Anti – Cancer Activity, Isolation and Characterization of Alkaloid from the Ethanolic Extract of the Medicinal Plant *Pavetta indica* Linn.

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ABSTRACT

In this study, the ethanolic extract of plant *Pavetta indica* Linn. showed a significant anti - cancer activity on Tryphan blue dye exclusion assay. The phytochemical analysis reveals that to isolate alkaloid type of compound from the plant by using chloroform, acetone and toluene buffer mixture. Characterization of the compound by using chemical and spectroscopic methods.

Key words: *Pavetta indica* Linn, ethanolic extract, isolation, anti - cancer activity.

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INTRODUCTION

Cancer cells usually invade and destroy normal cells. These cells are produced due to imbalance in the body while correcting this imbalance, the cancer may get treated. Ayurveda is the traditional Indian medical practices that use plant drugs successfully in use of natural drugs results in preventing or suppressing various types of tumours and cancers [1]. The synthetic anticancer remedies are found to be high in cost due to the expensive synthetic methodology to use herbal medicines (in the prevention and treatment of many cancerous diseases) they are comparatively economical [2]. *Pavetta indica* Linn.[3,4] (Tamil: Kattu thirani , Panna pavadai , Sirukonnai , Pavattai) is a shrub or small tree belongs to the family of Rubiaceace. It comprises about 350 species of trees, evergreen shrubs and sub-shrubs. It is found in woodlands, grasslands and thickets in sub-tropical and tropical Africa and Asian Countries [5]. The leaves very variable elliptic – oblong to elliptic – lanceolate and obovate – oblong, glossy – green flowers are white. The roots are said to possess purgative, aperient, diuretic and tonic properties and are prescribed in visceral

obstructions, jaundice, headache, urinary diseases and dropsical affections. The phytochemical investigation[6], chemical composition of essential oils[7] and physio – phytochemical screening[8] had been reported in this plant. The plant was studied anti – inflammatory potential[9], analgestic[10], antimicrobial[11], antipyretic activities[12], anti-oxidant[13], anti- diabetic[14], hepato protective[15], anthelmetic[16] and wound healing activities[17]. Linoleic acid, (9z,12z,15z)-octadeca-9,12,15-trienoic acid, proanthocyanadin, epicatachein and fercilic acid[18]. The compounds Chlorogenic acid, Ferlic acid, Salicine and Oleic acid[19] were isolated and characterised by chemical studies from this plant. The present study aims to isolate and characterise the alkaloid type of compound from the ethanolic extract of the plant *Pavetta indica* Linn. It also focuses on the anti - cancer activity of the crude extract.

MATERIALS AND METHODS

The leaves of *Pavetta indica* Linn. were collected from Narthamalai region (Near Pudukkottai District) from the month of July at 11.00 am. They were identified and authenticated by Dr. S. Soosairaj (SJCBOT 2474), Assistant Professor, Department of Botany and with Rapinet Herbarium, St.Joseph college (Autonomous)Tiruchirappalli -620002, Tamilnadu , India.

Sample preparation

The leaves of *Pavetta indica* Linn. were shade dried and powdered well. About 20g of the plant leaves were soaked in 100 mL of ethanol. It was left for 24 hours in order to extract the phytoconstituents- alkaloids, carbohydrate, tannins, steroidal glycosides, steroids, flavanoids, acids and others. The extract was filtered using Whatmann No.1filter paper to remove the residues.

In *Vitro* Anti – cancer activity [20-24]

Trypan blue exclusion method

Trypan blue is an azo dye that is used as a dye-stuff in anti-cancer activity studies. It is used as a vital stain to selectively colour of dead tissues which get stained to become blue cells. Live cells or tissues (having intact cell membranes) are not coloured

Trypan blue dye assay method was carried out to evaluate the in vitro cytotoxicity potentials of the ethanolic extract of the plant *Pavetta indica* Linn. Using the ethanolic extract of the plant, different concentrations - 10, 20, 50, 100 and 200µg/ml with distilled water were prepared. In a test tube, 100µl of plant extract was mixed with 800µl of phosphate buffer saline and 100µl ($1X10^6$ in 1ml) of Dalton's Ascitic Lymphoma (DAL) was added. Each concentration of the extract was tested in triplicate. All the samples were incubated at 37°C in an incubator for 30 mins. About 100µl of trypan blue dye (0.4%) was added to each of five different test tubes (which contained extracts in five different concentrations) and the

number of blue-coloured dead cells and the colourless live cells were counted in a haemocytometer under the microscope. Percentage of cytotoxicity was calculated by the following formula.

$$\text{Cytotoxicity (\% of dead cells)} = \frac{No.of\,Deadcells}{No.of\,Livecells + No.of\,Deadcells} \times 100$$

Dalton's Lymphoma Ascites (DLA) was maintained in Amala Cancer Research Center, Thrissur, Kerala, India. The cells were maintained *in vivo* in Swiss albino mice by intraperitoneal administration.

Isolation and Characterization

The above ethanoic extract is concentrated further by distillation. The chlorophyll present in the concentrated extracts was removed by treating with 4N dil. H_2SO_4 at 60 ºC on a water bath for 30-45 min and filtering it. The ethanoic extract was treated with 4N HCl. The homogeneous solution was further extracted with ether, labelled as ether layer - I. The resulting aqueous layer neutralized with 10% NaOH. Then it is again extracted with ether to get ether layer – II. The micro TLC is done using the plate (7.5 cm × 2.5 cm) coated with 100 micron silica gel (0.2 g/plate) as stationary phase and using suitable eluants. The compounds separated are noted down. The details of the micro TLC are given in the following Table No-1.

Table No-1 Details of the Thin Layer Chromatography

Extracts	Eluents	No.of compounds separated
Ether Layer – I (Concentrated)	Chloroform: Acetone : Toluene 6:4:10	2 (PIC_3, PIC_4)
Ether Layer – II (Concentrated)	Chloroform: Acetone : Toluene 4:8:8	2 (PIC_1, PIC_2)

The preparative TLC [25, 26] carried out using the plate (20 cm × 20 cm) coated with 100 micron silica gel (5 g/plate) and suitable eluant as given in the Table-1. The components separated as bands are isolated by extraction using acetone from the silica gel. The isolated components are purified by recrystallization using ethanol. Of the several components, the PIC_2 is taken for characterization as it is in large quantity (500 mg). The solubility of the compound (m.p. 188.6 ± 3ºC) was tested positively in solvents- in chloroform, acetone and ethanol. It decolorized bromine in alcohol indicating the presence of unsaturation. It showed a positive response with hydroxamic acid, by giving red coloration indicated presence of amide functional group and also it shows

positive response with chromic anhydride signifying the secondary alcohol functionality. It burns with a long sooty flame indicating the aromatic nature.

The molecular mass of the substance (PIC_2) was calculated to be 246.26 by the cryoscopic method using camphor solvent[27]. The UV-VIS spectrum was taken on the spectrophotometer, Lamda 35 model using spectroscopic grade ethanol. The FT-IR spectrum was recorded using the instrument Perkin-Elmer RXi spectrometer by KBr pellet method. The proton NMR and 13C NMR spectrum of the compound were taken on the 300 MHz Bruker model spectrometer using $CDCl_3$ solvent and TMS standard. The GC-MASS spectral study of the compound was done using spectrometer Schimadz U Japan. The data are shown in the Table No-2.

Table No – 2 The Spectral data of the compound

Spectroscopy	Experimental Data of the compound
UV-VIS Spectroscopy (λmax, nm)	198 (ε 10400), 217 (ε 48000) 249 (ε 16000) 230 (ε 630), 312 (ε 3500)
IR spectroscopy (νmax, cm-1)	3628,3598,3195,1220,1078,3443,3362,2958,2880,2846,1445,1441, 1386,1363,1264,1248,1039,1673,1539,3038,3023,3006,1506,1585, 1503,786,746,693
1H NMR spectroscopy (δppm)	1.904s,2.106s,3.902s,4.439s,6.611d(J8.8Hz),6.623d(J8.8Hz), 6.729d(J9.4Hz),6,889s,7,231d(J9.4Hz),8.132s
13C NMR (δppm)	26.79, 55.87, 120.82, 129.08, 138.57, 148.19, 151.37, 130.21, 131.02, 132.36, 158.69.
Mass spectroscopy (m/z values)	15.03,30.03,31.06,59.07,62.06,71.08,87.08,128.15,129.16,158.18,1 59.18,187.19,215.21,216.24,229.26,231.23,256.30,316.36,458.52,4 62.46

RESULTS AND DISCUSSION

In *Vitro* Anti-cancer activity

Table No - 3 Results of anti - cancer activity of ethanolic extract of Pavetta indica Linn.

Drug Concentrations (µg/ml)	Percentage of Death cell (DLA) (%)
	Ethanolic extract of the medicinal plant *Pavetta indica Linn.*
10	0
20	2
50	7
100	14
200	30

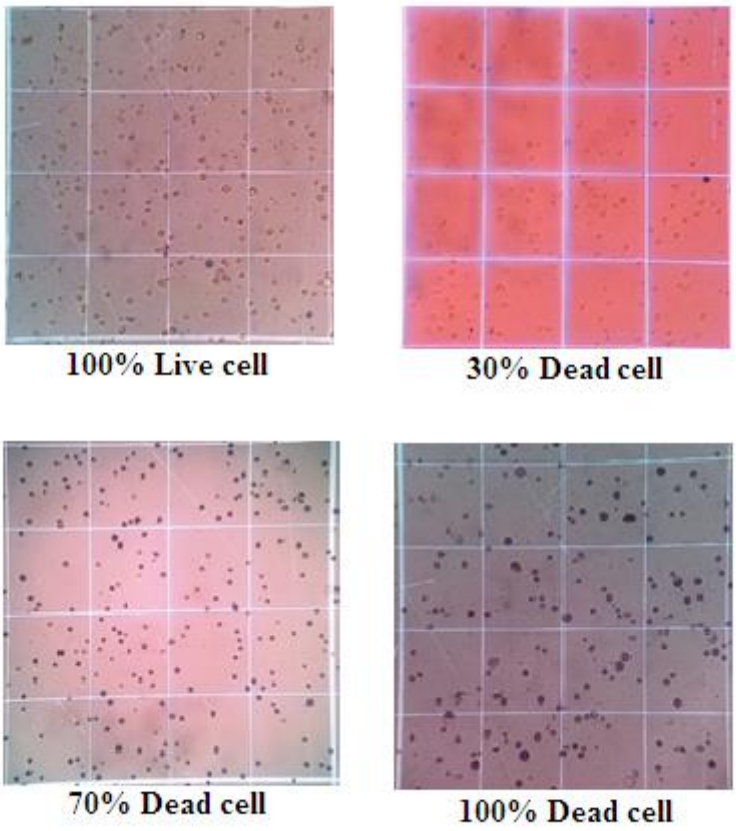


Figure 1. Percentage of dead cell in anticancer activity

The results given above the table showed the anti - cancer activity for the various concentrations (10, 20, 50, 100, 200 µg/mL) of ethanolic extracts of the medicinal plant *Pavetta indica*.Linn. It is observed that the plant extract of concentration at 200 µg/mL has a higher inhibition activity against cancer cells.

Isolation and Characterization[28, 29]

The isolated compound was purified and recrystallized out using ethanol. It was a pale yellow solid (m.p. 260-262 ºC and molecular mass 188.6 ± 3ºC). It was soluble in polar solvents like chloroform, acetone and ethanol, *etc*. By the tests with bromine in alcohol, indicating the presence of unsaturation. It showed a positive response with hydroxamic acid, by giving red coloration indicated presence of amide functional group and also it shows positive response with chromic anhydride signifying the secondary alcohol functionality.

Table No – 4 Results of UV- VIS Spectroscopy

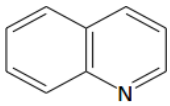
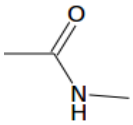
Chromophore	Electronic transition	Band at wavelength (nm)
	π- π^*	E-band 198 (ε 10400), 217 (ε 48000) Indicating the presence of C=C of double bond in conjucation.
	π- π^*	K-band 249 (ε 16000) and R-band 230 (ε 630) Indicating the presence of non – bonding electron in corbon atom.
		312 (ε 3500) Due to the presence of carbonyl group (double bond)

Table No –5 Results of Infra - Red Spectroscopy

Band at frequency (cm^{-1})	Vibration	Type of Bond
medium 3628, 3598 strong, broad 3195 medium 1220, 1078	stretching stretching bending	O-H (free) hydroxyl O-H (hydrogen bonded, intramolecular) O-H and C-O
strong, broad 3443, 3362	stretching	N-H
medium 2958, 2880, 2846 medium 1445, 1441, 1386, 1363 strong 1264 strong 1248, 1039	stretching bending stretching stretching	C – H of -CH_3 C – H of –CH_3 C-O-C C-O-C
strong 1673, 1539	stretching	>C=O of amide
weak 3038, 3023, 3006 medium 1506, 1585, 1503 medium 786, 746 strong 693	stretching stretching bending bending	-C=C-H of aromatic ring -C=C- of aromatic ring -C-H of aromatic ring -C=C- of aromatic ring

Table No – 6 Results of Proton NMR Spectroscopy

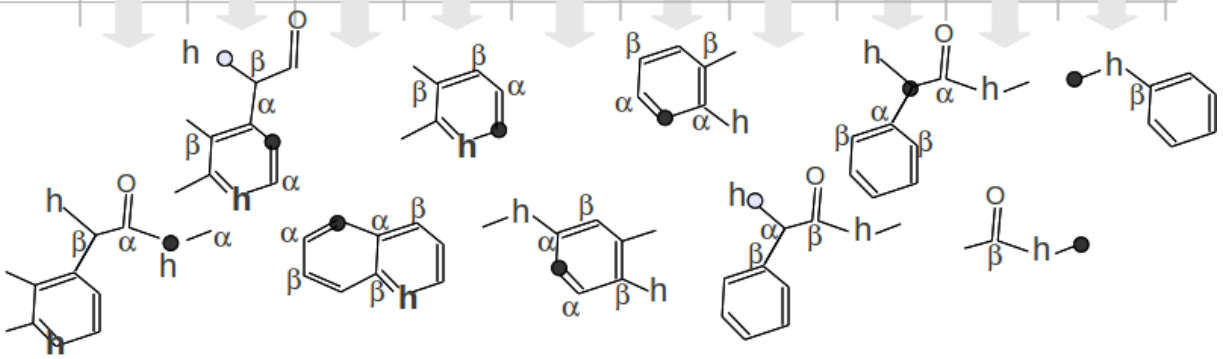
Chemical shift Signal pattern (Number of protons)	Environment of the Protons	Type of protons
1.904ppm singlet (3H)	Silghtly deshielded due to the bonding with the electronegative oxygen no neighbouring proton	C**H**$_3$-O
2.106ppm singlet (3H)	Significantly deshielded by the bonding with the electronegative nitrogen no neighbouring proton	C**H**$_3$-N
3.902ppm singlet (1H)	Observably deshielded by the attachment of the electronegative oxygen atom to the methynic carbon no neighbouring proton	-C**H**-O
4.439ppm singlet (1H)	Markedly deshielded by the attachment of the electronegative oxygen atom no neighbouring proton	-O**H**
6.611ppm doublet (1H) *J 8.8Hz*	Highly deshielded due to the pi-electron environment of the aromatic system one neighbouring proton	-[C**H**=CH]$_{ring}$-
6.623ppm doublet (1H) *J 8.8Hz*	Highly deshielded due to the pi-electron environment of the aromatic system one neighbouring proton	-[C**H**=CH]$_{ring}$-
6.729ppm doublet (1H) *J 9.4Hz*	Less Strongly deshielded due to the pi-electron environment of the aromatic system one neighbouring proton	-[C**H**=CH]$_{ring}$-
6.889ppm singlet (1H)	Strongly deshielded due to the pi-electron environment of the aromatic system and the presence of electronegative oxygen in a close proximity no neighbouring proton	-[C**H**=C-]$_{ring}$- **O**
7.231ppm doublet (1H) *J 9.4Hz*	Very strongly deshielded by the delocalised pi-bonding of the aromatic ring and the presence of electronegative nitrogen one neighbouring proton	-[N=**H**C=CH]$_{ring}$-
8.132ppm singlet (1H)	Very significantly deshielded by the bonding with the electronegative nitrogen atom and the presence of electron-withdrawing carbonyl group one neighbouring proton	–(C=O)–**NH**–

Table No – 7 Results of Carbon - 13 NMR Spectroscopy

Chemical shift ppm	Environment of the Carbons	Type of Carbon
26.79	Silghtly deshielded by the bonding with the electronegative nitrogen *N-Methyl part*	**CH_3**-N
55.87	Markedly deshielded by the bonding with the electronegative oxygen *Methoxy part*	**CH_3**-O
120.82, 129.08, 138.57, 148.19, 151.37, 130.21, 131.02, 132.36, 158.69,	Highly deshielded by the pi-bonded electrons Two of them are very highly deshielded due to bonding with electronegative nitrogen One of them is acutely deshielded by the bonding with electronegative oxygen atom *aromatic part of two fused rings having nitrogen atom at the nucleus of one ring* nine carbon atoms	**-[CH=CH]$_{ring}$-[CH=N-CH]$_{ring}$-**

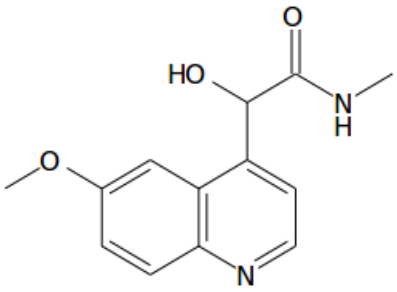
Table No - 8 The HMBC Spectral data of the compound

	7.132d 1H	7.231d 1H	6.889s 1H	6.729d 1H	6.623d 1H	6.611d 1H	4.439s 1H	4.011s 1H	2.106s 3H	1.904s 3H
26.79	α								bond	
55.87		β								bond
113.81	β	β					α	bond		
120.82		bond		α				β		
129.08		β	α			β		β		
130.21					α	bond				
131.02			bond		β	β				
132.36			β		bond	α				
138.57		α	β	β			β	α		
148.19			β	β	β	α				
151.37		α		bond						
158.69					α					β
168.63	α						β	α	β	



CONCLUSION

The Anti-cancer activity of ethanolic extract of the plant *Pavetta indica* Linn.showed the higher concentration (200 µg/mL) had a higher inhibition activity against cancer cells. The characterization study on the phytoconstituent PIC_2 involving UV, IR, H – NMR, C13 - NMR and Mass spectral studies revealed the presence of alkaloid type of compound. The final aspect of the structural characteristics of PIC2 was understood from the HMBC Correlation studies. Thus, the structure of the compound is deciphered to be a quinolone type of compound and the tentative structure is proposed, as shown here.



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