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"FORMULATION AND EVALUATION OF HERBAL OINTMENT FOR ANTIMICROBIALACTIVITY"

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Abstract:

Ointments are semisolid systems which usually behave as viscous elastic materials when shear stress is applied. They generally contain medicaments and are intended to be applied externally to the body or to the mucous membrane. Many medicaments meant for topical application to intact or broken skin or to mucous membranes, have been presented in the form of semisolid consistency variously designated as ointments, creams, salves, pastes etc used mainly as protective or emollient for the skin. The first step towards this goal is the screening of plants used in popular medicine. Along with other dosage forms, herbal drugs are also formulated in the form of ointment. The phyto chemical analysis of Jasminum officinale indicated the presence of alkaloids, flavonoids, tannins, glycosides, and steroids. Pharmacological studies revealed that the plant exerted antimicrobial and antibacterial effects. This research described and discussed the chemical constituents and pharmacological effects of Jasminum officinale.

KEYWORDS: Jasminum officinale, Methanolic extract, Antimicrobial, Antibacterial activity, E-coli, Staphylococcus aureus.

INTRODUCTION:

Ointments are semisolid systems which usually behave as viscoelastic materials when shear stress is applied. They generally contain medicaments and are intended to be applied externally to the body or to the mucous membrane. Semisolids constitute a significant proportion of pharmaceutical dosage forms. They serve as carriers for drugs that are topically delivered by way of the skin, cornea, rectal tissue, nasal mucosa, vagina, buckle tissue, urethral membrane, and external ear lining [1]. A semisolid dosage form is advantageous in terms of its easy application, rapid formulation, and ability to topically deliver a wide variety of drug molecules. Semisolids are available as a wide range of dosage forms, each having unique characteristics [2] Ointments are semisolid preparations for external application to skin or mucous membranes. Their composition softens but

does not melt upon application to the skin. [3] Therapeutically, ointments function as skin protective's and emollients, but they are used primarily as vehicles for the topical application of drug substances. [5] The aim of this study was to use herbal powder of jasmine leaves in the therapy of mouth ulcer in pharmaceutical Ointment .Jasmine is botanically known as Jasminum officinale or Jasmininie and belongs to the olive family of Oleaceae. [6]This plant is clinging plant. [4] The branches are striped and leaves are upward facing and uneven. Jasmine leaves show analgesic, antidepressant, antiseptic, expectorant, sedative, stomachic, diuretic, astringent, stimulating, anthelmintic, antioxidizing, anti inflammatory property. [7] The leaf of plant present ascorbic acid, anthranilic acid and its glycoside, indol oxygenase, alkaloid, salicylic acid. [8] The leaves of plant contain main chemical called as Jasmininie. The oil contains benzyl acetate, methyle thynylate and equal. It cure kapha and pitta and different disorders. Medicinal plant has valuable part in both medicinal and economical. Now a days herbal medicine uses are increased and their safety, quality and efficacy also increased. [9] Herbal medicine illustrate greater results as compare to other medicinal like allopathic medicine and herbal medicines avoiding typical side effects and gives better results to patients. [10] In the present situation people are targeting on herbal medicine and using different natural products to cure and prevent disease.

Figure no 1. Jasminum officinale

MATERIALS AND METHODS

Soxhlet Extraction:

In this method, finely ground sample was placed in a porous bag or thimble, made from a strong the filter paper or cellulose. Extraction solvent i.e, methanol was heated in the bottom flask vaporize in to the sample thimble condenses in the condenser and drip back. Theliquid contents emptied into the bottom flask again andthe process was continued. [11] The final methanolic extract is collected.

Figure no 2 Soxhlet extraction apparatus

Preparation of Methanolic extract:

The powdered plant materials 25gm was packed in cellulose thimble placed in the extraction tube of a soxhlet apparatus and extracted with chloroform 250ml for 6hr. [12] The extract were concentrate under the vaccum and dried.

Preliminary Phytochemical screening of Jasmium officinalis :

Phytochemicals Test: It involves various chemicals tests to identify different phytochemicalsfor example, alkaloids can be detected by Mayers reagent etc. observation and results ofqualitative chemical constituents of T. Bellerica a combined knowledge of phytochemicalscreening. [13]

1)TestforAlkaloid: [14]

a) Dragandroff's test

b) Hanger's test.

c) Myer's test.

d) Wagner's test:

2) Testforflavonoid: [15]

a) SchinodaTest.

3) **Test for Saponins** [16]

4)TestforTannins: [17]

a) IodineTest

b) FerricchlorideTest

c) Testforsteroids

d) Salkowshi test

e) libermannBurchard test

5)Testforglycoside: [18]

a) Bortangerstest

b) killerkillanitest.

c) Baljettest:

6)Testforprotein: [19]

a) Biurrettest

b) xanthoproteictest:

Evaluation of Excipients

Cetostearyl alcohol:-soluble in ether soluble in ethanol and enlight petroleum practically insoluble in water and melted it is miscible with fixed oil and with liquid paraffin. [20]

Wool fat:-soluble in chloroform and in ether slightly soluble in boiling ethanol (95%) partially in soluble in water [21]

Yellow Soft paraffin:-soluble in chlorof or man dinetheran din light petroleum 40 degree 60 degree the solution sometimes you are light light appliances partial in soluble in ethanol on and in water. [23]

Hard Paraffin: soluble in chloroform and in ether practically in soluble in ethanol and in water. [23]

Formulation of ointment base: [24]

Table no 1

Sr.No.	Name of Ingredient	Quality to be taken
1.	Wool fat	0.63g
2.	Cetosterol alcohol	0.52g
3.	Hard paraffin	0.59g
4.	Yellow soft paraffin	9g

Procedure for Preparation of Ointment base: [25]

1. Fusion, in which ingredient are melted together add stirred to ensure homogeneity.
2. Trituration, in which finely subdivided insoluble medicament are evenly distributed by grinding with a small amount of the base or one of its ingredient followed by dilution with gradually increasing amount of the base. [26]

Formulation of Herbal ointment:

Table no 2.

Sr.No	NameofIngredient	Qualitytobetaken
1.	PreparedJasmineextract	0.06g
2.	Ointment	0.06g

Procedure for Preparation of herbal ointment [27]

a) Initially ointment base was prepared by weighing accurately grated Hard paraffin whichwas place in evaporating dish on water bath. After melting of hard paraffin wax remaining ingredients were added and stir gently to aid melting and mixing homogenous followed by cooling of ointment base. [28]

b) Herbal treatment was prepared by mixing accurately weighed jasminum extract to theointment base by levigation method to prepare a smooth paste. With 2 or 3 times its weight of base, gradually

incorporating more base until two form homogenous ointment , finally transferred in a suitable container. [29]

FormulationTable:

Table no 3.Composition of various ointment formulation. [30]

Ingredient	**Quantity in molar gm**			
	F1	F2	F3	F4
Cetosterolalcohol	0.5	0.52	0.51	0.55
Hardparaffin	0.59	0.52	0.58	0.54
Woolfat	0.63	0.53	0.59	0.59
YellowSoftParaffin	9	9	9.48	9.80
Jasminum	0.6	1.12	1.6	1.8

EVALUATIONOFOINTMENT: [31]

1. **Determinationofconsistency**:

Consistency of the formulation was checked by its application on outer surface of the skin.

2. **Determination of percentage yield**

Weigh the empty container which the environment formulation was feel for the story. The Other in weight of that container with gel formulation was note to obtainthepractical in surprise the weight of the empty container with the failed containerofgelformulationinthiswaywehad calculatethepercentageyield oftheproduct.Percentageyield=(practicalyield/theoreticalyield)*100

3) Determination of Ph measurement:

Ph of the formulation is determined by using digital pH metre .we had measured 2 gof appointment and dissolve into 20 ml distilled water and keep aside for two hoursthe measurement of pH is performed by dipping the glass electrode completely in to the ointment system three time and average value are noted.

4) Determination of Homogenecity:

All prepaid share all prepared ointment formulation we are examined for by visual inspection after the ointment have been settled into the particular container it was observed that the ointment our presence and its appearance to for maggregate.

5) Determination of viscosity:

Brookfield viscometer was used to determine the viscosity of the formulation withspindle number one at 25° C the ointment was rotated at speed 0.3,0.6,1.5 rotation per minute and its speed the corresponding dial reading was noted.

6) Determination of Spreadability:

Spreadability was determined by spreading that ointment on the hand surface.

S=M *L/T

Where,

S=Spreability

M=WeighttidetotheupperslideL=lengthoglassslide

T=Timetakentoseparatetheslide

7) Determination of Extrudability:

Formulated ointment was packed in a standard capped collapsible aluminum tube and Seal by creeping to the end ointment the moment of the extruded ointment was collected and wave accessibility was determined by measuring the percentage of rating ointment.

8) Determination of Clarity :

Its was determined by visual inspection.

Determination of antimicrobial and antibacterial activity

Test microorganism:

Bacteria : E-coli. , Staphylococcus aureus.

Antimicrobial and antibacterial activity

The agar well diffusion method described by the Zaria (1955) was adopted for the anti microbial sensitivity tests. for antibacterial studies ,the microbial strain Escherichia coli and for antimicrobial study the microbial strain Staphylococcus aureus was collected from Manoharbhaipatel Institute of pharmacy, Gondia.

Preparation of inoculum

Bacteria suspension were prepared from overnight culture by the direct colony method. Colonies were taken directly from the plate and suspended through this free culture broth. These pre-culture broth were 31 allowed to stand overnight in a Rotary Shaker at 37 degree Celsius after which this culture where maintain on growth increase for further use.

Preparation of growth media:

Nutrient Agar was used for preparation of medium for growth of above said organism. Nutrient agar taken (2.3gm width 100 ml of distilled water) for preparation of growth media. Prepared nutrient agar was autoclaved at 121 degree Celsius and 15 lb .pressure and the nnutrient agar was poured in petriplates under the laminar flow with suitable sterile condition.

After solidification, plates were kepts in incubator at 24hours for checking of contamination in media ,followed by using the plates for further testing the antibiotics susceptibility of the isolated strain.

Preparation of zone of inhibition

Antibacterial and antimicrobial activity was checked by agar well diffusion method. In this method a previously liquefied medium was inoculated with 0.2 ml of fungal and bacterial suspension having a uniform turbidity at temperature of 400 C.20 ml of culture medium was poured into the sterile petri dish having an internal diameter of 8.5 cm .Care was taken for the unjform thickness of the layer of medium in different plates. After complete solidification of liquefied inoculated medium , the wells

were made aseptically with cork better having 6mm diameter.In each of these plate gel solution was placed carefully .plates were kepts for pre diffusion for 30 mins .
After it normalized to room temperature , the plates were incubated at 37 degree celsicus for 24 hours to case bacteria and at 27 dgreecelsicus for 48 hours in case of fungi After incubation periods was over , the zone of inhibition was measured with the help of Hi –antibiotics zone scale.

Observations

A) Studyofthemacroscopiccharacteristicsofdrug

Table no 4.

Colour	Green
Odour	Pleasant
Taste	Pungent

A) Preliminary Phytochemicals Test

B) Table no 5. Preliminary Phytochemicals screening of Jasminum Officinale

Sr.on	Plant constituents	Test/Reagents	Aqueouse xtract	Methanol extract
1	Alkaloidtest	Dragendroff'stest	+	+
		Hager'stest	+	+
		Mayer'stest	-	-
		Wagner'stest	+	+
2	Flavonoidstest	Shinodatest	-	+
		Leadacetatetest	-	+
3	Tannintest	IodineTest	-	+
		Ferricchloridetest	-	+
4	Steroidstest	Salkowskitest	-	+
		Liebermanntest	-	+
5	Glycosidestest	Borntrager'stest	-	+
		KillerKillanitest	-	+
		Baljettest	-	+
6.	Proteintest	Biurettest	+	-
		Xanthoproteictest	+	-

A) Evaluation of Excipients:

Table no 6. Solubility test for Excipients

Excipients	Chloroform	Ether	Ethanol
Cetostearylalcohol	Soluble	Freelysoluble	Soluble
HardParaffin	Soluble	Soluble	Insoluble
WoolFat	Soluble	Slightlysoluble	Insoluble
YellowsoftParaffin	Soluble	Slightlysoluble	Insoluble

1) Physicalevaluation.

Table no 7. Physical evaluation of ointment formulation

Formulation	Colour	consistency	odor
F1	Lightgreen	Good	characteristic
F2	Lightgreen	Good	characteristic
F3	Lightgreen	Good	characteristic
F4	Lightgreen	Good	characteristic

2) Percentageyield

Table no 8. Percentage yield of the formulation

Formulation	Percentage yield%
F1	94.12
F2	93.44
F3	91.35
F4	95.56

3) ph

Table no 9. ph of ointment formulation

Formulation	PH
F1	6.7
F2	6.9
F3	7.2
F4	7.0

Homogeneityandspreadability

Table no 10. Homogeneity of ointment formulation

Formulation	**Homogeneity /spreadability**
F1	Good
F2	Good
F3	Good
F4	Good

1) Viscosity

Table no 11. Viscosity of ointment formulation

Formulation	**viscosity**
F1	4400
F2	4600
F3	4200
F4	4500

A) EvaluationofAntimicrobial&Antibacterialactivity:

Table no 12 Zone of Inhibition, diameter in mm

Formulations	**Diameter of zone of inhibition of Staphylococcus aureus**
Formulated ointment	19.5mm
Marketed preparation	20.1mm
Jasminum leaves extract	21.2mm

Table no 13. Zone of inhibition, diameter in mm

Formulations	**Diameter of zone of inhibition of *e.coli***
Formulated ointment	22.15
Marketed preparation	18mm
Jasminum leaves extract	20mm

DISCUSSION

Jasminum of ficinale is one of the most well-famous fragrant plants worldwide and has been prescribed in folk medicines in many countries according to its multipurpose actions. In addition, Jasmine tea is the most famous scented tea in many countries including China, Japan, and Thailand. Never the less, the chemical constituents and pharmacological activities of J.officinale have been rarely reported.

Our phytochemicals analysis indicated that the methanolic leaf extract contained the mixtures of glycosides, flavonoids, steroids, alkaloids, and tannins. Therefore, the various therapeutic actions used in different traditional medicines are definitely attributed to the mixtures of active ingredients in the Jasmine leaves.

we first described that the extract of leaves of J.officinale showed positive results for various phytochemicals as shown in table1.

The result from the present study suggests that the Jasmine leaf extract exert antibacterial and antimicrobial activity due to presence of mixture of chemical constituent.

CONCLUSION

Formulation of Herbal ointment was successfully developed that met the relevant pharmaceutical characteristics. The extract contain different Phytochemicals which is responsible for its antimicrobial & antibacterial activity the prepared formulations showed good spreadability, no evidence of phases separation and good consistency during the study period. Stability parameters like visual appearance, nature, viscosity and pH of the formulations showed that there was no significant variation during the study period. The prepared formulations showed proper range that is approximately pH 7. The ointement was found to be stable during stability study. From the presentstudy it can be concluded that it is possible to develop ointments containing herbal extracts and can be used as they are more potent healers because they promote the repair mechanism in the natural way. F3 formulation showed the best activity among all the formulation.

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