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PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION ON THE ROOTS OF *MUSA ACUMINATA*

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ABSTRACT

The process of new drugs development is enforced by the success of herbal drugs in pharmaceutical market. Another approach to natural product drug discovery is to utilize the information derived. From industrial view point regarding sufficient supply of natural product derived active constituents. Musa Acuminata has been used of the management of wound healing, fever, cough, bronchitis, sexually transmitted infection, allergic infection, dysentery, and non communicable diseases. This study was designed to assess its phytochemical and wound healing property on the male Wister rats. The roots of Musa Acuminata is extract in soxhlet extraction in three deferent methods i) Chloroform extract ii) Petroleum ether extract iii) Ethanol extract. And for pharmacological activity five group ware created each group contain four animals which weight between 150-180gm. Group-1 is treated on normal control, Group-2 is treated on topically with hydrophilic ointment base, Group-3 is treated topically with framycetin sulphate cream 1% w/w, Brand name is soframycin, Group-4 is treated on ethanol extract, Group-5 is treat on chloroform extract and study the wound healing activity.

KEYWORDS: Musa Acuminata, Wound Healing, Soxhlet extract, framycetin sulphate, Soframycine.

1. INTRODUCTION

Wounds are visible results of individual cell death or damage and can be classified by size, depth causation (surgery, accident, or circulatory failure). Wound healing is process, which fundamentally connective tissue response.[1,2] Initial states of process involves an acute inflammatory phase followed by the synthesis of collagen & other cellular macromolecules which are letter remodelled to from scar. Two phenomena play essential rule in initial wound closing (a) formation and contraction of granulation tissue (b) movement and replication of epithelia cells over wound area (epithelisation). It is followed by development vessels producing vascular giving red granular appearance, hence lown by term granulation tissue.[3,4] Epithelial cells at the margin enlarge & begin to migrate down walls of wounds. Migration proliferation together result contraction of wound surface in open excised wound. This movement of the edges towards of wound is brought about contraction of the fibroblasts called myofibroblasts. Some important local factor influencing wound healing are infection as well as poor local blood supply, defect in collagen formation, excess of blood clot in wound area when drugs possessing wound healing property are applied on surface wound, the formation of granulation tissue & epithelial cells over the wound area become rapid than that in the normal process.[5,6,7] In the present study have evaluated by wound healing activity by topical application of the menthol extract of the roots of *Musa acuminata* is applied Viz- Excision wounds method, incision wound method and tissue histo pathological study against the standard drug framycetic sulphate (1% w/w). The traditional healers chhatisgarh use this herb unique medicinal to beal all types wounds and bolis very less time also in we evaluate successfully anti- microbial property in previous sector. All these finding from strong hasis evaluating the wound healing potency of extract.[8,9,10]

2. MATERIALS AND METHOD

2.1. Collection and Authentication of plant material

The roots of *Musa Acuminata* were collected in the month of march 2022 from the garden of *Musa Acuminata* in the district of Purba Medinipur, Saridaspur, Patashpur, West Bengal.

Fig. 1

2.1. *Preparation of Plant material*

The roots of the plant was collected and shade dried following by drying in hot air oven for 3hour. At low temperature then it was powdered by hand grinder and passed through sieve no 30 and collected.

2.2. Extraction

On the basis of polarity different solvents like petroleum ether, chloroform, ethanol and distil water are chosen for successive soxhlet extraction. Petroleum ether, chloroform, ether, a non-polar solvent used for separation of chlorophyll and fats from the plant material. Chloroform are medium polar solvent, ethanol a more polar solvent. The aim of choosing so many polar solvents is to separate the different secondary plant metabolites of different polarities.

Fig. 2

2.3. Experimental Model

The study of wound healing effect was evaluated by in vivo method. Male Wister rats, weight between 150-200gm were individual house in a clean polypropylene cages and maintained under standard environmental condition of temperature (23±10c) and fed on normal pellet diet & tap water. Animals were acclimatized to laboratory conditions before were carried out. Rats were

divided into five group and each group contain four animals.[11,12]

Group -1: Untreated control
Group -2: Treated topically with hydrophilic ointment.
Group -3: Treated topically with framycetic sulphate cream 1% w/w. Brand name Soframycin.
Group -4: Treated topically with ethanol extract.
Group -5: Treated topically with chloroform extract.

2.4. Preparation of hydrophilic ointment base

Stearyl alcohol: 25% w/w
White petrolatum: 25% w/w
Sodium lauryl sulphate: 1% w/w
Methyl paraben: 0.025% w/w
Propyl paraben: 0.015% w/w
Water: 37% w/w

Stearyl alcohol & white petrolatum were melted on a stream bath and warmed to 750c the measured amount of Sodium lauryl sulphate, propyxlene glycol, methyl paraben & propyl ben were dissolved in 37gm. Of purified water & warmed to 750c. The aqueous solution was add slowly to the alcoholpetrolatum melt. The mixture was stirred until congealed. To about 3m each of the two above preparation was taken and to them 1gm and 2gm ethanol extract was added and stirred until mixed properly. Thus all the control and test drugs were prepared. All other chemicals like formaldehyde solution, acetone, benzene & paraffin wax (58 c) were purchased from Ranbaxy Laboratories Ltd. India. All other chemical were purchased om either E.Merck, India Ltd. Or Ranbaxy labotories Ltd. and were of analytical grade.[13,14,15]

2.5. Excision wound method

Excision injuries were foisted in rats as described by Morton & Malone under light ether nesthise rats. The shaved skin of the impressed area was gutted to the full consistence to gain a crack area of about 300mm^2 of all the rates. The parameters observed were chance crack osure and compression time. The chance crack check was recorded on and if day till injuries were fully healed. The scar shape and crack area were traced and measured by graphed transparent wastes. The crack size of 300mm^2 was taken as 100 & scar ses expressed as a of the original crack area.[16,17,18,19]

3. Phytochemical Investigation

3.1. Test for alkaloids

3.1.1. Wagner's Reagents

With alkaloid it shows reddish-brown precipitate. is prepared by dissolving 1.27 gm of iodine and 2gm of potassium iodide in 5 ml of water and the final volume make to 200 ml. Mayer's Reagents: It is another method of detecting alkaloids. With alkaloids, shows white to buff precipitate. To prepare the reagent, 1.36 gm of mercuric chloride is dissolved in distilled water. In another part dissolve 5 gm of potassium iodide in 60ml of distilled water. both were mixed properly and volume was adjusted to 200ml.[20,21,22]

3.1.2. Dragendroff's Reagents

With alkaloids, this reagent gives orange-brown colored precipitate. To prepare this reagent, 14gm of sodium iodide was boiled with 5.2 gm of bismuth carbonate in 50 ml of glacial acid for a few minutes. Then it was allowed to stand for over-night and the precipitate of sodium acetate was filtered out. To 40ml of filtrate 160ml of acetate and 1 ml of water was added. The stock solution was stored in amber-colored bottle. During experiment, to 10ml of this stock solution, after adding 20 ml acetic acid solution final volume was made up.[23,24]

3.1.3. Hager's Reagents

This reagent shows characteristic crystalline precipitate with many alkaloids. In this case a saturated aqueous picric acid was used for detection of alkaloids.[25,26]

3.2. Test for carbohydrates

3.2.1. Fehling's Test

In this method to about 2ml of Fehling's solution A and 2ml of Fehling's solution B, 2ml of the extract was boiled. The presence of reducing sugar is conformed if yellow or brick red ppt appears.[27,28,29]

3.2.2. Molisch's Test

When the aqueous or alcoholic solution of the extract and 10% alcoholic solution of a napthol were shaken and conc. Sulphuric acid was added along the side of the test tube, a violet ring at the junction of two liquids conforms presence of carbohydrates.[30,31]

3.3. Test for glycoside

3.3.1. Test for cardiac glycoside

Keller- Killiani Test

To an extract of the drug in glacial acetic acid, few drops of ferric chloride and conc. H_2SO, acid are added. A reddish brown colour is formed at the junction of two layers and upper layer turns bluish green.[32,33,34]

3.3.2. Legal Test

To a solution of glycoside in pyridine, sodium nitroprusside solution and sodium hydroxide solution were added. A pink to red colour will conform the presence of glycosides.[35]

3.4. Test for anthraquinone glycosides

3.4.1. Brontrager's Test

To perform this test, 0.1gm of the powdered drug was boiled with 5ml of 10% sulphuric seid for 2 min. It was filtered while hot, then cooled and the filtrate was shaken with equal volume of benzene. The benzene layer was allowed to separate completely from the lower layer, which was pipetted out and transferred to a clean test tube. Then half of its volume of aqueous ammonia (10%) was added and shaken gently and the layers were allowed to separate. The lower ammonia layer will so

show red pink colour due to presence of free Anthraquinones.[36,37]

3.4.2. Modified Borntrager's Test

The C-Glycosides of Anthraquinones requires more drastic conditions for hydrolysis and thus a modification of the above test is to use ferric chloride and hydrochloric acid to affect oxidative hydrolysis. When 0.1 gm of the drug. 5ml of dilute. HCL and 5 ml of 5% solution of ferric chloride were added and boiled for few minutes and the subsequently cooled and filtered part is shaken with benzene: the separated benzene layer and then add equal volume of dilute solution of ammonia which shows pink colour.[38,39,40]

3.5. Test for gums and mucilages

3.5.1. Molisch's Test

When aqueous or alcoholic solution of the extract and 10% alcoholic solution of a napthol were shaken and conc. H_2SO, added to the side of the test tube, appearance of violet ring at the junction of two liquids indicates the presence of carbohydrates, gums and mucilage's.[41,42]

3.5.2. Precipitated with 95% Alcohol

When 95% of alcohol added to the extract, gums and precipitate outs being insoluble in alcohol.

3.6. Test for proteins and amino acid

3.6.1. Biuret Test

When 2ml of the extract, 2ml 10% NaOH solution and 2-3 drops of 1% $CuSO_4$, solution were mixed, the appearance of violet or purple colour conforms the presence of proteins.[43,44]

3.6.2. Ninhydrin Test

When 0.5ml of ninhydrin solution if added to 2 ml of the extract and boiled for 2 minutes and then cooled, appearance of blue colour conforms the presence of proteins.

3.6.3. Xanthoproteic Test

When 2ml of extract and 1 ml of conc. HNO, were boiled and cooled and subsequently 40% NaOH solution added drop by drop to it, appearance of coloured solution indicates the presence of proteins.[45,46]

3.6.4. Millon's Test

2ml of millon's reagent and 2ml of the drug extract add properly and boiled, after completely these process add few drops of $NaNO_2$, red colour indicate present of proteins.[47,48]

3.7. Test for tannins and phenolic compounds

3.7.1. With Lead Acetate

Tannins are precipitated with lead acetate solution.

3.7.2. With Ferric Chloride

Generally phenols were precipitated with 5% w/v solution of ferric chloride in 90% alcohol and thus phenols are detected.

3.7.3. With Gelatine Solution

To a solution of tannins (0.5-1%) aqueous solution of gelatins (1%) and sodium chloride (10%) were added. A white buff precipitate conforms the compounds.

3.8. Test for steroids and sterols

3.8.1. Salkowski's Test

In this test to 5 ml of the solution of extract in chloroform in a dry test tube, equal volume of cone. H_2SO, was added along the side of the test tube ad the upper chloroform layer and lower acid layer were observed. The presence of steroids or sterols are conformed by the upper layer showing a play of colours first from bluish red to gradually violet and lower acid layer showing yellow colour with green fluorescence.

3.8.2. Libermann Burchard Reagent

2ml chloroform extract were dry, after drying 2ml acetic anhydride and 2-3 drops of con H_2So_4 were add stand for some time and a emerald green colour is observed.

3.9. Test for Triterpenoids

3.9.1. Tin and Thinly Chloride

For detection of triterpenoids the extract was dissolved in chloroform. A piece of metallic tin and I drop of thionyl chloride was added to it. Pink colour conforms the result.

3.10. Test for saponins

3.10.1. Foam test

2ml drug extract and alcohol dilute separately and make up the volume of 10ml shake the solution after 30 min saponins are separate.

3.11. Test for flavonoids

3.11.1. With NaOH

For the detection of flavonoids, the extract was first dissolved with water. It was filtered and the filtrate was treated with sodium hydroxide. A yellow colour conforms the presence of flavonoids.

3.11.2. With Sulphuric Acid

A drop of sulphuric acid when added to the above, the yellow colour disappears.

3.11.3. With Mg & HCL

In this method of detection, the extract to be tested was dissolved in water. It was then filtered and the filtrate with magnesium, after that, a few drops of cone. HCL was added to it. A pink colour confirmed the presence of flavonoids.[49,50]

3.12. Test for ascorbic acid

To 2ml of 2%W/V solution, add 2ml of water, 0.1 gm of sodium bicarbonate and about 20 mg of ferrous sulphate, shake and allow to stand; a deep violet colour is

produced and 5 ml of dilute sulphuric acid, the colour disappear. Dilute 1 ml of a 2% W/V solution add with 5 ml water & add one drop of a freshly prepared 5% w/v solution of sodium nitropruside & 2 ml dilute NaOH Solution & stir, the yellow colour turns blue.[51,52]

3.13. Test for vitamin-E

Disssolve 2gm in 2ml of ethyl alcohol add 0.2ml of nitric acid and heat carefully on a water bath for five minutes The colour changes from yellow to brick red.[53,54]

Fig. 3

4. RESULT AND DISCUSSION

The results of preliminary phytochemical tests for presence of secondary phytoconstituents is as follow;

SL. NO	Test/Reagent used	Extract (+) Sign for present and (-) Sign for absent		
		Pet. Ether extract	Chloroform extract	Ethanol Extract
1.	Alkaloids:-			
	Mayer's Reagent	-	-	-
	Dragendroff's Reagent	-	-	-
	Wagner's Reagent	-	-	-
	Hager's Reagent	-	-	-
2.	Carbohydrates:-			
	Molisch's test	-	+	+
	Fehling's Test	-	+	+
	Benedict's reagent	-	+	+
	Barfoid's Reagent	-	+	+
	Iodine Test	-	+	+
3.	Glycosides:-			
	Keller-Killiani Test	-	+	+
	Legal Test	-		+
	Modified Borntrager's Test	-	+	+
	Borntrager's Test	-	+	+
4.	Proteins and Amino Acids:-			
	Ninhydrine Test	-	-	+
	Biuret Test	-	-	+
	Millon's Test	-	-	+
	Xanthoproteic Test	-	-	+
5.	Tannin:-			
	Ferric chloride sol	-	-	+
	Gelatin solution	-	-	+
	Lead acetate solution	-	-	+
6.	TriTerpenoids	+	-	-
7.	Saponin			
	Foam Test	-	-	+
	With NaHCO3	-	-	+
8.	Flavonoids			
	With NaOH	-	-	+
	With H2SO4	-	-	+
	With Mg/HCl	-	-	+
9.	Steroids:-			
	Liebermann's Test	-	+	+
	Salkowski Test	-	+	-

5. DISCUSSION

From the below data it was clear that the petroleum ether shows positive test for triterpenoids, chloroform extract shows positive test for carbohydrate, cardiac glycosides, tannins, flavonoids, and saponins, and the ethanol extract show presents of vitamin C & E.[38,39,40]

Post wound healing (Days)	Wound healing area in(mm^2)				
	Group-1	Group-2	Group-3	Group-4	Group-5
0	302±3.2(0)	304 ± 4.6(0)	303 ± 2.9 (0)	303 ± 2.4 (0)	306 ± 3.5 (0)
3	276 ± 4.6 (8.6)	265 ± 4.6 (8.6)	250 ± 2.5 (17.5)	257 ± 3.74 (15.9)	260 ± 3.2 (14.2)
6	236 ± 4.6 (8.6)	228 ± 4 (24.8)	187 ± 3.7 (38.3)	200 ± 2.7 (34.7)	206 ± 4.6 (31.9)
9	193 ± 5.6 (36.1)	166 ± 2.4 (45.3)	111 ± 4.9 (45.3)	125 ± 3.1 (60.2)	136 ± 2.05 (55.2)
12	141.2 ± 4.3 (53.3)	112 ± 2.8 (63.2)	42 ± 4.6 (86.2)	56 ± 2.55 (82.5)	81 ± 2.2 (73.1)
15	92 ± 5.6 (69.5)	74 ± 2.2 (75.5)	0 (100)	26 ± 2.45 (91.6)	49 ± 2.5 (85.2)
18	59 ± 3.1(80.6)	38 ± 2.05(87.6)	0 (100)	0 (100)	12 ± 3.2 (96.1)

WOUND RECOVERY AREA GRAPH

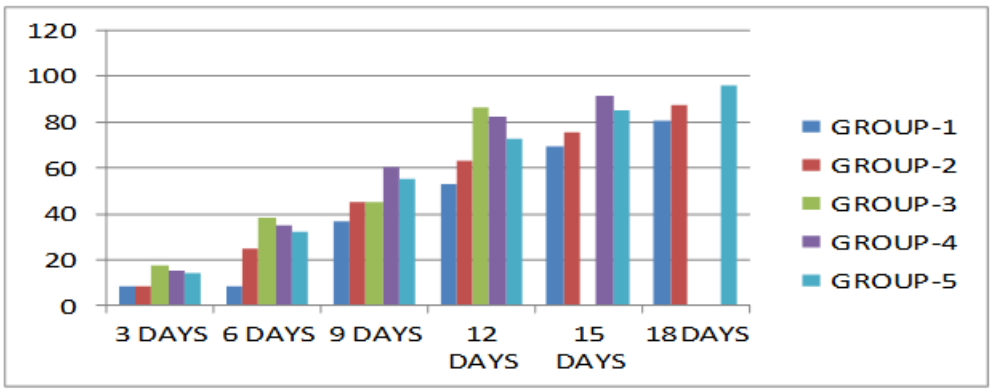

Fig-4

6. CONCLUSION

After performing all the assessment systematically, a conclusion is drawn on the basis of the results from various phytochemical & pharmacological studies on the trunk The different extracts obtained by soxheation of the roots of the plant are collected. The qualitative phytochemical analysis shows presence of secondary phyto constituents in the different extracts. The petroleum ether extract contains triterpinoids. The Chloroform extract shows presence of glycoside,

mucilage, carbohydrates and steroid &sterols. The Ethanol extract shows presence of carbohydrate, glycosides, Tannins, Steroids, Saponnins & Flavonoids. And in pharmacological evaluation ethanol extract shows good activity then chloroform extract and petroleum extract.

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