

RESEARCH ARTICLE

ISOLATION AND CHARACTERIZATION OF FLAVONE GLYCOSIDE VITEXIN FROM PEPPEROMIA PELLUCIDA LINN***PAPPACHEN LEENA.K.^{1,2}, Dr. CHACKO ANNAM³**¹Research Scholar, Karpagam University, Eeachanari Post, Coimbatore²Amrita School of Pharmacy, Amrita Viswa Vidyapeetham University, AIMS Health Sciences Campus, Kochi³St. Josephs College of Pharmacy, Cherthala, Alappuzha**Corresponding author's E-mail: leenabijudaniel@gmail.com***ABSTRACT**

Pepperomia pellucida Linn, widely used in traditional medicine belongs to the family Piperaceae. It has been used for treating abdominal pain, acne, boils, colic, fatigue, gout, headache, renal disorders, and rheumatic joint pain. The whole plant after collection, authentication and drying, was extracted with petroleum ether, chloroform, methanol and water using soxhlet extractor. The aim of the study was to isolate the phytoconstituents from the methanolic extract of *pepperomia pellucida* by Preparative-Thin Layer Chromatography (P-TLC) and characterized by spectroscopic techniques (UV-visible spectroscopy, infrared spectroscopy, ¹H and ¹³C NMR spectroscopy and mass spectrometry). A flavonoidal structure was isolated from the methanolic extract of the plant. The light yellow coloured compound which solubilizes in methanol, ethanol and water having m.p. 203-204°C and R_f value 0.61 in mobile phase acetonitrile : water: acetic acid (6:4:0.1) was identified by the various spectroscopic methods. So from this study, it is concluded that the isolated compound may be vitexin which is responsible for various pharmacological activities of the plants.

Keywords: *Pepperomia pellucida*, Vitexin, Thin Layer Chromatography, Phytoconstituent.**INTRODUCTION**

Traditional herbal remedies have always been valued since time immemorial. The plant derived substances have the advantage of creating therapeutic, prophylactic and diagnostic substances with specific functions and minimum side effects. *Pepperomia pellucida* is an annual, shallow - rooted herb usually growing to a height of about 15 to 45 cm. It is characterized by succulent stems, shiny, heart shaped fleshy leaves and tiny, dot like seeds attached to several fruiting spikes¹. It has a mustard – like odour when crushed. *Pepperomia pellucida* has been used as a food item as well as medicinal herb. *P. pellucida* has been used for treating abdominal pain, abscesses, acne, boils, colic, fatigue, gout, headache, renal disorders, and rheumatic pain, and to treat breast cancer, impotence, measles, mental disorders, and smallpox. It has been used in salads or as a cooked vegetable to help relieve rheumatic joint pain.^{2,3}

The plant is also used as topical medicine for skin related problems such as acne, pimples and abnormal complexion. Pounded whole plant is used as warm poultice for boils, pustules and pimples (De Fatima *et al.*, 2004). In the other parts of Asia such as Bangladesh, the aerial parts including leaves of the plant have been used by local traditional healers to treat the excited mental disorders (Khan *et al.*, 2008). Manila Medical Society reported that *P. pellucida* is useful to relieve rheumatoid arthritic joint pains, but it may manifest some CNS depression side effects (Khan *et al.*, 2008). Several scientific studies reported the anti-bacterial and analgesic activities of *P. pellucida* (Aziba *et al.*, 2001; Khan and Omoloso, 2002)^{2,3} and some other studies reported the isolation of bioactive phytoconstituents with strong anti-fungal and anti-cancer

properties from *P. pellucida* (Ragasa *et al.*, 1998; Xu *et al.*, 2006)^{4,5}.

The plant *Pepperomia pellucida* was found to have variety of chemical constituents. Phytochemical screening revealed the presence of alkaloids, cardenolides, flavonoids, terpenoids, saponins, tannins etc. The present research was therefore, undertaken to the isolation and characterization of flavone glycoside, vitexin from the methanolic extract of *Pepperomia pellucida linn*.

MATERIAL AND METHOD:**Collection & Authentication:**

Pepperomia pellucida was collected from Kollam district of Kerala(India) and was authenticated by Dr.V.J.Dominic , Sacred Heart College,Thevara. The whole plant was washed thoroughly with sufficient water. It was dried in shade for 3 weeks and powdered using mechanical grinder and sieved to get the powder of uniform size.

Chemicals and instruments:

All the chemicals and re-agents used for the experiments were of analytical grade.

Isolation of Phytoconstituent:

Hundred grams of dry powder of the whole plant of *pepperomia pellucida* were successively extracted with petroleum ether and chloroform for the removal of fatty material, steroids etc. The remaining powder was exhaustively extracted in a Soxhlet apparatus with methanol. After the evaporation of methanol under vacuum, the extract was dried. The dried residues were

dissolved in methanol and subjected to Thin Layer Chromatography. Silica gel was used as the stationary phase and acetonitrile : water: acetic acid (6:4:0.1) as the mobile phase in the TLC.

On the chromatograms the light yellow bands of vitexin with R_f value of 0.61 were located. These bands were carefully eluted and extracted with methanol. The solvent evaporated from the resulting extracts and residue thus obtained was subjected to various physical and spectral analysis⁶.

Chemical identification of Constituents: A little amount of the isolated constituent was dissolved in alcohol and subjected to the following tests⁷.

Shinoda Test (Magnesium Hydrochloride reduction test): To the test solution, add few fragments of Magnesium ribbon and concentrated Hydrochloric acid drop wise .Observe the colour.

Ammonia test: Filter paper dipped in alcoholic solution of the drug was exposed to ammonia vapour. Formation of yellow spot on the filter paper observed.

Zinc Hydrochloride Reduction Test: To the test solution add a mixture of Zinc dust and conc. Hydrochloric acid. Heat the solution and observe the color.

Vanillin HCl test: Vanillin HCl was added to the alcoholic solution of the drug. Formation of pink colour due to the presence of flavonoid glycosides observed.

RESULTS AND DISCUSSION:

Characterization of the compound:

The light yellow coloured compound, melting point 203-204°C, have been isolated. The compound is soluble in methanol, ethanol and water and shows the spectra at particular wavelength.

UV Spectra:

The UV spectra of the peaks were typical of flavonoid (UV maxima at 260–270nm and 330–370 nm). Peak showed UV absorption with maximum at 269 nm and 338nm.

IR Values:

FT-IR data showed that the isolated compound presented the characteristic intensities of C=O absorption band at 1624 (carbonyl), 3610, 3558(hydroxyl) 2935, 1149, 1097(C-H stretch)and 1568, 1501 cm⁻¹ (aromatic).

NMR values:

The ¹ H NMR [400 MHz, d₆ -DMSO] spectrum showed a chelated hydroxyl proton (d 13.18, 1H, s), an aromatic singlet (d 6.28, 1H, s), a downfield signal (d 6.79, 1H, s), suggestive of a flavonoid⁸ and an AA'BB' spin system [d 8.03, (2H, d, J=8.5 Hz) and 6.09 (2H, d, J=8.5 Hz)], attributable to a para disubstituted phenyl unit. In addition,

the ¹ H NMR spectrum showed a series of signals between d 3.19 and 3.98 (6H), characteristic of a sugar unit. The absence of usual O-glycosidic anomeric proton signal and the presence of signal at d 4.69 (1H, d, J=9.88 Hz) indicated the presence of a C-glycoside.

Mass Spectrum: Mass spectra of isolated constituent show Molecular ion m/z 433. The above physical and spectral data of compound represent that the isolated constituent may be vitexin⁹.

CONCLUSION:

In the above study, vitexin was isolated and characterized from methanolic extract of *peperomia pellucida* Linn and this is a flavonoidal constituent. Vitexins are lignan compounds, induce apoptosis and suppress tumor growth, has potent anti oxidant effect and it can be effectively used for the prevention of UV-induced adverse skin reactions such as free radical production and skin cell damage¹⁰.

Vitexin has potent hypotensive effects, by its ganglionic blocking properties .Anti-inflammatory effects of vitexin were indicated by its anti- histaminic, anti -bradykinin and anti -serotonin properties.

Additional clinical uses include anti-viral, anti -thyroidal, anti-arteriosclerotic and anti-hepatotoxic effects. The ability of vitexin to inhibit super oxide radicals , leads the potential application of its anti-ageing effects in cosmetic field.

Conflict of interest: We declare that we have no conflict of interest.

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