Nishanthi M. *et al*. / *International Journal of Phytopharmacology*. 3(2), 2012, 173-177.

e- ISSN 0975 – 9328
Print ISSN 2229 – 7472

International Journal of Phytopharmacology

Journal homepage: www.onlineijp.com

IJP

EXTRACTION AND PRELIMINARY PHYTOCHEMICAL INVESTIGATION ON WHOLE PLANT OF *PEPEROMIA TETRAPHYLLA* (G.Forst.Hook & Arn)

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ABSTRACT

The Plant *Peperomia tetraphylla* (G.Forst) Hook & Arn. belonging to family (Piperaceae) has been claimed to possess various medicinal properties. A juice of the whole plant is employed in treatment of convulsions, skin diseases, cough, asthma like symptoms and kidney disorders. *Peperomia tetraphylla* (G.Forst.Hook & Arn).is an important plant described in ayurveda. This plant is used for treatment of a number of ailments like urinary disorders and cardiac problems. The whole plant of *Peperomia tetraphylla* (G.Forst.Hook & Arn) was extracted with different organic solvents in increasing order of polarity. The results of the preliminary investigation revealed the presence of alkaloids, steroids, flavonoids, terpenoids, glycosides, & carbohydrates. The Methanol and Acetone extracts were studied phytochemical compounds were isolated by using thin layer and column chromatography. The chemical structures of the isolated compounds were established by spectroscopic techniques such as UV, IR and NMR, MASS spectroscopy. This was again confirmed by co TLC with standard sample. The aim of the present study was focused on the pharmacognostical, physicochemical and phytochemical properties were carried out, which would like to facilitate quick identification and selection of the drug from various adulterates.

Keywords: -*Peperomia tetraphylla*, Flavonoids, Chromatography, Terpenoids and Spectroscopy.

INTRODUCTION

Pharmacognostical study is the preliminary step in the standardization of crude drugs. The detailed pharmacognostical evaluation gives valuable information regarding the morphology, microscopical and physical characteristics of the crude drugs. Pharmacognostic studies have been done on many important drugs, and the resulting observations have been incorporated in various pharmacopoeias (Indian Pharmacopoeia, 1985). There are a number of crude drugs where the plant source has not yet been scientifically identified. Hence pharmacognostic study gives the scientific information regarding the purity and quality of the plant drugs (Kirtikar KR *et al*., 1995).

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Plants have an almost limitless ability to synthesize aromatic substances mainly secondary metabolites, of which at least 12,000 have been isolated, a number estimated to be less than 10% of the total. In many cases, these substances serve as the molecules of plant defense against predation by microorganisms, insects, and herbivores. Further, some of which may involve in plant odour (terpenoids), pigmentation (tannins and quinines), and flavor (capsacin). However, several of these molecules possess medicinal properties. *Peperomia tetraphylla* is an important medicinal plant, used in Ayurveda, Unani, Siddha, and in folk medicine for treating several ailments including microbial infections, diarrhoea and diabetes (Massiot G *et al*., 1992; Adinolfi M *et al*., 1994; Gamble JS *et al*., 1958; Seetharam YN *et al*., 2000; Dey PM *et al*., 1987)

The Plant *Peperomia tetraphylla* (G.ForstHook & Arn). belonging to family Piperaceae. A juice of the whole plant is employed in treatment of convulsions, skin diseases, cough, asthma like symptoms and kidney disorders. The whole plant of *Peperomia tetraphylla* (G.Forst.Hook & Arn). was extracted with different organic solvents in increasing order of polarity. The aim of the present study was focused on the Pharmacognostical, Physicochemical and Phytochemical properties were carried out, which would like to facilitate quick identification and selection of the drug from various adulterates.

MATERIALS AND METHODS

Collection and Identification of Plants

The plant specimen was collected from Kozzhi Hills. The plant material was authenticated by Dr. P.Jayaraman, Plant Anatomy Research Centre, Pharmacognosy Institute.

Preparation of Plant Extract

The shade dried plant material was powdered using mixer grinder, and subjected to soxhelet extraction with petroleum ether, n-Hexane, Acetone chloroform, 95% ethanol, and distilled water for 18 hrs in the order of increasing polarity of solvents.

Preliminary screening of Secondary Metabolites

The shade dried plant material was powdered using mixer grinder, and subjected to soxhelet extraction with petroleum ether, chloroform, 95% ethanol, and distilled water for 18 hrs in the order of increasing polarity of solvents. The condensed extracts were used for preliminary screening of phytochemicals such as alkaloids (Iodine, Wagner and Dragendorff's tests), flavonoids (Pew's, Shinoda and NaOH tests), glycosides (Keller-Kiliani, conc.H_2SO_4, and Molish tests), Lignins (Labat and Lignin tests), phenols (ellagic acid and phenol tests), saponins (foam and haemolysis tests), sterols (Liberman-Burchard, and Salkowski tests), tannins (gelatin test) were carried out. (Kokate CK *et al*., 1994; Ikan R *et al*., 1981)

Quantitative estimation of secondary metabolites

The presence of secondary metabolites from whole plant powder of *Peperomia tetraphylla* were quantitatively determined by adopting standard protocols. Alkaloids by Ikan's method, (Swain T *et al*., 1959) flavonoids by Swain and Hillis method, (Bray HG *et al*., 1964) phenols by Bray and Thorpe method, (Schanderi SH *et al*., 1970) tannins by Folin-Denis method, (Sanchez GL *et al*., 1972) and saponins by Sanchez method. (Wagner R *et al*., 1996).

Separation of secondary metabolites by thin layer chromatography

For the thin layer chromatographic studies of secondary metabolites, precoated Alugram®Sil G/UV254nm (Machery – Nagel GmbH,Germany) aluminum plates (20 X 20cm) were used.

TLC Study of alkaloids

The powdered whole plant powder of *Peperomia tetraphylla* were wetted with a half diluted NH4OH and lixiviated with EtOAc for 24h at RT. The organic phase was separated from the acidified filtrate and basified with NH_4OH (pH 11-12). It was extracted with chloroform (3X), condensed by evaporation and used for chromatography. The alkaloid spots were separated using the solvent mixture chloroform and methanol (15:1). The colour and hRf values of the separated alkaloids were recorded both under ultraviolet (UV-254nm) and visible light after spraying with Dragendorff's reagent. (Harborne JB *et al*., 1998; Nuzillard JM *et al*., 1996; Thongphasuk P *et al*., 2004; Brandt V *et al*., 2001; Evans WC *et al*., 1996).

TLC Study of flavoniods

One gram powder of *Peperomia tetraphylla* was extracted with 10ml methanol on water bath (60^0C/ 5min). The filtrate was condensed by evaporation, added a Phytochemical Studies of Medicinal Plant 512 mixture of water and EtOAc (10:1 mL), and mixed thoroughly. The EtOAc phase thus retained was used for chromatography.

The flavonoid spots were separated using chloroform and methanol (19:1) solvent mixture. The colour and hRf values of these spots were recorded under ultraviolet (UV254nm) light (Brain KR *et al*., 1975).

RESULTS AND DISCUSSION

The coarsely powdered dried whole plant of *Peperomia tetraphylla* Powder was extracted with petroleum ether, chloroform, acetone, methanolic and

aqueous extracts, and their colour and consistency were studied (Table 1). The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, sterols etc. Thus the preliminary screening tests may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development.

Further, these tests facilitate their quantitative estimation and qualitative separation of pharmacologically active chemical compounds. The Preliminary phytochemical analysis were made clearly indicated the presence of alkaloids, carbhohydrates, terpenoides, tannins, aminoacids, flavanoids, gums, and mucilage The results are shown in (Table 2).

The isolated compound PTC1 was crystalline in nature, white in colour, odourless and bitter in taste with melting point (180^0- 185^0 C), soluble in water and in organic solvents. The isolated compound PTC1 gave R_f value of 0.89 when subjected to Thin Layer Chromatography in Table 3.

The isolated compound PTC1 gave R_f value of 0.89 when subjected to Thin Layer Chromatography .UV Spectra of the crystalline compound PTC1 showed λ max of 220.26 nm with absorbance of 1.60666 shown in Fig.2. IR Spectra of the crystalline compound PTC1 showed characteristic absorption (in cm^{-1}) at 3948.92 - Alcohol and Phenols (C-H- stretching), sharp peak at 1639, 1382 – presence of carbonyl group with ring oxygen or lactone ring shown in Fig.3. May be possess NMR spectra shows the Isoprenyl group (3.319-3.334), active methylene group triplet (3.5-3.7) presence shown in Fig.4. From the above spectral analysis it is concluded that the isolated compound PTC1 may be terpenoids because of isoprenyl group present and active methylene group appeared in multi triplet (3.5 -3.7).

Table 1. Successive Solvent Extraction of *Peperomia tetraphylla*

Plant Name	Parts Used	Method of Extraction	Solvents	Colour & Consistency	Average Extractive Value (% w/w)
Peperomia tetraphylla	Whole Plant	Continuous hot Percolation by Soxhlet Apparatus	Petroleum ether	Dark green	1.29
			n-Hexane	Green & sticky with oil mass	0.56
			Chloroform	Light green	0.46
			Acetone	Yellowish green	1.35
			Alcohol	Brownish green	1.11
		Cold Maceration	Aqueous	Brown	4.52

Table 2. Data Showing the Preliminary Phytochemical Screening of *Peperomia tetraphylla*

Phyto constituents	Petroleum ether extract	n-hexane extract	Chloroform extract	Acetone extract	Alcoholic extract	Aqueous extract
Alkaloids	(-)	(-)	(+)	(+)	(+)	(+)
Carbohydrate	(+)	(+)	(+)	(+)	(+)	(+)
Glycosides	(-)	(+)	(+)	(+)	(+)	(+)
Flavonoids	(-)	(-)	(+)	(+)	(+)	(-)
Phytosterols	(+)	(+)	(+)	(+)	(+)	(-)
Fixedoils& Fats	(+)	(+)	(+)	(-)	(-)	(-)
Saponins	(-)	(-)	(-)	(-)	(-)	(-)
Phenolic Compounds and Tannins	(-)	(-)	(+)	(+)	(+)	(+)
Lignins	(+)	(+)	(+)	(+)	(+)	(+)
Proteins & Amino Acids	(-)	(-)	(-)	(-)	(-)	(-)
Gums & Mucilage	(-)	(-)	(-)	(-)	(-)	(-)

Presence (+) **Absence (-)**

Table 3. Data showing the Thin Layer Chromatography of the Various Extracts of *Peperomia tetraphylla*

S.No	Extracts	Solvent System	No.of Spots	Colour and Consistancy	Iodine Chamber	UV Light
1	Petroleum Ether Extract	A	2	Yellow	0.72	0.79
		B	3	Green	0.85	0.92
		C	2	Green	0.73	0.82
		D	2	Dark brown	0.93	0.9
		E	2	Yellow	0.74	0.76
2	Acetone Extract	A	3	Green	0.82	0.85
		B	2	Brown	0.85	0.76
		C	2	Dark green	0.92	0.98
		D	3	Yellowish green	0.76	0.82
		E	1	Yellow	0.78	0.76
3	Chloroform Extract	A	2	Light green	0.89	0.88
		B	3	Yellow	0.92	0.90
		C	1	Greenish yellow	0.87	0.86
		D	2	Dark brown	0.90	0.86
		E	1	Yellow	0.86	0.97
4	Methanol Extract	A	2	Dark green	0.82	0.86
		B	2	Yellow colour	0.76	0.79
		C	3	Yellowish green	0.78	0.75
		D	2	Yellow colour	0.83	0.86
		E	2	Green colour	0.85	0.88
		F				

Fig 1. Whole Plant of *Peperomia tetraphylla*

Fig 2. Ultra Violet - Visible Spectroscopy of PTC1

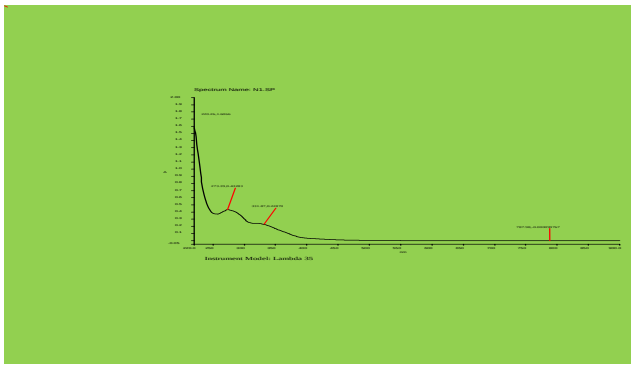


Fig 3. Infra Red Spectroscopy of PTC1

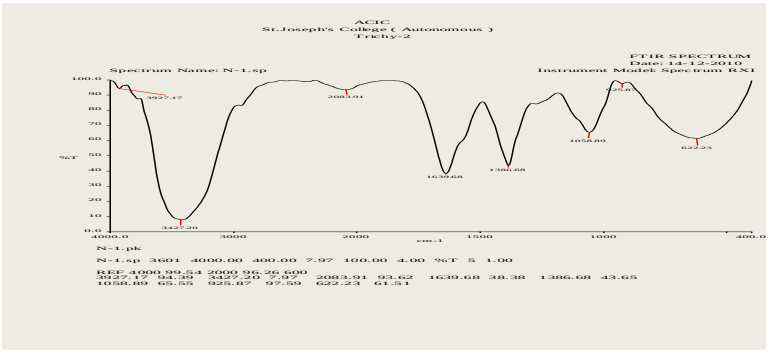
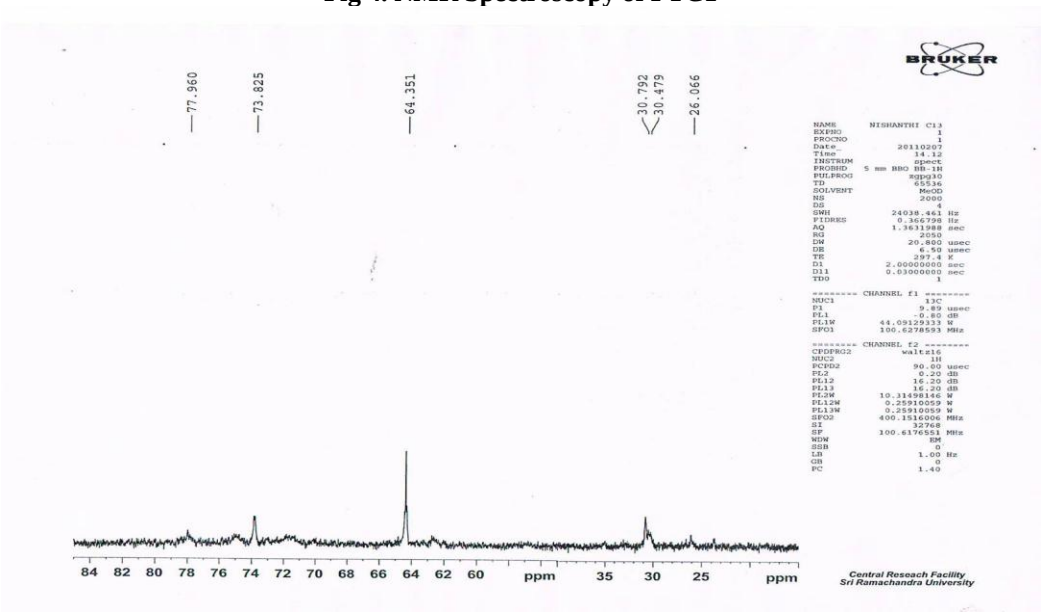


Fig 4. NMR Spectroscopy of PTC1



CONCLUSION

The preliminary phytochemical evaluation and spectral evaluation with UV, IR, NMR was done on extracts of *Peperomia tetraphylla*. The data generated from these experiments have provided the chemical basis for the wide use of this plant as therapeutic agent for treating various ailments. However, there is need to carry out further advanced spectroscopic studies in order to elucidate the structure of these compounds. Further work therefore needs to be carried out on the fractions in order to isolate, purify and characterize the active chemical compounds which could be subjected to further toxicological analysis.

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