm in diameter which proves the antibacterial property of *S. cumini* leaves.

The previous study also showed the higher activity of methanolic extract which may be due to presence of tannins and phenols. Similarly, the previous study of petroleum ether showed activity only against *E.coli* where no study was found against diethyl ether. The previous study shows the potential of leaves of *S.cumini* to be potent antimicrobial agent as per Elfadil, et al.

The Methanolic extract showed activity against *Candida albican*. The concentration used was 12.5mg/ml, 25mg/ml, 50mg/ml and 100mg/ml where cycloheximide was used as standard. The antifungal activity was may be due to tannin and phenol present which is similar to the study done by Elfadil, et al. where methanol showed activity against *candida albican.*

With the excellent antibacterial and antifungal activity of *S. cumini* leaves gel and ointment were formulated from Methanolic extract of plant. Gel of 20% concentration was prepared using Carbopol. The content of herbal based gel was propylene glycol as plasticizer, Triethanolamine as neutralizer, distilled water and carbopol as gelling agent. The physiological properties of the prepared gel were evaluated for physical appearance, solubility, p^H, spread ability, antimicrobial activity which shows satisfactory results. Gel was dark brownish in color with translucent appearance which showed excellent gelling property and also the gel did not produce any irritation upon application to the skin. The pH was 6.9 which lie in the normal range of the skin. Spreadability was found to be 12.22 gm*cm/s. From the result, it is concluded that formulated gels were, stable and complied with the guidelines.

Also, Ointment of Methanolic extract of leaves of *S. cumini* was prepared through fusion method using Polyethylene glycol (PEG 400 AND PEG 4000) and subjected to evaluation of physiological parameters. The herbal ointment was found to be good in characteristics with respect to pH, solubility, antimicrobial activity. Ointment was light brown in color with good appearance having the pH 6.6 which lies in normal range of skin. Spreadability was found to be 11gm*cm/s. Formulated ointments were stable and complied with the guidelines.

Zone of inhibition of gel and ointment formulation of same plant extracts was almost similar to that of isolated plant extract activity. The formulated gel and ointment were tested against *S.aureus, MRSA* which showed result comparable to plant extract and against *Candida albican.* The zone of inhibition of ointment was lesser than gel which may be due to the excipients used, Ointment being little fatty diffusion problem may have raised. It clearly demonstrated that plant extract of gel and ointment formulation has almost similar antimicrobial properties with respect to plant extract.

Similarly, the advantage of the use of topical antimicrobials is their ability to deliver high local concentrations of antibiotic irrespective of vascular supply. Further benefits include the absence of adverse systemic effects, and a low incidence of resistance.

Thus, the present research work suggests that herbal gel and ointment formulation holds a tremendous potential against wound healing and can prove to be a safe and efficacious remedy for treating skin infection. However an elaborate protocol for the clinical trials is needed to be designed and implemented to check the activity on human volunteers for safety and acceptability.

Conclusion

Antimicrobial activity test of different extract of *S. cumini* leaves i.e petroleum ether, diethyl ether, and methanol and aqueous was performed. It was found that the plant possessed antimicrobial properties which are very important with the future prospects. The Methanolic extracts of plant have significant antimicrobial activity rather than petroleum ether, diethyl ether and aqueous extract. The activity of Methanolic extract was seen against *S. aureus, MRSA, P. aeruginosa, K. pneumonia* (MDR) and *Candida albican*. With the antimicrobial activity of Methanolic extract topical preparation herbal gel and ointment were formulated. Hence gel and ointment from Methanolic extract of *S. cumini* leave showed comparable result with extract when tested against *S.aureus, MRSA* and *Candida albican*.

Topical preparations from plant extract are better acceptable and compatible. Formulation of topical preparation from plant extract may be thus beneficial to industrial and human civilization.

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Declaration

No conflict of interest associated with this research work.

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