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Phytochemical and Wound Healing Activity of Tubers of *Momordica cymbalaria*



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ABSTRACT

The aim of this study was to carry out the phytochemical screening of the *Momordica cymbalaria*. Phytochemical screening of the aqueous and various different solvent extracts of the tubers revealed the presence of alkaloids, flavonoids, steroids, Triterpence saponins etc the tubers of *Momordica cymbalaria*, the wound healing activity of the selected Indian medicinal plant studied for its effects on wound in rats using excision method, the Methanolic extract for wound healing activity through topical route on excision wound model. The activity was compared with standard drug Nitrofurazone (0.2% w/w). *Momordica cymbalaria* Methanolic extract was found to have better and faster wound healing effect than standard drug Nitrofurazone ointment on excision wound model.



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INTRODUCTION

Momordica cymbalaria (Hook, Fenzl ex Naud.) is a vine of the *Momordica* genus found in the Indian states of Andhra Pradesh, Karnataka, Madhya Pradesh, Maharashtra and Tamil Nadu.^[1] It is used in the local folk medicine as an abortifacient and for the treatment of diabetes mellitus. It is a relative of the bitter melon plant (*M. charantia*) which is also used against diabetes. The plant has also been named *Luffa tuberosa* (Roxb.) or *Momordica tuberosa* (Roxb.) Pharmacological studies indicate possible action of extracts of the plant on several medical conditions. The water extract was reported to have hypoglycemic activity in diabetic rabbits but not in normal rabbits.^[2] The ethanol extract was reported to protect rats from isoproterenol induced myocardial injury.^[3]

Plants are used medicinally in different countries and are source of many potent and powerful drugs ^[4] Traditional medicine using plant extracts continues to provide health coverage for over 80% of the world's population, especially in the developing world ^[5]. Analyses of phytochemicals from *M. charantia* revealed the presence of active components like momorcharins, momordenol, momordicilin, momordicins, momordicinin, momordin, momordolol, charantin, charine, cryptoxanthin, cucurbitins, cucurbitacins, cucurbitanes, cycloartenols, diosgenin, elaeostearic acids, erythrodiol, galacturonic acids, gentisic acid, goyaglycosides, goyasaponins and multiflorenol ^[6-7].

Momordica cymbalaria Hook. F. belongs to the Cucurbitaceous family. The plant is a perennial herbaceous climber either allowed to trail on the ground or to climb on supports with the aid of tendrils. It is found in the south Indian states of Andhra Pradesh, Karnataka, Madhya Pradesh, Maharashtra and Tamil Nadu as a weed. The nutritional studies of the fruits of *M. cymbalaria* have reported that they possess a high level of calcium, potassium and vitamin C, in addition to its high crude fiber content.^[8] The fruit extracts of *M. cymbalaria* were shown to have antidiabetic, hypolipidemic ^[9-10] .anti-diarrhoeal^[11], and antiulcer activity ^[12]. The roots of the plant are used for menstrual irregularities, anti-fertility, antiovolatory, abortifacient ^[13], and hepatoprotective ^[14] activity. No researcher has yet reported antimicrobial activities of leaves of this plant. Therefore, it is worth conducting an investigation on the antimicrobial activities of extract of *M. cymbalaria* leaves.

MATERIALS AND METHODS

Collection of plant material

The fresh Plant of *Momordica cymbalaria* has Collected Chittoor (district) during the month of April 2016. The plant material was identified and authenticated at, Sri Krishna devaraya University, By Dr. S. Thimma Naik, Department of Botany. The fresh plant material was dried under shade. Dried plant material was powdered using mechanical grinder and passed through sieve no.60 to get the powder of desired coarseness. Powdered material was preserved in an air tight container.

Preliminary phytochemical screening:

Preliminary Phytochemical screening was done according to the phytochemical methods described. Following chemical tests were carried out for different extracts of *Momordica cymbalaria Tubers* to identify the presence of various phytochemical constituents.

Test for Carbohydrates:

Molisch's test (*General test*): To 2-3mL. Aqueous extract, add few drops of alpha-naphthol solution in alcohol, shake and add Conc. H₂SO₄ from sides of test tube. Violet ring is formed at the junction of two liquids.

Tests for Reducing Sugars:

a) *Fehling's test*: Mix 1mL. Fehling's A solution and 1mL. Fehling's B solutions boil for one minute. Add equal volume of test solution. Heating (in boiling water bath), for 5-10 min. First a yellow, then brick red precipitate is observed.

b) *Benedict's test*: Mix equal volume of Benedict's reagent and test solution in test tube. Heating (in boiling water bath), for 5 min. Solution appears green, yellow or red depending on amount of reducing sugar present in test solution.

Test for Monosaccharides:

Barfoed's test: Mix equal volume of Barfoed's reagent and test solution. Heat for 1-2 min. (In boiling water bath) and cool. Red precipitate is observed.

Test for Pentose Sugars:

Bial's Orcinol test: To boiling Bial's reagent add few drops of test solution. Green or purple coloration appears.

Aniline acetate test: Boil test solution in test tube. Hold filter paper soaked in aniline acetate in the vapor. Filter paper turns pink.

Mix equal amount of test solution and HCl. Heat, add a crystal of phloroglucinol. Red color appears.

Test for Hexose Sugars:

Seliwanoff's test (for ketohexose like fructose): Heat 3mL. Seliwanoff's reagent solution and 1mL Test solution in bearing water bath for 1-2 min. Red color is formed.

Tollen's phloroglucinol test for galactose: Mix 2.5mL conc. HCl and 4mL. 0.5% phloroglucinol. Add 1-2mL, test solution and heat. Yellow to red color appears.

Cobalt-chloride test: mix 3mL, test solution with 2mL Cobalt chloride. Boil and cool. Add few drops of NaOH solution. Solution appears greenish blue (glucose) or purplish (fructose) or upper layer greenish blue and lower layer purplish (mixture of glucose and fructose).

Test for Non-Reducing Sugars:

Test solution does not give response to Fehling's and Benedict's tests.

Hydrolyze test solution. Fehling's and Benedict's tests are positive.

Test for Non-Reducing Polysaccharides (Starch):

Iodine's test: Mix 3mL, test solution and few drops of dilute Iodine solution. Blue color appears; it disappears on boiling and reappears on cooling.

Tannic acid test for starch: With 20% tannic acid, test solution gives precipitate.

Test for Gums:

Hydrolyze test solution using dilute HCl. Perform Fehling's or Benedict's test. Red color is developed.

Test for Mucilage:

Powdered drug material shows red color with ruthenium red.

Powdered drug swells in water or aqueous KOH.

Test for Proteins:

Biuret test (General test): To 3mL test solution add 4% NaOH and few drop dos 1% CuSO₄ solution. Violet or pink color appears.

Million's test (for proteins): Mix 3mL test solution with 5mL Million's reagent solution. White precipitate is obtained; warm it then the precipitate turns to brick red or the precipitate dissolves giving red colored solution.

Xanthoproteic test (for protein containing tyrosine or tryptophan): Mix 3mL test solution with 1mL. Conc.H₂SO₄. White precipitate is formed boil. Precipitate turns yellow. Add NH₄OH, precipitate turns orange.

Test for proteins containing sulfur: Mix 5mL test solution with 2mL. 40% NaOH and 2 drops 10% lead acetate solution, boil. Solution turns black or brownish due to PbS formation.

Precipitation test: The test solution gives white colloidal precipitate with following reagents: absolute alcohol, ii) 5% HgCl₂ solution, iii) 5% CuSO₄, iv) 5% lead acetate, v) 5% ammonium sulphate.

Tests for Steroids: Liebermann – Burchard reaction:

Salkowski reaction: To 2mL. of extract, add 2mL.chloroform and 2mL.Conc. H₂SO₄, shake well. Chloroform layer appears red and acid layer shows greenish yellow fluorescence.

Mix 2mL. Extract with chloroform. Add 1-2mL acetic anhydride and 2 drops of Conc. H₂SO₄, from the sides of the test tube. First red, then blue and finally green color appears.

Liebermann's reaction: Mix 3mL. Extract with 3 mL acetic anhydride. Heat and cool. Add few drops of Conc. H₂SO₄. Blue color appears.

Tests for Volatile Oils:

Hydrodistillate material Separate volatile oil from distillate and perform the following tests:

1. Volatile oils have characteristic odor.
2. Filter paper is not permanently stained with volatile oil.
3. *Solubility test:* Volatile oils are soluble in 90% alcohol.

Tests for Glycosides:

Determine free sugar content of the extract. Hydrolyze the extract with mineral acid (Dil. HCl/Dil.H₂SO₄). Again determine the total sugar content of the hydrolysed extract. Increase in sugar content indicates presence of glycoside in the extract.

1) Tests for Cardiac Glycosides:

- a) *Baljet test:* A thick section shows yellow to orange color with sodium picrate.
- b) *Legal's test* (test for cardenolides): To aqueous or alcoholic extract, add 1mL Pyridine and 1mL Sodium nitroprusside. Pink to red color appears.
- c) Test for deoxysugars (*Keller – killiani test*): To 2mL. Extract, add glacial acetic acid, one drop 5% FeCl₃ and Conc. H₂SO₄. Reddish brown color appears at the junction of two liquid layers and upper layer appears bluish green.
- d) Liebermann's test (Test for bufadienolides): Mix 3mL. Extract with 3mL Acetic anhydride. Heat and cool. Add few drops of Conc. H₂SO₄. Blue color appears.

2) Tests for Anthraquinone Glycosides:

1. *Borntrager's test for anthraquinone glycosides:* To 3mL. Extract, add Dil. H₂SO₄. Boil and filter. To cold filtrate, add equal volume benzene or chloroform. Shake well. Separate the organic solvent. Add ammonia. Ammonical layer turns to pink or red.

2. *Modified Borntrager's test for C – glycosides:* To 5mL. Extract add 5mL. 5% FeCl₃ and 5mL. Dil. HCl. Heat for 5 min. in boiling water bath. Cool and add benzene or any organic solvent. Shake well, separate organic layer, add equal volume Dil. Ammonia. Ammonical layer shows pinkish red color.

3) Tests for Saponin Glycosides:

Foam test: Shake the drug extract or dry powder vigorously with water. Persistent foam is observed.

Hemolysis test: Add drug extract or dry powder to one drop of blood placed on glass slide. Hemolytic zone appears.

4) Tests for Flavonoids:

a) *Shinoda test:* To dry powder or extract, add 5mL. 95% ethanol, add few drops of Conc. HCl and 0.5g magnesium turnings. Pink color is observed.

b) To small quantity of residue, add lead acetate solution. Yellow colored precipitate is formed.

c) Addition of increasing amount of NaOH to the residue shows yellow colouration, which decolorizes after addition.

5) Tests for Alkaloids:

Evaporate the aqueous, alcoholic and chloroform extracts separately. To residue, add dilute HCl. Shake well and filter. With filtrate, perform following tests:

a. *Dragendroff's test:* To 2-3mL of filtrate, add few drops of Dragendroff's reagent. Orange brown precipitate is formed.

b. *Mayer's test:* To 2-3mL of filtrate add few drops of Mayer's reagent which give a precipitate.

c. *Hager's test:* To 2-3mL of filtrate add Hager's reagent which gives a yellow precipitate.

d. *Wagner's test:* To 2-3mL. of filtrate add few drops of Wagner's reagent which give reddish brown precipitate.

e. *Murexide test for purine alkaloids*: To 3-4mL. of test solution, add 3-4 drops of Conc. HNO₃. Evaporate to dryness. Cool and add 2 drops of NH₄OH. Purple color is observed.

Table: 1 Qualitative chemical examination of various extracts of *Momordica cymbalaria* (Tubers)

S.No	Test	Methanol	Petroleum ether	Benzene	Ethyl acetate	Aqueous
1	Alkaloids	+	+	+	+	+
2	Carbo hydrates	-	-	-	-	-
3	Phytosterols	+	-	+	-	-
4	Saponins	-	-	-	+	+
6	Proteins and amino acids	-	-	-	-	-
7	Terpenoid	-	+	-	+	-
8	Flavanoids	+	-	-	-	+
9	Steroids	+	+	-	+	-

+ denotes the presence of the respective class of compounds.

- denotes the absence of the respective class of compounds

Wound healing activity:

Excision wound model

The rats were inflicted with excision wound as described by ^[15-16]. An excision wound was inflicted by cutting away 5 mm² full thickness of a pre-determined area on the depilated back of the rat. The rats were divided into four groups of six animals each. Group 1 (control) animals were topically applied with simple ointment base, Group 2 animals were topically

applied with Nitrofurazone ointment and the remaining groups were topically treated with 200mg kg⁻¹ b.w of test substances mixed with ointment base. Treatments were given once daily till the wound was completely healed. Epithelialization period was noted as the number of days after wounding required for the dead tissue remnants to fall off leaving no raw wound behind. Wound contraction rate was monitored by planimetric measurement of the wound area on alternate days. This was achieved by tracing the wound on a graph paper. Reduction in the wound area was expressed as percentage of the original wound size^[17].

RESULTS AND DISCUSSION

Excision wound model:

The experimental animals were anesthetized using Lignocaine 2% injection over the local selected region. The rats were depilated over the region excision wound was infected by cutting away of 5 mm square thickness of skin from the predetermine area, the wound was left and rest to the open environment then the drugs reference standard (0.2% w/w Nitrofurazone ointment) control (simple ointment BP) only *Momordica cymbalaria* in methanol extract is applied. (10% w/w simple ointment) were applied till the wound was healed. This model was used to monitor by calculating the decreasing area.

General formula as follows:

$$RWH = \frac{\text{Size of wound in surface area (mm}^2\text{) at Day 9}}{\text{Size of Wound in surface area (mm}^2\text{) at Day 1}} \times 100\%$$
 Reduction in Healing = 100 – RWH

The wound healing activity was studied by using four groups. The groups are, Group I negative control simple ointment, In Group II positive control Nitrofurazone 0.2% w/v, Group III MCME.

The size of the wound in surface area, as follows:

On the Day 1: (35.25), (35.25), (35.25), (35.25).

On the Day 3: (35.25), (26.46), (22.32), (28.12).

On the Day 5: (35.25), (15.24), (15.12) (20.14).

On the Day 7: (24.12) (2.24) (4.12) (8.24).

On the Day 9: (20.42) (0.482) (3.12) (2.18).

The mean percentage closure of excision wound model on Day 9 (72.62) (98.63) (91.14) (93.37).

Contraction of the excision wound was promoted from Day 1 of the treatment till Day 9. The epithelization of wound in case of mice treated with extract was found to be quite earlier than control. It is also comparable with the marketed preparation. It suggests that the stem extract of *Momordica cymbalaria* promoted wound healing activity. The excision wound model showed significant wound healing property of the stem extract of *Momordica cymbalaria*, which was well compared with standard drug. The results are shown in **Table 1. (Figures 1-6)**

Table 1: Effect of topically applied *Momordica cymbalaria tubers* extracts on excision wound in mice.

Group	Avg. wt	Drug/ Formulation	Size of wound in surfaces area Day 1 (mm ²)	Day 3 (mm ²)	Day 5 (mm ²)	Day 7 (mm ²)	Day 9 (mm ²)	Percentage of wound healing
I	170-210 gms	Control	35.25	35.24	35.24	24.12	20.42	72.62
II		Nitrofurazone	35.25	26.46	15.24	2.24	0.482	98.63
III		MCCE	35.25	22.32	15.12	4.12	3.12	91.14
IV		MCME	35.25	28.12	20.14	8.24	2.18	93.37

MCCE: *Momordica cymbalaria* Chloroform extract, **MCME:** *Momordica cymbalaria* Methanolic extract,

Excision Wound Model Percentage of wound healing

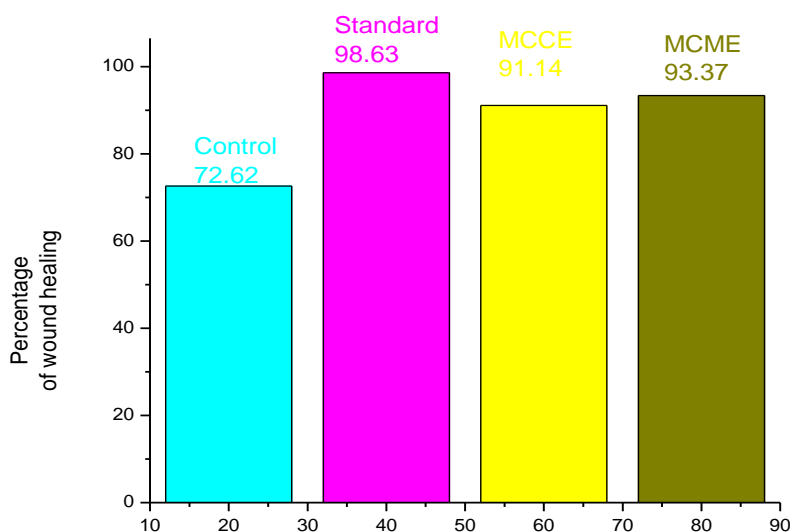


Figure:- 1: Effect of Chloroform extract of *Momordica cymbalaria* and its fractions on percentage of wound contraction in excision wound model .

(All the values represent the Mean \pm of data obtained from six different animals)

CONCLUSION

The phytochemical investigation of the *Momordica cymbalaria* tubers extract showed that the presence of Steroids, saponins, Flavonoids, Alkaloids, Tri terpenes and Steroids. It can be concluded that when the Methanolic extracts of *Momordica cymbalaria* was separately applied externally on albino Wistar rats by topical route through excision wound model then it showed faster as well as better wound closure and wound contraction as compared to standard marketed formulation called as Nitrofurazone ointment.

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