ORIGINAL ARTICLE

Development and evaluation of antimicrobial herbal formulations containing the methanolic extract of *Samadera indica* for skin diseases

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ABSTRACT

Samadera indica Gaetrn (Simaroubaceae) is claimed to possess various pharmacological activities like antioxidant, antifungal, antitumor, antiviral, and so on, but its taste is bitter. The aim of the present study is to investigate the toxicity of the methanolic extract and to develop suitable herbal formulations of the methanolic extract of *Samadera indica,* having efficient antimicrobial activity. The methanolic extract prepared from the dried leaves of *Samadera indica* by continuous hot percolation, were used to examine the toxicity, according to the OECD 423 guidelines, in Swiss Albino mice. Topical formulations were prepared by incorporating *Samadera indica* (5% w / w) in an emulsifying ointment and a carbopol gel base and evaluated for physical parameters and *in-vitro* antimicrobial activity (*S. aureus, P. aeruginosa* and *C. albicans)*. The study reveals that no animals under the study showed any clinical signs of toxicity or mortality when administered a dose of 5 – 2000 mg / kg body weight. Therefore, the maximum tolerated dose of the methanolic extract of *Samadera indica* was above 2000 mg / kg body weight. The formulated ointment and gel had acceptable physical parameters that showed that they were compatible with the skin, and in addition to this, these formulations passed the short-term stability studies. The *in-vitro* antimicrobial activity studies showed that the formulated ointment showed significantly strong ($p < 0.05$) activity against *S. aureus, P. aeruginosa* and *C. albicans* than the formulated gel. Thus, the present study concludes that the formulated ointment and gel are safe and efficient antimicrobial formulations for the topical delivery of the methanolic extract of *Samadera indica*.

Key words: Acute toxicity, antifungal activity, antibacterial activity, gel, ointment, *Samadera indica*

INTRODUCTION

Since ancient times, herbal- or plant-based medicines have served as a platform for the prevention and cure of diseases and to date many more constituents of these natural sources are yet to be explored. This has enlightened scientists to find out newer compounds from the herbal source to treat many infectious diseases. Reports show that most of the medicinal plants possess antimicrobial, antioxidant, and anti-inflammatory properties, which have paved a way in the prevention of many infectious diseases, and also have potential benefits for the society.[1] The present scenario of infectious diseases shows that there has been an alarming increase in the incidence of new and re-emerging infectious diseases.[2] Another important concern is the development of resistance to the antibiotic in clinical use. Hence, there is a pressing need to develop a natural formulation, which can act against the microorganisms causing skin diseases.

Previous pharmacological data showed that although it

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tasted bitter,[3] *Samadera indica* Gaetrn (Simaroubaceae) had antitumor,[4] antifeedant,[5] phytotoxic,[6] antiviral,[7] anthelmintic,[8] and anti-malarial activities,[9] growth regulating activities,[10] and antioxidant[11] and antimicrobial[2] activities. It was used to vitiate diseases such as *vata, kapha,* arthritis, constipation, and skin diseases like leprosy, scabies, puritus, and erysipelas.[3] Therefore, the present study was aimed to develop and evaluate herbal formulations containing the methanolic extracts of *Samadera indica*.

MATERIALS AND METHODS

Plant Material

The plant was collected from the locally growing area, mostly from Ernakulam district, Kerala, during the month of February. It was then botanically authenticated by Dr. V.J. Dominic, Head, Department of Botany, Sacred Heart College, Kochi, and a voucher specimen has currently been deposited in the Department of Pharmacognosy, Amrita School of Pharmacy, Kochi.(SI / 028 / 2009) The leaves were separated, dried, coarsely powdered, passed through sieve No. 40, and stored in a closed container for further use.[12]

Chemicals Used

The chemicals used in the study were: Sabouraud fluid media and Sabouraud's Dextrose Agar media (Himedia, Mumbai), Dimethyl Sulfoxide (E. Merck Ltd., Mumbai, India), and Carbopol 934P (Lobachemie Pvt. Ltd., Mumbai). Emulsifying wax, white soft paraffin, liquid paraffin, and methanol were procured from Nice Chemicals, Kochi.

Monographic Analysis of Herbs

The herb was evaluated for extractive and ash value, to confirm their standard specifications according to the Indian pharmacopeia.

Preparation of Crude Extract

The coarse powder of the leaves (50 g) was extracted by a continuous hot percolation process.[2,11] The powder was first defatted with n-hexane and then allowed to dry. The marc thus obtained was extracted for 72 hours with methanol as a solvent. The resulting solvent was then removed under reduced pressure, and thus formed a semisolid, which was vacuum dried using a rotary flash evaporator, to get a solid residue. This residue was the Methanol Extract of *Samadera indica* (MESI). The dried extract thus obtained was used for the formulation.

Phytochemical Screening

The dried methanolic extract was analyzed for various phytoconstituents like alkaloids, proteins, steroids, saponins, flavonoids, phenolic compounds and tannins, and gums and mucilages.[13]

Acute Toxicity Studies

The Institutional Animals Ethics Committee approved the use of animals for the present study (Ethical Clearance number: P.Col/48/2010/IAEC/VMPC) and the acute toxicity was carried out as per the OECD 423 guidelines. Twelve female Swiss Albino Mice weighing between 20 and 25 g and between age eight and twelve weeks were procured for the experimental trial. The animals were maintained under controlled environmental conditions (30 – 70% humidity and temperature – 22 ± 3ºC) and were exposed to a photoperiod of 12 hours of daylight and 12 hours of night, in an animal house, approved by the committee for the purpose of control and supervision of experiments on animals. The selected animals were fed with standard feed and drinking water and monitored on a regular basis.

The animals were selected randomly and grouped, three animals per group. They were kept fasting four hours prior to the treatment and the test substance was administered in a single dose by the oral route. Subsequently, the dose was gradually increased with each step, starting from 5 mg / kg then to 50, 300, 2000 mg / kg. After the substance had been administered, the food could be withheld for a further one-to-two hours, in mice.

The animals were observed for changes in skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic, and central nervous systems, and somatic motor activity and behavior pattern. They were noted individually after dosing, at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first four hours, daily, and thereafter for a total of 14 days.[14,15]

Formulation

Formulation of the ointment

Required quantities of emulsifying wax, white soft paraffin, and liquid paraffin, accurately weighed, were heated up to 70 – 75ºC and stirred until a uniform mass was obtained The extracts were incorporated into the ointment base and the composition of the ointment base and the ointment formulations[16] are given in Table 1.

Formulation of gel

Gels were prepared by dispersing the polymer in a mixture of water and glycerol with methyl paraben as the preservative. The extract was incorporated into it. The dispersion was then neutralized and made viscous by the addition of triethanolamine.[17] The composition of gel formulations are given in Table 2.

Table 1: Formulation composition of the ointment

Ingredients	Quantity (%)
Methanolic extract of *Samadera indica*	5
Emulsifying wax	30
White soft paraffin	50
Liquid paraffin	20

Physical evaluation of the formulation

The formulations were inspected visually for their color, homogeneity, consistency, and phase separation.[18]

Measurement of pH

The pH was measured using a pH meter, which was calibrated before each use with standard buffer solutions at pH 4, 7, 9. The electrode was inserted into the sample 10 minutes prior to taking the reading at room temperature.[18]

Viscosity

The viscosity of the formulations was checked using a Brookfield Viscometer (DV-I PRIME, USA). The gels were rotated at 0.3, 0.6, 1.5 rotations per minute. The viscosity of the gel was obtained by multiplying the corresponding dial reading with the factor given in the Brookfield Viscometer catalog.[19]

Spreadability

Spreadability is expressed in terms of time in seconds taken by two slides to slip off from the gel when placed in between the slides under the direction of a certain load. The excess amount of sample was placed between the two glass slides and a definite amount of weight was placed on these glass slides to compress the glass slides of uniform thickness.[20] A weight of 70 g was added and the time required to separate the two slides was noted. Spreabability was calculated using the formula

S = M.L / T

Where, M = wt tied to upper slide, L = length of glass slides, T = time taken to separate the slides.

Extrudability

The formulations were filled in collapsible tubes after the ointments were set in the container. Extrudability of the different ointment formulations was determined in terms of weight in grams required to extrude a 0.5 cm ribbon of ointment in 10 seconds.[21]

Microbial contamination

Microbial contamination of ointment and gel with bacteria and moulds, respectively, was determined by spreading a thin loopful of the material withdrawn from the depth of the bulk product on a nutrient and Sabouraud agars, and incubating for 24 to 48 hours, at 37^{0}C.

Table 2: Composition of herbal gel

Ingredients	Quantity (%)
Methanolic extracts of *Samadera indica*	5
Carbopol gel	1
Triethanolamine	0.5
Propylene glycol	10
Ethanol	2.5
Water	q.s to 100 ml

In order to assess the degree of contamination, 1 g of material was dispersed in 4 ml of sterile Ringer solution, containing 0.25% Tween 80. Appropriate dilutions were made in the same dispersion vehicle and 0.5 ml was plated out on the appropriate solid medium using the surface viable method. Emergent colonies were counted after necessary incubation.

Antimicrobial evaluation

In-vitro antibacterial activity

Staphylococcus aureus and *Pseudomonas aeruginosa* are the commonly occurring gram-negative and gram-positive bacteria causing skin infection, hence, were selected for the study.

Test organism

Clinical microbial extracts of gram-negative (*Pseudomonas aeruginosa*) and gram-positive (*Staphylococcus aureus*) were used as antibacterial agents.

Antibacterial activity

In-vitro antibacterial activity was evaluated using the agar well diffusion technique. Muller-Hinton agar was used as the medium.[21] The sterile agar was inoculated with the bacteria culture (*S. aureus, P. aeruginosa*) for 48 hours, at 37°C. Wells were bored by using a sterile borer, and standard formulations (1000 µg / ml was prepared by dissolving the test sample in DMSO) were placed into them. Plates were kept for two hours in the refrigerator to enable prediffusion of the extracts into the agar. Next, the plates were incubated overnight (24 hours) at 37°C. The spectrum of activities of the extracts were compared with the marketed formulation, betadine ointment.[22-26]

In-vitro antifungal activity

A preliminary study of the *in-vitro* antifungal activity of the methanolic extract of *Samadera indica* against various fungal species like *Candida albicans, Aspergillus niger,* and *Aspergillus fumigates* showed that *Samadera indica* showed activity only against *Candida albicans* at a minimum inhibitory concentration of 250 µg / ml. Therefore, in the present study *Candida albicans* was used for the study.[2]

Test organism

The microbial culture *Candida albicans* was used, which was procured from National Center for Industrial Microorganisms (NCIM), Pune, India.

Antifungal activity

Sterile Sabouraud Dextrose Agar plates were prepared and 0.1 ml of inoculums from the standardized culture of the test organism was spread uniformly. Wells were prepared by using a sterile borer of diameter 10 mm. The test substance of 100 µl,, prepared by dissolving in dimethyl sulfoxide (1000 µg / ml) and the solvent control (DMSO) were added in each well separately. The plates were placed for 1 hour, at 4^{0}C, to allow the diffusion of the test solution into the medium; and the plates were incubated at 28^{0}C

for 48 hours, a period of time sufficient for the growth of at least 10 to 15 generations. The zone of inhibition around the well was measured in millimeters and compared with clotrimazole cream (marketed formulation).[25,26]

Stability

The stability studies were carried out for all the formulations. The formulations were kept at two different temperature 4 ± 2ºC and 30 ± 2ºC, 65 RH, for three months. The pH and the viscosity of the formulations, which were determined after three months, were compared with the initial pH and viscosity.

Statistical analysis

All experimental measurements were carried out in triplicate and were expressed as an average of three analyses ± standard deviation. Statistical analyzes was performed by the t-test.

RESULTS AND DISCUSSION

Monographic Analysis of Herbs

Ash value is a criterion to judge the identity or purity of a crude drug. The total ash value, water soluble ash, and acid insoluble ash of the herb are represented in Table 3.

The extractive values of the extracts obtained from the crude drug depend upon the chemical nature and properties of the contents of drugs and the various solvents used. The extractive value of *Samadera indica* in alcohol is represented in Table 4.

Phytochemical Screening

The preliminary phytochemical analysis of the methanolic extracts of *Samadera indica* revealed the presence of alkaloids, tannins, phenolic compounds, triterpenes, carbohydrate, steroids, proteins, and flavanoids.

Acute Toxicity Studies

The acute toxicity studies of the extract were studied and all the animals tolerated the maximum test doses of the extract, as there were no clinical signs of toxicity or mortality of the animals at a dose from 5 to 2000 mg / kg body weight. The animals could tolerate a dose greater than 2000 mg / kg body weight. The absence of the clinical signs of toxicity in treated animals suggests that the extract offers no potential ill effects to animals [Table 5].

Physical Evaluations of Formulations

The methanolic extracts of *Samadera indica* were formulated into a gel and ointment; these formulations did not show a considerable change in characters like color, odor, and consistency, and there was no phase separation observed during the course of the study.

The results of pH, spreadability, and viscosity of the formulations are recorded in Table 6. The results from Table 6 reveal that the pH of the developed formulations, such as the ointment and gel, are 6.97 ± 0.012 and 6.737 ± 0.045, respectively. The result shows that the formulations are compatible with the skin.

The viscosity of the formulations was measured using a Brookfield viscometer. The viscosities of the ointment and gel were 9664 ± 16.371 cps and 6462 ± 194 cps, respectively. The values are represented in the Table 6. This showed that in the case of a gel, the consistency depended on the ratio of the solid fraction, which produced the structure of the liquid fraction.

The spreadability of the formulation is recorded in Table 6. The spreadability of the formulations was acceptable.

The extrusion of the topical formulation from the tube is important during the application, as also patient acceptance. The study showed that the extrudability of the gel was comparatively better than the ointment.

Table 3: Determination of the ash value

Physical value	Values
Total ash	6.36733 ± 0.00751
Water soluble ash	1.66 ± 0.32512
Water insoluble ash	2.14533 ± 0.00351

*Value is expressed as Mean ± SD, n = 3

Table 4: Determination of the extractive value

Solvent	Extractive value
Alcohol	2.32 ± 0.2286

*Value is expressed as Mean ± SD, n = 3

Table 5: Response after treatment of animal

Response	Head		Body		Tail	
	Before	After	Before	After	Before	After
Alertness	Normal	Normal	Normal	Normal	Normal	Normal
Grooming	Absent	Absent	Absent	Absent	Absent	Absent
Touch response	Absent	Absent	Absent	Absent	Absent	Absent
Torch response	Normal	Normal	Normal	Normal	Normal	Normal
Pain response	Normal	Normal	Normal	Normal	Normal	Normal
Tremors	Absent	Absent	Absent	Absent	Absent	Absent
Convulsion	Absent	Absent	Absent	Absent	Absent	Absent
Righting reflux	Normal	Normal	Normal	Normal	Normal	Normal
Gripping strength	Normal	Normal	Normal	Normal	Normal	Normal
Pinna reflux	Present	Present	Present	Present	Present	Present
Corneal reflux	Present	Present	Present	Present	Present	Present
Writhing	Absent	Absent	Absent	Absent	Absent	Absent
Pupils	Normal	Normal	Normal	Normal	Normal	Normal
Urination	Normal	Normal	Normal	Normal	Normal	Normal
Salivation	Normal	Normal	Normal	Normal	Normal	Normal
Skin colour	Normal	Normal	Normal	Normal	Normal	Normal
Lacrimation	Normal	Normal	Normal	Normal	Normal	Normal

Microbial Contamination

The microbial contamination of the ointment and gels after 24 hours was found to be 1.45 CFU (Colony Forming Unit) and 2.12 CFU for fungi, at a temperature of 37°C, and 3.21 and 2.89 CFU for bacteria, at the end of five days.

Antimicrobial Activity

The antimicrobial activities (by measuring the zone of inhibition) of the formulated formulations were compared with the marketed formulation.

Antibacterial Activity

Table 7 shows the antibacterial activity of the formulations of the methanolic extract of *Samadera indica* and the marketed formulation against various strains of bacteria such as *S. aureus* and *Pseudomonas aeruginosa*. The table represents that the formulations had antibacterial activity against both gram-negative and gram-positive bacteria. The ointment of the methanolic extract of *Samadera indica* had comparatively more activity than the gel formulation ($P < 0.05$).

Antifungal Activity

The antifungal activity of the formulations were carried out against *Candida albicans*, this is represented in Table 8. The ointment of the methanolic extract of *Samadera indica* had comparatively more activity than the gel ($P < 0.05$).

Stability Studies

The stability studies showed that the formulations were stable during the study period as these formulations did not show any physical instability, and the pH before and after the study did not show any significant change. Hence, the formulations were found to be stable [Table 9].

CONCLUSION

From the present study, it was concluded that the methanolic extract of *Samadera indica* was safe and non-toxic. The maximum tolerable dose of extract was greater than 2000 mg / kg body weight. The extract did not show any clinical signs, and hence, was potentially non-toxic. The formulated formulations showed acceptable physical properties, and hence, were compatible with the skin. The *in-vitro* antimicrobial activity showed that the formulated ointment of the methanolic extract of *Samadera indica* showed comparatively more against the bacterial and fungal species rather than the formulated gel. In addition, the formulated formulations passed the short-term stability, indicating the physical and chemical stability of the product. Hence, the formulated formulations of the methanolic extracts of *Samadera indica* were safe and efficient carriers, with potent antimicrobial activity.

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Table 6: Physical parameters of the formulation

Formulations	pH*	Viscosity (cps)*	Spreadability (gcm/s)*
Samadera ointment (SO)	6.97 ± 0.011	9664 ± 16.37	41.37 ± 0.341
Samadera gel (SG)	6.74 ± 0.045	6462 ± 194	22.6 ± 1.276

*The value is expressed as Mean ± SD, n = 3

Table 7: *In-vitro* antibacterial activity

Organism	Zone of inhibition(mm)		
	Samadera ointment	Samadera gel	Marketed formulation (betadine)
Staphylococcus aureus	22 ± 1.000	18 ± 0.60	23 ± 0.60
Pseudomonas aeruginosa	22 ± 0.50	19 ± 1.00	23 ± 0.70

*The value is expressed as Mean ± SD, n = 3

Table 8: *In-vitro* antifungal activity

Organism	Zone of inhibition (mm)		
	Samadera ointment	Samadera gel	Marketed formulation (clotrimazole)
Candida albicans	23 ± 1.00	20 ± 1.00	25 ± 0.60

*The value is expressed as Mean ± SD, n = 3

Table 9: Stability studies of the formulations

Formulations	pH		Viscosity	
	Initial	Final	Initial	Final
Samadera ointment	6.85 ± 0.021	6.78 ± 0.016	9664 ± 16.37	9775 ± 28.79
Samadera gel	6.49 ± 0.045	6.51 ± 0.034	6462 ± 194	6478 ± 89.45

*The value is expressed as Mean ± SD, n = 3

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