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PRELIMINARY PHYTOCHEMICAL AND *IN VITRO* ANTIMICROBIAL EVALUATION OF *KEDROSTIS ROSTRATA* PLANT EXTRACTS

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ABSTRACT

The present study was designed with the object of preliminary phytochemical evaluation and the study of antimicrobial activity of the extracts of the aerial parts of *Kedrostis rostrata* plant. In this study, the whole plant *Kedrostis rostrata* was collected authenticated, the aerial parts was separated and dried for powdering. The dried plant 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 subjected to 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, flavanones and flavonoids, carbohydrates, terpenoids, sterols and saponins. A significant presence of majority of phytochemicals were found in the methanol and ethyl acetate extracts 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 *E. coli*, *S. aureus*, *P. aeruginosa* and *Candida sp*.,. Outcome of this study is beneficial and further investigation in the future may give more significant results.

KEYWORDS: *Kedrostis rostrata*, preliminary phytochemical evaluation, antimicrobial evaluation.

INTRODUCTION

The use of plants as source of remedies for the treatment of diseases can be traced back to the prehistoric times.[1] India has one of the oldest, richest and most diverse cultural traditions associated with the use of medicinal plants.[2] Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases.[3]

Recently, traditional herbal medicines are getting significant attention in global health debates. Between 1983 and 1994, 78% of the new drugs approved by the FDA correspond to those derived from unmodified natural products or drugs semi-synthetically obtained from natural sources.[4] Many hope traditional herbal medicine research will play a critical role in global health. Industry has also invested millions of US dollars looking for promising medicinal herbs and novel chemical compounds.[5]

A wide range of medicinal plant parts like root, stem, flower, fruit, twigs exudates and modified plant organs has been used for extraction of raw drugs. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. These chemicals are termed as phyto-chemicals[1] that includes alkaloids, glycosides, saponins, resins, oleoresins, sesquiterpene, lactones and oils, both essential and fixed. Herbal medicines are safer than synthetic medicines because the phyto-chemicals in the plant extract target the biochemical pathway.

The high demand for drugs from plant sources therefore requires systematic evaluation of plants used in traditional medicine for various ailments. Hence, it is necessary to evaluate medicinal plants for promising biological activity.[6] With this view, the present study was aimed to evaluate the phytochemical nature and antimicrobial activity of the extracts of the plant *Kedrostis rostrata*, an attempt to provide a platform for further research.

MATERIALS AND METHODS

Plant collection and identification

The whole plant of *Kedrostis rostrata* including tubers was collected from the Kumarakovil, a hilly area nearer to Thuckalay, Kanyakumari District, Tamil Nadu, India. Identification and authentication of collected plant were done 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 procedure.[6] The aerial part of the collected plant was separated and dried in shade for about two weeks, powdered the dried material by using mechanical grinder and stored in the airtight container for further researches. Initially, the powdered material was extracted with the solvents of increasing polarity viz., petroleum ether, chloroform, ethyl acetate and methanol in the soxhlet apparatus assembly. For that, about 30g of dried coarse powder was weighed, moistened with the selected solvents, packed in the extracting apparatus and extracted with 500ml of each solvent individually. After each extraction, subsequent extraction was done by using the same dried marc. Each extract was filtered, distilled off the solvent to obtain the dried extract. The percentage yield of each dried extracts obtained was calculated.

Preliminary phytochemical screening

Preliminary phytochemical analysis of the prepared extracts was done in reference with the procedure.[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 procedure.[7,8] The test cultures were procured from MTCC, Chandigarh, India.

Evaluation of antibacterial activity
Antibacterial activity of the extracts of *K. rostrata* was tested against six pathogenic bacteria viz., *Staphylococcus aureus, Shigella boydii, Enterococcus faecalis, Enterobacter aerogens, Bacillus subtilis, E. coli, Klebsiella pneumoniae, Proteus vulgaris* and *Pseudomonas aeruginosa*. 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.

***In vitro* antifungal activity**

The antifungal activity of prepared extracts of *K. rostrata* were tested against three fungal pathogens viz., *Aspergillus sp., Candida sp., and Mucur* sp., The Sabouraud Dextrose Agar (SDA) was used as the media.. A swab of test culture was inoculated in the SDA 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 whole plant *Kedrostis rostrata* was collected and the aerials parts of the collected material was made into coarse powder after proper drying and extracted with solvents of increasing polarity viz., Petroleum ether, Chloroform, Ethyl acetate and Methanol. Regarding the percentage yield of dried extracts obtained, 3.6gm of dried extract was obtained from the petroleum ether extract. The chloroform extract gave 4.2gm of dried extract. In case of ethyl acetate and methanol extract, 5.6 and 8.3gm of dried extract was obtained respectively.

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

Table1: Preliminary phytochemical evaluation of the extracts of *Kedrostis rostrata.*

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**				
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 *K. rostrata* extracts, it was able to identify a significant presence of presence of alkaloids, terpenoids, flavonoids, sterols, saponins and phenolic compounds. In case of alkaloids a significant positive results was found in the ethyl acetate and methanol extracts which was in accordance with the results of previous literatures.[9,10] The anticancer property of alkaloids was documented in several reports.[11] In the present study a significant presence of terpenoids was found in the chloroform, ethyl acetate and methanol extracts. Similar results were found in the previous reports.[12] Anti-allergic, anti-inflammatroy, anti-microbial, anti-hyperglycemic, anti-spasmodic and immunomodulatory properties of terpenoids were documented in various studies.[13] The presence of flavones and flavonoids was identified in the tested extracts, particularly, in the chloroform, ethyl acetate and methanol extracts which was well-known for their anti-oxidant and anti-cancer activity and beneficial effects on heart disease. Anti-inflammatory, anti-diabetic and anti-microbial properties also reported in several studies.[11,14] The significant presence of sterols in the chloroform, ethyl acetate and methanol extracts was found in the results. Cardiotonic, anti-microbial and insecticidal properties of sterols was reported in the several previous studies. Saponins were found in the methanol extract of the present study. Anti-inflammatory property of saponins was documented in the previous studies.[15] A significant presence of phenolic compounds was found in the ethyl acetate and methanol extracts of the tested extracts. The antioxidant property of the phenolics was reported in several studies.[16] In this study, among the phytochemicals identified the majority were showed their presence in the ethyl acetate and methanol extracts which may be due to their high polarity.[17]

Evaluation of antibacterial activity of *K. rostrata* extracts revealed the activity in selected organisms in different extent (Table 2). From the results it was found that the methanol extract showed a significant activity against *E. coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. The result of antifungal evaluation is shown in Table 3. The results revealed the antifungal activity of tested extracts, particularly, the ethyl acetate and methanol extracts. From the results it was found that the methanol extract showed a significant activity against *Candida sp.* The presence of terpenoids, flavanones and flavonoids and sterols found in our preliminary phytochemical evaluationan may be responsible for the antimicrobial activity of the methanol extract of *K. rostrata*.

Table 2: Antibacterial activity of extracts of *Kedrostis rostrata.*

Extracts	**Bacterial culture**								
	1	2	3	4	5	6	7	8	9
	Zone of inhibition (Diameter/mm)								
Petroleum ether	Nil	Nil	09	Nil	11	14	Nil	Nil	12
Chloroform	10	Nil	12	Nil	10	15	Nil	Nil	12
Ethyl acetate	13	Nil	10	Nil	14	17	10	Nil	14
Methanol	17	15	14	Nil	16	19	13	10	17

1–Staphylococcus aureus; 2–Shigella boydii; 3–Enterococcus faecalis; 4–Enterobacter aerogens; 5–Bacillus subtilis; 6–E. coli; 7–Klebsiella pneumoniae; 8–Proteus mirabilis; 9–Pseudomonas aeruginosa

Table 3: Antifungal activity of extracts of *Kedrostis rostrata.*

Extracts	**Fungal culture**		
	1	2	3
	Zone of inhibition (Diameter/mm)		
Petroleum ether	Nil	12	Nil
Chloroform	Nil	15	12
Ethyl acetate	10	16	10
Methanol	14	19	14

1–Aspergillus sp., 2–Candida sp., 3–Mucur sp

CONCLUSION

The medicinal value of *Kedrostis* plants were reported in different studies. With this view, the plant *Kedrostis rostrata* was selected as the candidate for our research. In the present study, the extracts of aerial parts of the *Kedrostis rostrata* were subjected to preliminary phytochemical and antimicrobial evaluation successfully. Our further studies directed towards the evaluation of different pharmacological activities of these extracts and the studies on the tubers isolated from this plant may give more significant results.

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