Development and Evaluation of Antimicrobial Herbal Ointment of Root of Clinacanthus Nutans

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Abstract: Antimicrobial sensitivity causes a serious problem in the skin infection, for this treatment various formulation are available in the market day by day research has been developed in the pharmaceutical industry. But now a days many pharmaceutical industry moving towards the herbal formulation to this is an attempt of Clinacanthus nutans showed excellent activity against Staphylococcus aureus and Bacillus subtilis ($p < 0.05$). Most of these organisms are natural flora of the skin and also known etiologic agents of several skin and mucous membranes infections of man. The extract did not show any activity against Pseudomonas contrary to observations F3 compared more favorably with Gentamicin ointment for its antibacterial activity against S. aureus and B. subtilis. Formulation 3 showed a slightly higher activity than Gentamicin ointment against S. aureus, and lower activity against B. subtilis. Clinacanthus nutans herbal ointment did not show any activity against Pseudomonas while the standard drug Gentamicin ointment showed relativelyhigh activity against E. coli and P. aeruginosa. The prepared formulations show a smooth and homogeneous appearance. The pH values of all the prepared formulations ranged from 6.1 to 7.3, which are considered acceptable to avoid the risk of irritation upon application to the skin.

Keywords: Herbal ointment, Clinacanthus nutans, Gentamicin ointment, *P. aeruginosa,* spreadability.

INTRODUCTION

Herbal medicines are being used by about 80% of the world population primarily in the developing countries for primary health care.[1] They have stood the test of time for their safety, efficacy, cultural acceptability and lesser side effects. Ancient literature also mentions herbal medicines for age-related diseases namely memory loss, osteoporosis, osteoarthritis, diabetes, immune and liver disorders, etc.[2] for which no modern medicine or only palliative therapy is available.[3] The chemical constituents present in them are a part of the physiological functions of living flora and hence they are believed to have better compatibility with the human body[4]. There are over 1.5 million practitioners of traditional medicinal system using medicinal plants in preventive, promotional and curative applications.[5]

According to survey report by WHO, about 25 per cent of prescribed human medicines are derived from plants and 80 per cent people still depend on traditional system of medicines.[6] The herbal wealth of India and the knowledge of their medicinal properties have a long tradition, as referred in Rig veda and other ancient literature. The topography of India in the tropical belt with its varied climatic zones made it a vast storehouse of medicinal plants.[7] The quality assessment of herbal formulations is of paramount importance in order to justify their acceptability in modern system of medicine. One of the major problems faced by the herbal industry is the unavailability of rigid quality control profiles for herbal materials and their formulations. Regulatory bodies have laid down the standardization procedures and specifications for Ayurvedic preparations.[8] The World Health Organization (WHO) has appreciated the importance of medicinal plants for public health care in developing nations and has evolved guidelines to support the member states in their efforts to formulate national policies on traditional medicine and to study their potential usefulness including evaluation, safety, and efficacy.[9]

Fig.No.1. Formulated herbal ointment

Material and method

The roots of *Clinacanthus nutans will be* collected from Gondia district, Maharashtra, India. The herbarium sheet of plant specimen will be authenticated by Department of Botany, DB Science Gondia and notated as identification voucher No. HS- ACC/18.09.2022/7885. The roots of *Clinacanthus nutans* were cleaned from its debris, dried and coarsely powdered. It was stored in well-stopper container for furtherwork.

EXTRACTION OF ROOTS OF *Clinacanthus nutans*

The sun-dried roots of *Clinacanthus nutans* was powdered using a laboratory mill (Kenwood Ltd, Hertfordshire). 140g of milled leaves of *Clinacanthus nutans* was extracted with methanol by maceration for 48 hr. The extract was filtered and concentrated using a Buchi V-801 rotary evaporator at 35°C to obtain semisolid extract. The extract was stored in a refrigerator.[10] A stock concentration of 400mg/ml was prepared from which working concentrations of 200mg/ml, 150mg/ml, 100mg/ml and 50mg/ml were prepared.

Characteristics of extracts:

Characteristics of extracts is shown in Table 1

Table 1: Characteristics of Extracts

Sr. No.	Solvent	Colour	% Yield
1.	Methanol	Brown	6.39

Preliminary Phytochemical screening of *Clinacanthus Nutans.*

Phytochemical Screening

The analysis was focused on the dried methanolic extract of ground dried C. alata leaves. Briefly, the maceration of 20 g plant material was done in 125 ml of methanol 80° at room temperature for 48 hours. After filtration using cotton wool, the methanolic extract was dried at 40°C in the oven.

Table 2: Preliminary phytochemical screening of *Clinacanthus nutans*

Secondary metabolites	Reagents	RESULTS
Saponins	Foam index	++
Sterols & polyterpenes	Liebermann	-
Gallic tannins	FeCl3 1%	++
Flavonoids	Shinoda	-
Catechic tannins	FeCl3 1%	-
Anthocyanosides	H_2SO_4 &NH4OH	-
Anthracenosides	Borntraeger	+++
Alkaloids	Dragendorff	-

- : Absence; +: Presence; ++: Abundant; +++: very abundant. Preliminaryphytochemical screening showed the presence Anthracenosides

ANTIMICROBIAL ACTIVITY ROOTS OF *Clinacanthus nutans*

The antibacterial activity of the methanolic extract of the leaves of *Clinacanthusnutans* at concentrations of 50mg/ml, 100mg/ml, 150mg/ml and 200mg/ml were determined using the cup plate method. A molten Mueller Hinton agar stabilizedat 45°C was seeded with 0.1 ml of a 24 h broth culture of the test organism (*B. subtilis, E. coli, P. aeruginosa and S. aureus*) containing approximately 10 cfu / ml in a sterile petri dish and allowed to set. Wells of 6mm diameter were created with a sterile cork borer and filled to about three-quarters full with solutions of the methanolic extract of the leaves of *Clinacanthus nutans*. The plates were pre-incubated for 1 h at room temperature to allow for diffusion of the solution and then incubated for 24 h. The zones of inhibition were measured (mean, *n=2*). Streptomycin and propylene glycol were used as positive and negative controls respectively. The *in vitro* bacterial response to the extract was evaluated using the diameter of the zones of inhibition as follows; resistant: 10 mm and below, intermediate: 11-15mm and susceptible 16mm and above.

Results of antimicrobial activity of *Clinacanthus nutans*

The preliminary *in vitro* antimicrobial activity of the methanolic extract of *Clinacanthus nutans* presented in Table.3 showed excellent activity against *Staphylococcusaureus and Bacillus subtilis.*

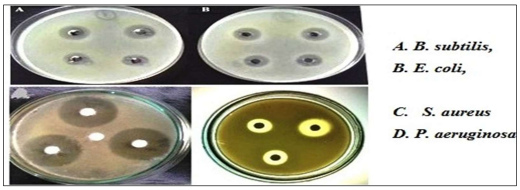

Fig. 2: Antibacterial activity of roots of *Clinacanthus nutans*

Table. 3 : Preliminary *in vitro* antibacterial activity of methanolicextract of *Clinacanthus nutans* (Zone of inhibition in mm)

Test organism	Concentration of Extract				Standard
	50mg/ml	100mg/ml	150mg/ml	200mg/ml	Streptomycin
S. aureus	16.50±0.58	17.00±0.00	18.00±1.41	19.00 ± .00	21.00 ±0.00
B. subtilis	12.50±0.50	12.00±1.29	13.00±0.50	16.00±0.50	20.00 ±0.50
E. coli	10.50±0.00	9.00±1.20	10.20±0.5	11.25±0.5	27.00± 0.00
P.aeruginosa	0.00	0.00	0.00	0.00	30.00± 1.00

Clinacanthus nutans has antibacterial activity and has high potential as antibacterial agent when formulated as ointment for topical use and could therefore explain the successes claimed in the folk use of the plant in the treatment of common skin conditions. The potency of the *Clinacanthus nutans* herbal ointment against *Staphylococcus aureus* could be harnessed in the containment of the organism implicated as the commonest etiologic agent of boils, carbuncles, infantile- impetigo and wounds.

PREPARATION OF OINTMENTS

Three topical ointment bases of varying degrees of aqueous/anhydrous character, namely: simple ointment BP, emulsifying ointment BP and aqueous cream BP were prepared by fusion method. In this method the constituents of the base were placed together in a melting pan and allowed to melt together at 70°C. After melting, the ingredients were stirred gently maintaining temperature of 70°C for about 5 minutes and then cooled with continuous stirring. Formulation of ointment was done by incorporating 10 g of the semisolid methanolic extract of *Clinacanthus nutans* into the various bases by triturating in a ceramic mortar with a pestle to obtain 100 g of herbal ointments containing 10 % w/w of *Clinacanthus nutans* extract. The prepared herbal ointments were put in ointment jars, labeled and were stored at room temperature pending the evaluation.

Table.4 :Preparation ointment of medicated formulations with methanolic extract of *Clinacanthus nutans*

Formulations	Ingredients	Concentration (%w/w)
Formulation 1	Extract	10
	Wool fat	5
	Cetostearyl alcohol	5
	Hard paraffin	5
	White soft paraffin	85
Formulation 2	Extract	10
	Liquid paraffin	20
	Emulsifying wax	30
	White soft paraffin	50
Formulation 3	Extract	10
	Emulsifying ointment	30
	Chlorocresol	0.1
	Purified water	69.9

Physical evaluation of formulated Ointments

Colour and Odour

Physical parameters like colour and odour were examined by visual examination.

Consistency

Smooth and no greediness is observed.

pH

P^H of prepared herbal ointment was measured by using digital P^H meter. The solutionof ointment was prepared by using 100ml of distilled water and set aside for 2hrs. P^H was determined in triplicate for the solution and average value was calculated.

Spreadability

The spreadability was determined by placing excess of sample in between two slides which was compressed to uniform thickness by placing a definite weight for definite time. The time

required to separate the two slides was measured as spreadability. Lesser the time taken for separation of two slides results better spreadability. Spreadability was calculated by following formula

$$S = M * L/T$$

Where,

S= Spreadability

M= Weight tide to the upper slide

L= Length of glassslide

T= Time taken to separate the slides

Extrudability

The formulation was filled in collapsible tube container. The extrudability was determined in terms of weight of ointment required to extrude 0.5cm of ribbon of ointment in 10 seconds.

Diffusion study

The diffusion study was carried out by preparing agar nutrient medium. A whole board at the center of medium and ointment was by placed in it. The time taken by ointment to get diffused through was noted. (After 60minutes)

LOD

LOD was determined by placing the formulation in petri-dish on water bath and dried for the temperature 105^{o}C.

Solubility

Soluble in boiling water, miscible with alcohol, ether, chloroform.

Wash ability

Formulation was applied on the skin and then ease extend of washing with water was checked.

Non irritancy Test

Herbal ointment prepared was applied to the skin of human being and observed forthe effect

Stability study

Physical stability test of the herbal ointment was carried out for four weeks at various temperature conditions like 2^{o}C, 25^{o}C and 37^{o}C. The herbal ointment was found to be physically stable at different temperature i.e. 2^{o}C, 25^{o}C, 37^{o}C within four weeks.

Result of Physical evaluation of formulated Ointments

Table. 5 Physical evaluation of formulated Ointments methanolic extractof *Cassiaalata For F1, F2 & F3 Formulation*

Physicochemical parameters	**Observation**
Colour	Yellow
Odour	Characteristic
Consistency	Smooth
pH	6.4
Spreadability(seconds)	7
Extrudability	0.4 gm
Diffusion study (after 60min)	0. 7 cm
Loss on drying	35%
Solubility	Soluble in boiling water, miscible with alcohol, ether,chloroform
Washability	Good
Non irritancy	Non irritant
Stability study (2 C, 25 C, 37 C)	Stable

***IN-VITRO* ANTIBACTERIAL EFFICACY OF FORMULATED OINTMENTS**

Formulation 1 (*Clinacanthus nutans* 10% w/w in simple ointment B.P),

Formulation 2 (*Clinacanthus nutans* in emulsifying ointment B.P)

Formulation 3 (*Clinacanthus nutans* 10%w/w in aqueous cream B.P).

The cup-plate method was used to assess the relative antibacterial efficacy of the formulated herbal ointments prepared with the extract of *Clinacanthus nutans* culture of the test organism (*Bacillus subtilis* and *Staphylococcus aureus*) andcontaining approximately 10 cfu / ml was used. Wells of 6mm diameter were created and filled to three-quarters full with the topical products of *Clinacanthus nutans* extract. The plates were pre-incubated for 1 hr at room temperature to ensure adequate diffusion and finally incubated at 37°C for 24 h. A commercial brand of Gentamicin ointment (Drug field Pharmaceutical Ltd, Lagos) was used as standard while blank ointment base was used as control. The experiments were run in duplicate and the zones of inhibition were determined and recorded (mean± SD,*n = 2*).

Result In vitro antibacterial efficacy of formulated ointments

Table .6 Result In vitro antibacterial efficacy of formulated ointmentsmethanolicextract of *Clinacanthus nutans.*

	Formulation. 1		**Formulation. 2**		**Formulationn. 3**	
Test Organism	100mg/ml	150mg/ml	100mg/ml	150mg/ml	100mg/ml	150mg/ml
S. aureus	19.00±0.5	20.00±0.00	19.00±0.00	20.00±0.00	23.00±0.00	24.50±1.91
B. subtilis	16.00±1.00	16.50±0.58	16.50±1.41	17.00±0.50	19.00 ±0.55	21.00±0.33
E. coli	2.60	4.00±1.41	3.40	6.50±0.00	6.50 ± 0.00	8.50±0.50
P. aeruginosa	0.00	0.00	0.00	0.00	0.00	0.00

CONCLUSION

The antibacterial activity of the methanolic extract of Clinacanthus nutans roots was evaluated. This activity was maintained when the extract was incorporated into the ointment base for topical use in the treatment of common skin condition. The ointment was stable after two months. Further the extract can be used for the commercial production of Clinacanthus nutans ointment. Similarly, this ointment can be tested for skin related bacterial infections. This study shows that Clinacanthus nutans has antibacterial activity and has high potential as antibacterial agent when formulated as ointment for topical use and could therefore explain the successes claimed in the folk use of the plant in the treatment of commosn skin conditions. The potency of the Clinacanthus nutans herbal ointment against Staphylococcus aureus could be harnessed in the containment of the organism implicated as the commonest etiologic agent of boils, carbuncles, infantile- impetigo and wounds. This investigation suggests the use hydrophobic ointments containing Clinacanthus nutans as antibacterial ointment preparation. Aqueous cream, a water-miscible topical base, was possibly a better vehicle for the release of the antibacterial compounds present in Clinacanthus nutans extract.

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