



RESEARCH ARTICLE

SECONDARY METABOLITE PROFILING OF *PIPER VELAYUDHANII* E. S.S. Kumar and S.P. Mathew

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ABSTRACT

Piper velayudhanii E. S. S. Kumar and S.P. Mathew is an endemic species confined mainly to upper Nilgiris, Tamilnadu, India. The plant has not been found a place either in ethnomedicinal literature or phytochemical research. Hence the present work is aimed at secondary metabolite profiling of leaf and fruit to investigate the scope of bio prospecting one of the more than 2000 species of *Piper*. The study observed that leaf contains alkaloids, tannins, steroids, triterpenoids, saponins and Gum and Mucilages. Notably flavonoids could not make into the list. However, the fruit is demonstrated to contain alkaloids, tannins, triterpenoids, saponins, and gum and mucilages and fixed oil including steroids, flavonoids and glycosides. The FT – IR analysis of the leaf as well as the fruit exposed the array of bioactive functional groups associate with the secondary metabolites in *Piper velayudhanii* making the species worth pursuing pharmacological studies.

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INTRODUCTION

The genus *Piper* L. belongs to Piperaceae and has over 2000 species (Singh et al., 2011) distributed in both hemispheres. *Piper* species, widely distributed in the tropical and subtropical regions of the world are used medicinally in various manners. Plants belonging to the genus *Piper* are reputed in the Indian Ayurvedic system of medicine for their medicinal properties and in folklore medicine of Latin America and West Indies (Parmar et al., 1997). *Piper velayudhani* is an endemic wild species found in the upper Nilgiris of the Nilgiri Biosphere Reserve, 1700 meter above mean sea level. The extensive literature surveys did not account for any previous report on the uses of *Piper velayudhani*. Hence the present study is designed to demonstrate the secondary metabolite profile to analyze the medicinal potential, if proved to elevate the status of the species there by forest managers can plan to handle the threat to the species.

MATERIALS AND METHODS

Piper velayudhani (Fig.1) is collected from Lamb's Rock, Coonoor, Nilgiris. The plant is identified with Botanical

Survey of India, Coimbatore. Freshly collected specimens, both leaves and fruits, are shade dried and powdered with a mortar and pestle. The successive extraction of the leaf and fruit are carried out according to the method suggested by Das et al., 2010. Secondary metabolite screening has been performed for phytochemicals such as alkaloids (Waldi 1965, Wanger 1993, 1996 and Evans, 1997), flavonoids (Trease and Evans, 2002), tannins (Segelman et al., 1969), steroids and triterpenoids (Finar, 1986), saponins (Kokate, 1999), Glycosides (Camporese et al., 2003) and fixed oils (Kokate, 1999). The FT-IR analysis was performed based on KBr pellet (or Alkali halide disc) method as described by Stuart (2004).

RESULTS AND DISCUSSION

The results of the secondary metabolite analysis of leaf (Table 1) and fruit (Table 2) are given below. Phytochemicals such as alkaloids, tannins, steroids, triterpenoids, saponins and Gum and Mucilages are observed in the leaf of *Piper velayudhanii*. The ethanol extract yielded much of the metabolites except flavanoids, triterpenoids and fixed oil. However, the water extract contained all the above mentioned compounds such as alkaloids, tannins, steroids and triterpenoids. It is worth mentioning that flavonoids are absent in the leaves.

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Figure 1. *Piper velayudhanii*

When petroleum ether extracted steroids, gums and mucilage and fixed oil the ethyl acetate fraction contained steroids and fixed oil. Unlike leaf *P. velayudhanii* fruit on successive extraction yielded an array of secondary metabolites. Phytocompounds such as alkaloids, flavonoidstannins, steroids, triterpenoids, saponins, glycosides and gum and mucilages and fixed oil are extracted into various solvents. The most successful extract is found to be ethanol which yielded alkaloids, flavonoids tannins, triterpenoids, and gum and mucilages and fixed oil except steroids, saponins and glycosides whereas water yielded only triterpenoids, saponins and fixed oil. Ethyl Acetate is observed to be productive in having alkaloids, steroids, triterpenoids, glycosides and fixed oil. Except tannins, flavanoids and saponis all other compounds are found to be present in the fruit of *P. velayudhanii*. The secondary metabolite screening of both leaf and fruit of *P. velayudhanii* demonstrate a significant result that secondary metabolites vary in composition as well as structure and properties between leaf and fruit. This observation requires further elaboration and characterization. The FT- IR spectrum obtained for leaf and fruit are illustrated in Figure 2 and 3 respectively. The respective functional groups corresponding to the IR absorption of the leaf powder with metabolites are listed in Table 3. The functional groups in turn represent the secondary metabolites identified in the screening. The functional groups corresponding to the respective IR spectra are interpreted by following Bassler and Morrill (2007).

Table 1. Secondary metabolite screening of successive extracts of *P. velayudhanii* leaves

S. No.	Chemical Constituents	Tests	Leaf			
			Organic solvents			Water
			PE	EA	Eth	
1	Alkaloids	a) Dragendorff's test	-	-	+	+
		b) Mayer's test	-	-	+	+
		c) Wagner's test	-	-	+	+
		d) Hager's test	-	-	+	+
2	Tannins	5% FeCl ₃ test	-	-	+	+
3	Steroids	Liebermann - Burchard's test	+	+	+	+
4	Triterpenoids	a) Liebermann - Burchard's test	-	-	-	+
5	Saponins	Foam test	-	-	+	-
6	Gum & Mucilages	Whistler and BeMiller test	+	-	+	-
7	Fixed oils	Spot test	+	+	-	-

PE – Petroleum Ether; EA – Ethyl acetate; Eth – Ethanol;

“+” Indicates presence of compounds; “-” Indicates absence of compounds

Table 2. Secondary metabolite screening of successive extracts of *P. velayudhanii* Fruit

No.	Chemical Constituents	Tests	Fruit			
			Organic solvents			Water
			PE	EA	Eth	
1	Alkaloids	a) Dragendorff's test	-	+	+	-
		b) Mayer's test	-	-	+	-
		c) Wagner's test	+	+	+	-
		d) Hager's test	-	-	+	-
2	Flavonoids	10% HCl & 5% NaOH test	-	-	+	-
3	Tannins	5% FeCl ₃ test	-	-	+	-
4	Steroids	Liebermann - Burchard's test	+	+	-	-
5	Triterpenoids	a) Liebermann - Burchard's test	+	+	+	+
6	Saponins	Foam test	-	-	-	+
7	Glycosides	Keller - Kiliani test	+	+	-	-
8	Gum & Mucilages	Whistler and BeMiller test	+	-	+	-
9	Fixed oils	Spot test	+	+	+	+

PE – Petroleum Ether; EA – Ethyl acetate; Eth – Ethanol;

“+” Indicates presence of compounds; “-” Indicates absence of compounds

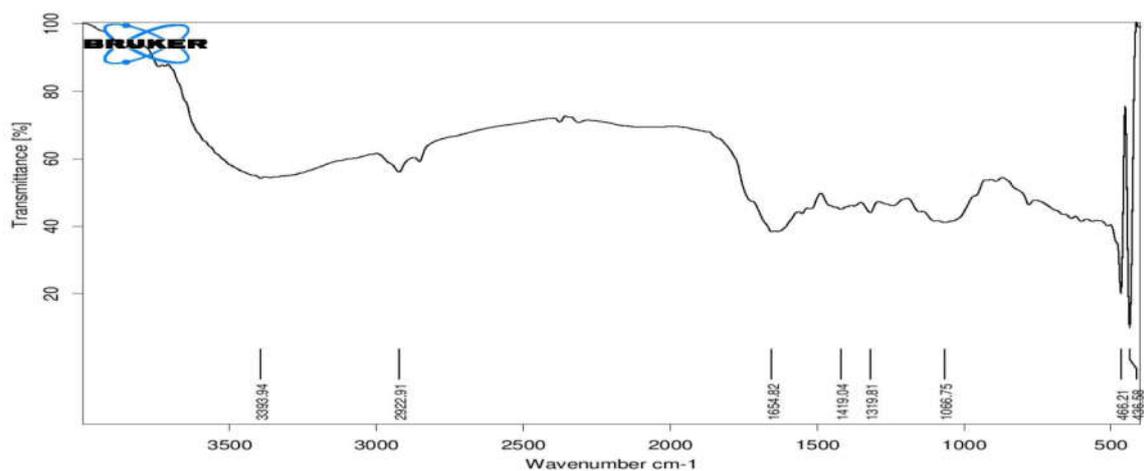


Fig.2. FT- IR Spectrum of leaf powder

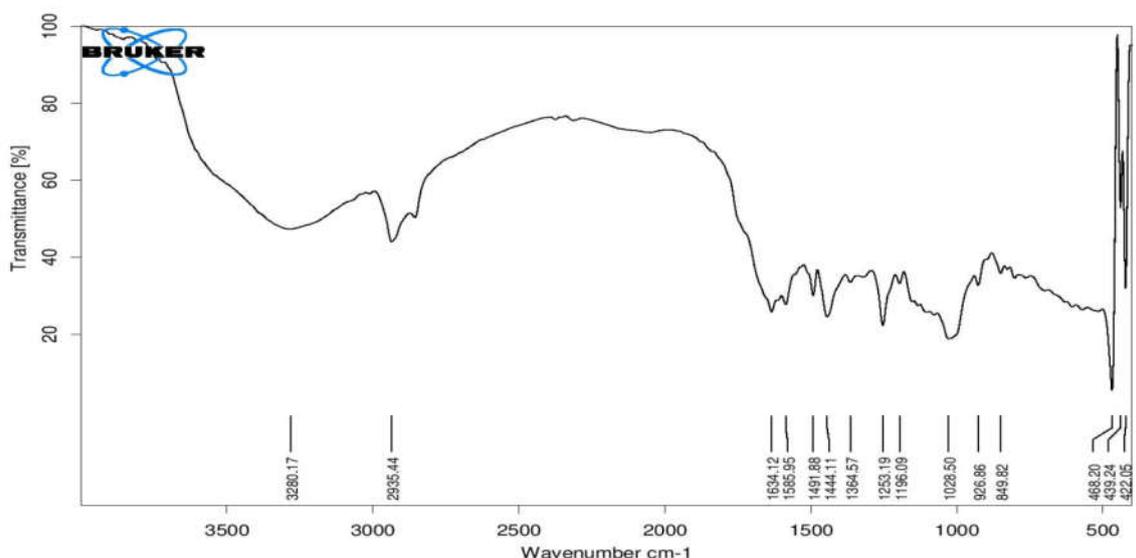


Fig.3. FTIR Spectrum of fruit powder

Table 3. Functional groups corresponding to the IR Spectrum of leaf powder

Wave Number cm^{-1}	Range	Functional Group
3393.94	3100–3500	N - H
2922.91	3000–2840	C-H
1654.82	1620 - 1680	C=C
1419.04	1400 - 1600	C = C
1319.81	1200 - 1450	O - H
1066.75	1050-1150	C - O

Table 4. Functional groups corresponding to the IR Spectrum of fruit powder

WaveNumber cm^{-1}	Range	Functional Group
3280.17	3100–3500	N - H
2935.44	2840 - 3000	C-H
1634.12	1620 - 1680	C = C
1585.95	1400 - 1600	C = C
1491.88	1400 - 1600	C = C
1444.11	1350– 1480	-C - H
1364.57	1350– 1480	-C - H
1253.19	1080 - 1360	C - N
1196.09	1180 - 1200	C - O - C
1028.50	1050 - 1150	C - O
926.86	900 - 950	OH
849.82	675 - 1000	= C - H

The figure 3 represents the FT- IR spectrum obtained for fruit and demonstrate a large array of functional groups in correspondence with secondary metabolite screening of the fruit. The various functional groups obtained during FT – IR analysis of the fruit powder indicate that large number of critical functional groups capable of initiating bioactivity are sufficiently present in the *Pipervelayudhanii* fruit. FTIR spectroscopy of the current research proved that is a reliable and sensitive method for detection of bioactive compounds. The FTIR spectral range is not only used to determine functional groups of a molecule, but it also provides a characteristic fingerprint region that can be used to uniquely identify the compounds. The FTIR spectroscopy allows the analysis of a relevant amount of compositional and structural information in plants and is a time-saving method to characterize and identify the functional groups (Grube *et al.*, 2008). The advantage of the infrared technique is that it can be nondestructive, requires a relatively small amount of sample, is fast and is accurate (Stuar, 2004; Lin *et al.*, 2011). Infrared technique does not require a reagent, so this method is more eco-friendly.

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