WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 10, Issue 12, 2033-2038. Research Article ISSN 2277– 7105

SCREENING OF ANTIHYPERLPIDEMIC ACTIVITY ON WHOLE PLANT OF *PAVONIA ODORATA* WILLD

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Article Received on
14 August 2021,

Revised on 05 Sept. 2021,
Accepted on 26 Sept. 2021

DOI: 10.20959/wjpr202112-21899

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ABSTRACT

Pavonia odorata Willd is a medicinal plant found in the moist deciduous forest region of the world where it is used by local people to treat fever, dysentery, ulcers and bleeding disorders. The present study is aimed at investigating the antihyperlipidemic activity on methanolic extract of P.odorata *Willd.* The preliminary phytochemical screening of the methanolic extract showed presence of alkaloids, phenolic compounds, steroids, mucilage and flavonoids. The antihyperlipidemic activity was determined through Oil Red O Staining technique. Throughout the studies the plant extract showed potent antihyperlipidemic activity and the activity was found to be concentration dependent which may be attributed to the various phyto constituents of the plant.

KEYWORDS: *Pavonia odorata* Willd, Antihyperlipidemia, Oil Red O Staining, 3T3L1 Pre adipocyte cells.

1. INTRODUCTION

Herbal products play a very important role in the treatment of various disease conditions. These products can be obtained through different process like extraction of any parts of plants, marine organism or from micro-organism fermentation. Substances which are obtained from plant source are getting very much attention from the scientist and researchers due to the increasing number of applications. All plants have medicinal properties which are

the rich source of drugs used in nutraceuticals, medicine, modern medicines, folk medicines, food nutrients, pharmaceutical excipients and chemical entities for synthetic drugs.[1]

Pavonia odorata Willd, commonly known as Sugandhabala belongs to the family Malvaceae. They are erect herb reaches a height of about 45-90cm. *P. odorata* Willd is widely distributed in Indian subcontinent, Africa, Sri Lanka, Pakistan and Myanmar.[2] In India it is mainly found in deciduous forest up to an altitude of 1000 m. They are commonly found in the warmer parts like Andhra Pradesh, Bihar, Karnataka, Kerala, Orissa, Maharashtra Punjab, Rajasthan, Tamilnadu, Uttar Pradesh and West Bengal. In Ayurvedic system of medicine it is used as a cooling, diaphoretic, diuretic, and demulcent. In combination with other plant drugs, it is prescribed as an astringent, tonic for fever, inflammation and hemorrhage. It has been reported for anti- inflammatory, anti-microbial, anticancer, antioxidant, antifungal, antibacterial, antidiabetic, antiulcer, antitumor immunomodulatory, and cardiovascular activity.[3]

Hyperlipidemia is characterized by increased level of cholesterol in the form of low-density lipoprotein (LDL), chylomicrons, VLDL, etc. It is characterized by hypercholestremia is the most prevalent indicators of cardiovascular diseases.[4]

2. MATERIALS AND METHODS

2.1 Plant collection and Preparation of extract

The whole plants of *Pavonia odorata* Willd were collected from the natural habitat in and around Wayanad district, Kerala on January 2020. The plant was identified and authenticated by Dr.P. Sreeja, Dept. of Botany and Research Centre Sir Syed College Talipramba, Kannur Kerala, India. The whole plants were washed and shade dried. After drying, it was ground to yield fine powder for further analyses. The extraction was carried out by hot percolation method. The solvent used was methanol. The extract was concentrated to dryness under controlled temperature.

2.2 Preliminary Phytochemical Analysis

For preliminary phytochemical tests, 35 g of powdered material was extracted using methanol. The presence of different phytoconstituents viz., alkaloids, carbohydrates, proteins, amino acid, fat and oils, terpenoids, steroids, saponins, tannins, flavonoids, phenol, anthraquinones and glycosides were determined using standard procedure.[5-6]

2.3 Antihyperlipidemic activity using Oil Red O Staining

3T3 L1 (Pre adipocyte cells) cell line was initially procured from National Centre for Cell Sciences (NCCS), Pune, India and maintained Dulbecos modified Eagles medium (Gibco, Invitrogen). The cell line was cultured in 25 cm^2 tissue culture flask with DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate and antibiotic solution containing: Penicillin (100U/ml), Streptomycin (100µg/ml), and Amphoteracin B (2.5µg/ml). Cultured cell lines were kept at 37ºC in a humidified 5% CO_2 incubator (NBS Eppendorf, Germany).

Reagent Preparation

1) Oil Red-O stock: 0.5gms of Oil Red-O powder (Fisher Biotech) was added into 100 ml of isopropanol and stirred overnight in a glass bottle. Then the mixture was filtered through Two Layer Whatman Papers.

2) Fresh Oil Red-O working solution: It was prepared by adding 6.0ml of stock to 4.0ml of double distilled water. The mixture was filtered through. 22um syringe filter and used for further studies.

3) Fixative Solution- 11.0ml of 37% formaldehyde + 29.0ml PBS; final concentration is 10%.

Procedure

Cells were grown at an initial density of 10^5 cells/well in a 24-well plate and treated with 1mM FFAs for 24 h. Cells were then washed three times with iced PBS and fixed with 4% paraformaldehyde for 30 minutes. After fixation, cells were washed three times and stained with Oil Red O solution working solution for 15 min at room temperature. Cells were washed again with phosphate-buffered saline (PBS) to remove unbound staining. To quantify Oil Red O content levels, dimethyl sulfoxide was added to each sample; after shaking at room temperature for 5 min, the density of samples were read at 510 nm on a spectrophotometer (Cary 60, Agilent).

CALCULATION

$$\textbf{Percentage Cholesterol Transport: } \frac{(\text{Palmitic Acid Control OD} - \text{Sample OD})}{\text{Palmitic Acid Control OD}} \textbf{ X 100}$$

3. RESULTS AND DISCUSSION

3.1 Preliminary phytochemical screening

Table 1: Phytochemical composition of whole plants of *P.odorata*.

Sn	Phytochemicals	Methanolic extract
1	Alkaloids	+
2	Carbohydrates	-
3	Flavonoids	+
4	Fats and oils	+
5	Glycosides	-
6	Saponins	-
7	Mucilage	+
8	Phenolic compounds	+
9	Steroids and Terpenoids	+

3.2 Antihyperlipidemic activity

Table 2: Effect of methanolic extract of *P.odorata* on 3T3L1 cells.

Concentration(µg/mL)	OD AVERAGE	Percentage Cholesterol Transport
Palmitic acid	0.2131	0.00
Methanolic extract		
25	0.1889	11.36
50	0.1799	15.56
100	0.1428	32.99

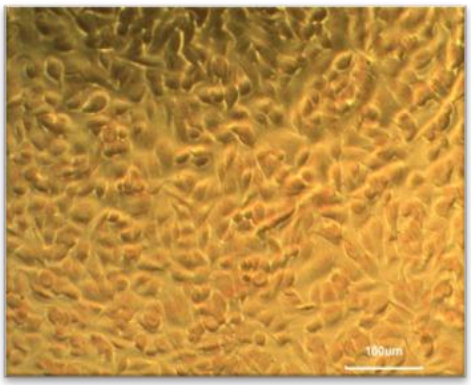

Fig. 1: Control

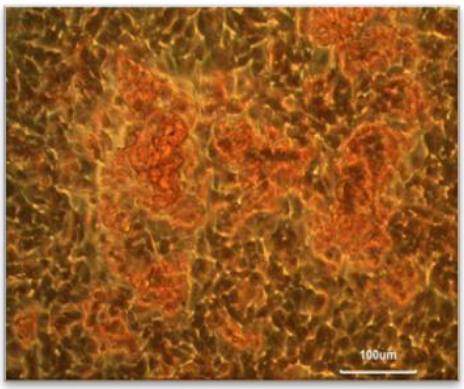

Fig. 2: Palmitic acid

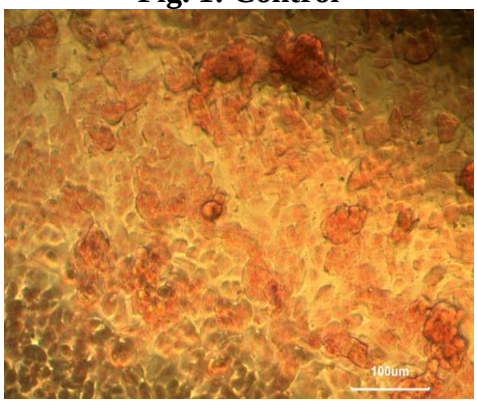

Fig. 3: Conc - 25 µg/mL

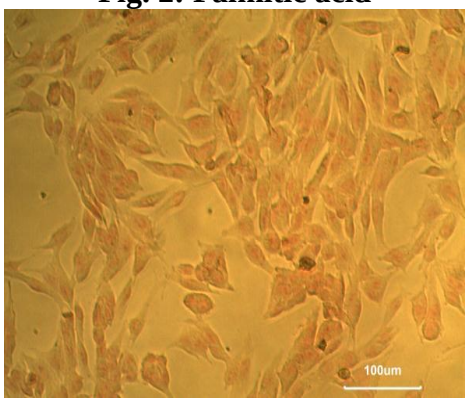

Fig. 4: Conc -50 µg/mL

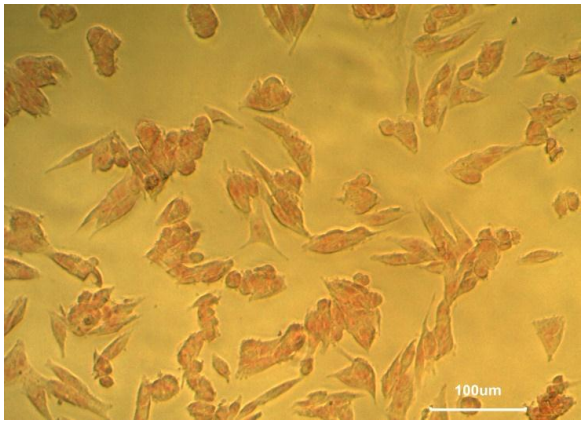

Fig. 5: Conc - 100µg/ml.

The methanolic extract of whole plant of *P.odorata* Willd exhibited anti hyperlipidemic activity in 3T3L1 cell lines. The extracts showed good anti hyprerlipidemic potential with the inhibition activities ranging between 11 to 32 percent. Compairing with the standard palmitic acid the percentage of cholesterol transport was higher in methanolic extract. The preliminary phytochemical screening of the plant extract showed the presence of chief secondary metabolites like alkaloids, flavonoids, poly phenolic compounds, tannins etc. Flavonoids are well documented to have potent antihyperlipidemic activity. Polyphenolic compounds and tannins are proven good natural anti-hyperlipidemic agents. These results suggest novel uses of P.odorata by showing that the whole plants are rich in flavonoids and flavonoid derivatives with an antihyperlipidemic effect in hepatic cells.

4. CONCLUSION

From the above results the present study was concluded that the alcoholic extract of the whole plant of *Pavonia odorata* Willd exhibits anti-hyperlipidemic activity in 3T3L1cell lines. The anti-hyperlipidemic activity exhibited by *Pavonia odorata* Willd could be due to the presence of flavonoids, poly phenolic compounds and tannins.

5. ACKNOWLEDGEMENT

All authors have no conflict of interest. This research did not receive any specific grant from any funding agency.

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