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Preliminary Phytochemical Screening of *Azima tetracantha*. Lam root

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ABSTRACT

***Azima tetracantha*, family Salvadoraceae, is indication for the rheumatism, diarrhea and other inflammatory conditions in traditional medicine. Siddha texts mentioned the diuretic activity of root indication for rheumatism and dropsy. In *Siddha* system, *Mulankaalvatham is* related to Knee joint pain. With reference to Siddha text Gunapadam Moolikaivakuppu, Part - I, written by Murukeshamudaliyar, *Mulankaalvatham* treats with internal medicine *IyankamverKudineer.* Single herbal ingredient *Iyankam Ver* (Root of*A.tetracantha*). Though root of *A.tetracantha* is as part of a long- established customarily used for anti-*vatha* property, either preclinical or clinical studies were not conducted yet. This study was taken up to assess its phytochemical analysis and its pharmacological action practiced for treatment of *Mulankaalvatham.* Root of*A.tetracantha*was freshly collected in and around Kaithady, Jaffna district, Sri lanka(Latitude: 9°40'8.04" Longitude: 80°7'14.51"). It was identified and authenticated by the experts of *Siddha* medicine, Department of Gunapadam, Unit of Siddha Medicine, University of Jaffna. The roots were washed, shade-dried, powdered, and extracted with distilled water. Presence of phyto constituents such as carbohydrates, proteins, flavonoids, glycosides, steroids, alkaloids, tannin and phenolic compounds were qualitatively analyzed by using water extracts of *A.tetracantha* root. The screening revealed the presence of flavonoids, tannin, terpene, glycoside, reducing sugar and Steroids. Phytoconstituents present in the extract may be responsible for its biological/ pharmacological properties and therefore may be developed as future drug candidates.**

Keywords: About four key words or phrases in alphabetical order, separated by commas.

INTRODUCTION

Azima tetracantha, family Salvadoraceae, is indication for the rheumatism, diarrhea and other inflammatory conditions in traditional medicine. Siddha texts mentioned the diuretic activity of root indication for rheumatism and dropsy. In *Siddha* system, *Mulankaalvatham is* related to Knee joint pain. With reference to Siddha text Gunapadam Moolikaivakuppu, Part - I, written by Murukeshamudaliyar, *Mulankaalvatham* treats with internal medicine *IyankamverKudineer.* Single herbal ingredient *Iyankam Ver* (Root of*A.tetracantha*). Though root of *A.tetracantha* is as part of a long- established customarily used for anti-*vatha* property, either preclinical or clinical studies were not conducted yet. This study was taken up to assess its phytochemical analysis and its pharmacological action practiced for treatment of *Mulankaalvatham.* Root of*A.tetracantha*was freshly collected in and around Kaithady, Jaffna district, Sri lanka(Latitude: 9°40'8.04" Longitude: 80°7'14.51") and was identified and authenticated by the experts of *Siddha* medicine, Department of Gunapadam, Unit of Siddha Medicine, University of Jaffna. The roots were washed, shade-dried, powdered, and extracted with distilled water. Presence of phyto constituents such as carbohydrates, proteins, flavonoids, glycosides, steroids, alkaloids, tannin and phenolic compounds were qualitatively analyzed by using water extracts of *A.tetracantha* root. The screening revealed the presence of flavonoids, tannin, terpene, glycoside, reducing sugar and Steroids. Phytoconstituents present in the extract may be responsible for its biological/ pharmacological properties and therefore may be developed as future drug candidates.

METHEDOLOGY

Selection of ingredient of *Iyangamverkudineer*

In this study, *Iyangamverkudineer* has been selected for *Mulankaalvatham* on oral ulcers from the classical Siddha literatures.

Collection of the ingredients of *Iyangamverkudineer*

Root of*A.tetracantha*was freshly collected from in and around Kaithady, Jaffna district, Sri lanka (Latitude: 9°40'8.04" and Longitude: 80°7'14.51)

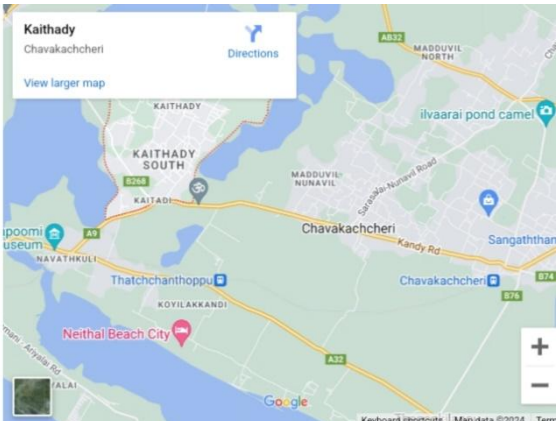

Figure 1: Google map of Kaithady area

Identification and authentication of ingredients of *Iyangamverkudineer*

Root of *Iyangamverkudineer* was identified and authenticated by the experts of *Siddha* medicine, Department of Gunapadam, Faculty of Siddha Medicine, University of Jaffna. The purification of these raw materials was done as illustrated in *Gunapadam Mooligaivaguppu*.

Extraction of root and phytochemical analysis

The root of *Azima tetracantha* were collected carefully washed the sand, shade-dried, powdered, and extracted with distilled water.

Qualitative phytochemical analysis

Presence of phyto constituents such as carbohydrates, proteins, flavonoids, glycosides, steroids, alkaloids, tannin and phenolic compounds were qualitatively analyzed by using water extracts of *A.tetracantha* root as follows:

Test for Alkaloids: Dilute hydrochloric acid was added in to the extract, shaken well then, filtered. The filtrate was tested for the alkaloid.

1. **Hager's test:** Few drops of Hager's reagent were treated with two milliliters of root extract. A yellow precipitate was not formed, shows the absence of alkaloids.
2. **Wagner's test:** Few drops of Wagner's reagent were tested with two milliliters of extract. A reddish-brown precipitate was not formed; it shows the absence of alkaloids.

Test for Flavonoids:

1. **Lead Acetate test:** Few drops of Lead acetate solution were treated with root extract.
   A yellow precipitate was found, it shows the presence of alkaloids.
2. **Alkaline reagent test:** 2ml of root extract was treated with two to three drops of sodium hydroxide. In initial stage, a deep yellow colour formed but it gradually changed colourless by adding few drops of dilute HCL, point to that flavonoids were present.

Test for Tannin and Phenolic compounds:

1. **Ferric Chloride test:** 1 ml of root extract was added to 2ml of 5% neutral ferric chloride solution, formation of dark blue colour shows the presence of phenolic compounds and tannins.
2. **Lead Acetate test:** Root extract was treated with few drops of Lead acetate solution. A formation of white precipitation indicating the existence of Phenolic compound.
3. **Gelatin test:** 2 ml of 1% gelatin solution containing 10% sodium chloride was added in to the aqueous solution of root extract, white colour precipitation was found. Precipitations point to that the phenolic compounds.
4. **Dilute Iodine Solution test:** 2 ml of root extract were treated with few drops of iodine solution. Transient red colour was found is point to that the phenolic compound.

Test for Saponins:

Froth test: A drop of Na2CO3 solution was added to 5 mL of extract in a test tube and shakes vigorously, kept the test tube for 5minutes. Form was not found. It indicated the absence of saponins.

Test for Triterpenoids and Steroids:

1. **Salkowski's test:** Added 3ml of chloroform with 0.5 ml of the extract and filtrated. Few drops of concentrated sulphuric acid was added along the sides of the test tube, test tube was shake and kept for few minutes. Red colour was found in lower showed the presence of steroids. Golden yellow colour was found in bottom layer was showed the presence of triterpenoids.
2. **Liebermann** - Burchard's test: Chloroform was treated with root extract. Few drops of acetic anhydride were added in to the chloroform root extract mixture. It was boiled and kept to cool. After cooling, Con. sulphuric acid was added through the sides of the test tube. At the junction of two layers, brown ring was formed. Upper layer was turned in to green colour was indicated the presence of steroids and upper layer changed in to deep red in colour was showed the existence of triterpenoids.

Test for Reducing Sugar:

1. **Fehling's test:** 1 ml of root extract was added in to 2 ml Fehling's solution. The mixture containing test tube was heated on water bath for 10 minutes. Red colour precipitation was formed. It was showed the presence of reducing sugar.
2. **Benedict's test:** Each same volume of root extract and Benedict's reagent were mixed in to boiling tube. Boiling tube was heated on water bath for 5- 10 minutes. Green, yellow, orange or red colours were observed. It was indicated the existence of reducing sugar.

Test for Proteins and Amino acids:

1. **Biuret's test:** Root extract was treated with 1 ml of 10% sodium hydroxide solution in to boiling tube and heated. In to the mixture, a drop of 0.7% copper sulphate was added to Violet / pink colour was not seen. It was indicated the absence of proteins.
2. **Ninhydrine test:** Root extract was treated with 5% ninhydrin solution in to boiling tube. It was heated with on a water bath for 10 minutes. Bluish colour not form. It was indicated the absence of amino acids.

Test for Glycosides:

1. **Legal's test:** In to the root extract, 1 ml of sodium nitroprusside solution was added. By using of 10% sodium hydroxide solution, mixture was made as alkaline. Pink to blood red colour was formed. It was indicated the existences of cardiac glycosides.
2. **Keller- Killiani test:** In to the 2 ml of extract, 1 drop of 5% ferric chloride and 3ml of glacial acetic acid were added. By the sides of the test tube, 0.5 ml of Con sulphuric acid was added carefully. In the acetic acid layer, blue colour was formed. It was showed the presence of cardiac glycosides.

Test for Fats and Oils:

1. Solubility test: In to 2ml of water extract, few drops of chloroform was added and observed its solubility.
2. In to 2ml of water extract, few drops of 90% ethanol was added and observed its solubility.

RESULTS AND DISCUSSION

Table 1: Presence of phytochemical constituents

Phytochemical	Result
Alkaloids	-
Flavonoids	+
Phenol	-
Tannin	+
Saponin	-
Terpene	+
Glycoside	+
Anthocyanin	-
Reducing sugar	+
Protein	-
Steroids	+

Siddha medicine says the traditional aspect of medicinal value, phytochemical and pharmacological properties of *A.tetracantha*in authentic siddha texts. Various plant parts extracts of *A.tetracantha were* used in several siddha preparations for prevention purpose or the illness management especially rheumatism diarrhea and other inflammatory disorders. Results of phyto chemical investigation of *A.tetracantha*root extract was shown the presence of many phyto chemical components in root extract such as flavonoids, tannin, terpene, glycoside, reducing sugar and steroids. The results was tabulated in Table 1, presence of phytochemical indicated by Positive sign (+) whereas absence of phytochemical indicated by negative sign (-). Water extract of the root containing phytochemical compounds may be the reason for its pharmacological properties and therefore, in future further studies should be done on drug candidate.

Screening of phytochemical of the medicinal plants are usually guide the further phytochemical analysis such as isolation, purification and characterization. These chemical compounds are responsible for bio activity. It can be used in pharmaceutical drug development. By the result of this study, presence of phytochemicals could be the reason for versatile pharmacological activities related to *Mulankaalvatham.*

CONCLUSION

Current study revealed that, the water extract of *A.tetracantha*root showed the presence of various phytochemicals. Water extract of the root containing phytochemical compounds may be the reason for its pharmacological properties, which may be used in the treatment of *Mulankaalvatham*. It could be a sufficiently great inventiveness to start the correlation between the phytochemical components and their bioactivity. Existence of phytochemical constituents in the *A.tetracantha* root extract can be prospect for their bioactivity, which may serve its ability as probable remedy for the Mulankaal*vatham*.

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