INDO AMERICAN JOURNAL OF PHARMACEUTICAL RESEARCH

PAVETTA INDICA: BIOCHEMICAL ANALYSIS AND EVALUATION OF ANTIOXIDANT AND CYTOTOXIC EFFECT OF ITS AQUEOUS AND METHANOLIC EXTRACTS.

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ARTICLE INFO

Article history
Received 01/02/2020
Available online
31/01/2021

Keywords
Pavtta Indica;
Antioxidant;
Cytotoxic;

ABSTRACT

Pavetta indica is used in traditional and ayurvedic medicines. Its biochemical, antioxidant and cytotoxic properties were evaluated in the present study. Methanol and aqueous extract of *Pavetta indica* showed a significant antioxidant activity. Cytotoxic effect on normal cell lines was negligible. So it can be used in function food as an antioxidant agent.

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*Please cite this article in press as **Thara K.M** et al. Pavetta Indica: Biochemical Analysis and Evaluation of Antioxidant and Cytotoxic Effect of its Aqueous and Methanolic Extracts. Indo American Journal of Pharmaceutical Research.2021:11(01).*

INTRODUCTION

Details of the role of plants in curing the diseases have been documented as materia medica in Chinese, German etc. Ayurveda and Unani keep account of the medicinal aspects of the indigenous flora. Sumarian clay tablets of around 4000 years ago have record of plant remedies for various illnesses. Around 100 plant remedies have been listed on ancient Egyptian papyrus about 3000 years ago. There are growing apprehension on the recent views that allopathic drugs, largely based on synthetic chemicals**,** though are therapeutically effective than other types, cause adverse side effects. This has made man to think of other alternatives, preferably of natural types like the plant -based, which are not only harmless but also cheaper, replenishable and of wider range in occurrence [1].

The Ayurvedic preparations contain 75 to 80% of natural products . It is believed that Ayurvedic products have the capacity to purify the cells and tissues and also to prevent or remove the toxic products from the live cells [2].

Medicinal plants contain wide range of bioactive components which are mainly responsible for their biological activities that help treat chronic as well as acute infectious diseases. The activity may be due to a single entity or synergistic effect of different components. These compounds are generally classified as phenolic, flavanoids, alkaloids, saponins, terpenoids, glycosides etc [3,4]

Antioxidant compounds are able to neutralize the excess of ROS or RNS and, as a consequence of this activity, it has been suggested that they play an important role in prevention of many diseases, e.g. atherosclerosis, cardiovascular and neurological diseases and cancer. So a study on the antioxidant activities of the plant extract is very important along with its other properties [5,6,7].

The phenolic substances present in the plants and plant-based foods posses strong antioxidant properties and thus are being increasingly investigated. Plant tissue contains a net work of compounds that can control the level of reactive oxygen species including phenol compounds, vitamin C and E, glutathione and several enzymes. Phenol compounds widely distributed in the plant tissues include flavanoids, tannins, hydroxyl cinnammate esters and lignin. Adverse effects caused by over exposure to synthetic chemicals increased the public awareness and this led to the research for green solutions [8,9].

Flavones, flavonoids, flavonols**,** chalcones xanthones, biflavones, isoflavones and aglycones etc come under the class of flavanoids [10]. Flavanoids are a group of plant bioactive compounds studied widely. Around 4000 compounds have been characterized and classified as flavanoids.It has the general formula C_6-C_3-C_6. Flavanoids are ubiquitous in plant cells where photosynthesis is carried out. It is also commonly found in fruits, vegetables, nuts, seeds, stems, flowers, tea, wine, propolis and honey. For centuries, preparations containing these compounds were used as food [11].

Evidences suggest that cellular damage or oxidative injury arising from production of free radicals or reactive oxygen species (ROS) are critical causative factors in the pathogenesis of many neurodegenerative disorders, inflammatory conditions, auto-immune diseases, atherosclerosis, aging diabetes, cancer and gastrointestinal disorders[12,13,14]. Results from biological and phytochemical studies indicate that medicinal plants have profound antioxidant potential that can be exploited further in the prevention and treatment of these devastating disorders. This was reported by different workers [15,16,17]. Many polyphenolic compounds as flavonoids and phenolic acid from plant materials such as herb extract have shown the antioxidant activity against ROS [18,19,20,21]. People have studied the use of medicinal plants and its use in traditional medicines as antioxidants[22].

Antioxidant compounds are cancer preventive in nature. Many molecular and cellular targets of chemopreventive compounds have been identified. Some of the major signaling pathways of membrane associated receptor such as tyrosine kinases are cell signaling kinases like membrane associated protein kinases (MAPK), phosphatidylinositol 3-kinase (PI3K)/Akt, and necrosis factor, NF-_B (transcription factor). The above cell signaling kinases and transcription factors are also important targets of certain dietary antioxidants. Chemopreventive effects of dietry agents may be due to their ability to modulate many signal transduction pathways in a manner which prevents carcinogenesis [23,24]

CYTOTOXICITY

Cytotoxicity is an important factor which determines the success of a drug in the market. A biological active compound should be less cytotoxic to human cells and organs. Then only it can be used as a drug to treat a particular disease. Many compounds have failed in the market due its cytotoxicity and other side effects. World Health Organization has put forward some guidelines to determine the cytotoxicity of a product.

Pavetta indica is a plant belongs to the family Rubiaceae. Common names are ‘Pavatta’ ‘Papat’ and ‘Tiyakphala’ and it is seen as shrub in forest. The plant is found distributed throughout India and its leaves and roots have medicinal properties.

MATERIALS AND METHODS

Plant material was collected from Palaghat district, Kerala, India and identified from the Botany Department, University of Calicut (Voucher no 6765). Seeds were used in the present study. Dried and 10gm of the powder was used for the extraction purpose. Solvent extraction was done using the method Bioassay guided fractionation based on the polarity of the solvent [25,26]

Bioassay- guided fractionation

In this method, plant extract was prepared using different solvents sequentially, according to their increasing order of polarity. 10 gm of the powdered plant material (weight varies depending on plant material) was first mixed with the solvent of the least polarity (100ml) and the mixture was kept on an orbitory shaker at 200 rpm for 18 hrs. Supernatant was collected by filtering and evaporating to dryness. The residue thus obtained was weighed and stored. It was then mixed with solvent of next higher polarity and the process was repeated for different solvents, depending on their order of polarity. The different fractions thus collected on evaporation were dissolved in respective solvents at required concentration or in dimethylsulphoxide (DMSO) or in sterile water (if soluble). Concentration of the prepared extract was 10mg/ml (stock solution). The extract was stored under refrigeration (-20^{0}C) till its use for further analysis. The biological activities of the extracts were tested after filter sterilization[26,27].

Biochemical analysis for flavanoids (Ferric chloride method), phenolic acid (Folin's method), alkaloids (Nessler's reagent), terpenoids, glycosides etc were done as per the method described earlier [27,28,29].

Estimation of total phenolic content:

Total phenolic content of the extracts were estimated using Folin-Ciocalteu method [30, 31]

Estimation of total flavanoids:

The total flavanoid content was estimated by the method described by earlier[28,29]

Reverse Phase High pressure liquid chromatography: RP-HPLC (Li et al., 2010)

Plant extracts which showed significant antimicrobial, antioxidant activities were analysed using HPLC. RP-HPLC-profiling was done using general method, changing the solvent system according to the plant material [28,29].

HPLC SYSTEM:

HPLC unit with dual pump, rheo dyne injector, SPD photodiode array detector in combination with 6.12 SP5 integration software was used. The following chromatographic conditions were given for the present experiment.

Column: Lichrosper RP 18 e 5µm (Merck).

Detector: **SPD PDA**, Flow rate: 1ml/min, Injection volume: 20µl.

The mobile system and wavelength were selected according to the sample. The mobile phase used for different samples were as follows

Pavetta indica:- Acetonitrile: methanol (95:5)

Determination of antioxidant activity.

Diphenyl di picryl phenyl hydrazyl (**DPPH) method**:

This method was used to determine the antioxidant activity of different extracts [30,31,32].

Extract preparation

Table.1. Percentage yield for each plant extract.

Name of the plant	Plant part used for the extraction *	Solvent used	Dry mass (gm)	%yield **	Extraction method used ***	Designated as
Pavetta indica	R	Methanol	2.5	2		
		Ethanol	0.1	0.4	C	PI1
		Water	1.5	1	C	PI2
		Chloroform	ND	ND		

There was 2% yield of methanol fraction while it was 1.5% in the case where water was used as solvent.

Table 2.Various metabolites present in the extracts.

Name of the plant	Extract tested	Phenolic acids	Flavanoids	Alkaloids	Saponins	Glycosides	Terpenoids	Protein
Pavetta indica	PI1	+	+++	-	-	+	-	-
	PI3	+	+	-	-	-	-	-

A maximum flavanoid content was obtained in the methanol fraction of the *Pavetta indica*.

Table 3. Total phenolics and flavanoids in the extracts.

Name of the plant	Extract used for the estimation	Total phenolics as Gallic acid equivalents	Total flavanoids as Quercetin equivalents
Pavetta indica	PI1	35.5 + 4.1	5.3
		20+ 2	2.5

The total phenolic content was 35.5 gallic acid equivalent and flavanoid contant was equivalent to 2.5 gm quercetin.

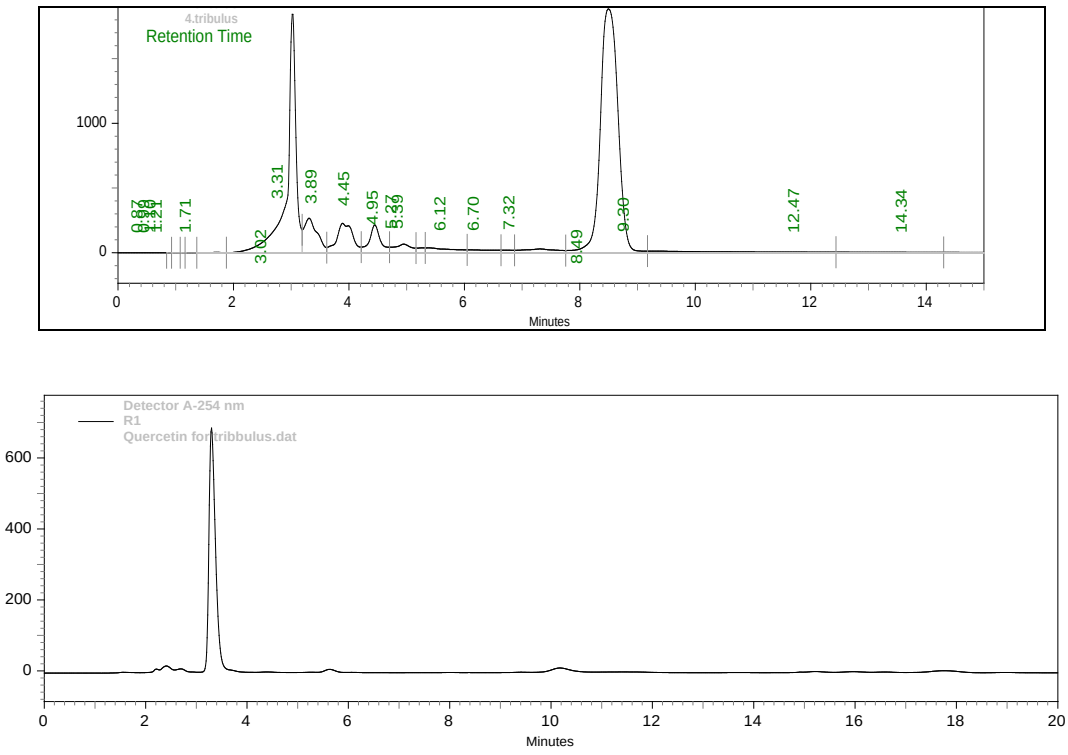
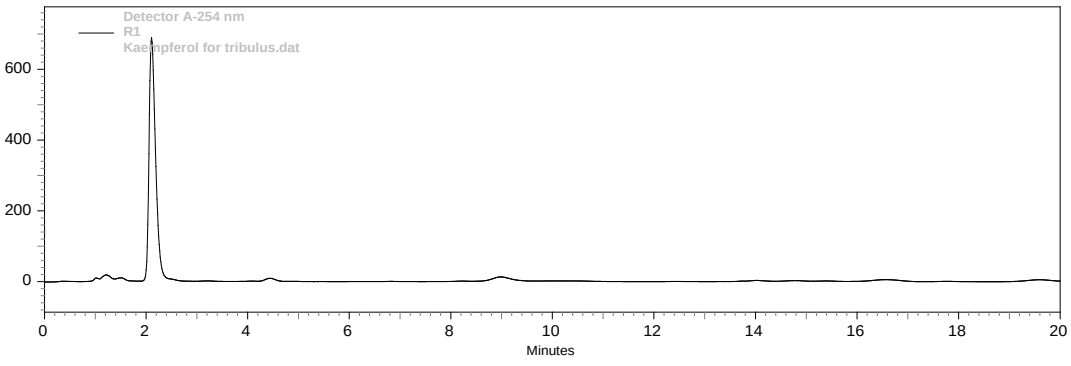


Figure 1. HPLC Chromatogram of *Pavetta indica* aqueous extract.

Quercetin
Kaempferol



Quantification of Quercetin and Kaempferol in *Pavetta indica*
Concentration of Sample: 20mg/ml (with respect to (wrt) dry extract weight)
Concentration of Std Quercetin: 100ug/ml (1 mg dissolved in 10ml)
Concentration of Std Kaempferol: 100ug/ml (1 mg dissolved in 10ml)
Retention time of Quercetin: 3.3
Retention Time of Kaempferol: 2.1
Percentage purity of injected Quercetin: 98%
Percentage purity of injected Kaempferol: 99%
Area given by Standard Quercetin in the standard profile: 45896217
Area given by Standard Kaempferol in Standard profile: 69852471
Area given by Quercetin in sample profile: 1072971
Area given by Kaempferol in sample profile: 33 (310nm)
Result:

The percentage of Quercetin in the sample : 0.26 % (w/w)
The percentage of Kaempferol in the sample : Traces
0.26 % of quercetin was found to present in the methanol extract of *Pavetta indica.*

Table 4. Antioxidant activity of extracts (Figure: 26a, b).

Name of the plant	Extract used for the estimation	IC_{50}DPPH activity (µg/ml)
Pavetta indica	PI1	49.51
	PI3	56.0

On testing antioxidant activity methanol extract showed an IC_{50} value of 49.5µg/ ml while it was 56.0µg/ ml for water extract.

Table 5. Cytotoxicity assay for various extracts.

		Normal Mice spleen cells % inhibition	IC_{50} (concentration for 50% inhibition)
Pavetta indica	PI1	12	1742.74
	PI3	3	2365

The cytotoxicity value was 12% for methanol extract and 3% for water extract. So aqueous extract was less toxic.

Pavetta indica:
It showed the presence of phenolic (35.5GAE) flavanoid (5.3QE) and glycoside compounds. Presence of 0.85 % quercetin and 0.00065% kaempherol has been noticed. The antioxidant activity shown was significant comparing with ascorbic acid as standard. The extract was not very toxic on normal mice spleen cells, the cell inhibition being 12%. The medicinal properties of the extract may be due to the presence of the flavanoids, Quercetin, Kaempherol etc. The anti-inflammatory activity of *Pavetta indica* was reported earlier [33].

CONCLUSIONS

Pavetta indica is a good source of phenolic and flavanoid compounds and can be used as an antioxidant agent. Cell toxicity was negligible. Further biochemical analysis has to be conducted for the identification of active components present. *Pavetta indica* can be used in preventive medicines as an antioxidant agent after clinical verification.

ACKNOWLEDGEMENT
I acknowledge all those who helped me to carry out this work.
There is no conflict of interest.

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