Isolation and Identification of compounds from Root extract of *Pavetta Indica linn*

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ABSTRACT

Objective: ***The main aim of the present study to isolate & characterization of compounds from root extract of Pavetta indica linn. Material & methods: Root of the plant material was successively extracted with petroleum ether, chloroform and ethanol. The crude extract partitioned with different solvent system by increasing their polarities (Petroleum ether, benzene, chloroform, ethyl acetate and ethanol). The compounds were fractioned by using column chromatography & thin layer chromatography technique. The compounds have been characterized on the basis of spectral analysis (IR, ^{1}H NMR and Mass spectroscopy).Result: on the bases of result ethanolic extract of plant pavetta Indica linn isolate four compounds namely, Chlorogenic acid, Fercilic acid, Salicine and Oleic acid are being reported first time from roots part. Conclusion: As this is the first attempt of any phytochemicals investigation of root of pavetta indica linn further isolation & purification of other fraction of this plant is recommended which could yield some novel and bioactive compounds.***

Key Words: ***Pavetta indica linn, Petroleum ether, Benzene, Chloroform, Ethyl Acetate Ethanol, Chlorogenic acid, Fercilic acid, Salicine, Oleic acid***

INTRODUCTION

All Medicinal plants and their derived medicines are widely used as natural alternatives to synthetic chemicals in traditional cultures in the world and they are becoming most popular in modern society **(1).** In the herbal medicine field of there has been an exponential development in last few decades in developed and developing countries. Herbal medicine is increasingly most popular due to its natural origin and lesser side effects **(2).** Historically, all medicinal preparations are originate from plants, either in the simple form of raw plant materials otherwise in the refined form of crude extracts, mixtures, etc **(3).** The main goal of medicinal plants and traditional health systems is solving the health care problems of the world are gaining increased attention. Because of this resurgence of interest, the research work on plants of medicinal importance is increasing phenomenally at the international level. Most of the developing countries have assumed traditional medical practice as an integral part of their culture. Phytochemical are the basic source for the establishment of several pharmaceutical industries **(4).** The plant *Pavetta Indica linn* belonging *Rubiaceae.* 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 Asia (5). Stems contain a green resin, starch, an organic acid and a bitter glycoside resembling salicin. Root contains glucoside and is rich in D-mannitol. β-Sitosterol, α-amyrin, quercetin, caffeic acid, chlorogenic acid**(6).** The earlier literature surve revels that the *pavetta indica linn* having Diuretic activity (7), antimicrobial activity (**8),** antioxidant activity **(9**), antidibetic activity (10), hepatoprotective activity

(11), antinflamatory activity (12), Analgesic activity (13), Anthelmintic activity (14), anticorrosive nature (15) and Wound healing activity (16).

MATERIAL AND METHODS

General Experimental Procedure

Melting point of the isolated compounds was found out by open capillary tube method. TLC was performed on pre coated plates (Silica gel G 100 mesh) by using different solvent system. By using capillary tube, the fractions were spotted on TLC plates and the chromatogram was run in different solvent system. The compounds were developed related to their affinity towards different solvent system. The different spot developed in each solvent system were identified in the iodine chamber and calculated the R_F value. The compounds were subjected to IR spectroscopy in KBr (PwrkinElmer Spectrum Version 10.03.06), 1H and C13 NMR (Bruker DRX-300) and mass spectroscopy (ESI-HRMS, Agilent 6520 Q-Tof).

Collection and Authentification of the Plant Material

The roots of *pavetta indica linn* were collected from Bhopal (Vindya herbal medicinal garden) during the month of October and identified by Dr. Ziaul Hasan, HOD, Department of Botany, Safia College of Science and Education Bhopal (MP) India. A voucher specimen of plant was specified as 550/Bot/Safia/14b.The roots were then washed with water to remove soil and other extraneous matter. The roots were cut into small pieces and were dried under shade for 20 days. Then the dried material was homogenized to coarse powder and was stored in airtight container.

Extraction of Plant Material

About 400 gm of dry coarse powder was soaked with petroleum ether (2500ml) for two days. After this, soaked materials were extracted with petroleum ether (40˚C- 60˚C) by continuous hot percolation method for 72 hrs. The petroleum ether extracts were filtered and concentrated under reduced pressure. A green- black residue was obtained. The mark left after the petroleum ether extraction then dried and extracted with chloroform (2600 ml) for 72 hrs. The chloroform extract were also filtered and concentrated under reduced pressure. A dark black residue was obtained. The mark left after the chloroform extraction then dried and extracted with methanol (2600 ml) for 72 hrs. A dark black residue was obtained. Crude extracts were stored in desiccators.

Isolation of compounds

10 gms of ethanolic crude extract of root of pavetta indica linn was chromatographed over about 300 gms of silica gel column. The solvent used were petroleum ether, benzene, chloroform, ethyl acetate, ethanol and their mixtures in the order of increasing polarity. The column was packed by using the suspension of silica gel in petroleum ether. Each 100 ml of the elutes were collected and concentrated. Each fraction was tested for the presence of various constituents and checked on TLC for number and type of constituents.

RESULT AND DISCUSSION

Column chromatography of ehanolic root extracts of *pavetta indica linn* furnished four compounds namely PIR1, PIR2, PIR3 and PIR4. The structures were determined by spectral data.

Compound PIR 1 (Chlorogenic acid)

This compound was obtained from the elution of column with Ethyl Acetate and Chloroform fraction (08:02) yielded dark brown semisolid 100mg residue which is soluble in Chloroform, Ethyl

Acetate, Benzene & Methanol. The Rf was 0.43(), Melting Point 155˚C. Its IR spectrum data showed the band at 3447.60 cm^{-1} O-H Stretching; 2924.87, 2854.06 cm^{-1} C-H Stretching; 1702 cm^{-1} C = O Stretching; 1459 cm^{-1} C-H Bending, NMR single ($CDCl_3$) (δ ppm) showed at 0.821-0.947 R-CH_3 proton; 1.083-1.250 R-CH_2 Proton, 1.321-1.531 R-CH Proton; 1.589-1.922 Alylic proton; 2.011-2.234 Ester Proton ; 3.903-3.99 Phenolic Proton &7.275 Aromatic Proton. In Mass the largest fragment ion appears to be posses an m/2 413. M 90, M 111, M 112, M 146, M 190, M 230 and M 264 peaks were observed. Therefore likely fragment masses are 90, 111, 112, 146, 190, 230 and 264.

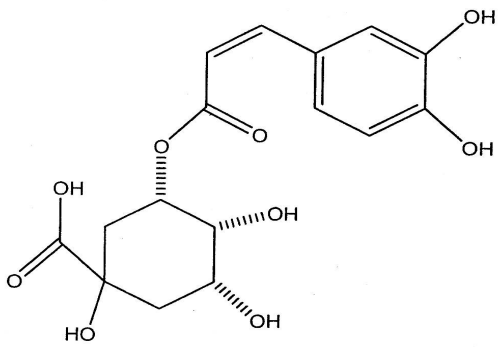

Fig 1 Chlorogenic Acid

Compound PIR 2 (Fercilic Acid)

Compound PIR2 was obtained from Ethyl Acetate and Chloroform fraction (07:03) yielded dark brown semisolid 85mg residue which is soluble in Chloroform, Ethyl Acetate, & Methanol. The Rf Value was 0.40 (), Melting Point 170˚C. Its IR spectrum data showed the band at 3448.23 cm-1 O-H Stretching; 2926.46, 2855 cm-1 C-H Stretching; 1734 C=O Stretching; 1460, 1378 C-H Bending; NMR single ($CDCl_3$) (δ ppm) showed at 0.821-0.942 R– CH3 Proton; 1.002-1.265 R– CH2 Proton; 1.296-1.593 R–CH Proton; 1.694 Alylic Proton; 2.319 carbonyl group; 3.679 OR Proton (hydroxyl) & 7.274 Aromatic Proton. In Mass spectrum the parent ion mass as 407, the data showed the peaks at M-42, M 106, M-202, and M -204.

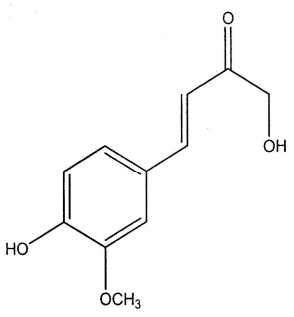

Fig 2 Fercilic Acid

Compound PIR 3 (Salicine)

Compound PIR3 was obtained from the elution of column with Hexane: Methanol (07:03) yielded dark brown semisolid 140mg residue which is soluble in Chloroform, Ethyl Acetate, & Methanol. The Rf Value was 0.45 (), Melting Point 245˚C. Its IR spectrum data showed the band at 3433cm^{-1} O-H Stretching; 2920, 2851 cm^{-1} C-H Stretching; 2363.26 extended Resonance; 1725 C=O Stretching; 1463, 1348 C-H Bending; 1261 C-O stretching. The NMR single ($CDCl_3$) (δ ppm) 0.792-0.948 R–CH_3 Proton, 0.968-1.297 R- – CH_2 Proton, 1.345-1.534 R – CH_2 Proton, 1.627-2.023 Alylic Proton, 2.187-2.314 Ester Proton, 3.679 OR (Hydroxyl), 7.274 Aromatic Proton. In Mass spectrum the largest fragment ion appears to be posses an m/2 of about 302. In ethanol fraction larger m/2 forming molecule are being extracted. We get M-55; M-71 and M-99 peaks are discernable.

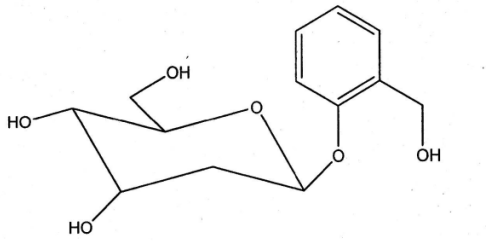

Fig 3 Salicine

Compound PIR 4 (Oleic Acid)

Compound PIR4 was obtained from the elution of column with Butenol : Acetic Acid : Water (4.5:5:0.5) yielded Yellowish Green semisolid 30mg residue which is soluble in Chloroform, Ethyl Acetate, & Methanol. The Rf Value was 0.25 (), Melting Point 200˚C. Its IR spectrum data showed the band at 3452^{-1} O-H Stretching; 2921, cm^{-1} C-H Stretching; 2852 extended Resonance; 1737.74 C=O Stretching, 1461.94 C-H Bending, 1259 C-O stretching.The NMR single ($CDCl_3$) (δ ppm) 0.71R–CH_3 Proton, 1.28 R – CH_2 Proton, 3.09 OR (Hydroxyl), 4.52 OPh Proton. 8.84 Carboxylic proton. In Mass spectrum data showed molecular weight peak at m/z 368 appeared as (M-1)+. Study showed molecular ion peak at m/z 746 (M+) and the base peak at m/z 663 for the fragment [$(CH2)_{45}CH_2OH$]+(M++2)

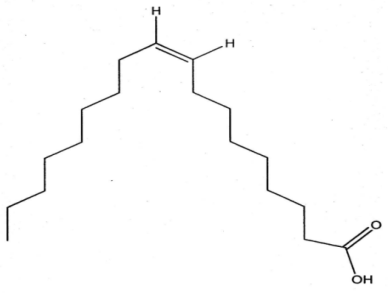

Fig 4 Oleic acid

CONCLUSION

Pavetta indica linn belonging to family Rubeacea. The roots of the plant is traditionally clamed for it traditional medicinal value (Diuretic and purgative). Petroleum ether, chloroform and ethanol extracts of the roots were prepared. The ethanol crude extract was subjected for column chromatography by using increasing polarities (Petroleum eter, benzene, chloroform, ethyl acetate and ethanol) which isolate four compounds namely Chlorogenic acid, Fercilic acid, Salicine and Oleic acid. These compounds were isolated first time from root of *pavetta indica linn.* As this is the first attempt of any phytochemical investigation of root of *pavetta indica*, further isolation & purification of other fraction of this plant is recommended which could yield some novel and bioactive compounds.

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