Anti-cancer Activity, Isolation and Characterization of Terpenoid from the Ethanolic Extract of the Medicinal Plant *Pavetta indica* Linn.

S. Suresh
Department of Chemistry
Vetri Vinayaha College of Engineering & Technology, Trichy
G. Pradheesh
Department of Chemistry, SNS College of Technology, Coimbatore
&
V. Alex Ramani
Department of Chemistry, St. Joseph's College, Trichy

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 Terpenoid type of compound from the plant by using benzene, acetone and toluene buffer mixture. Characterization of the compound by using chemical and spectroscopic methods.

Keywords: *Pavetta indica* Linn, ethanolic extract, isolation, anti-cancer activity

1. 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.

2. 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 a.m. They were identified and authenticated by Dr. S. Soosairaj (SJCBOT 2474), Assistant Professor, Department of Botany and with Rapinet Herbarium, St. Joseph's College, Tiruchirappalli, Tamil Nadu, India.

2.1 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.

2.2 *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 200 µg/ml with distilled water were prepared. In a test tube, 100 µl of plant extract was mixed with 800 µl of phosphate buffer saline and 100 µl (1×106 in 1 ml) 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:

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

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.

2.3 Isolation and Characterization

The above ethanoic extract is concentrated further by distillation. The chlorophyll present in the concentrated extracts was removed by treating with 4 N 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-1.

Table-1: Details of the micro Thin Layer Chromatography

Extracts	Eluents	No. of compounds separated
Ether Layer-I	Benzene : Acetone (4:1)	2 (PIC_3, PIC_4)
Ether Layer-II	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 Chloroform from the silica gel. The isolated components are purified by recrystallization using ethanol. Of the several components, the PIC_3 is taken for characterization as it is in large quantity (500 mg). The solubility of the compound (m.p. **** ºC) was tested positively in solvents- in chloroform, benzene and acetone. It decolorized bromine in alcohol indicating the presence of unsaturation. 0.5 ml of extract was dissolved in 1ml of chloroform. The mixture was treated with Conc. H_2SO_4. It gave red coloration and also it shows positive response with chromic anhydride signifying the alcohol functionality. It burns does not burns with a long sooty flame indicating the aliphatic nature.

The molecular mass of the substance (PIC_3) was calculated to be 288.47 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-2.

Table-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)	744, 906, 911, 929, 1018, 1198, 1371, 1394, 1448, 1375, 1381, 1448, 1453, 1469, 1649, 1656, 2846, 2853, 2863, 2872, 2915, 2924, 2941, 2958, 3614
1H NMR spectroscopy (δppm)	0.961s(3H)J 5Hz, 1.008s(3H), 1.217d(2H)J11.1, 7.1Hz, 1.398d(2H)J9.1, 6.2, 5.4Hz, 1.498d(2H)J11.1, 7.1Hz, 1.693t, 5.863d(1H)J 8.3, 2.6 Hz, 5.991s(1H), 3.206d(1H)J11.1, 7.3Hz, 4.381s(1H)
13C NMR (δppm)	17.72, 18.79, 19.24, 28.97, 37.61, 43.18, 44.01, 46.01, 47.81, 62.23, 63.93, 75.79, 79.25, 109.18, 118.72, 120.33, 147.59, 148.18,
Mass spectroscopy (m/z values)	15.03, 17.01, 28.05, 30.07, 42.08, 42.08, 43.09, 68.07, 70.09, 104.15, 110.15, 133.21, 140.22, 142.19, 187.30, 245.38, 246.39, 258.40, 271.46, 273.43, 288.47, 374.60, 546.86.

3. Results and Discussion

3.1 In *Vitro* Anti-cancer activity

Table-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

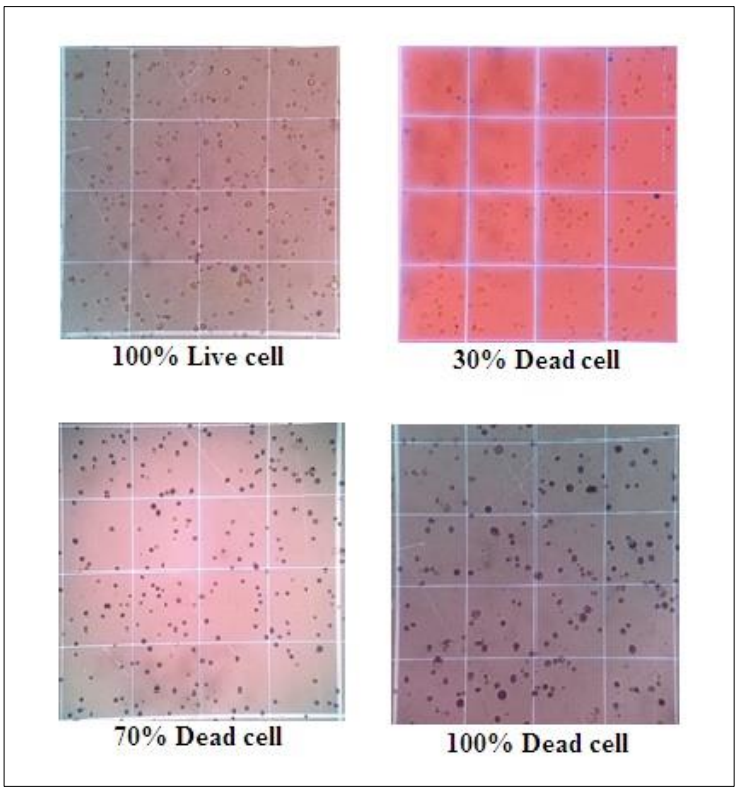


Fig. 1: Anti cancer activity of ethanolic extract of *Pavetta indica* Linn.

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.

3.2 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 288.5). 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-4a: Intrepretation of UV- VIS Spectral Data

Chromophore	Electronic transition	Band at wavelength (nm)
	π- π^*	E-band 198 (ε 10400), 217 (ε 48000) indicating the presence of C=C double bond of ethylenic chromophore

Table-4b: Intrepretation of Infra-Red Spectral Data

Band at frequency (cm^{-1})	Vibration	Type of Bond
Weak 3614 Strong, Broad Medium 1198	Stretching Stretching Bending	O-H free (Hydroxyl group) O-H free (H bonded) Intra molecular O-H group
Strong 1018	Bending	C-O bond
Strong 2863, 2872, 2941, 2958, 744 Medium 1375, 1381, 1448, 1453 Strong 2891 Medium 929, 1371, 1394	Stretching Bending Stretching Bending	C-H of CH_3 C-H of CH_3 C-H of CH_3 C-H of CH_3
Weak 3051, Strong 3048 Medium 1649, 1656 Strong 906, 911 Strong 2853, 2924 Strong 2846, 2915 Strong 1448, 1469	Stretching Stretching Bending Stretching Stretching Bending	C=C-H of ring C=C of ring C-H of ring C-C-H of ring C-H of CH_3 C-H of CH_3

Table-4c: Interpretation of Proton NMR Spectral Data

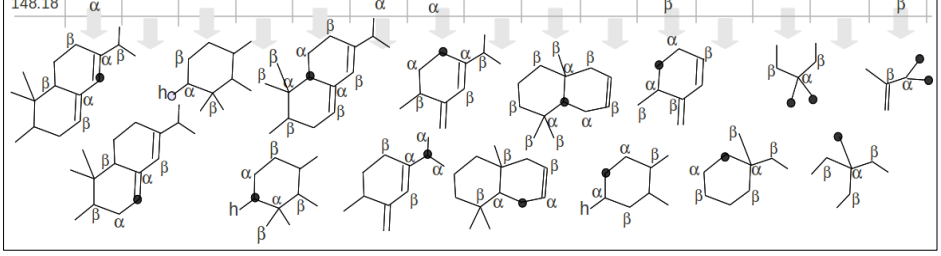
Chemical shift Signal pattern (Number of protons)	Environment of the Protons	Type of protons
0.961 ppm singlet (3H) J 5Hz	Slightly shielded protons of methyl group one neighboring proton	$-CH_3$
1.008 ppm singlet (3H)	Less shielded proton in methyl part with no neighboring proton	$-CH_3$
1.217 ppm doublet (2H) J11.1, 7.1 Hz	Markedly shielded proton in methylenic part with three neighboring proton	$-CH_2$
1.398 ppm doublet (2H) J 9.1, 6.2, 5.4 Hz	Observably shielded methylenic proton. Three neighboring proton	$-CH_2$
1.498 ppm doublet (2H) J11.1, 7.1 Hz	Remarkably shielded methylene environment with three neighboring proton	$-CH_2$
1.693 ppm triplet	Slightly shielded proton environment. Three neighboring proton	-C-C-
5.863 ppm doublet (1H) J 8.3, 2.6 Hz	Deshielded proton attachment with π-electron system with two neighboring proton	-C=C-
5.991 ppm singlet (1H)	Markedly deshielded proton due to the attachment with π-electron system with no neighboring proton	-C=C-
3.206 ppm doublet (1H) J 11.1, 7.3 Hz	Significantly deshielded by the attachment of electronegative oxygen atom to methylenic carbon. Two neighboring proton	-CH-O
4.381 ppm singlet (1H)	Markedly deshielded by the attachment of the electronegative oxygen atom with no neighboring proton	-OH

Table-4d: Interpretation of Carbon - 13 NMR Spectral Data

Chemical shift (ppm)	Environment of the Carbons	Type of Carbon
17.72, 18.79, 19.24	Observably shielded carbon in methyl part	$-CH_3$
28.97, 37.61, 43.18, 44.01, 46.01, 47.81	Slightly deshielded carbon in Methylene group	$-CH_2$
62.23, 63.93, 75.79, 79.25	Markedly deshielded by the carbon in methylenic part	-CH
147.59, 148.18, 118.72, 120.33	Carbon in highly deshielded environment due to the attachment in π-electron system.	-C=C-
109.18	Highly deshielded carbon due to attachment with more electronegative oxygen atom	-C-O

Table-4e: Interpretation of HMBC data of the compound

	5.991s 1H	5.863m 1H	4.381s 1H	3.206m 1H	2.301m 1H	2.287m 1H	2.011m 2H	1.996m 1H	1.693m 1H	1.493m 2H	1.398m 2H	1.217m 2H	1.008s 6H	0.982s 3H	0.961s 6H
17.72				β					β				bond		
18.79					β				β			β		bond	
19.24						α									bond
28.97					α		α				bond				
37.61				β	β				β	α		bond		β	
43.18			β	α						bond		α			
44.01	β				β	β	bond				α				
46.07		α						bond	α			α			
47.81	β					bond	β								α
62.23					α			β	α	β	β	β		α	
63.93			β	α				β	α				α		
75.79	β	β			bond		β		β	β	α	β		β	
76.82		β		β	β			α	bond				β	β	
109.18			α	bond					β	α		β	β		
118.72	bond	β			β	β	β								
120.33	β	bond			β			α	β						
147.59	α	α			α			β			β				
148.18	α					α	α				β				β



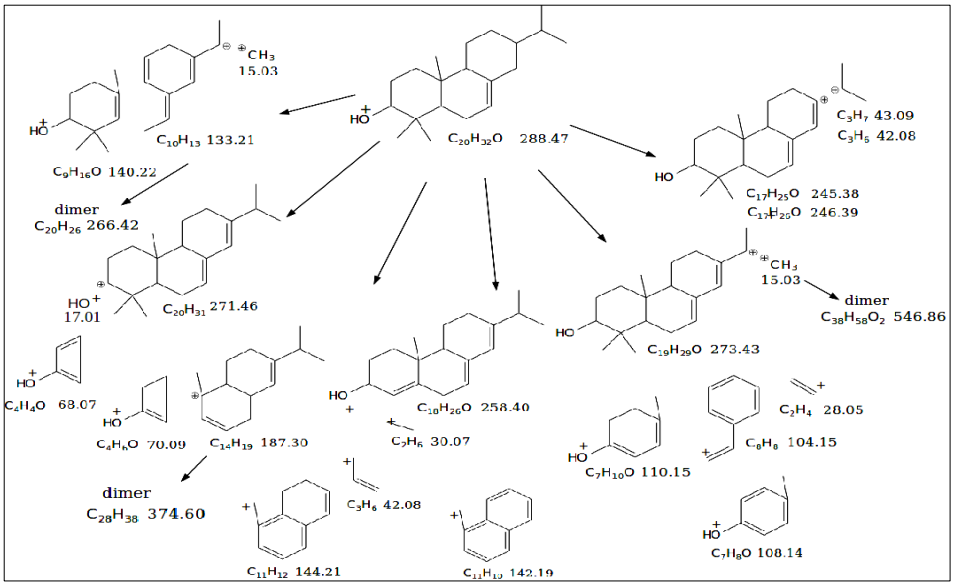
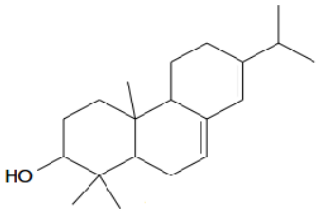


Fig. 2: Mass spectral Fragmentation Pattern of the compound - PIC_1

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_1 involving UV, IR, H–NMR, C13-NMR and Mass spectral studies revealed the presence of terpenoid type of compound. The final aspect of the structural characteristics of PIC_1 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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