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INVITRO STUDY OF WOUNDHEALING ACTIVITY OF PLANT EXTRACT ON ANISOMELES MALABARICA LEAVES

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ABSTRACT

AIM: The aim of the study is to analysis the invitro wound healing activity of chloroform, acetone, and aqueous extracts of Anisomeles malabarica leaves.

METHODS: The leaves was collected and shade dried, made into a coarse powder. The powdered plant material was subjected to successive solvent extraction by Soxhlet extraction method using chloroform, acetone, and water as a solvent. The further extract was determined by woundhealing activity.

CONCLUSION: The present study reveals the wound healing activity of Anisomeles malabarica of leaf extract.

Keywords: Anisomeles Malabarica, Phytochemical Screening Aqueous, Chloroform, Acetone, Wound Healing Activity.

I. INTRODUCTION

Anisomeles malabarica, more commonly known as the **Malabar catmint**, is a species of herbaceous shrub in the family Lamiaceae. It is native to tropical and subtropical regions of India, and Sri Lanka, but can also be found in Malaysia, Bangladesh, Myanmar, Andaman Islands and Reunion.

Growing up to 2 m high, it has narrow green leaves 3–8 cm in length, and 1.5–3 cm wide.

It is pollinated by sunbirds and carpenter bees, and bears purple flowers in mid spring, though it may also bear the flowers throughout the year. Originally used in Sri Lankan and Hindi folk medicine, the current main uses are medicinal, aromatics and cosmetics.

Taxonomical Classification

- KINGDOM : Plantae
- PHYLUM : Streptophyta
- CLASS : Equisetopsida
- SUB-CLASS : Magnoliidae
- ORDER : Lamiales
- FAMILY : Lamiaceae
- GENUS : Anisomeles
- SPECIES : Anisomeles malabarica

Fig 1: Anisomeles malabarica

II. MATERIALS AND METHODS

Leaves materials: The leaves of Anisomeles malabarica were collected from the Sungarahalli village, Dharmapuri District. The gathered leaves were transferred to the laboratory after being firmly packaged in a polythene bag. After that it was left at room temperature at a dark environment for 2 weeks. After that, a blender was used to turn it into a coarse powder.

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Materials:

- Soxhlet extractor
- Extraction thimble
- Solvents (Chloroform, Acetone, Water)
- Sample (coarse powder)
- Heating mantle
- Condenser.

PREPARATION OF EXTRACTS:

Preparation of the extracts of the powdered leaves Anisomeles malabarica is done by using following solvents.

- Aqueous extracts
- Chloroform extracts
- Acetone extracts

1. **Aqueous extract:** The shade dried coarse powder of leaves 100gm was packed well in Soxhlet apparatus and subjected to continuous hot extraction with 500ml of Distilled water for 18hrs. The extract was distilled in vaccum under pressure in order to remove the solvent completely. It was dried and kept in dedicator till experimentation obtained extract was weighed and percentage yield was calculated in terms of air dried powdered crude drug.
2. **Chloroform extract:** The shade dried coarse powder of leaves 90gm was packed well in Soxhlet apparatus and subjected to continuous hot extraction with 350ml of Distilled water for 18hrs. The extract was distilled in vaccum under pressure in order to remove the solvent completely. It was dried and kept in dedicator till experimentation obtained extract was weighed and percentage yield was calculated in terms of air dried powdered crude drug.
3. **iii)Acetone extract**: The shade dried coarse powder of leaves 80gm was packed well in Soxhlet apparatus and subjected to continuous hot extraction with 350ml of Distilled water for 18hrs. The extract was distilled in vaccum under pressure in order to remove the solvent completely. It was dried and kept in dedicator till experimentation obtained extract was weighed and percentage yield was calculated in terms of air dried powdered crude drug.

Table 1: Extractive values of Aqueous extract, Chloroform extract, Acetone extract of Anisomeles malabarica leaves.

SPECIES	EXTRACTS	PERCENTAGE YIELD
Tamil Nadu	Aqueous extract	0.33%
Tamil Nadu	Chloroform extract	0.25%
Tamil Nadu	Acetone extract	0.26%

Fig 2: Soxhlet extraction

III. PHYTOCHEMICAL ANALYSIS OF ANISOMELES MALABARICA LEAF EXTRACTS

TEST FOR CARBOHYDRATES

Molish test

To 2-3ml of the test solution, add few drops of molish reagent solution and shaken, con. Sulphuric acid was added from the side of the test tube produces the violet ring was formed.

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TEST FOR ALKALOIDS

Preparation of test solution: The test solution was prepared by dissolving the extracts in dil. hydrochloric acid, the solution was filtered and the filtrate was subjected to the following tests for the detection of the presence of the alkaloids.

Dragendroff's test

A few ml test solution was added with 1-2ml of Dragendroff's reagent produce a reddish brown precipitate.

TEST FOR PROTEIN

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

Biuret test

To 3ml of test solution, 40% sodium hydroxide solution and few drops of 1% copper sulphate solution was added which produce blue color.

TEST FOR STEROID

Preparation of test solution: The plant extract was dissolved in chloroform and subjected into following tests.

Salkowski test

The test solution was added with concentrated sulphuric acid and allowed to sometime and this leads to formation of red color in the lower layer leads to presence of steroids.

TEST FOR TRITERPENOIDS

Preparation of test solution: The plant extract was dissolved in chloroform and subjected into following tests.

Libermann-burchard test

The test solution was added with acetic anhydride and boiled and cooled and added with concentrated sulphuric acid along the test tube leads to formation of brown ring at the junction of two layers and the formation of red intense color indicates the presence of triterpenoids.

TEST FOR SAPONINS

Preparation of test solution: The plant extract is dissolved in water and making it as a aqueous solution.

Foam test

To the 0.5 gm of test solution, add 2ml of water and vigorously shake to produce persistent foam for 10 minutes.

TEST FOR FLAVANOIDS

Preparation of test solution: The plant extract is dissolved in 2M Hydrochloric acid and heated Up to 30-40 mins at 100^{o} celsius and cool down and added with ethyl acetate and furtherly concentrated to dryness and ready too used as a test sample.

Lead acetate test

The test solution was added with lead acetate solution to from the yellow color indicates the presence of flavanoids.

TEST FOR REDUCING SUGAR

Fehling's test

A few ml of test solution was added with 1ml each of Fehling's solutions A & B and boil with water bath to produce the red precipitate.

TEST FOR TANNINS

Preparation of test solution: The test solution was prepared by dissolving the extract in water and alcohol.

Ferric chloride test

To 1ml of the test solution, ferric chloride solution was added leads to the formation of the dark blue or greenish black color shows the presence of tannins.

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TEST FOR GLYCOSIDES

Preparation of test solution:

The test solution was prepared by the sample dissolved in alcohol.

Killer killain test

To the test solution, few drop of ferric chloride solution was added and concentrated sulphuric acid was also added with it to form the two Layer occur, lower layer of reddish brown color and upper layer of bluish green color simultaneously.

TEST FOR AMINOACIDS

Preparation of test solution: The test solution was prepared by dissolving the plant extract in the water.

Ninhydrin test

The 3ml of test solution was heated and 3 drops of 5% ninhydrin solution was added in boiling water and was boiled for 10 minutes to form the purple or bluish colour to be formed.

TEST FOR FAT & OIL

Solubility test

Oils are soluble in ether, benzene, and chloroform but insoluble inn ethanol and water.

TEST FOR COUMARIN

Sodium hydroxide test

The plant extract is dissolved in 10% NaOH and chloroform to produce yellow colour.

TEST FOR RESIN

Acetic anhydride test

To the 1ml of plant extract is added to acetic anhydride solution and 1ml of conc. sulphuric acid to produce orange to yellow.

TEST FOR CHOLESTROL

To the 2ml of plant extract, 2ml of chloroform and 10 drops of acetic anhydride solution was added and add 2-3 drops of conc H_2SO_4 to produce red – rose colour.

TEST FOR QUINONES

Hydrochloric acid test

The plant extract is added to conc. HCl to produce green colour.

TEST FOR DITERPENES

Copper acetate test

The plant extract is dissolved in in distilled water and 2-4 drops of copper acetate solution to produce an emerald green colour.

Table 2: Phyto constituent of Aqueous, Chloroform and Acetone extracts of leaves of Anisomeles malabarica

S.NO	PHYTOCONSTITUENTS	AQUEOUS	CHLOROFORM	ACETONE
1.	ALKALOIDS	+	+	+
2.	Glycosides	+	+	+
3.	Carbohydrates	_	+	+
4.	Tannis	+	+	+
5.	Flavanoids	_	+	+
6.	Phytosterols	_	_	_
7.	Proteins	_	_	_
8.	Cholestrol	+	_	_
9.	Terpenoids	_	+	_

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Phytoconstituent: Presence (+), Absences (-).

IV. WOUND HEALING ACTIVITY

Inhibition of protein denaturation was evaluated by the method of Mizushima and Kobayashi 1968 and Sakat *et al*. 2010 with slight modification. 500µL of 1% bovine serum albumin was added to 10, 20, 30, 40 and 50µL of sample. This mixture was kept at room temperature for 10 minutes, followed by heating at 51°C for 20 minutes. The resulting solution was cooled down to room temperature and absorbance was recorded at 660 nm. Aspirin using as a standard. The experiment was carried out in triplicates and percent inhibition for protein denaturation was calculated using:

$$\text{Percentage inhibition} = \frac{100 - (\text{O.D. of test} - \text{O.D. of product control}) \times 100}{\text{O.D. of Control}}$$

Table 3: % Inhibition of Standard (Aspirin), Chloroform, Acetone.

Concentration	% Inhibition of Standard (Aspirin)	% of Inhibition Chloroform	% of Inhibition Acetone
10 µl	33%	27%	21 %
20 µl	43%	30 %	28 %
30 µl	58%	47 %	40 %
40 µl	79%	56 %	52 %
50 µl	88%	72 %	65 %

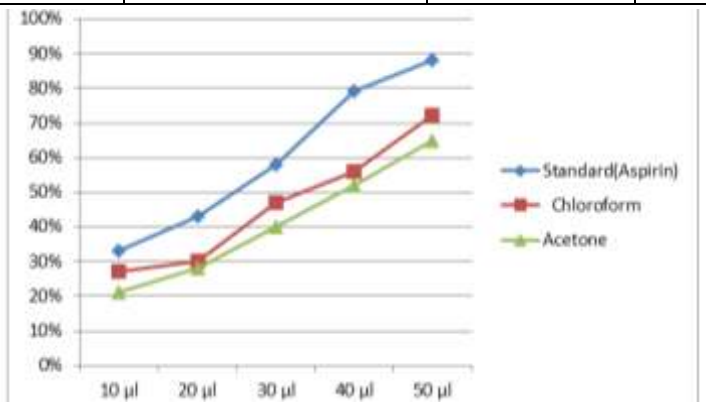

Fig 3: Graphical Representation of Standard, Chloroform, Acetone.

V. CONCLUSION

The higher concentration of 50 µg/ml of the Chloroform extract shows 72 % and Acetone 65 %. The 50 µg/ml standard Aspirin drug shows 88 %.

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