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Simple Isolation and characterization of P-coumaric acid from *Cynodon dactylon* Linn. (Pers)

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ABSTRACT

P-coumaric acid is a non-flavonoid phenolic acid and is a major constituent of the species *Cynodon dactylon* Linn. (Pers.). In this study isolation of p-coumaric acid was achieved by preparative TLC and the compound thus isolated was characterised by Ultraviolet, Mass and H^1 NMR spectral analysis. Mass spectral data shows molecular ion peak of m/z=164.2. A simple method of isolation was reported in this study for identification of P-coumaric acid as a potent antioxidant and anticancer agent prevailed in different commonly available plant species.

INTRODUCTION

This study was aimed at development of suitable extraction and isolation procedure which aid to get the purified material and to develop an UV method for estimation of p-coumaric acid in methanolic extract of druva grass. Further the study also deals with characterisation of p-coumaric acid in the sample extract by Mass and H^1 NMR spectral analysis for structure identification [1, 2].

Objective

P-coumaric acid is a non-flavonoid phenolic acid and is a major constituent of the species *Cynodon dactylon* Linn. (Pers.). In this study isolation of p-coumaric acid was achieved by preparative TLC and the compound thus isolated was characterised by Ultraviolet, Mass and H^1 NMR spectral analysis. Mass spectral data shows molecular ion peak of m/z=164.2. A simple method of isolation was reported in this study for identification of P-coumaric acid as a potent antioxidant and anticancer agent prevailed in different commonly available plant species.

Experimental methods

The UV spectrum of the purified compound was recorded from 190 to 600 nm on a *ELICO* double beam spectrophotometer UV-visible spectrophotometer. ESI mass spectra was acquired from isolated compound and characterised. Proton nuclear magnetic resonance spectra were acquired using 400MH_Z NMR spectrometer employing TMS

as an internal standard and deutrated methanol was used as solvent.

Physicochemical constants

Determination of Physicochemical constants is performed as per the standard protocol followed in the Ayurvedic Pharmacopoeia. The values are tabulated in Table 3 and 4.

Table 1: Extractive values

Extractive value	Values in % w/w
Alcohol soluble extraction	0.07 %
Water soluble extraction	0.14%

Table-2Ash values

S.No	Constituents	Values
1	Total ash	2.78%w/w
2	Acid insoluble ash	0.05%w/w
3	Water soluble ash	0.08%w/w
4	Sulphated ash	0.08%w/w
5	Moisture content	1%w/w
6	Foreign organic mater	0.06gm

PRELIMINARY PHYTOCHEMICAL SCREENING

Table-3

Name of the phyto constituents	Pet. ether	n-hexane	Ethanol
Carbohydrates	+	+	+
Gums/Mucilage	_	_	+
Proteins/Amino acids	_	_	+ 7.1.3.
Fats/Oils	_	_	_
Steroids	+	+	_
Glycosides	_	_	_
Alkaloids	_	_	_
Flavonoids	_	_	+
Phenol/Tannins	_	_	+
Oxalic acid	_	_	_
Malic acid	_	_	_
Saponins	+	_	_
Vit-A / Vit- C	+	_	_
Coumarin derivatives	_	_	_

Complete extraction of p-coumaric acid was achieved by successive solvent extraction with ethanol. Preliminary phytochemical study reveals that the extract may contain phenolic compounds which may be non-flavanoid in nature. Several mobile phase combinations were tried and Chloroform: Methanol: Formic acid (85:10:5v/v) was found optimum for separation of p-coumaric acid from ethanolic extract of druva grass. The R_f values of standard and sample compound matches each other and the R_f value was found as **0.52**. Isolation of p-coumaric acid from the extract was achieved by preparative thin layer chromatography using the same chromatographic conditions followed for identification of active constituent. TLC profile of compound was represented.

Characterisation of isolated compound was done by studying ultraviolet, mass and H^1NMR spectra. P-coumaric acid shows UV absorption at about at 345nm in ethanol indicates the presence of conjugation and hydroxyl auxochrome which shifts the absorption maximum towards visible side of the spectrum and it was represented and Mass spectral data shows molecular ion peak m/z=164.2 which has a moderate abundance …….. H^1 NMR spectra show different kinds of protons in the compound which was seen in the spectra and its assignment corresponds each type of hydrogen resembles the complete structure of p-coumaric acid.

Fig-1 Ethanolic extract Druva grass

Comparitive TLC plate of Standard and sample

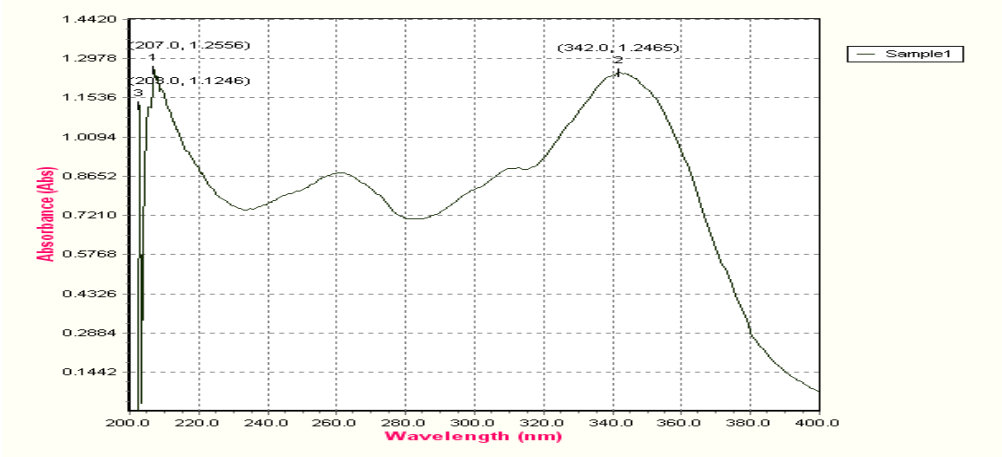

Fig-2 UVabsorption spectrum of p-coumaric acid in methanol.

(Isolated from ethanolic extract by preparative TLC)

Respective data of mass spectroscopy

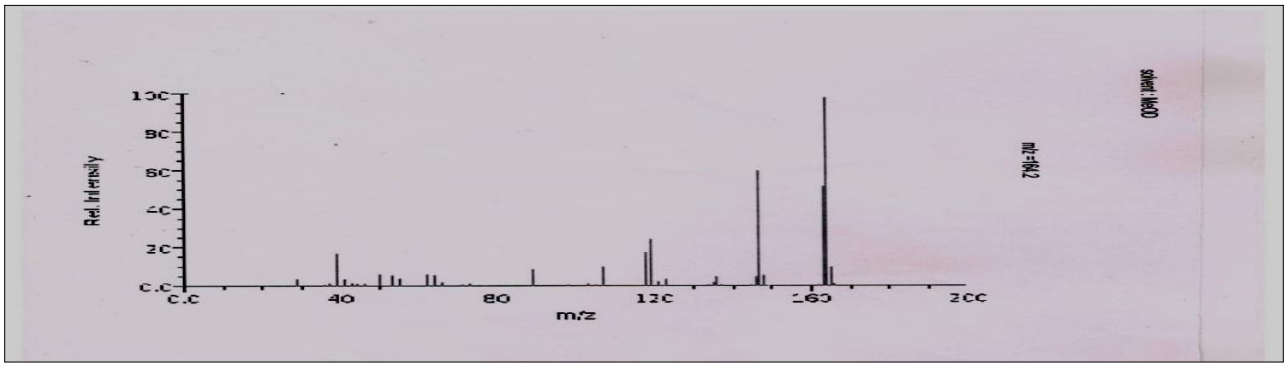

Fig-3 Mass Spectra of P-Coumaric acid

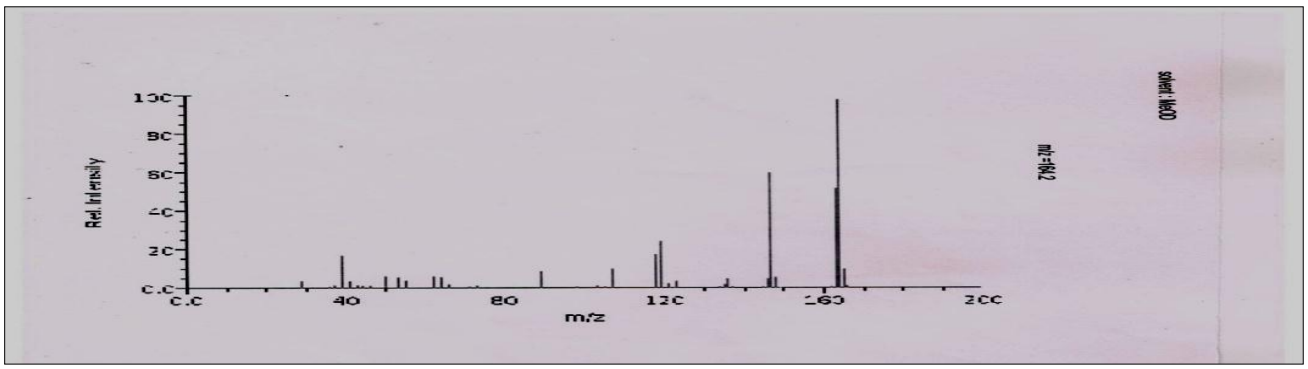

Fig-4 Mass Spectra of Ethanolic extract of durva grass

Respective data for NMR Spectroscopy

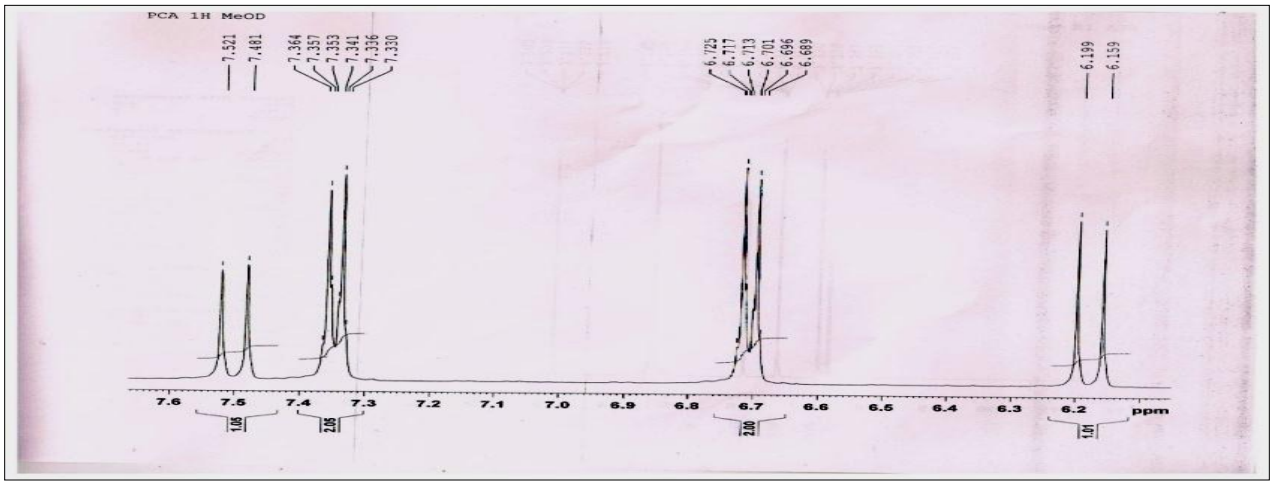

Figure No. 20: NMR S pectra of P-Coumaric acid

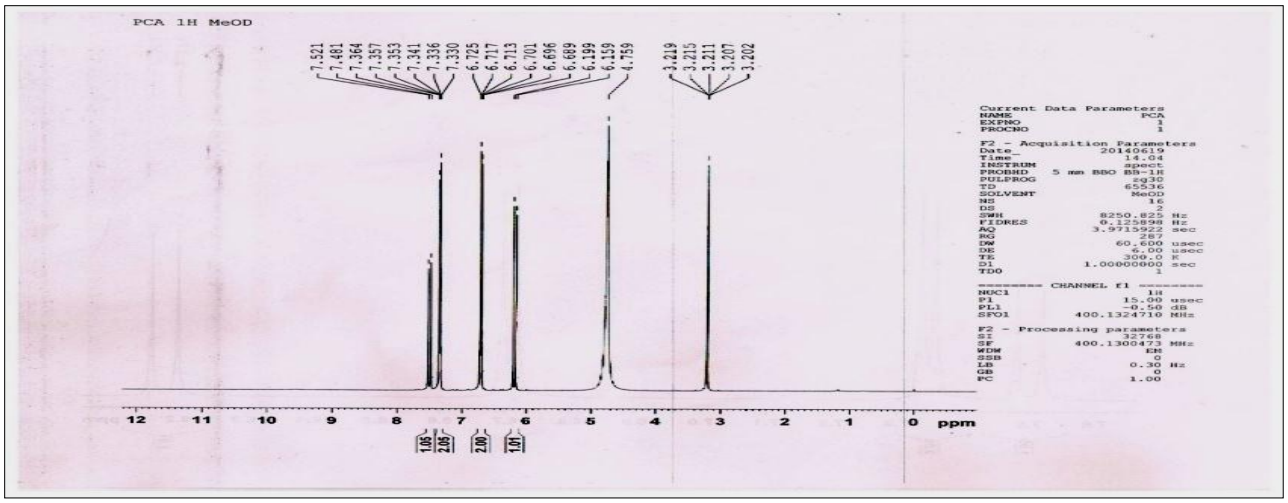

Fig-4: NMR Spectra of P-Coumaric Acid

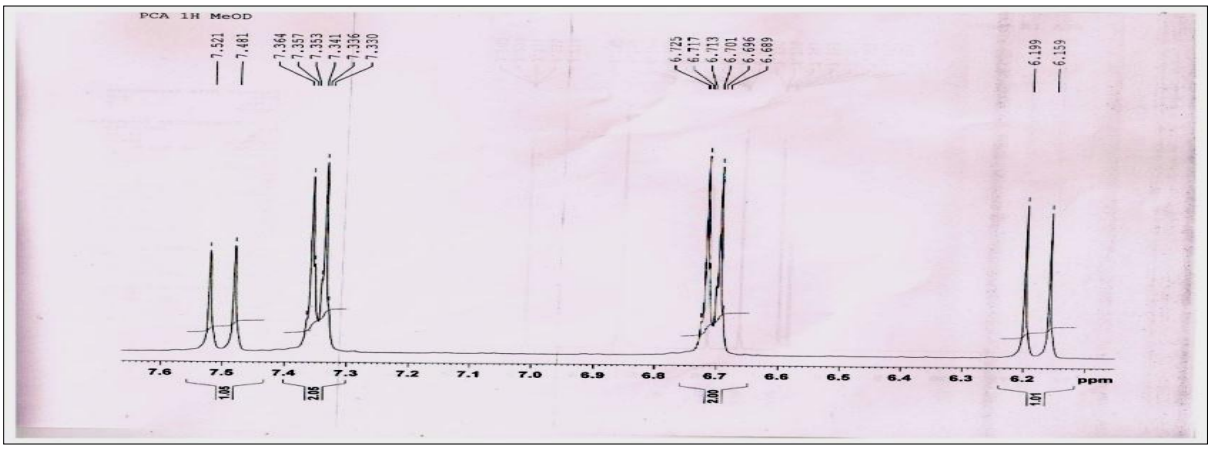

Fig-5: NMR Spectra of methanoloic extract of Durva grass

Table-4 Chemical shifts of *p*-coumaric acid in H^1 NMR spectra, δ (ppm)

Protons	Chemical shift (δ_{ppm}) Experimental (Isolated compound)
1	--
2	7.341
3	6.689
4	3.219
5	6.725
6	7.364
7	6.199
8	7.521

PHARMACOLOGICAL EVALUATION *IN VITRO*

Table 5: Antioxidant activity of ethanolic extract of cynodon dactylon in p-Nitroso Dimethyl Aniline method

S. No.	Name of the Drug	Concentration (µg/ml)	% Radical scavenging (mean ± S.E.M)
1.	Ethanolic extract	10	70.29 ± 0.0115
		20	71.16 ± 0.0145
		40	77.95 ± 0.0115
		80	78.57 ± 0.0145
2.	Ascorbic acid	10	74.30 ± 0.0145
		20	76.40 ± 0.0145
		40	78.20 ± 0.0115
		80	79.01 ± 0.0115

CONCLUSION

Isolation, identification and characterisation of p-coumaric acid was achieved successfully which will be helpful for the standardisation of herbal formulations containing this active constituent.

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