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"Formulation and Evalution of Antibacterial Ointment by Using Phyllanthus Amarus Extract"

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ABSTRACT

The aim of present research work is to "formulation and evalution of antibacterial ointment by using phyllanthusamarus extract" the composition of herbal Ointment are leaves of Phyllanthusamarus plant , methanol extract of Phyllanthusamarus ,woolfat used as emulsifying agent,soft paraffin and hard paraffin used as emoliant,cetostearyl alcohol used as a stabilizer.for extraction we use decoction method . ointment was prepared having antibacterial activity fr methanol extract of Phyllanthusamarus.the ointment was evaluated for some parameters like spreadability test, washabilitytest,irritancytest,Test of penetration, test of rheological properties,test of preservative efficacy,pH determination test.the result of above test are as following.the ointment physical character is green in colour and bitter in taste .pH is (4.5-6.2)observed and neutralize by buffer solution.thespreadability test (50)Is obtained.thewashability test in that slightly washable and sticky is observed.The test of penetration (20 mg drug absorbed) is observed. The test of rheological properties (viscous in nature) is observed.test of preservative efficacy is no preservatives is observed.no skin irritation .the formulation was clear had good antibacterial activity.and result showed the production of stable herbal Ointment with no sign of skin irritation and no change in antibacterial activity.And will not only give antibacterial activity but also give good emoliantactivity.Further research and development Is required to improve its quality and safety.

Keywords: Herbal antibacterial ointment, decoction method, natural composition, antibacterial activity

I. INTRODUCTION

Fig .no 1 .Phyllanthusamarus leaves

ointment is a viscous semisolid preparation used topically on a variety of body surfaces. Phyllanthusamarusointment in which Phyllanthusamarus is also known as "bhuiAmla" and is belonging to the family phyllanthaceae.phyllanthusamarus is an important plant of Indian Ayurvedic system of medicine which is used in the problems of stomach, genitourinary system, liver, kidney and spleen. It is bitter, astringent, stomachic, diuretic, febrifuge, and antiseptic..some Ointment are cause skin irritation but this Phyllanthusamarus ointment does not cause any skin irritation.this ointment also work as a emoliant to the skin .and had good antibacterial activity.

The selection of active ingredient for skin care is based on the ability of the ingredients to prevent skin damage as well as improve the skin quality by nourishing the skin and protecting the skin .the most advantage of this ointment is that it has no side effects on the skin .it contains woolfat,soft paraffin and hard paraffin, cetostearyl alcohol which protect skin .Phyllanthusamarus ointment has good antibacterial properties which is obtained from nature.

Skin swelling, diarrhoea,fever,skin flushing, soreness,allthise are actually caused by

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bacteria.and these called as bacterial infection.presence of bacteria causes skin swelling, diarrhoea,fever, chills etc .this Phyllanthusamarus ointment has good antibacterial properties.and this Phyllanthusamarus ointment cure this antibacterial infections.

II. MATERIAL AND METHOD

Materials:-

The powder of phyllanthusamarus collected locally from Gondia and authentication with the help of authentic herbarium species at botany department , D. B. Science college Gondia. The powder of phyllanthusamarus was stored in well-stoppered container. The dried material of the plant then used for further work.

Phyllanthusamarusdrug ,Methanol,Wool fat ,Cetostearyl alcohol ,Hard paraffin ,Soft paraffin.

Method:-**Extraction of phyllanthus amarus by decoction method**

Decoction method is the process of extraction , we have performed for seperation of medically active portion of plant of phyllanthusamarus from the inactive or inert components. For the Extraction process, we have required the dried leaves of plant phyllanthusamarus, solvent and soxlet apparatus.

In the decoction method , we have to performed such following steps to obtained quality extract from phyllanthusamarus . In the first we collect the powder of phyllanthusamarus.

Then in next steps we performed Extraction , so for Extraction we set up the soxlet apparatus, condenser , round bottom flask and heater system , in proper manner . Powder of phyllanthusamarus can be in Soxlet condenser in required quantity or may be upto 50 gm , and in round bottom flask , we used the 250 ml of methanol solvent for the Extraction . The solvent methanol heated on 70°-80°C continuously . Then the process takes time upto 4-5 hr , to bring Extract . Excess solvent was removed by the performing evaporation and distillation method and the further concentrated in a vacuumoven .

Identification test-

1. Detection of Alkaloids

Preparation of test solution:

The test solution was prepared by dissolving the extract in dilute hydrochloric acid .the solution was filtered .the was then subjected to the following tests for the detection of the presence of alkaloids.

1) dragondorffs test :- in 3 ml of filtrate ,few drops of dragondorff reagents (potassium bismuth iodide solution) were added.formation of orange brown coloured ppt shows presence of alkaloids

2) mayers test

Few drops of mayers reagent(potassium mercuric iodide solution) were added in 3 ml of solution . formation of cream coloured ppt indicates presence of alkaloids

3.hagers test

Small quantity of hagers reagent(saturated aqueous solution of picric acid) was added in filtrate . formation of yellow coloured ppt shows presence of alkaloids

4.wagners test

Few drops of Wagner's reagent (iodine in potassium iodide) were added in 3 ml of filtrate , formation of reddish brown coloured ppt indicates presence of alkaloids

2.Detection of glycosides

Preparation of test solution :

it was prepared by dissolving sample in alcohol

1)Borntragers test (anthaquinone glycoside)

To about 3 ml extract , dilute sulphuric acid was added.to cold extract equal volume of benzene or chloroform was added .after shaking , organic solvents were well separated .add ammonium then ammonical layer turned pink it indicates presence of glycoside

2.killer killiani test (cardiac glycoside):

to the test solution few drops of ferric chloride solution and concentrated sulphuric acid was added then formation of two layers occur ,lower layer of reddish brown colour and upper layer of bluish green colour simultaneously

3.baljet test : sodium picrate was added to the test solution then the colour of solution changed from yellow to orange colour it indicates presence of glycoside

3.Detection ofFlavonoids

Preparation of test solution:

To small amount of extract equal amount of 2 M hydrochloric acid was added and heated for about 30-40 min at 100°C .the extract was cool down and again extracted with ethyl acetate which was further concentrated to dryness and ready to be used as test sample

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1. **Shinoda test**
5 ml of (90% v/v) ethanol was added in the extract and then few drops of concentrated hydrochloric acid and 0.5 g magnesium turnings were added.then pink colour shows the presence of flavonoids

2.**lead acetate test**
To small quantity of extract ,lead acetate solution was added, yellow coloured ppt formation shows the presence of flavonoids

3.**Sodium Hydroxide Test :**
Addition of large amount of sodium hydroxide to the extract then shows yellow coloration ,which decolourized after some time it indicates presence of flavonoids

4.Detection of Triterpenoids
Preparation of test solution:
the test solution was prepared by dissolving the extract in chloroform and subjected to the following test

1. Salkowski test
A few drops of concentrated sulphuric acid were added to the solution and allowed to stand for some time,the formation of red colour in the lower layer indicates the presence of steroids .and formation of yellow colour in lower layer indicates presence of triterpenoids

2.libberman burchard test
Some drops of acetic anhydride were added to the test solution ; the contents were boiled and cooled. Then concentrated sulfuric acid was added from the side of the tube.then formation of brown ring in the junction of two layers .the upper layer turns green indicates the presence of steroids.and formation of red indicates presence of triterpenoids
5.Detection of Saponinsdetection of saponins

Preparation of test solution :
it was prepared by dissolving extract in water .and making it aqueous extract.

1.Foam test
The drug extract vigorously shaken with water.persistant foam formation indicates presence of saponin

2.Libermann-Burchard Test :
To drug extracts few drops of glacial acetic acid and two drops of concentrated sulfuric acid were added.

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Formulation of Phyllanthusamarus Ointment

Sr no	Ingredients	Quantity
1	Woolfat	1 gm
2	Soft paraffin	16gm
3	Hard paraffin	1gm
4	Cetostearyl alcohol	1gm
5	Phyllanthusamarus extract	1gm
		Total – 20 gm

Preparation of Phyllanthusamarus ointment

Preparation of Phyllanthusamarus ointment by using fusion method

All the glassware dry them properly as per SOP.

1.Weigh all the ingredient properly.

2.Take hard paraffin and cetostearyl alcohol melt them in procelain dish and kept on water bath.

3.To above melted mixture add wool fat, white soft paraffin and extract then stir well.

4.After melting all ingredints remove from procelain dish.

5.After into a air tight container and placed in cool and dry place.

III. RESULT

Preliminary Phytochemical Screening of Herbal Extracts

In the phytochemical screening of extract of P. amarus we perform the chemical test and determine the presence of alkaloids, glycosides, flavonoids, terpenoids, saponins.

Phytoconstituent	Test	Present
Alkaloid	Mayers test Dragondroff test Wagner's test Hagers test	+ + + +
Glycoside	Borntragers test Killer killiani test Baljet test	+ + +
Flavonoids	Shinoda test Lead acetate test Sodium hydroxide test	+ + +
Terpenoids	Libermannburchard test	+

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	Salkowski test	+
Saponin	Foam test	+

Evaluation: Evaluation Parameters of Herbal Ointment

In the evaluation parameter of herbal ointment, we perform different types of evaluation tests of ointment.

Sr no	Test	Result
1	Spreadability test	50
2	Washability test	Slightly washable
3	Irritancy test	Non irritant
4	Identification test Colour Odour Thickness	 Green Bitter Viscous
5	Test of penetration	20mg drug absorbed
6	Test of rheological properties	Viscous
7	Test of preservative efficacy	No preservatives
8	pH value	4.5-6.2

TLC of phyllanthusamarus

The pharmacologically active methanolic extract obtained from powder of phyllanthusamarus was subject to thin layer chromatography to find out number of compound present in it.

Sr no	Solvent system	UV light	No of components	PhyllanthusamarusRf value
1	Methanol:water (9:1)	280nm	1	0.51
2	Cetostearyl alcohol: ethyl alcohol (5:5)	280nm	1	0.49
3	Ethyl alcohol: chloroform (8:1)	280nm	1	0.75

Antibacterial activity

Microbial activity (zone of inhibition in (nm) of streptomycin, methanol extract of P. amarus and ointment against clinical isolates.

Drug	Conc (%w/v)	E.coli	B.subtilis	S.aureus	A.niger
Streptomycin	0.1	18	15	16	13
Methanol extract of Phyllanthusamarus	0.1	08	06	06	05
Ointment (1)	0.1	07	05	05	05

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(2)	1.0	08	06	06	05

Fig no 2.zone of inhibition

Diffusion method

Impregnated disc absorb moisture from the agar and antibiotic diffuses into the agar medium and distance from the disc increases.antibiotic concentration decreases viable growth of bacteria occurs on the surface of agar where the concentration of antibiotic

Falls below the inhibitory level For the test strain .concentration of diffused antibiotic

At the interface of the growing and inhibited bacteria approximately.MIC obtained in dilution test .

A suspected antibacterial compound or treatment Is present within reservoir created in on inoculated plate of agar medium.above diffusion of the compound through the agar ,zone of inhibition forms where concentration of the diffused molecules are sufficient to inhibit growth of bacteria .the growth is blocked, resulting in the observed zone . Which extends outwards from the reservoir to the distance from the reservoir at which concentration required for inhibition exist .

The Phyllanthusamarus Ointment is was added in agar plate .and incubated for 24 hrs.The material having antibacterial activity.inhibit the growth of bacteria and clear distinct zone of inhibition was seen around the plate.

IV. DISCUSSION :

The ointment were prepared in college with the used of plant phyllanthusamarus . This plant containing antibacterial activity are reported in the literature for its biological activity . This will limit the amount of phytochemicals absorbed into systemic circulation , thus reducing system side effect of these phytochemicals.

In studied we were studied reasearch paper which has reported the antibacterial activity . Preparation of ointment will provide instant localised topical effect due to patient may relief with burn , wound and ect …

V. CONCLUSION

Phyllanthusamarus ointment has good antimicrobial properties and can be used for the treatment of wounds and skin infection caused by susceptible organisms.

The crude methanol extract of Phyllanthusamarus and Phyllanthusamarus extract formulated as an ointment have antibacterial activity against B. subtilis, S. aureus, E. coli, P. aeruginosa and A. niger. Our findings have justified the medicinal uses and speculations about the therapeutic values of this plant for combating topical infectious diseases. The activity against these organisms showed remarkable concentration dependence. The Phyllanthusamarus ointment can be used as a potential agent for the treatment of wounds and skin infections caused by these susceptible organisms. The streoptomycin solution of 0.1% conc. Have more activity in E. coli and less activity in A. niger. 0.1% of phyllanthusamarus extract and 1.0% of ointment has a greater activity on E.coli than A. niger. The ointment have more antibacterial activity against E. coli and less antimicrobialantibacterial activity against A. niger.

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