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COMPARATIVE STUDY ON PHYTOCHEMICAL PARAMETERS AND PHARMACOLOGICAL EVALUATION OF WOUND HEALING ACTIVITY ON THE FRUITS AND LEAVES OF GYNOCARDIA ODORATA ROXBURG

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ABSTARCT

The powdered leaves of Gynocardia odorata is green in color, mild bitter in taste and has characteristics odour while the powdered fruit is brown in colour and has a characteristics taste and odour. Both the powdered leaves and fruits were mounted with chloral hydrate, phloroglucinol, weak iodine and HCL and stained with saffranine, it showed the pressence of starch grains, fragments of vessels, fibres and calcium oxalate crystals. The physicochemical constants like ash value such as total ash, acid insoluble ash, water soluble ash, moisture content, extractive values such as water soluble extractive value and alcohol soluble extractive value were determined for both the leaves and fruits. These helped in formulating pharmacoepoeial standards of the drugs. The extracts obtained by successive solvent extraction of both the leaves and fruits were subjected to preliminary phytochemical analysis which revealed the presence of Flavanoids, Glycosides, Tannins, Saponins, Fixed oils, Fats, Steroids and Triterpenoids. The wound healing activity of aqueous extracts of both the leaves and fruits of the plant Gynocardia odorata was evaluated by Excision wound model in Wister albino Rats. It has been observed that the aqueous extracts of the fruit at a dose level of 10% w/w showed better wound healing activity than that of the aqueous extract of the leaves at the same dose level. This was evident by faster rate of wound closure and epithelization period in excision wound model, but was found to be lesser in comparision to the standard drug povidone iodine.

KEYWORDS: Gynocardia odorata, Leaves, Fruits, Wound, Povidone Iodine.

INTRODUCTION

" A medicinal plant is any plant which in one or more of each organ, contains substance that can be used for therapeutic purpose or which is a precursor for synthesis of useful drugs". This definition of medicinal plant has been formulated by WHO (World Health Organization).Now it has been established that the plants which naturally synthesizes and accumulate some secondary metabolites like alkaloids, glycosides, tannins, volatile oils and contain minerals and vitamins and posses medicinal properties.[1]

Use of Traditional Herbal Medicines

By definition, 'Traditional' use of herbal medicines implies substantial historical use, and this is certainly true for many products that are available as 'Traditional herbal medicines'. Traditional medicine (also known as indigenous or folk medicine) comprises unscientific knowledge system that developed over generations within various societies before the era of modern medicine. Practices known as traditional medicines include Herbal, Ayurveda, Siddha medicines, Unani, ancient Iranian medicine, Islamic medicine, traditional Chinese medicine, traditional Korean medicine, acupuncture, Muti, Ifa, traditional African medicine, and other pseudo medical knowledge and practice all over the globe. In the written record, the study of herbs dates back over 5,000 years to the Sumerians, who described well-established medicinal uses for such plants as laurel, caraway and thyme. Anciant egyptian medicine of 1,000 BC are known to have used garlic, opium, castor oil, coriander, mint, indigo and other herbs for medicine and the old testament also mentions herb use and cultivation, including mandrake, vetch, caraway, wheat, barley and rye.[2]

The plant Gynocardia *odorota Roxb.* (synonyms: Chalmugra, Chulmurga, Alasakapaha) which is to undergo pharmacognostical, phytochemical comparative study of leaves and fruits having a wide range of therapeutic effects on various disease like leprosy, secondary syphilis, rheumatism, scrofula, and in phthisis. The dose for an infant is 1 or 2 drops daily; for an adult, from 2 to 4 minims, repeated 3 times a day.[3] It has been reported that this plant is also used for treatment of Diarrhoea, gastric and in blood purification.[4] The oil of Gynocardia odorota Roxb has long been applied for stiff joints and sprains, psoriasis and other inflammatory diseases.[5] Apart from the pharmacognostical and phytochemical study, this plant is also studied for their pharmacological activity because of its therapeutic effects on various skin diseases. Due to having a tremendous therapeutic effects on skin; the possible

wound healing activity of this plant Gynocardia *odorota Roxb.* has been evaluated widely in this study.

Wound may be defined as loss or breaking of cellular and anatomic or functional continuity of living tissues. Wound repair is essential physiological process that is important for tissue homeostasis, but it can be impaired in disease and contribute to numerous pathogens. Wounds are inescapable event in life.[6, 7] Wound healing process holds steps which involve coagulation, inflammation, formation of Granulation tissue, matrix formation remodeling of connective tissue, collagianization and acquisition of wound strength. The normal healing response begins the moment the tissue is injured. Wound healing can be classified into any of three type- healing by first intention, healing by second intention or healing by third intention, depending on the nature of the edges of the healed wounds. In wounds healed by the first intention, the edges are smoothly closed at no scar is left. On the other hand wound healing by second intension involves the formation of granulation tissue, which fill up the gaps between the wound edges and is associated with significant loss of tissue, leaving little scars. Wounds healed by third intention, are usually those left open for three to five days until granulation bed falls before they are sutured, generally resulting in extensive scar formation.[8, 9]

MATERIALS AND METHODS

Collection, identification and preparation of the plant material

The plant material was collected in the month of July from Majhitar a place in the E. Sikkim district of Sikkim, then identified and authenticated at Botanical Survey of India, Gangtok, Sikkim. The taxonomical identification was carried out following standard literature.[10] The plant material were washed throughly with running water to remove the earthly material or adherent impurities and then shade dried. The dried material was powdered by means of mechanical grinder. The resulting powdered material was stored in air tight containers for further studies.

Phytochemical analysis[11]

The concentrated extracts were subjected to chemical test as per the methods mentioned below for the identification of the various constituents.

Detection of Alkaloid

Solvent free extract, 50mg is stirred with few ml of dilute hydrochloric acid & filtered. The filtrate is tested carefully with various alkaloidal reagents as follows.

- **Mayer's test:** To a few ml of filtrate, one or two drops of Mayer's reagent is added by the side of the test tube. A white or creamy precipitate indicates test as positive.
- **Wagner's test:** To a few ml of filtrate, few drops of Wagner's reagent is added by the side of the test tube. A reddish-brown precipitates indicates test as positive.
- **Hager's test:** To a few ml of filtrate, 1 or 2 ml of Hager's reagent is added by the side of the test tube. A prominent yellow precipitate indicates test as positive.
- **Dragondoff's reagent:** To a few ml of filtrate, 1 or 2 ml of Dragondoff's reagent is added by the side of the test tube. A prominent yellow precipitate indicates test as positive.

Detection of Carbohydrates

The extract (100 mg) is dissolved in 5ml of water & filtered. The filtrate is subjected to the following test.

- **Molisch's test:** To 2ml of filtrate, 2 drops of alcoholic solution of alpha-napthol is added, the mixture is shaken well & 1ml of conc. H_2SO_4 is added slowly along the side of the test tube & allowed to stand. A violet ring indicates the presence of carbohydrate.
- **Fehling's test:** 1ml of filtrate is boiled on water bath with 1ml each of Fehling solution A & Fehling solution B, a red precipitates indicates the presence of sugar.
- **Bendict's test:** To 0.5 ml of filtrate, 1ml of Benedict's reagent is added. The mixture is heated on a boiling water bath for 2 minutes. A characteristic colored precipitate indicates the presence of sugar.
- **Barfoed's test:** To 1 ml of filtrate, 1ml of Barfoed's reagent is added & heated on a water bath for 2minutes. Red precipitates indicate the presence of sugar.

Detection of Saponins

The extract (50 mg) is diluted with distilled water & made upto 20ml. the suspension is shaken for 15 minutes. A layer of 2cm of foam indicates the presence of saponin.

Detection of Phenolic compounds

- **Ferric chloride test:** The extract (50 mg) is dissolved in 5ml of distilled water. To this, few drops of natural 5% ferric chloride solution is added. A dark green color indicates the presence of Phenolic compounds.
- **Gelatin test:** The extract (50 mg) is dissolved in 5 ml of distilled water & 2ml of 10% sodium chloride solution is added to it. White precipitates indicate the presence of Phenolic compounds.
- **Lead Acetate test:** The extract (50 mg) is dissolved in distilled water and to this 3ml of 10% lead acetate solution is added. A bulky white precipitate indicates the presence of Phenolic compounds.

Detection of Glycosides

50gm of extract is hydrolyzed with concentrated hydrochloric acid for 2 hours on a water bath, filtered & the hydrolysate is subjected to the following test.

- **Borntrager's test:** To 2ml of filtered hydrolysate, 3ml of $CHCl_3$ is added & shaken, $CHCl_3$ layer is separated & 10% NH_3 solution is added to it, pink color indicates the presence of glycosides.
- **Legal test:** 50mg of the drug extract is dissolved in pyridine, sodium nitroprusside solution is added and make alkaline using 10% sodium hydroxide. Presence of glycoside is indicated by pink color.
- **Keller-Killiani test:** To an extract of drug in glacial acetic acid, few drops of ferric chloride and conc. sulphuric acid are added. A reddish brown colour is formed at the junction of the two layers and the upper layer turns bluish green.

Detection of Flavanoid

- **Magnesium and Hydrochloric acid reduction:** The extract (50 mg) is dissolved in 5 ml of alcohol & few fragment of magnesium ribbon & conc. HCl acid (dropwise) is added. If any pink to crimson color develops, presence of flavanol glycosides is inferred.
- **Alkaline reagent test:** An aqueous solution of the extract is treated with 10% NH_4OH solution. Yellow fluorescence indicates the presence of flavanoids.
- **Aqueous sodium hydroxide test:** An aqueous solution of the extract is treated with sodium hydroxide solution it gives blue to violet(anthocyanins), yellow(flavones),and yellow to orange(falvonones).

- **Concentrated sulphuric acid test:** An aqueous solution of the extract is treated with Conc. Sulphuric acid it gives yellowish orange (anthocyanins), orange to crimson (flavonones) and yellow to orange (flavones).

Detection of Proteins & Amino acids

The extract (100 mg) is dissolved in 10ml of distilled water & filter through Whatman filter paper no-1 & the filtrate is subjected to tests for proteins and amino acids.

- **Millon's test:** To 2ml filtrate, few drops of Millon's reagent is added. A white precipitates indicates the presence of proteins.
- **Biuret test:** An aliquot of filtrate is treated with one drop of 2% copper sulphate solution. To this 1ml of ethanol (95%) is added, followed by excess of potassium hydroxide palate. Pink color in the ethanolic layer indicates the presence of proteins.
- **Ninhydrin test:** Two drops of Ninhydrin solution (10mg of Ninhydrin in 200 ml of acetone) is added to 2ml of aqueous filtrate. A characteristic purple color indicates the presence of proteins.

Detection of Phytosterols

Libermann Burchard's test

The extract (50mg) is dissolved in 2 ml of acetic anhydride. To this solution, 1-2 drops of conc. Sulphuric acid are added slowly along the sides of the test tube. An array of color changes shows the presence of phytosterols.

Detection of Fixed oils and Fats

- **Spot test:** Press a small quantity of extract separately between two filter papers. Oil stains on the paper indicate the presence of fixed oil.
- **Saponificatiion test:** Add a few drops of 0.5 (N) alc. KOH to a small quantity of extract along with a drop of phenolphthalein. Heat the mixture on water bath for 1-2 hr. formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

Detection of Gums & Mucilage

Extract (100mg) is dissolved in 10ml of distilled water & to this 25ml of absolute alcohol is added with constant stirring. White or cloudy precipitate indicates the presence of gums & mucilage.

Chromatographic Technique

Chromatography is a technique of separation of mixture into individual component using a stationary phase and a mobile phase. For the separation of a component TLC technique is applied here.

Thin layer chromatography[12]

Preparation of Thin layer Chromatography Plates

About 30 grams of silica gel was weighed out and it was shaken with (10 ml) of distilled water to form a homogenous suspension. This suspension was poured into a Thin Layer chromatogram applicator, which adjusted to 0.25 mm thickness. The plates were kept in the hot air oven at 100° C for one hour to activate the silica gel. The plates were then stored in a dry atmosphere and used wherever required.

Principle of TLC

The principle of TLC is adsorption. One or more compounds are spotted on a thin layer of adsorbent coated on a chromatographic plate. The mobile phase solvent runs because of capillary action. The component move according to their affinities towards the adsoebent. The component move according to their affinity towards the stationary phase travels slower. Thus the components are separated on a Thin layer chromatographic plate based on the affinity of the plate of the components towards the stationary phase.

Practical requirements

1. Stationary phase
2. Glass plate
3. Preparation and activation of plate
4. Application of sample
5. Development tank
6. Mobile Phase
7. Development technique
8. Detecting and visualizing agent

Staionary Phase

There are several adsorbents which can be used as stationary phase. Some of the stationary phases, their composition and the ratio in which they have to be mixed with water or ether solvents to form slurry for preparing TLC plates. The stationary phases used are as follows-

- Silica gel H
- Silica gel G
- Silica gel GF
- Silica gel 60 F254
- Cellulose powder
- Keieselguhr

Glass plate

Glass plates which are specific dimensions like 20cm×5cm (full plate), 20cm×10cm (half plate), 20cm×5cm (quarter plate) can be used. These dimensions are used since the width of the commercially available TLC spreader is 20cm.

Preparation and activation of TLC plate

After preparing the slurry the TLC plate can be prepared by using any one of the following technique: pouring, dipping, spraying and spreading.

In **pouring** technique, the slurry is prepared and poured on to a glass plate which is maintained on a labeled surface. The slurry is spread uniformly on the surface of the glass plate. After setting, the plate is dried in an oven. The disadvantage is that uniformity in thickness cannot be ensured.

In **dipping** technique two plates are dipped into the slurry and are separated after moving from slurry and later dried. The disadvantage is that a larger quantity of slurry is required even for preparing fewer plates.

Spraying technique resembles that of using a perfume spray on a cloth. The suspension of adsorbent or slurry is sprayed on a glass plate using a sprayer. The disadvantage is that the layer thickness cannot be maintained uniformly all over the plate.

Spreading is the best technique where a TLC spreader is used. The glass plates are stacked on a base plate. The slurry after preparation is poured inside the reservoir of TLC spreader. The thickness of adsorbent layer is adjusted by using a knob in the spreader. Normally a thickness of 0.25 mm is used for analytical purpose and 2mm thickness for preparative purpose. Then the spreader is rolled only once on the plates. The plates are allowed for setting. This is done to avoid cracks on the surface of adsorbent. After setting, the plates are activated by keeping in an oven 100°C to 120°C for 1 hr.

Application of sample

Usually to get good spots, the concentration of the sample or standard solution has to be minimum 2-5µl of a 1% solution of either standard or test sample is spotted using a capillary tube or micropipette. The spots can be placed at random or equidistant from each other by using a template, with marking. The spot should be kept at least 2cm above the base of the plate. And spotting area should be immersed in the mobile phase in the development tank.

Mobile phase

Pure solvent or mixtures of solvents are used. The following gives a list of solvents (of increasing polarity)-Petroleum ether, carbon tetrachloride, cyclohexane, carbon disulfide, ether, acetone, benzene, toluene, ethyl acetate, chloroform alcohols, water, pyridine, organic acids etc.

Detecting or visualizing agents

After development of TLC plates, the spots should be visualized. Detecting coloured spots can be done visually. But the detecting colourless spots, any one of the following techniques can be used.

1) **Non specific method:** Where the no of spot can be detected, but not the exact nature or type of the compound.

(a) Iodine chamber method: Where brown or amber spots are observed when the TLC plates are kept in a tank with few iodine crystals at the bottom.

(b) Sulphuric acid spray reagent: 70-80% v/v of sulphuric acid with few mg of either potassium dichromate or potassium permanganate or few ml of nitric acid as oxidizing agent is used. This reagent after spraying on TLC plates is heated in an oven. Black spots are seen due to charring of compounds.

(c) UV chamber for fluorescent compounds: When compounds are viewed under UV chamber, at 254 nm or at 365 nm, fluorescent compound can be detected. Bright spots are seen under dark background.

(d) Using fluorescent stationary phase: When the compound is not fluorescent, a fluorescent stationary phase is used. When the plates are viewed under UV chamber, dark spots are seen on a fluorescent background. Example of such stationary phase is silica gel GF.

(e) Specific methods: Specific spray reagents or detecting agents or visualizing agents are used to find out the nature of compounds or for identification purposes. Example-

i. Ferric Chloride-for phenolic compounds, tannins and coumarin
ii. Ninhydrin in Acetone-for amino acids
iii. Dragondroff's Reagent-for alkaloids
iv. 3,5-dinitrobenzoic acid- for cardiac glycosides
v. Anisaldehyde-sulphuric acid reagent-for triterpenoids

Analysis

The **Rf (Retardation factor)** value is calculated for identifying the spots i.e. in qualitative analysis**. Rf value** is the ratio of distance traveeled by the solute to the distance travelled by the solvent front.

$$\mathbf{Rf} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent front}}$$

The **Rf value** range from 0 to 1 but ideal value are from 0.3 to 0.8. Rf value is specific and constant for every compound in a particular combination of stationary and mobile phase. When the Rf value of a sample and reference compound is same, the compound is identified by its standard.

PHARMACOLOGICAL ACTIVITY

Drugs formulation

Two types of ointment formulation were prepared from each of the extract, 5% (w/w), where 5g and 10g of both the leaf and the fruit extract were incorporated in 100gm of simple ointment base B. P. respectively. The formula for simple ointment B.P. is.

Sl No	Ingredients	Quantity
1	Wool Fat	5
2	Hard Paraffin	5
3	Cetostearyl Alcohol	5
4	White Soft Paraffin	85

Preparation of simple ointment

Hard paraffin and cetostearyl alcohol were melted on water bath. To this wool fat and white soft paraffin were incorporated, stirred until all ingredients were melted. Contents were examined for any foreign particles and decanted if required. Stirrer the mixture thoroughly until cold.

Acute dermal irritation test[13-16]

Skin irritation test for the different test substances was conducted on rabbits by using occluded dermal irritation test. The skin of each rabbit was shaved at two different positions on the dorsal side. The first area was kept as control, to which non medicated ointment was applied. The second area was applied with the test substance. On day one of the test period the preparations were evenly applied on the shaven area of the animals' skin. Immediately the area was covered by dressing gauze over which a plastic sheet (occlusive material) was placed and altogether the covering was loosely held in contact with the skin by means of a non –irritating adhesive tape. After 24 hrs of exposure period, the elastic bandage, the adhesive plaster, the plastic sheet and the gauze were removed taking care not to damage the skin and the test site was rinsed with distilled water. The animals were examined for the presence of erythema and edema according to Draize dermal irritation scoring system at grading intervals of 1, 24, 48 and 72 hrs. The degree of erythema and edema were determined based on the scores given in Table below.

Primary irritation index (PII) which is a parameter that indicates the potential of a given substance for skin irritation was also calculated for the different test substances by summing up all the erythema and oedema scores of all the 4 times intervals of grading (1, 24, 48 and 72 hrs) and dividing by the number of test sites (2) mulitiplied by the grading interval. According to Draize classification substances scoring PII of<2 are mildly irritant, 2-5 moderately irritant,>5 severely irritant.

Table. 1: Draize dermal irritation scoring system.

Erythema and scar formation	Value	Edema formation	Value
No erythema	0	No oedema	0
Very slight erythema	1	Very slight oedema	1
Well defind erythema	2	Slight oedema	2
Moderate to severe erythema	3	Moderate oedema	3

Evaluation of wound healing activity[17-24]

The wound healing efficiency of *Gynocardia odorata Roxb.* Leaf extract and fruit extract were evaluated employing one wound animal model viz., Excision wound model.

Animals

The healthy Wister albino rats of either sex, weighing 150-220g were used. All animals were housed, fed and treated in accordance with the in-house guidelines for animals protection.

The animals were housed under standard environmental conditions of temperature and humidity (25±0.50°C) and were fed with standard pellet diet and water *ad libitum* throughout experimentation period. Ethical clearance for handling the animals was obtained from the Institutional animal ethical prior to the beginning of the project work.

Excision wound model

The animals were divided into 6 groups each with 6 animals.

Group A: Control, applied topically 0.5g, simple ointment.

Group B: Standard, applied topically 0.5g, 5% w/w Povidine iodine ointment.

Group C: Treated with *Gynocardia odorata Roxb.* Leaf extract 5% w/w ointment 0.5g, topically.

Group D: Treated with *Gynocardia odorata Roxb.* Leaf extract 10% w/w ointment 0.5g, topically.

Group E: Treated with *Gynocardia odorata Roxb.* Fruit extract 5% w/w ointment 0.5g, topically.

Group F: Treated with *Gynocardia odorata Roxb.* Fruit extract 10% w/w ointment 0.5g topically.

The rats were inflicted with excision wounds are described by Morton and Malone(1972). In this model a standard wound is made by cutting a circular skin in dorsal thoracic region of the experimental animals. The hairs were removed from the dorsal thoracic region of the rats using depilitor and veet hair removing cream. An area of 500mm² was marked on the shaved area with an indelible ink and rubber seal. The area was washed to normal saline. A full thickness excision wound of circular area of 500 mm² was created along the markings under light ether anesthesia. The rats were kept in individually in separate cages. The physical attributes of wound healing viz. wound closure (contraction) and epithelization were recorded. The wound contraction was studied by tracing the raw wound area on a transparent paper on 4^{th}, 8^{th}, 12^{th} and 16^{th} day. The criterion for complete epithelisation was fixed as formation of scar with absence of raw wound area. The wound area was measured planimetrically with the help of sq. mm scale graph paper.

The percentage wound closure was calculated by using the following formula

$$\% \text{ of wound contraction} = \frac{wound\ area\ of\ day\ zero - wound\ area\ on\ corresponding\ days}{wound\ area\ of\ day\ zero} \times 100$$

Statistical analysis

The data obtained from the experiment i. e. means of wound area measurement and epithelisation period between different groups (Control, standard, test treated) were subjected to one way ANOVA followed by DUNNETT's multiple comparison test. The 'P' values were analysed and recorded in respected tables.

RESULTS

Phytochemical analysis

The extracts were subjected to preliminary phytochemical analysis and the results are represented in the table below.

Table 2: Phytochemical analysis of Leaves extract of Gyanocardia *odorata Roxb.*

Test	P	B	C	A	M	E	C.W
Alkaloids	−	−	−	−	−	−	−
Carbohydrate	−	−	−	−	+	+	+
Glycosides	−	−	−	−	+	+	+
Flavonoids	−	−	−	−	+	+	+
Fixed and oils	+	−	−	−	+	+	+
Phenolic compounds and Tannins	−	−	−	−	+	+	+
Saponins	−	−	−	−	+	+	+
Proteins and amino acids	−	−	−	−	−	−	−
Phytosterols	+	+	+	+	−	−	−
Gums and mucilage	−	−	−	−	−	−	−

(+) - Present (−) - Absent

P - Petroleum Ether, A -Acetone, B - Benzene, M - Methanol

E- Ethanol, C -Chloroform, C.W - Chloroform water.

Table. 3: Phytochemical analysis of Fruits extract of Gyanocardia *odorata Roxb.*

Test	P	B	C	A	E	C.W
Alkaloids	−	−	−	−	−	−
Carbohydrate	−	−	−	−	+	+
Glycosides	+	−	−	−	+	+
Flavonoids	−	−	−	−	+	+
Fixed and oils	+	−	−	−	+	−
Phenolic compounds and Tannins	−	−	−	−	+	+
Saponins	−	−	−	+	+	+
Proteins and amino acids	−	−	−	−	−	−
Phytosterols	+	+	+	+	−	−
Gums and mucilage	−	−	−	−	−	−

(+) - Present (−) - Absent

P - Petroleum Ether, A -Acetone, B - Benzene,

E -Ethanol, C - Chloroform, C.W - Chloroform water.

Thin Layer Chromatography

The TLC study confirms the presence of some compounds in aqueous extract of leaf and fruit like flavanoids, triterpenoids and glycosides. Result of TLC study for aqueous extract of both leaf and fruit is given in the table below.

Table. 4: Rf Values.

Compound	Mobile Phase	Ratio	Rf value (leaf)	Rf value (fruit)
Flavanoids	N- Butanol: Acetic Acid: water	3:6:9	0.61	0.63
Terpenoids	Toluene: Ethyl Acetate	93:7	0.60	0.58
Glycosides	Ethyl Acetate: Methanol: water	81:11:8	0.66	0.69

Pharmacological Study

Skin- irritation test

In skin irritation test, no irritation symptoms were developed over the test period. Neither erythema formation nor skin swelling were developed during a 72 hr time period for all test substances. Hence the PII is found to be zero. This indicates that all the test substances from the leaves and fruits of Gynocardia *odorata* do not have irritant property.

Wound healing activity

Effect of topical application of aqueous leaf extract ointment and aqueous fruit extract ointment of *Gynocardia odorata* in excision wound model

Topical application of *Gynocardia odorata* leaf extract ointment at a concentration of 10% w/w extract in simple ointment has demonstrated significantly reduction in the wound area and faster rate of epithelisation time when compared with the control (simple ointment treated group) as well that of 5% w/w leaf extract ointment, and 5% fruit extract ointment but it is lesser than that of standard drug 5% Providone Iodine. The wound healing activity of the 10% w/w leaf extract ointment was found to be also lesser than that of 10% w/w aqueous fruit extract which showed better result than that of the 10% leaf extract ointment. Moreover, the 10% aqueous fruit extract ointment showed significant reduction in the wound area and faster rate of epithelisation time when compared with the control (simple ointment treated group), and 5% fruit extract ointment as well as that of 5%w/w aqueous leaf extract ointment

and 10% w/w leaf extract ointment. But, the wound healing activity of the 10%w/w fruit extract ointment was found to be lesser than the standard drug, 5% Providone Iodine ointment treated group. The results are depicted in the below table.

Table 5: Percentage wound contraction in excisional wound healing model.

Drug Treatment	4th day	8th day	12th day	16th day	Period of epithelization(Days)
Control	16.70±0.6015	54.46±3.713	68.24±3.932	77.44±3.188	22.40±0.5099
Standard	*** 44.62±0.4817	*** 85.28±1.984	*** 96.50±3.341	-	*** 13.80±0.5831
5% L	* 28.89±1.182	* 68.02±3.119	* 78.62±3.302	84.21±2.839	20.80±0.5831
10% L	*** 37.76±1.838	*** 75.55±3.653	** 87.50±3.313	** 92.75±2.849	*** 18.60±0.6000
5%F	** 35.24±2.369	** 73.44±2.213	* 82.00±3.982	* 88.47±2.494	** 19.60±0.8124
10%F	*** 43.66±5.232	*** 82.93±3.613	*** 91.88±3.165	*** 96.28±2.676	*** 17.60±0.5099

The values are expressed as Mean ± SEM, n= 6 in each group. If * P<0.05, ** P<0.01 and ***P<0.001 vs. control where, 5% L = 5% leaves extract ointment, 10% L = 10% leaves extract ointment, 5%F = 5% Fruit extract ointment, 10% F = 10% Fruit extract ointment.

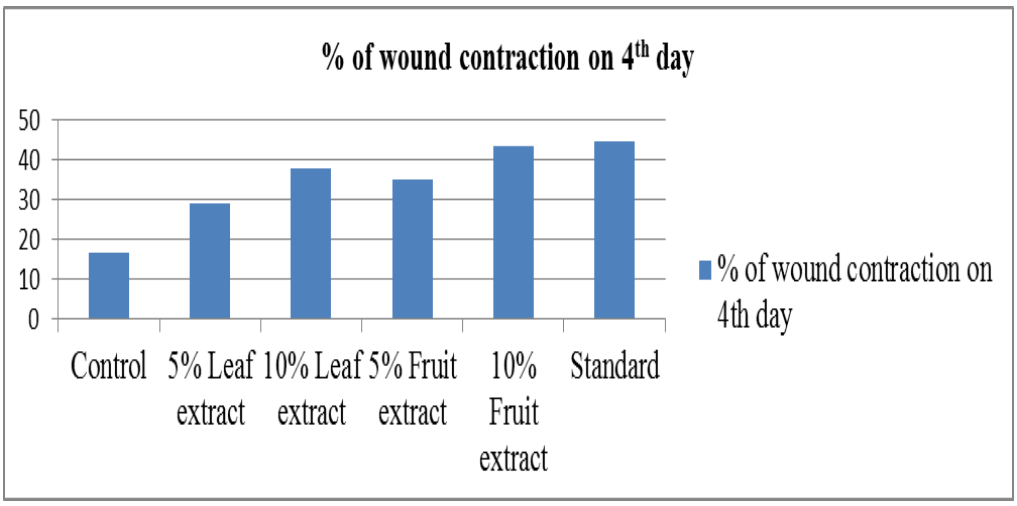

Fig. 1: % of wound contraction on 4th day.

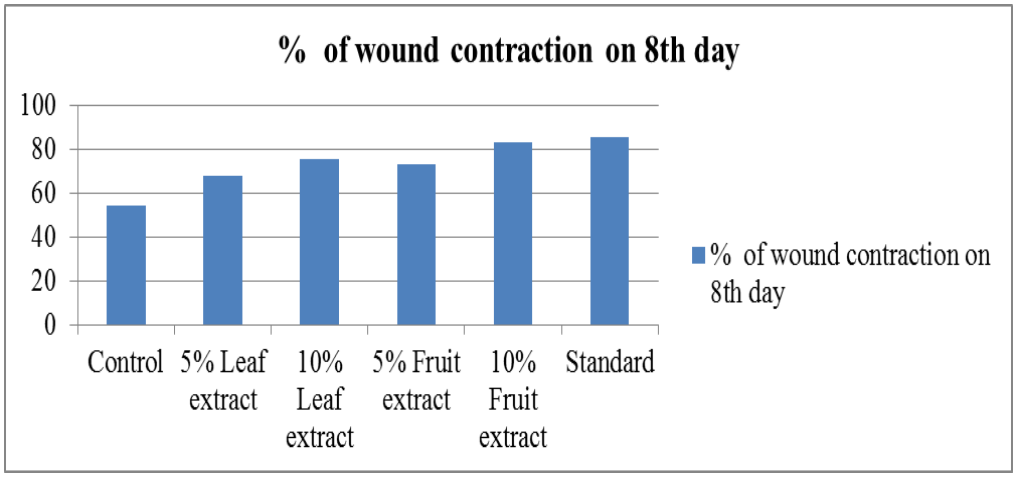

Fig. 2: % of wound contraction on 8th day.

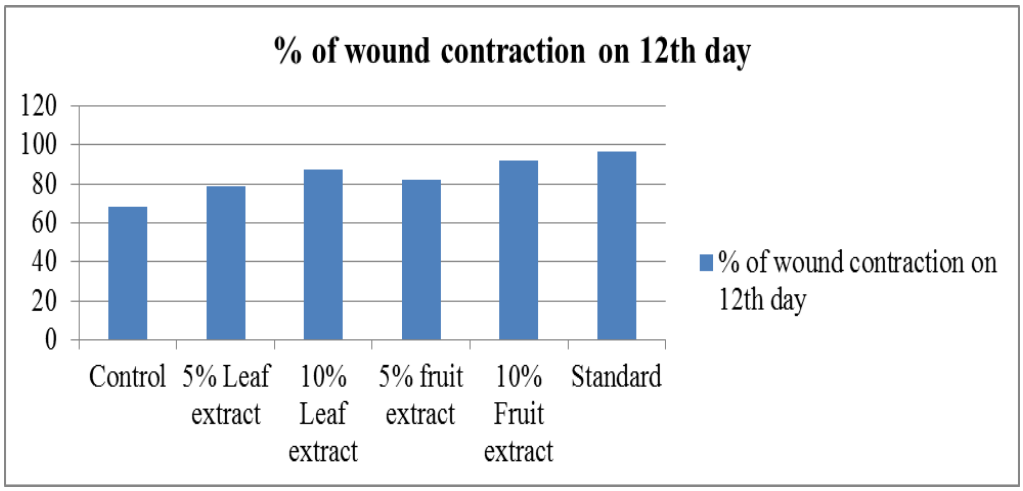

Fig. 3: % of wound contraction on 12th day.

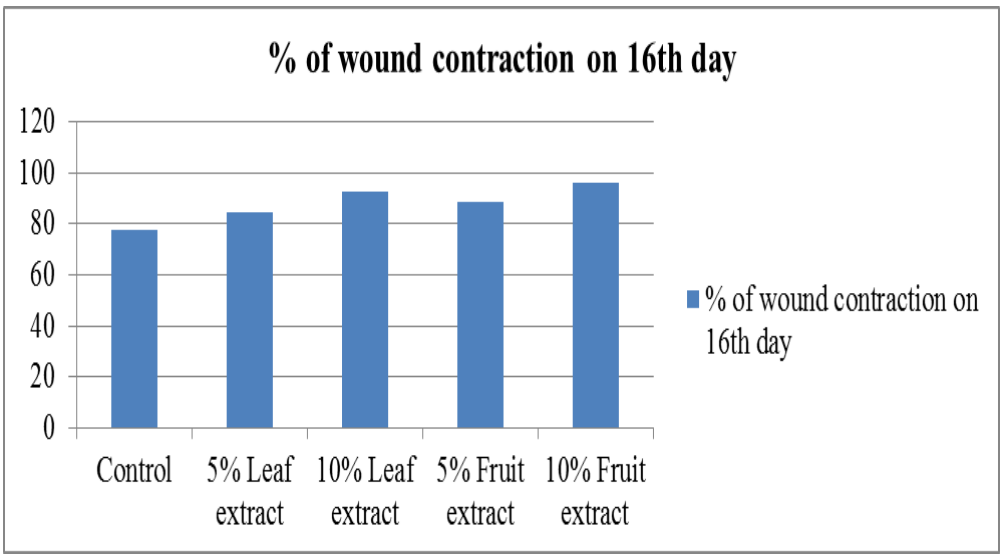

Fig. 4: % of wound contraction on 16th day.

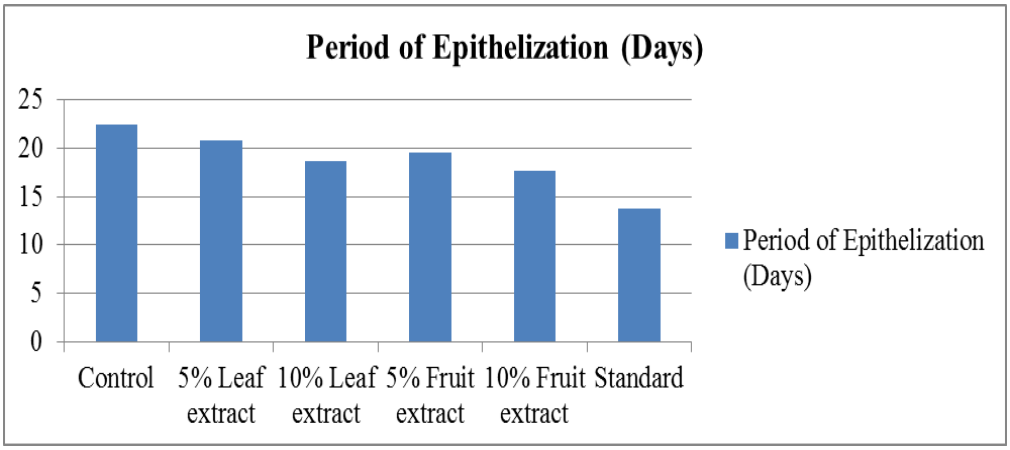

Fig. 5: Period of Epithelization.

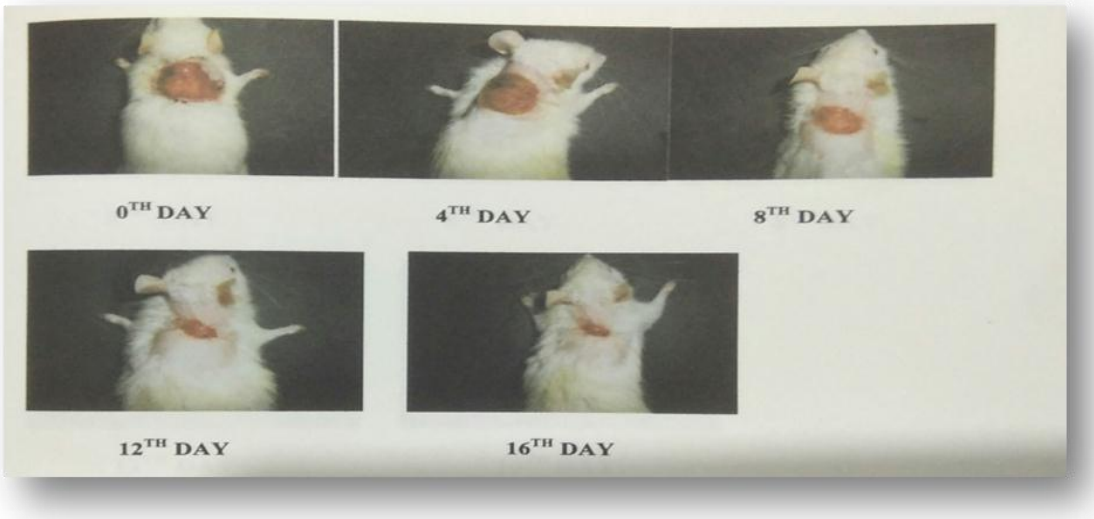

Fig. 6: Wound healing pattern of the leaf extract (5%) ointment of Gynocardia odorata in Rats.

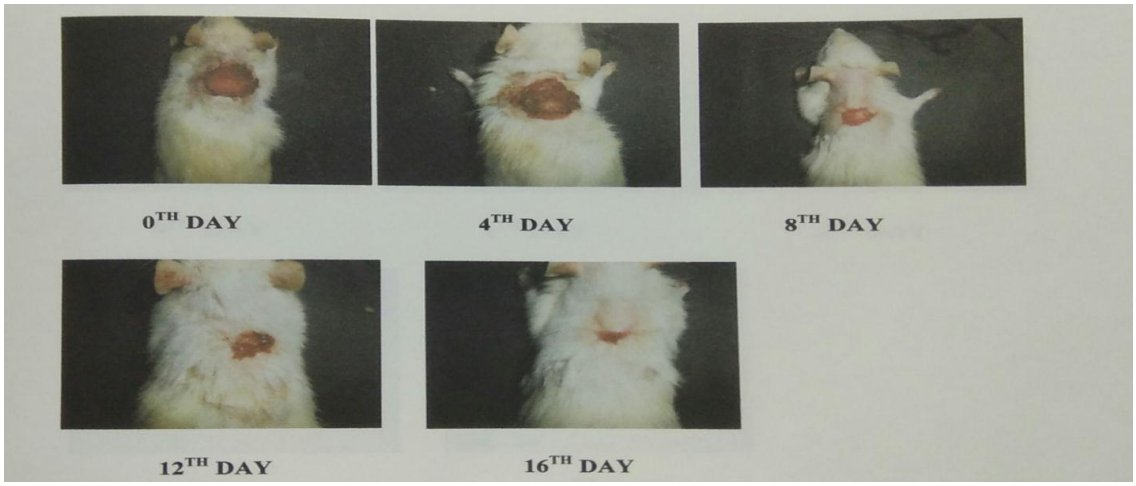

Fig. 7: Wound healing pattern of the fruit extract (5%) ointment of Gynocardia odorata in Rats.

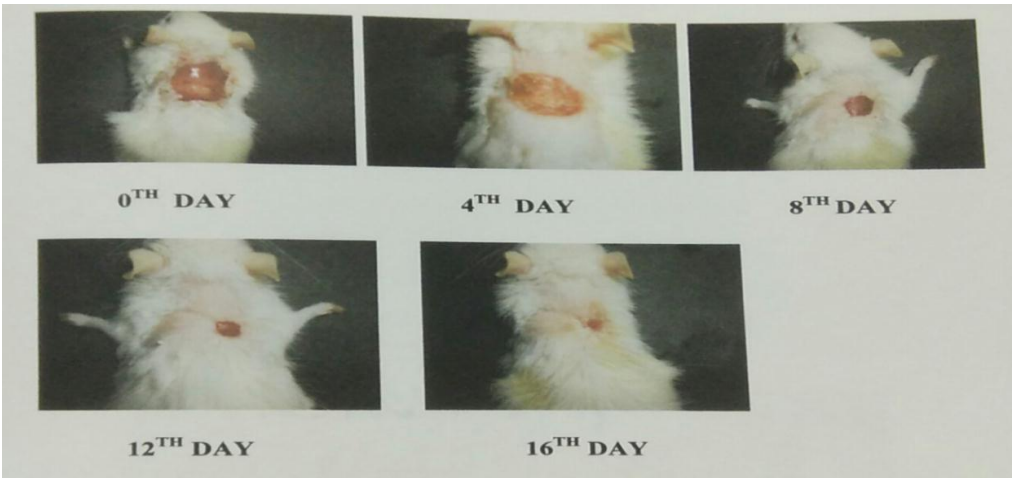

Fig. 8: Wound healing pattern of the leaf extract (10%) ointment of Gynocardia odorata in Rats.

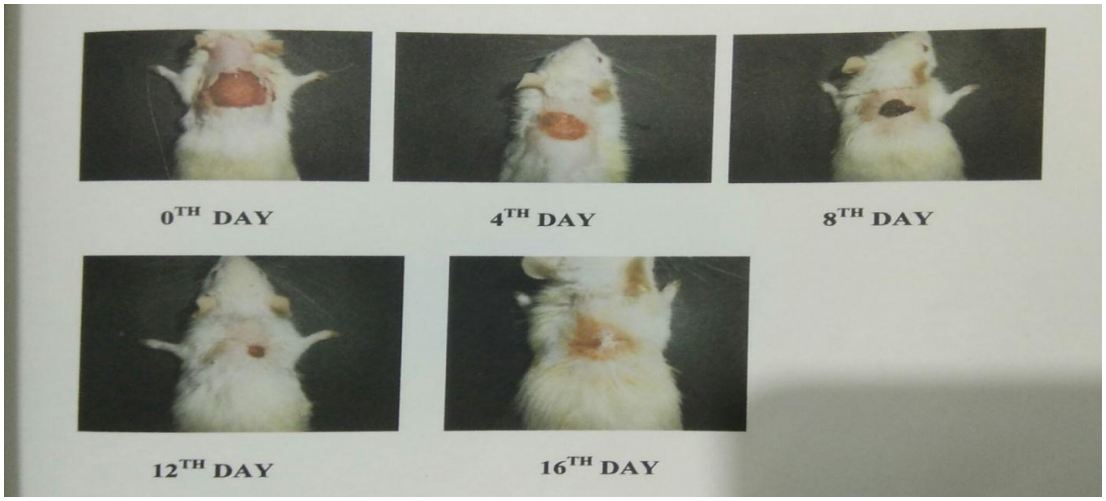

Fig. 9: Wound healing pattern of the fruit extract (10%) ointment of Gynocardia odorata in Rats.

DISCUSSION

The powdered leaves of Gynocardia odorata is green in color, mild bitter in taste and has characteristics odour while the powdered fruit is brown in colour and has a characteristics taste and odour. Both the powdered leaves and fruits were mounted with chloral hydrate, phloroglucinol, weak iodine and HCL and stained with saffranine, it showed the pressence of starch grains, fragments of vessels, fibres and calcium oxalate crystals.

Fluorescence analysis of both the powdered leaves and fruits were carried out by treating with different solvents and observing under different wavelength such as visible light, uv rays(254 nm) and (365 nm) and also the behaivours of both the powdered drug of fruit and

leaves of Gynocardia odorata treated with different solvents were observed. This study helped in distinguishing the drug in powdered form.

The extracts of the plant material both of the powdered leaves and the fruits were obtained by successive solvent extraction which was done by using petroleum ether, benzene, chloroform, acetone, methanol, ethanol and water. The extracts of the leaves obtained from petroleum ether, benzene, chloroform and acetone were dark green in colour while that of the fruit were light yellowish in brown in colour. The extracts of both the leaves and fruits obtained from methanol, ethanol and water were dark brown in colour. All the extracts were sticky in nature. The quantity of extracts of the leaves were found to be maximum in case of water extract while it is minimum incase of chloroform extracts while the quantity of extracts of the fruit was found to be maximum incase of water extract while it is minimum incase of petroleum ether extract. The extracts obtained by successive solvent extraction of both the leaves and fruits were subjected to preliminary phytochemical analysis which revealed the presence of Flavanoids, Glycosides, Tannins, Saponins, Fixed oils, Fats, Steroids and Triterpenoids.

The TLC Analysis showed in naked eyes and under different wavelengths and by using different mobile phase of both the leaf extract and fruit extract showed the presence of some compound like Flavanoid, Triterpenoids and their significant Rf values were found.

The wound healing activity of aqueous extracts of both the leaves and fruits of the plant Gynocardia odorata was evaluated by Excision wound model in Wister albino Rats. It has been observed that the aqueous extracts of the fruit at a dose level of 10% w/w showed better wound healing activity than that of the aqueous extract of the leaves at the same dose level. This was evident by faster rate of wound closure and epithelization period in excision wound model, but was found to be lesser in comparision to the standard drug povidone iodine.

Flavanoids are known to promote the wound healing process mainly due to their astringent and antimicrobial property, which seems to be responsible for wound contraction and increase rate of epithelization. Similar types of wound healing activity were also documented on other medicinal plants in the literature. In the present research work all the surgical interventions were carried out under sterile conditions and rats were closely observed for any signs of infections, since during the present study no animals showed visible signs of

infection. Hence this is very important to note that the control microbial infection is necessary for better wound healing and its management.

Further, tannins promote the wound healing through several cellular mechanism, chelation of free radicals and reactive species of oxygen, promoting contraction of the wound and increasing the formation of capillary vessels and fibroblasts and including keratinocyte proliferation, but do not act on the differentiation towards cornified cells. However, or results revealed that tannins are one of the important phytoconstituents responsible for wound healing mainly due to their astringent and antimicrobial property.

Therefore, it can be inferred that, in present investigation significant wound healing potential of the Gynocardia odorata leaves and fruits may be due to flavanoids and tannins content, which were confirmed by preliminary phytochemical screening.

CONCLUSION

In this present work the comparative phytochemical studies of Gynocardia odorata Roxb. leaves and fruits from sikkim region have been carried out. A scientific evidence on the comparative studies in this plant is not available.

The result of the phytochemical evaluation was found by using pharmacopoeial standards. The qualitative phytochemical investigation produced valuable information about different phytoconstituents present in the various extracts, which will help the future investigators for isolating the active principle and developing newer medicinal properties.

TLC studies of the aqueous extract of the leaf and fruit showed the pressence of Flavanoids, Triterpenoids derivatives etc.

The result of the pharmacological activity showed that the aqueous extracts of fruit and leaf of Gynocardia odorata has significant wound healing activity. Therefore, the pharmacological activity that was carried out based on the traditional reports of the use of the plant was found to be correct and was scientifically proven.

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