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PHARMACOGNOSTIC STUDIES AND EVALUATION OF ANTIOXIDANT, ANTI-INFLAMMATORY ACTIVITY OF VARIOUS EXTRACTS OF *CHASSALIA CURVIFLORA* (WALL.) THWAITES ROOT

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ABSTRACT

The roots of the plant *Chassalia curviflora* (Rubiaceae) have been used by *Kani* tribes for the treatment of colic pain and to heal wounds and pimples. Oil boiled with the juice of the leaves is used for eye and ear disease, ulcer and sore throat. Decoction of root is given as a remedy in phlegm, rheumatism and pneumonia. The work highlights pharmacognostic studies and evaluation of antioxidant, anti-inflammatory activity of various extracts of the roots of plant *Chassalia curviflora*. The pharmacognostic studies like loss on drying, ash values total ash value, extractive values, crude fibre content were done on the plant proved to be a tool for the authentication and identification of the plant The antioxidant activity studies using Iron chelating assay, DPPH radical scavenging assay and total antioxidant assay showed that ethyl acetate extract could be a promising source of natural antioxidants. The anti inflammatory activities by inhibition of protein denaturation and proteinase inhibitory assay showed ethyl acetate extract to possess potent anti inflammatory activity. Thus the roots of the plant *Chassalia curviflora* can be suggested to be used in diseases like phlegm, rheumatism and pneumonia due to its antioxidant and anti inflammatory properties.

Key words: *Chassalia curviflora*, antioxidant, anti inflammatory

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INTRODUCTION

Herbal drugs have an active role in traditional medicines for healing and health benefits. A key obstacle, which has hindered the promotion in use of Alternative medicine in the developed countries is the absence of quality control measures. It becomes extremely important to make sure that the Standardization of the plant and parts of the plant to be used as medicine. Pharmacognostical and pharmacological studies are some of the techniques used for the unique identification and standardization of the plant material (Fig-1).The present work highlights pharmacognostic studies and evaluation of antioxidant, anti-inflammatory activity of various extracts of the roots of plant *Chassalia curviflora* (1, 2).

The roots of the plant *Chassalia curviflora* (Rubiaceae) have been used by *Kani* tribes for the treatment of colic pain and to heal wounds and pimples. Oil boiled with the juice of the leaves is used for eye and ear disease, ulcer and sore throat.

Decoction of root is given as a remedy in phlegm, rheumatism and pneumonia (3).

Fig-1 ***Chassalia curviflora***

MATERIALS AND METHODS

Plant Collection

Chassalia curviflora roots were collected from Cheruvandoor, Kottayam district, Kerala and authenticated. The roots were collected washed, dried in shade and coarsely powdered.

Extraction

Successive solvent extraction was performed with different solvents on roots The extracts obtained were weighed. Its percentage yield was calculated in terms of the air-dried weight of the plant material. The colour and consistency of the extract were noted and tabulated.

Pharmacognostic Studies (8, 9)

Different pharmacognostic studies include evaluation of macroscopy,microscopy, physicochemical parameters such as determination of ash values, extractive values, crude fibre content, loss on drying.

Macrocopy

The colour, odour, shape and other external characteristics of *C.curviflora* root were studied.

Microscopy

Transverse section of the softened root samples was taken, stained with toluidine blue and mounted.

Powder Microscopy

Powdered material was cleared with NaOH and mounted in glycerine medium after staining with phloroglucinol and Con.Hydrochloric acid. Different cell components were studied and measured

Physicochemical Evaluation of the Crude Drug

Loss on drying

The air dried powdered roots of *C.curviflora* was weighed and dried in a hot air oven at 100^0C until a constant weight was obtained. The loss in weight was recorded as loss on drying.

Determination of Ash Values of the Crude Drug

Total Ash Value

2g of accurately weighed drug was incinerated at a temperature not exceeding 450ºC until free from carbon, cooled and weighed.

Acid Insoluble Ash value

The total ash obtained by above method was boiled with 25 ml of 2N hydrochloric acid and filtered. The filtrate along with the ash less filter paper was incinerated, cooled and weighed.

Water Soluble Ash Value

The total ash obtained was boiled with 25ml of water for 5minutes. The insoluble matter was collected in an ash less filter paper, incinerated, cooled and weighed. From that water insoluble ash value, the water soluble ash value was calculated.

Determination of Extractive Values

Alcohol Soluble Extractive Value

The air dried and coarsely powdered roots (5g) were macerated with 100ml of 95% alcohol in a stoppered conical flask for 24 hours. It was shaken frequently for six hours and allowed to stand for 18 hours. This extract was filtered; 25ml of the filtrate was concentrated in a tared petridish and weighed out.

Water Soluble Extractive Value

The above procedure was repeated using distilled water instead of alcohol.

Determination of crude fibre content

About 2g of powdered drug was heated with 50ml of 10%V/V HNO3 and filtered. The residue was mixed with 50ml of 2.5%V/V NaOH solution and boiled for 30 sec with constant stirring. The residue was then strained and washed with hot water. It was then transferred to a clean dry crucible, weighed and percentage crude fibre content was calculated.

ANTIOXIDANT ACTIVITY STUDIES (4-6)

Iron Chelating Assay

Different concentrations of extracts and standard (ascorbic acid) solution were incubated with 1ml *O*-phenanthroline solution in methanol and 2ml ferric chloride solution at ambient temperature for 10 minutes. After incubation, the absorbance of solutions was measured at 510 nm. IC_{50} value was calculated from the graph of inhibition percentage plotted against extract concentration.

DPPH radical scavenging assay

Various concentrations of the extract and standard (ascorbic acid) in methanol were added to 1ml of a methanolic solution of DPPH (0.135mM). The mixture was vigorously shaken and then allowed to stand at room temperature for 30 min in the dark. The absorbance of the mixture was measured at 517 nm. The scavenging activity on the DPPH radical was expressed as inhibition percentage and IC_{50} value was calculated was calculated.

ANTI- INFLAMMATORY ACTIVITY STUDIES (7, 10, 11)

PROTEIN DENATURATION

5ml 0.2% w/v bovine serum albumin in Tris HCl buffer saline and different concentrations of extracts in methanol were taken in test tubes and heated at 72°C for 5 minutes, cooled for 10 minutes. The absorbance of these solutions was determined at 660nm. The experiment repeated with standard (prednisolone) also. The IC_{50} was calculated and compared with standard.

PROTEINASE IHIBITORY ACTION

The reaction mixtures (2ml) contained 0.06 mg trypsin, 1ml 25mM Tris HCl buffer (pH7.4) and 1ml aqueous solution of plant extracts of different concentration (100,200,300,400,500µg/ml). The mixtures were incubated at 37ºC for 5min.Then 1ml of 0.8%(w/v) casein was added. The mixtures were incubated for an additional 20 minutes. Then 2ml of 70 %(w/v) perchloric acid was added to terminate the reaction. The cloudy suspension was centrifuged. Absorbance of the supernatant was read at 280nm against buffer as blank. $IC_{50}$was calculated and compared with standard (prednisolone).

RESULT AND DISCUSSION

Extraction

The percentage yield, colour and consistency of the extract were studied and tabulated (table-1) below.

Table -1 Characteristic of extracts of roots of *Chassalia curviflora*

Sl. No.	Extracts	Colour and consistency	Yield (in gm)	Percentage Yield (%W/W)
1	Pet. Ether extract	Light green sticky mass	2.44g	3.75%
2	Chloroform extract	Brown sticky mass	0.432g	0.66%
3	Ethyl acetate extract	Brownish yellow mass	0.717g	1.10%
4	Alcoholic extract	Reddish Brown mass	3.43g	5.2%
5	Aqueous extract	Brown dry mass	3.82g	5.8%
6	Total ethanolic extract	Reddish brown sticky mass	4.3g	7.3%

Pharmacognostic Studies

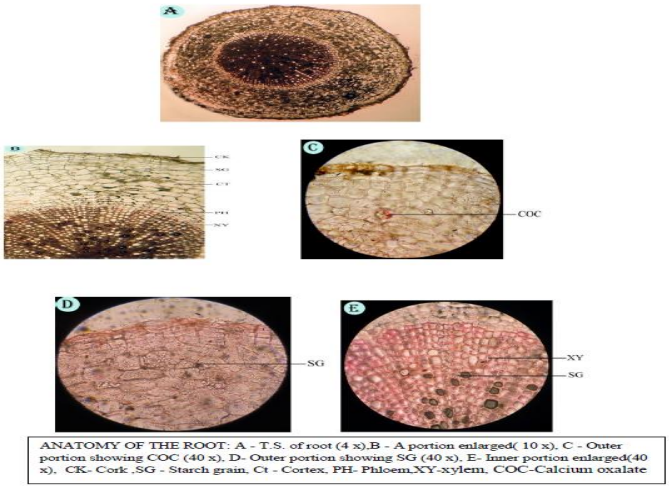
Morphological studies

Root is yellow in colour, aromatic odour, astringent taste, short and subcylindrical shape (Fig-2)

Fig- 2 Dried roots of *Chassalia curviflora*

Microscopy

The T.S of *Chassalia curviflora* roots were taken and shown in the figure-3.



ANATOMY OF THE ROOT: A - T.S. of root (4 x),B - A portion enlarged(10 x), C - Outer portion showing COC (40 x), D- Outer portion showing SG (40 x), E- Inner portion enlarged(40 x), CK- Cork ,SG - Starch grain, Ct - Cortex, PH- Phloem,XY-xylem, COC-Calcium oxalate

Fig-3 Anatomy of *Chassalia curviflora roots*

Powder Microscopy

The powder study of *Chassalia curviflora* roots were perfomed and shown in figure-4.

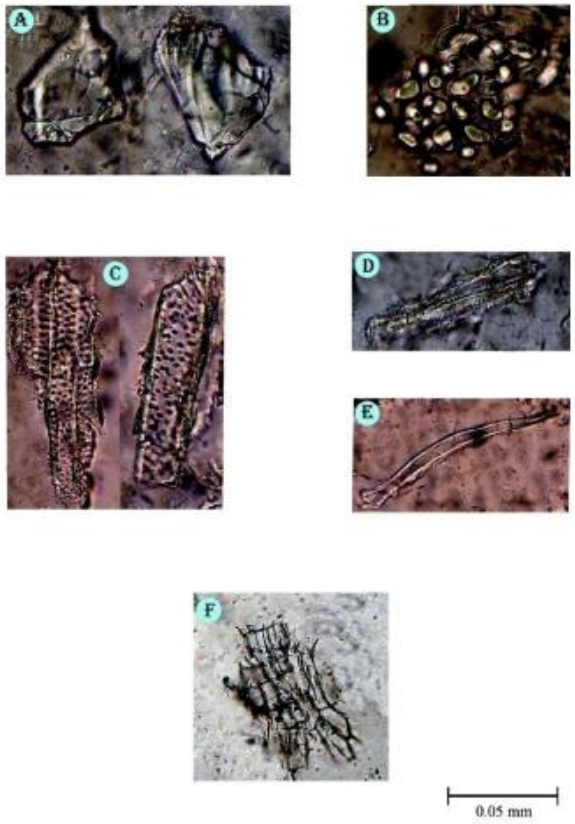


Fig-4 Powder microscopy of *Chassalia curviflora*

Physicochemical Evaluation

The phytochemical studies on the roots were carried out and tabulated (table-2).

Table-2 physicochemical evaluation of roots of *Chassalia curviflora*

S No	PARAMETRES	VALUE(%w/w)
1	Loss of dryng	03.99
2	Total ash	05.64
3	Acid insoluble ash	03.92
4	Water soluble ash	02.67
5	Alcohol soluble extractive value	09.13
6	Water soluble extractive value	13.53
7	Crude fibre content	47.15

Antioxidant Activity Studies

The antioxidant studies of roots were carried out and the IC_{50} Values of each extracts was calculated and tabulated (table -3 and fig-5) and compared with standard.

Table-3 Antioxidant studies on various extracts on roots of *Chassalia curviflora*

S.No	Extract/ Standard	IC_{50} Values (mcg/ml)	
		Iron Chelating Assay	DPPH radical scavenging activity
1	Ascorbic acid	4	42
2	Pet. ether extract	433	185
3	Chloroform extract	34	101
4	Ethyl acetate extract	26	79
5	Alcoholic extract	139	133
6	Aqueous extract	202	157

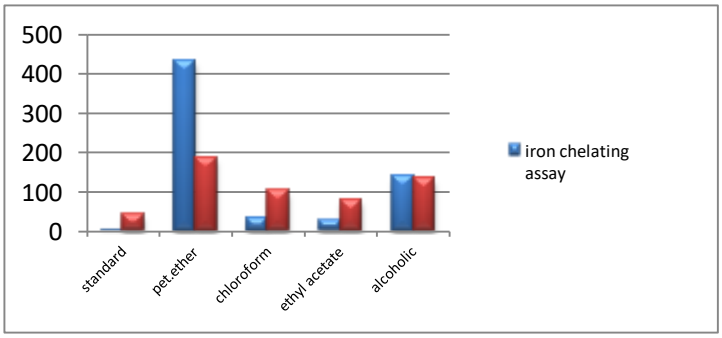


Fig-5 Graphical representation of antioxidant studies on various extracts on roots of *Chassalia curviflora*

Anti- Inflammatory Studies

The antioxidant studies of roots were carried out and the IC_{50} Values of each extracts was calculated and tabulated (table-4 and fig-6) and compared with standard.

Table-4 Antiinflammatory studies on various extracts on roots of *Chassalia curviflora*

S No	Extract/ Standard	IC_{50} Values (mcg/ml)	
		Protein Denaturation Method.	**Proteinase Inhibitory Action**
1	Prednisolone	93	80
2	Pet. ether extract	412	420
3	Chloroform extract	136	215
4	Ethyl acetate extract	103	119
5	Alcoholic extract	277	316
6	Aqueous extract	355	387

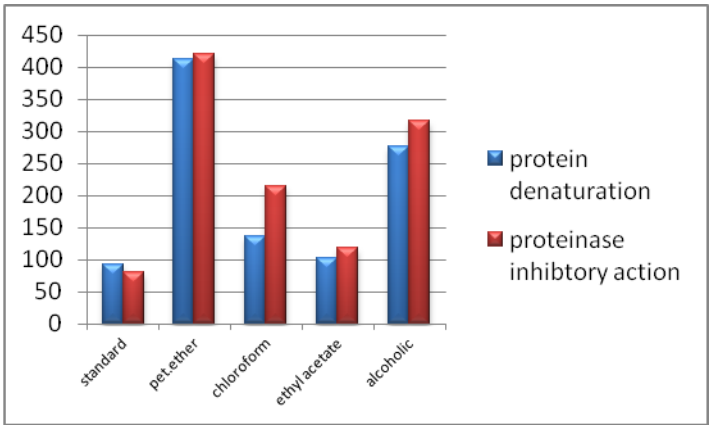


Fig-6 Graphical representation of antiinflammatory studies on various extracts on roots of *Chassalia curviflora*

CONCLUSION

The roots of the plant *Chassalia curviflora* (Rubiaceae) was found to be having antioxidant and anti inflammatory properties. The antioxidant activity studies using Iron chelating assay, DPPH radical scavenging assay and total antioxidant assay showed that ethyl acetate extract could be a promising source of natural antioxidants. The anti inflammatory activities by inhibition of protein denaturation and proteinase inhibitory assay showed ethyl acetate extract to possess potent anti inflammatory activity. The pharmacognostic studies done on the plant proved to be a tool for the authentication and identification of the plant. Thus the roots of the plant *Chassalia curviflora* can be suggested to be used in diseases like phlegm, rheumatism and pneumonia due to its antioxidant and anti inflammatory properties.

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