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PRELIMINARY PHYTOCHEMICAL ANALYSIS OF *NEURACANTHUS SPHAEROSTACHYUS* DALZ LEAVES EXTRACTS

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ABSTRACT

Objective: *Neuracanthus sphaerostachyus* has been traditionally used to treat skin diseases, cough, and asthma. Plant has shown significant anti-inflammatory, anti-asthmatic, anti-colitic activity and lacks of sufficient phytochemical scientific evidences indicating the utility of this plant prompted us to investigate preliminary phytochemical analysis of the plant.

Methods: Preliminary phytochemical analysis was performed for four extracts and were underwent the qualitative phytochemical tests for different active plant constituents like alkaloids, flavonoids, saponins, carbohydrates, steroids, glycosides, phenolic compounds, tannins, proteins, and Triterpenoids.

Results: Many of the phytochemicals such as flavonoids, phenolic compounds, triterpenoids, tannins, saponins, steroids, proteins and carbohydrates were found in methanolic and aqueous extract while only steroids and triterpenoids were present in hexane and chloroform extract.

Conclusion: The results acquired from preliminary phytochemical analysis shows that methanolic and aqueous extract of plant has quality number of good active chemical components.

Keywords: Extracts, Phytochemicals, Flavonoids, Tannins, *Neuracanthus sphaerostachyus*

INTRODUCTION

Neuracanthus sphaerostachyus Dalz. is known as Pincushion plant due to its floral structure and commonly known as Putliyo (Hindi), Golgonda (Marathi), and Ganthera–Gandharo (Gujarati). It is native to Indian regions and widely distributed in the Western Ghats (Goa), Deccan, and throughout the Gujarat **[1]**. This plant is traditionally used in different areas of the Western Ghats. The mixture of ash of the whole plant with jaggery or honey is used for 2–3 times a day orally to cure a cough and asthma **[2, 3]**. Root paste is applied to ringworm. Literature review suggests that it has anti-inflammatory **[4]**, anti-asthmatic **[5]**, anti-colitic **[6]**, anti-cholinergic & anti-histaminic **[7]** potential. *Neuracanthus sphaerostachyus* shows the presence of vanillic acid, syringic acid, melilotic acid and 6-OH luteolin **[8]**.

The scientific literature survey reveals no report on the preliminary phytochemical analysis of different extracts of *N. sphaerostachyus* leaves prompted us to evaluate it.

MATERIALS AND METHODS

Collection and authentication of plant

Neuracanthus sphaerostachyus Dalz leaves were collected from girnar forest region of Junagadh, Gujarat. Plant material was authenticated by the National Institute of Science Communication and Information Resources (NISCAIR)-Council of Scientific and Industrial Research, New Delhi (NISCAIR/RHMD/Consult/2016/2987-14).

Extraction of plant material

Extractive values of crude drugs were used to determine the number of active constituents extracted with solvents from a given amount of medicinal plant material. The successive extraction was carried out in soxhlet apparatus with a known quantity of powder in different organic solvents such as hexane, chloroform, methanol, and then water. After exhaustive extraction, the solvent was filtered and concentrated under reduced pressure at 50–55 °C **[9]**.

Preliminary phytochemical analysis

All four extracts were underwent the qualitative phytochemical tests for different active plant constituents like alkaloids, flavonoids, saponins, carbohydrates, steroids, glycosides, phenolic compounds, tannins, proteins, and Triterpenoids **[10, 11, 12]**.

(i) Test for alkaloids

Various extracts were tested for their alkaloids. In chloroform extract, 10 mg chloroform residue was processed with 2 % HCl. End solution was filtered and filtrates were separately tested with wagner's reagent,

mayer's reagent, hager's reagent and dragendroff's reagent.

In methanolic extract, extract of about 10 mg was macerated with 2% HCl, resulting solution was filtered and treated with 25% NH_4OH and further extracted with chloroform and evaporated, dissolved in 2% HCl and further tested as same as chloroform extract.

In hexane extract, 10 mg hexane residue was macerated with 2% HCl, content was filtered and further processed with 25% NH_4OH. It was extracted with chloroform and evaporated. Further it was dissolved in 2% HCl and tested as same as chloroform extract.

In aqueous extract, 10 mg of water residue was dissolved in 1.5 ml distilled water, 25% NH_4OH and extracted with chloroform, evaporated, dissolved in 10% HCl and further tested as same as chloroform extract.

A positive (+) sign is presented with a opaque turbidity, + + sign with a light opalescent precipitate, and + + + sign with a heavy yellowish-orange precipitate with Dragendorff's reagent, a heavy yellow precipitate with Hager's reagent, a heavy yellowish-white precipitate with Mayer's reagent and a heavy reddish brown precipitate with Wagner's reagent.

(ii) Test for carbohydrates

Different extracts in small quantity were dissolved in separate test tube with 5 ml distilled water followed by filtration. The resultant filtrate was used for further tests:

Molisch test - The filtrate received was reacted with 0.2 ml of 20 % α-naphthol in ethanol. Appearance of violet coloured ring while addition of conc. H_2SO_4 win inclined test tube was observed.

Fehling's test - It was performed for the detection of reducing sugars. Fehling's solutions A & B was mixed in equal proportions and mixed with extract, heated for 30 min forming cuprous oxide precipitates of brick red colour near to boiling point due to the reduction. Reducing sugars may have all mono and disaccharides like lactose, maltose, cellobiose and gentiobiose.

A positive (+) sign for a slight violet coloured ring or precipitate, + + sign with a medium violet coloured ring or precipitate, and + + + with a heavy violet coloured ring or precipitate.

(iii) Test for Glycosides and Anthraquinones

Borntrager's test - It was performed to detect free and combined anthraquinones in different extracts. Different extracts were incorporated in benzene to separate out benzene layer. Later, dilute solution of NH_4 was added to observe reddish pink colour.

A small quantity of different extracts was macerated with HCl, kept on water bath for

next 2-3 hrs and further extraction was performed with benzene. Reddish pink colour was observed when benzene layer was processed with dilute NH_4 solution.

A positive (+) sign is represented for a light red, violet or pink colour, + + sign with a medium light red, violet or pink colour, and + + + sign with a strong light red, violet or pink colour.

Keller- Killiani test – The extracts were dissolved with few drops of $FeCl_3$ in glacial acetic acid, filtered. 1 ml concentrated H_2SO_4 was added to filtrate. Pale Red or brown colour was formed which turned blue colour by the end of reaction.

A + is represented sign for a light colour, + + sign with a medium colour, and + + + with a strong colour.

Legal test: The extracts were made alkaline with small quantity of 10% NaOH and mixed with pyridine and sodium nitroprusside, blue colour was observed.

A + sign is represented for a light colour, + + sign with a medium colour, and + + + with a strong colour.

(iv) Test for flavonoids

Shinoda test - Approximately 10 mg of different extracts was mixed with suitable solvent, for further treatment with magnesium ribbon (metallic). Later, 0.2 ml of concentrated HCl was added.

Ammonia test - Strips of filter paper was prepared by dipping it in to various extracts. Strips were reacted with ammonia and reported white to yellowish colour change.

A + sign is represented for a light yellow or red colour, + + sign for a faint yellow or red colour, and + + + sign for a pale yellow or red colour.

(v) Test for tannins and phenolic compounds

Approximately 10 mg of powdered extract was dissolved in their respective solvents and divided it in to 3 separate portions. First portion was mixed with small quantity of sodium chloride for each extract. Second portion was mixed with 1% gelatine and third portion with gelatine salt reagent. Presence of tannins was indicated by precipitates while treatment with both gelatine solution and reagent. Further each portion was confirmed by addition of 0.1 ml $FeCl_3$ showing blue or green black colour.

A positive (+) sign is represented with light precipitates, + + with a medium precipitates, and + + + with a turbid precipitates.

(vi) Test for proteins and amino acids

Small quantities of different extracts were mixed with distilled water followed by filtration. The resultant filtrates were processed for following tests:

Biuret's tests – filtrate was alkalized and reacted with ammonia. Red or light violet colour observed on addition of 0.2 ml copper sulphate.

Millon's test – Few drops of filtrate was reacted with Millon's reagent. Red precipitates were produced.

Ninhydrin test – Minute quantity of filtrate was mixed with solution of lead acetate. Precipitates were filtered. To a chromatogram (paper), a drop of filtrate was placed. Ninhydrin reagent was sprayed, followed by heating period for 5 minutes, produced violet red colour.

Xanthoprotein test – 0.1 ml of conc. HNO_3 was mixed with small quantity of filtrate from the sides of the test tube, produced yellow colour.

A + sign is represented a light colour, + + sign with a medium colour, and + + + with a strong colour.

(vii) Test for saponins

Foam test - A minute quantity of different extracts was macerated with acetone and petroleum ether. Water was added to the filtrate to shake it vigorously. Honeycomb froth was observed for 30 min.

A + is represented with 0.5 cm height, + + sign with 1 cm height, + + + sign with more than 1 cm of a height.

(viii) Test for sterols and or triterpenes

For saponification, different extracts were reacted with KOH. The mixture was mixed with distilled water; diethyl ether was used for further extraction. The extract was dried and evaporated for following tests.

Libermann-Burchard test - The remnant was mixed with $CHCl_3$, further 0.1 ml acetic anhydride was added. To the solution, from the sides 0.1 ml concentrated sulphuric acid was added. Red colour was detected.

Salkowski's reaction – Few drops of concentrated sulphuric acid was added to various extracts. Addition of acid formed yellow colour ring at the junction of two liquids.

Hesses reaction – To chloroform, extract was added in equal portion followed by addition of concentrated H_2SO_4 by the sides of the test tube. Pink coloured ring was formed.

Hersch's Sohn's reaction: 5 ml of tri-chloroacetic acid was dissolved with extract. Further, heating formed violet colour.

A + sign is represented with light colour, + + sign with medium colour, and + + + with strong colour.

RESULTS

The various dried extracts were tested for their active principle like alkaloids, glycosides, flavonoids, tannins/phenolic components, reducing sugars, protein/amino acids, steroids/ triterpenoids and results are placed in **Table 1**.

Table 1: Results of preliminary phytochemical analysis of various extracts of *Neuracanthus sphaerostachyus*

Type of Constituent	Hexane	$CHCl_3$	Methanol	Aqueous
Alkaloids	-	-	-	-
Phenolic Compounds & Tannins	-	-	++	+
Flavonoids	-	-	+	+
Saponins	-	-	+	-
Steroids	++	++	+++	-
Triterpenoids	++	+	+++	-
Proteins	-	-	++	+++
Carbohydrates	-	-	+	+++
Glycosides	-	-	-	-

Note: (+, ++, +++) represents intensity of colour; (-) represents absent

DISCUSSION

Herbal plants are used since many years due to the presence of active metabolites responsible for its pharmacological properties. These metabolites are synthesized in some or all parts of the plant. Four different leaves extract of *Neuracanthus sphaerostachyus* shows the presence of many active chemical components. Many of the phytochemicals such as flavonoids, phenolic compounds, triterpenoids, tannins, saponins, steroids, proteins and carbohydrates were found in methanolic and aqueous extract while only steroids and triterpenoids were present in hexane and chloroform extract.

Terpenoids are attributed for its analgesic and anti-inflammatory activities. While flavonoids are liable for a wide range of properties like anti-inflammatory, antiallergic, antitumor, estrogenic, antimicrobial, antioxidant, vascular and cytotoxic activity. Saponins are responsible for antibacterial activity **[13]**.

CONCLUSION

The results acquired from preliminary phytochemical analysis shows that methanolic and aqueous extract of plant have quality number of good active chemical components. Phytochemical profile of plant supports previous findings from Daniel M 1987. Presence of phenols, tannins, flavonoids, steroids and triterpenoids individually or due to synergistic effect, could be responsible for their biological activity. Further isolation and identification of each bioactive compound may be needed to extrapolate the active chemical components to formulate new drugs to treat a variety of disorders.

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REFERENCES

[1] Khare CP. Indian Medicinal Plants: An Illustrated Dictionary. New York: Springer Publication; 2007. pp. 372-3.

[2] Punjani BL, Kumar V. Traditional medicinal plant remedies to treat cough and asthmatic disorders in the Aravalli

ranges in North Gujarat, India. J Nat Remedies 2002; 2:173-8.

[3] Dangar DK, Patel NJ. Pharmacognostic studies on *Neuracanthus sphaerostachyus* Dalz. (Acanthaceae) leaves. Journal of Ayurveda and Integrative Medicine. 2020; 11(4): 529-33.

[4] Dangar DK, Patel NJ. anti-inflammatory activity of *Neuracanthus sphaerostachyus* Dalz. leaves. Asian Journal of Pharmaceutical and Clinical Research. 2019;12(3): 227-9.

[5] Dangar DK, Patel NJ. Evaluation of anti-asthmatic activity of Dalz. leaves. *Neuracanthus sphaerostachyus* extract. Asian Journal of Pharmacy and Pharmacology. 2019;5(4): 744-49.

[6] Dangar DK, Patel NJ. Anti-Inflammatory Effect of *Neuracanthus sphaerostachyus* Dalz. Leaves on Experimental Colitis in Rats. Indo Global Journal of Pharmaceutical Sciences. 2021;11(1): 7-14.

[7] Dangar DK, Tank CJ, Jivani RR AND Kirtane SR. Evaluation of Anti-histaminic and Anti-cholinergic activities of *Neuracanthus sphaerostachyus* Dalz Leaves. International Journal of Biology, Pharmacy & Allied sciences. 2022;11(7): 960-67.

[8] Daniel M, Sabnis SD. Chemosystematics of some Indian members of the *Acanthaceae*. Indian Acad Sci (Plant Sci). 1987; 97:315-32.

[9] Singh RM. Indian Pharmacopoeia. 2nd ed. New Delhi: Government of India; 1966. pp. 33-4.

[10] Khandelwal K. Practical Pharmacognosy. Nirali prakashan, Pune, 2010, 20th ed., pp. 25.1-25.9.

[11] Harborne, JB., Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis. Chapman and Hall, London, 1988, pp.1-226

[12] Trease EG, Evans CW. Pharmacognosy, Eastbourne: English language Book society; 1985, 12th Ed., pp. 344.

[13] Harborne JB, Williams CA. Advances in flavonoid research since 1992, Phytochemistry, 2000, pp. 481-504.