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Bioactivity guided isolation of various extracts of *Coscinium fenestratum* for Antioxidant activity.

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Research Article

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ABSTRACT

The Plant *Coscinium fenestratum* belonging to the family *stercularaceae* contains alkaloids and flavonoids as the chief constituents. In The plant was collected from the region of Kerala and was authentified by Department of Botany in Kerala University. The 50g of crude drug was dried, pulverized and subjected for successive extraction using different solvents like petroleum ether, benzene, chloroform, ethanol, methanol and chloroform water. The extraction was carried out using soxhlet assembly for 6 – 8 hrs. The extract was concentrated and the colour of the extract was noted with the extractive yield. The various extracts were subjected for antioxidant activity using Reducing power method. In this method the increase in the absorbance signifies the antioxidant activity. The ethanol and the methanol extracts showed maximum absorbance 0.916 to 1.113 absorbance at 700nm. Hence these two extracts were selected for the isolation of compounds responsible for the activity. By using ethanol and methanol the extraction was carried out to extract the constituents present in it. The ethanolic extract was packed in column and the compounds were eluted with hexane acetone and glacial acetic acid in the ratio 75:2.0:0.5. The different fractions were collected and subjected for TLC studies. The TLC studies were carried out to find out the new compounds present in the extract. The fractions were spotted on plate with the standard compound known as berberine. The 14th fraction showed two other compounds which are not according to the berberine. Those two compounds were scraped removed and dissolved in solvents separately. Again these compounds were subjected for spectral studies and anti oxidant activity. The reducing power method was used to screen the antioxidant activity. The two compounds were named as CF_1 and CF_2. CF_2 showed more antioxidant activity than the CF_1.

INTRODUCTION

The Plant *Coscinium fenestratum* belonging to the family *stercularaceae* contains alkaloids and flavonoids as the chief constituents. In The plant was collected from the region of Kerala and was authentified by Department of Botany in Kerala University. *Coscinium fenestratum* which was commonly known as *Daruharidhra* or Arishina balli is a Critically endangered species. The berberine was found to be major a compound. The plant posses antidiabetic, anti-inflammatory, antihypertensive and hepatoprotective activities [1].

METHODOLOGY

The 50g of crude drug was dried, pulverized and subjected for successive extraction using different solvents like petroleum ether, benzene, chloroform, ethanol, methanol and chloroform water. The extraction was carried out using soxhlet assembly for 6 – 8 hrs. The extract was concentrated and the colour of the extract was

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noted with the extractive yield. The Preliminary Phytochemical Screening was carried out to know the different phytoconstituents reported in the drug. The TLC identity test was carried out for all the extracts using different mobile phases [2,17].

Column chromatography

The column was packed using silica gel and the sample was loaded in the column. The solvent system selected was hexane : acetone in the ratio 5:5. The first fraction was collected and subjected for TLC studies. After development the two prominent blue spots were observed under UV light [3].

Antioxidant activity of *Coscinium fenestratum* [4,15]

Reducing Power method

This method is based on the principle of increase in the absorbance of the reaction mixture. Increase in the absorbance indicates increase in the antioxidant activity [5].

Reagents

Phosphate buffer 0.2 M
1% potassium ferricyanide
10% trichloro acetic acid
0.1 % ferric chloride

Procedure

1ml of stock solution of petroleum ether, benzene, chloroform, ethanol and methanol extracts were taken in separate test tubes in the solutions in all test tubes were made upto 1ml in methanol. To these, 2.5 ml of phosphate buffer (0.2 M, PH 6.6) and 2.5 ml of 1% potassium ferricyanide were added. The mixtures were incubated for 20 min at 50^{0}C. At the end of the incubation, 2.5 ml of 10 % trichloroacetic acid was added to the mixtures followed by centrifugation at 5000 rpm for 10 min. 2.5 ml of upper layer was transferred to test tubes containing 2.5 ml of distilled water and 0.5 ml of 0.1 % ferric chlorideThe absorbance was measured at 700 nm. Increase in absorbance of the reaction mixture indicated the reducing power of the samples [6].

RESULTS AND DISCUSSION

The various extracts were used to evaluate the antioxidant activity. The extracts showing the maximum activity were selected for isolation of phytoconstituents. The ethanol and the methanol extracts showed the maximum activity and hence these two extracts were selected for isolation of phytocostituents.

Evaluation of Anti oxidant activity [7,13]

Sl No.	Extracts	Concentration	Absorbance
1	Petroleum ether	100mg	0.371
2	Benzene	100mg	0.469
3	Chloroform	100mg	0.770
4	Ethanol	100mg	0.916
5	Methanol	100mg	1.113

Blank was used the set the wavelength between 600 – 800 nm. The Ethanol and the methanol extract showed the moderated to maximum antioxidant activity when compared to other extracts. The ethanol and methanol extract was considered for the further studies to find the different compounds responsible for anti oxidant activity. By using ethanol and methanol the extraction was carried out to extract the constituents present in it. The ethanolic extract was packed in column and the compounds were eluted with hexane acetone and glacial acetic acid in the ratio 75:2.0:0.5. The different fractions were collected and subjected for TLC studies [8].

The TLC studies were carried out to find out the new compounds present in the extract. The fractions were spotted on plate with the standard compound known as berberine. The 14th fraction showed two other compounds which are not according to the berberine. Those two compounds were scraped removed and dissolved in solvents

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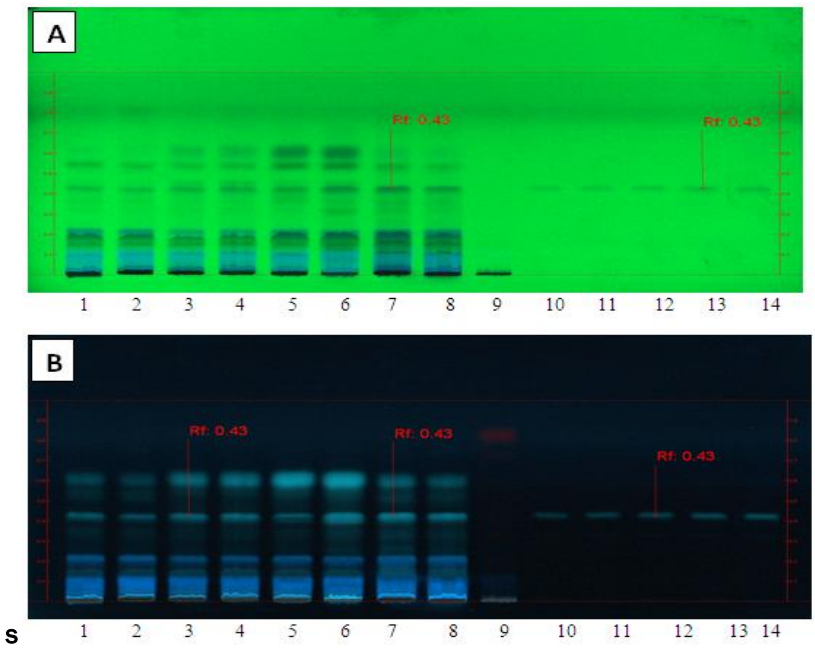
separately. Again these compounds will be subjected for spectral studies and anti oxidant activity. The reducing power method was used to screen the antioxidant activity [9,11]

Sl No.	Extract	Mobile Phase	Spot	Rf Value
1	Petroleum ether	Chloroform: Methanol: glacial acetic acid. (7.5:2.5:0.5)	Blue under UV	0.875
2	Benzene	----------	Yellow	0.95
3	Chloroform	----------	yellow	0.85
4	Ethanol	----------	Two bright yellow	0.90 0.825
5	Methanol	----------	One brown and one yellow	0.625 0.50
6	Petroleum ether, Benzene and Chloroform	Acetone :hexane (9:1)	3 spots2 spots1 spot	0.5, .87,0.9 0.5,0.1,0.57



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CONCLUSION

- The ethanolic and Methanolic extract showed moderate to maximum antioxidant activity.
- Hence these tow extracts were selected for the isolation of phytoconstituents using column chromatography, TLC, HPLC and HPTLC.
- The two new compounds apart from berberine were isolated and named as CF1 and CF2.
- These two compounds were again subjected for antioxidant activity in which CF2 showed more activity than CF1 [10].

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