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PRELIMINARY STUDIES ON PHYTOCHEMISTRY AND *IN VITRO* ANTIMICROBIAL ACTIVITY OF EXTRACTS OF *CNIDOSCOLUS ACONITIFOLIUS*

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ABSTRACT

The present study was aimed to evaluate the phytochemical and antimicrobial nature of the different extracts of the leaves of *Cnidoscolus aconitifolius* plant. Initially, the plant leaves was collected authenticated and dried for powdering. The dried material was powdered in mechanical grinder and the coarse powder thus obtained was extracted in soxhlet apparatus with the solvents of increasing polarity viz., petroleum ether, chloroform, ethyl acetate and methanol. The dried extracts thus obtained were used for the preliminary phytochemical evaluation and antimicrobial activity studies viz., antibacterial and antifungal evaluation. The results of preliminary phytochemical evaluation revealed the presence of alkaloids, glycosides, phenolic compounds and tannins, flavanones and flavonoids, terpenoids, and saponins. The methanol and ethyl acetate extracts showed a significant presence of majority of phytochemicals comparing with other two, the petroleum ether, chloroform extracts. In the evaluation of antimicrobial activity, the methanol extract showed a significant antibacterial and antifungal activity against tested pathogens particularly *P. vulgaris, S. aureus,* and *Aspergillus sp*. These results are useful for further investigation in the future.

KEYWORDS: *Cnidoscolus aconitifolius*, preliminary phytochemical evaluation, antimicrobial evaluation.

INTRODUCTION

The use of herbs as medicine is the oldest form of healthcare known to humanity and has been used in all cultures throughout history. Early humans recognized their dependence on nature for a healthy life and since that time humanity has depended on the diversity of plant resources for food, clothing, shelter, and medicine to cure myriads of ailments.[1] A vast knowledge of how to use the plants against different illnesses may be expected to have accumulated in areas where the use of plants is still of great importance. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavanoids, tannins and phenolic compounds.[2]

Cnidoscolus aconitifolius is a shrub or small tree native to southern Mexico and Central America, commonly known as chaya, tree spinach, or spinach tree is a cultivated, fast growing perennial plant belonging to the family Euphorbiaceae has a long history of cultivation for its edible leaves and therapeutic purposes, dating back to the Mayan civilization. It is believed to have its origins in the Yucatan Peninsula, spreading due to domestication. It has been widely introduced as a cultivated plant in warmer parts of the world. The genus *Cnidoscolus* comprises at least 40 species and this genus plants have a wide variety of claims for its medicinal efficacy for numerous ailments such as insomnia, gout and alcoholism, boosting low blood volume, lowering blood cholesterol, management and treatment of Diabetes mellitus.[3] With this view, the present study was designed to evaluate the phytochemical nature and the antimicrobial activity of the extracts of *Cnidoscolus aconitifolius*, an attempt to provide a direction for further research.

MATERIALS AND METHODS

Collection and identification of plant material

The leaves of *Cnidoscolus aconitifolius* was collected from in and around the Thriuvananthapuram District, Kerala, India. The collected material was identified and authenticated by Dr Chelladurai, Research Officer–Botany, (Scientist–C), (Rtd.)., Central Council for Research in Ayurveda & Siddha, Govt of India.

Preparation of powdered material and extraction

Powdering and extraction of collected plant material was done in reference with the previous literature.[4,5] The collected aerial part of the plant was dried in shade and powdered by using mechanical grinder and kept in the airtight container. Extraction of the coarse powdered material was done with the solvents of increasing polarity viz., petroleum ether, chloroform, ethyl acetate and methanol in the soxhlet apparatus assembly. For that, about 25g of dried coarse powder was moistened with

the respective solvent, packed in the soxhlet apparatus and extracted with 500ml of each solvent individually. The extract obtained was filtered and distilled off the solvent to obtain the dried extract. The percentage yield of each dried extracts was calculated.

Preliminary phytochemical screening
Preliminary phytochemical analysis of the extracts obtained was done in reference with the standard procedure.[4,6]

Chemical test for alkaloids
Little quantity of dried extract with alcohol was shaken with dilute hydrochloric acid and filtered. The acidified filtrate was used to detect the presence of alkaloids by following tests.

Mayer's test
The acidified filtrate (2ml) was treated with Mayer's reagent (1ml), shaken well and observed for the presence of creamy precipitate.

Wagner's test
The acidified filtrate (2ml) was treated with Wagner's reagent (1ml) and observed for the presence of reddish-brown precipitate.

Hager's test
The acidified filtrate (2ml) was treated with Hager's reagent (1ml) and observed for the presence of yellow precipitate.

Dragendorff's test
The acidified filtrate (2ml) was treated with Dragendorff's reagent (2ml) and observed for the presence of orange-red precipitate.

Chemical tests for glycosides
Little quantity of dried extract was hydrolyzed with dilute hydrochloric acid on a water bath for a few hours and the hydrolysate obtained was used to detect the presence of glycosides by following tests.

Legal test
The hydrolysate (2ml) was dissolved in pyridine (2ml). Freshly prepared sodium nitroprusside solution (2ml) was added to it. Made the mixture alkaline with sodium hydroxide solution and observed for the formation of pink colour.

Baljet test
The hydrolysate (2ml) was treated with sodium picrate solution (1ml) and observed for the formation of a yellow to orange colour.

Borntrager's test
A little quantity of the residue obtained from the evaporation of hydrolysate was mixed with water and shaken with an equal volume of chloroform. The chloroform layer was separated and equal quantity of dilute ammonia solution was added to it and shaken well and observed for the formation of pink colour in the ammonical layer.

Modified Borntrager's test
A little quantity of the residue obtained from the evaporation of hydrolysate was treated with ferric chloride and dilute hydrochloric acid. Then it was extracted with chloroform. The chloroform layer was separated and an equal quantity of dilute ammonia solution was added to it and shaken well and observed for the formation of pink colour.

Chemical tests for phenolic compounds and tannins
Ferric chloride test
A small quantity of the dried extract was mixed with water and treated with dilute ferric chloride solution (5%) and observed for the presence of blue colour.

Gelatin test
The dried extract dissolved in the water was filtered. To the filtrate, a 2% solution of gelatin containing 10% sodium chloride was added and observed for the presence of milky white precipitate.

Lead acetate test
The dried extract dissolved in the water was treated with a 10% lead acetate solution and observed for the presence of bulky white precipitate.

Decolourisation test
The dried extract dissolved in water was treated with dilute potassium permanganate solution and observed for the decolourisation of potassium permanganate.

Chemical tests for flavanones and flavonoids
Aqueous sodium hydroxide test
Aqueous sodium hydroxide solution was added to the little quantity of dried extract and observed for the yellow colouration of the solution.

Ammonia test
The filter paper wetted with a small quantity of alcoholic solution of the dried extract was exposed to ammonia vapour and observed for the formation of yellow colour.

Shinoda test
The dried extract mixed with alcohol was treated with magnesium or zinc and dilute hydrochloric acid and observed for the formation of orange-red or violet colour.

Chemical tests for carbohydrates
A small quantity of ethanolic extract was mixed with water or alcohol and filtered. The filtrate was subjected to the following tests to detect the presence of carbohydrates.

Molisch's test
The filtrate (2ml) was treated with a few drops of Molisch's reagent and concentrated sulphuric acid (2ml)

was added through the side of the test tube without shaking and observed for the presence of violet ring at the junction of two solutions.

Fehling's test
The filtrate (1ml) was treated with 1ml each of Fehling's solution A and B and boiled in a water bath and observed for the formation of a reddish precipitate.

Benedict's test
The filtrate (2ml) was treated with Benedict's reagent (2ml). Then the mixture was heated in a boiling water bath and observed for the presence of reddish precipitate.

Chemical tests for proteins and amino acids
Millon's test
Little quantity of dried extract was treated with of Millon's reagent (2ml) and observed for the formation of white precipitate, which on warming turn into a red coloured solution.

Biuret test
Little quantity of dried extract was treated with a few drops of 2% copper sulphate solution. To this excess of potassium hydroxide solution was added and observed for the formation of violet coloured solution.

Ninhydrin test
Little quantity of dried extract was treated with few drops of ninhydrin solution and heated on a water bath and observed for the presence of violet colour.

Chemical test for terpenoids
Salkowski test
Little quantity of dried extract was dissolved in chloroform. An equal volume of concentrated sulphuric acid was added to it and observed for the appearance of red colour in the chloroform layer and greenish-yellow fluorescence in the acid layer.

Chemical tests for sterols
A little quantity of the alcoholic extract was refluxed with alcoholic potassium hydroxide solution until the saponification was observed. The mixture was diluted and extracted with solvent ether. The ethereal extract was evaporated and the residue obtained was used in the tests for sterols.

Liebermann – Burchard test
The residue was taken with dry chloroform (1ml) and then it was mixed with 2ml of specially distilled acetic anhydride followed by a few drops of concentrated sulphuric acid through the sides of the test tube and observed for the formation of green colour in the upper portion which changes to bluish violet.

Salkowski test
The residue was dissolved in chloroform and an equal volume of concentrated sulphuric acid was added to it and observed for the red colour in the lower layer.

Chemical tests for saponins
Foam (Froth) test
A small quantity of dried extract was diluted with distilled water (20ml) in a graduated cylinder. The suspension was shaken for 15min and observed for the formation of froth.

Haemolysis test
A drop of blood was placed in a slide and mixed with a small quantity of dried extract and observed for haemolysis.

Chemical tests for gum and mucilage
Absolute alcohol (25ml) was added with an aqueous extract (10ml) with constant stirring. Filtered and the precipitate formed was dried in air and examined for swelling properties.

Chemical test for volatile oil
Powdered material (50gm) was subjected to hydro-distillation in volatile oil estimation apparatus (Clevenger apparatus). Collect the distillate and observed for the presence of volatile oil layer.

***In vitro* antimicrobial activity**
Antimicrobial activity of the prepared extracts was done in reference with the standard procedure.[7-9] Standard test cultures were used for the experiments.

Evaluation of antibacterial activity
Antibacterial activity of the test extracts was evaluated against six bacterial cultures viz., *E. coli, S. aureus, P. aeruginosa, K. pneumoniae, P. vulgaris* and *B. subtilis.* The inoculam of the experiment were prepared from 24h old culture in nutrient broth. The Muller Hinton agar plates were prepared. A swab of test culture was inoculated in the surface of the Muller Hinton agar plates so as to make a lawn. Sterile paper discs were made by using Whattmann No.1 filter paper, impregnated in the test extracts for 10min and placed in each plate and the inoculated plates were incubated at 37°C for 24hrs. Zones of inhibition in mm was measured and recorded.

Evaluation of antifungal activity
The antifungal activity of the test extracts was evaluated against *A. niger, A. flavus, C. albicans* and *Mucor* sp., The Potato Dextrose Agar was used as the media. A swab of test culture was inoculated in the PDA surface so as to make a lawn. Sterile paper discs of Whattmann No.1 filter paper were prepared, impregnated in the test extracts for 10min and placed in each plate. The inoculated plates were incubated in room temperature for 48h. Zones of inhibition in mm were measured and recorded.

RESULTS AND DISCUSSION
In the present study, the leaves of *Cnidoscolus aconitifolius* was collected and made into coarse powder after proper drying and extracted with different solvents such as Petroleum ether, Chloroform, Ethyl acetate and

Methanol. In case of percentage yield of dried extracts, the petroleum ether extract yielded 4.5gm of dried extract and the chloroform extract gave 4.8gm of dried extract. The ethyl acetate and methanol extract gave 6.2 and 7.5gm of dried extract respectively.

Presence of alkaloids, glycosides, phenolic compounds and tannins, flavanones and flavonoids, terpenoids, and saponins were found in the preliminary phytochemical evaluation of the extracts. The presence of alkaloids was identified in all the tested extracts but the ethyl acetate and methanol extracts showed a significant presence. In case of glycosides, the chloroform, ethyl acetate and methanol extracts showed its presence. However, the ethyl acetate and methanol extracts showed a significant presence of this constituent. The phenolic compounds and tannins were found in all the tested extracts but the ethyl acetate and methanol extracts showed its significant presence. Regarding with the flavanones and flavonoids, the results showed the presence of chloroform, ethyl acetate and methanol extracts with the significant presence in the last two extracts. The presence of terpenoids was found in all the tested extracts with a significant presence in chloroform, ethyl acetate and methanol extracts. Presence of saponins was found in the chloroform, ethyl acetate and methanol extracts with the significant presence in the last two extracts. A negative result was found in the test for sterols, carbohydrates, proteins and amino acids, gum and mucilage and volatile oil in all the four tested extracts (Table 1)

Table 1: Preliminary phytochemical analysis of *C. aconitifolius* extracts.

S. No.	Chemical Test	I	II	III	IV
1	**Alkaloids**				
a	Mayer's test	+	+	++	++
b	Wagner's test	+	+	++	++
c	Hager's test	+	+	++	++
d	Dragendorff's test	+	+	++	++
2	**Glycosides**				
a	Legal test	-	+	++	++
b	Baljet test	-	+	++	++
c	Borntrager's test	-	+	++	++
d	Modified Borntrager's test	-	+	++	++
3	**Phenolic compounds and tannins**				
a	Ferric chloride test	+	+	++	++
b	Lead acetate test	+	+	++	++
c	Gelatin test	+	+	++	++
4	**Flavanones and flavonoids**				
a	Aqueous NaoH test	-	+	++	++
b	Ammonia test	-	+	++	++
c	Shinoda test	-	+	++	++
5	**Carbohydrates**				
a	Molisch's test	-	-	-	-
b	Fehling's test	-	-	-	-
c	Benedict's test	-	-	-	-
6	**Proteins and Amino acids**				
a	Millon's test	-	-	-	-
b	Biuret test	-	-	-	-
c	Ninhydrin test	-	-	-	-
7	**Terpenoids**				
a	Salkowski test	+	++	++	++
8	**Sterols**				
a	Libermann-Burchard test	-	-	-	-
b	Salkowski test	-	-	-	-
9	**Saponins**				
a	Foams test/froth test	-	+	++	++
b	Haemolysis test	-	+	++	++
10	**Gum & mucilage**	-	-	-	-
11	**Volatile oil**	-	-	-	-

I – Petroleum ether extract; **II** – Chloroform extract; **III** – Ethyl acetate extract; **IV** – Methanol extract; (+) – presence of active constituents; (++) – significant presence of active constituents; (-) – absence of active constituents

In the preliminary phytochemical evaluation of the leaves of *Cnidoscolus aconitifolius* extracts, it was able to identify a significant presence of presence of alkaloids, glycosides, phenolic compounds and tannins,

flavanones and flavonoids, terpenoids, and saponins. The results of present study in case of alkaloids is in accordance with the results of a previous report.[10] The anticancer property of alkaloids was documented previous studies.[11] Similarly, some recent reports indicated the anticancer properties of glycosides apart from several biological activities including beneficial effects on heart disease.[12] The antioxidant property of the phenolics was reported in several studies.[13] The flavones and flavonoids were well-known for their anti-cancer anti-oxidant, anti-inflammatory, anti-diabetic and anti-microbial activity and beneficial effects on heart disease.[10,14] In case of terpenoids, various biological activities such as anti-allergic, anti-inflammatroy, anti-microbial, anti-hyperglycemic, anti-spasmodic and immunomodulatory activities were documented in various studies.[13] The saponins were well known for their anti-inflammatory property.[16] In this study, among the different phytochemicals identified, majority of them present in the ethyl acetate and methanol extracts which may be due to their high polarity. [4]

In the evaluation of antibacterial activity, it was found that the methanol extract showed a significant activity against the tested organisms (Table 2), particularly against the organism, *P. vulgaris* and *S. aureus*. The result of antifungal evaluation is presented in Table 3. The tested extracts showed the antifungal activity in the evaluation, particularly, the ethyl acetate and methanol extracts. From the results it was found that the methanol extract showed a significant activity against the *Aspergillus* species. The presence of terpenoids, flavanones and flavonoids in the extracts (Table 1) may be responsible for the antimicrobial activity of the methanol extract of *C. aconitifolius.*

Table 2: Antibacterial activity of *C. aconitifolius* extracts.

Extracts	Bacterial culture					
	1	2	3	4	5	6
	Zone of inhibition (Diameter/mm)					
Petroleum ether	Nil	13	09	Nil	11	12
Chloroform	12	10	12	Nil	15	16
Ethyl acetate	10	14	10	13	10	14
Methanol	15	17	14	15	18	17

1 – *E. coli*; 2–*S. aureus*; 3–*P. aeruginosa*; 4–*K. pneumoniae;* 5–*P. vulgaris*; 6–*B. subtilis*

Table 3: Antifungal activity of *C. aconitifolius* extracts.

Extracts	Fungal culture			
	1	2	3	4
	Zone of inhibition (Diameter/mm)			
Petroleum ether	Nil	Nil	10	Nil
Chloroform	Nil	Nil	12	Nil
Ethyl acetate	13	16	12	Nil
Methanol	17	19	16	13

1 – *A. niger*; 2– *A. flavus*; 3– *C. albicans*; 4– *Mucor* sp.

CONCLUSION

The therapeutic value of the plants of the genus *Cnidoscolus* were reported in several studies. With this view, the plant *Cnidoscolus aconitifolius* was selected as the candidate for our research. In this study, the extracts of leaves of *Cnidoscolus aconitifolius* were subjected to preliminary phytochemical and antimicrobial evaluation successfully. Our future studies directed towards the evaluation of diverse pharmacological activities of these extracts may give more significant results.

REFERENCES

1. Kunle OF, Egharevba HO, and Ahmadu PO. Standardization of herbal medicines-A review. Int. J. Biodvers. Conserv, 2012; 4(3): 101-12. Doi: 10.5897/IJBC11.163
2. Veeramuthu D, Muniappan A, Savarimuthu I. Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. BMC Complement Altern Med., 2006; 6: 35. Doi: 10.1186/1472-6882-6-35
3. Isola AO, Felix AS. Characterization of compounds from leaf extracts of tree spinach -*Cnidoscolus aconitifolius* (Miller) I. M. Johnston. International Journal of Scientific Research in Chemical Engineering, 2014; 1(5): 82-86. Doi: 10.12983/ijsrce-2014-p0082-0086
4. Anoob Kumar KI, Swamivel Manickam M, Sreejith M, Sebastin V. Preliminary phytochemical evaluation, isolation and spectroscopic characterization of constituents in the dried extracts of the whole plant *Crotalaria Biflora* (L). Int J Life Sci Pharma Res., 2020; 10(5): 124-33. Doi: 10.22376/ijpbs/lpr.2020.10.5.P124-133
5. Kulathuran Pillai K, Narayanan N, Chidambaranathan N, Jegan N. Phytochemical analysis of leaf extract of *Cnidoscolus chayamansa* McVaugh. Int J Pharmacogn Phytochem Res., 2014-15; 6(4): 741-45.
6. Sebastin V, Gopalakrishnan G, Sreejith M, Anoob Kumar KI. Preliminary phytochemical evaluation and spectral characterization of active constituents in the dried extracts of the whole plant *Argyreia imbricata* (Rox) Sant & Patel. Asian J Pharm Clin Res., 2019; 12(11): 56-60.
7. Mathan S, Anton Smith A, Kumaran J, Prakash S. Anticancer and antimicrobial activity of *Aspergillus protuberus* SP1 isolated from marine sediments of South Indian coast. Chin J Nat Medicines, 2011; 9(4): 286-292.
8. Sarita, M., Shisir, L., and Raj Kumar, D. *In vitro* antimicrobial activity of some medicinal plants against human pathogenic bacteria**.** J Trop Med., 2019; 1895340: 5. Doi: 10.1155/2019/1895340
9. Rahul P, Rakesh KJ, Lal Prasanth ML. Evaluation of antimicrobial activity of the extracts of selected marine microalgae against human pathogens. World Journal of Pharmaceutical and Medical Research, 2021; 7(1): 156-159.

10. Senguttuvan J, Paulsamy S, Karthika K. Phytochemical analysis and evaluation of leaf and root parts of the medicinal herb, *Hypochaeris radicata* L. for *in vitro* antioxidant activities. Asian Pac J Trop Biomed, 2014; 4(1): S359-367.
11. Balabhaskar R, Rajendra Kumar A, Selvarajan S, Faridha A, Gayathri G. Potential natural products with anticancer properties and their applications. Asian J Pharm Clin Res., 2019; 12(5): 27-33. Doi: 10.22159/ajpcr.2019.v12i5.32817
12. Khan H, Saeedi M, Nabavi SM. Mubarak MS, Bishayee A. Glycosides from medicinal plants as potential anticancer agents: Emerging trends towards future drugs. Curr Med Chem., 2019; 26: 2389-2406.
13. Pushpa, R., Mamta, A., and Subhanshi, S. Phyto-chemical and anti-oxidant properties of various extracts of M*ichelia champaca* leaves. Int J Pharm Pharm Sci., 2019; 11(5): 56-61. Doi: 10.22159/ijpps.2019v11i5.31745
14. Mohammed SA, Sanni S, Ismail AM, Kyari AS, Abdullahi S, Amina I. Preliminary phytochemical and elemental analysis of aqueous and fractionated pod extracts of *Acacia nilotica* (Thorn mimosa). Vet Res Forum, 2014; 5(2): 95-100.
15. Manjulika Y, Sanjukta C, Sharad Kumar G, Geeta W. Preliminary phytochemical screening of six medicinal plants used in traditional medicine. Int J Pharm Pharm Sci., 2014; 6(5): 539-542.
16. Anoob Kumar KI, Swamivel Manickam M, Sreejith M, Sebastin V. *In vitro* and *in vivo* anti-inflammatory evaluation of the whole plant extracts of *Crotalaria biflora* (L). Pharmacogn J., 2021; 13(3): 620-625. Doi: 10.5530/pj.2021.13.78