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HPTLC and GC-MS analysis of *Parthenocissus renukae* Anto & Pradeep

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Abstract
Parthenocissus renukae Anto & Pradeep belongs to family Vitaceae is a woody climber that spreads on rocks, grasslands and climb on large trees. It may badly affect the supporting tree by completely covering its canopy finally leading to the death of host tree. The study aims to find out the presence of alkaloid by HPTLC analysis with alkaloid as standard reference marker and to identify the secondary metabolites and bioactive compounds from the stem ethanol extract of *P. renukae*, by Gas chromatography and Mass spectroscopy (GC-MS). HPTLC analysis shows similar retention factor values and peaks between sample and alkaloid reference marker that confirms the presence of alkaloid. GC-MS shows the presence of alkaloids, fatty acid esters, flavonoids and alcohols and also other bioactive compounds like are 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione, Hexadecanoic acid, Hexadecanoic acid ethyl ester, Octadecanoic acid ethyl ester and Docosenamide. Further studies are needed to isolate active compounds of the extract as well as to reveal their exact mechanism of action in various diseases.

Keywords: *Parthenocissus renukae*, vitaceae, HPTLC analysis, GC-MS analysis

1. Introduction
Plant is an important source of medicine and plays a key role in world health [1]. Herbal medicines proved to be the major remedy in traditional system of medicine. They have been used extensively in medical practices since ancient times. This prompts the development in the practices of medicinal plants. The reasons are because of their biomedical benefits as well as place in cultural beliefs in many parts of world in the development of potent therapeutic agents [2]. The therapeutic potency of a medicinal plant is due to the presence of some bioactive components. These bioactive components are ascertained using phytochemical screening such as phytochemical tests and thin layer chromatography [3].
The species of Vitaceae family shows many medicinal properties like antiulcer activity, anxiolytic, anticonvulsant, antiradical and antibacterial activity. They are also used for the treatment of several diseases such as rheumatism, epilepsy, stroke and also in the treatment of diabetes [4]. *Parthenocissus renukae* member of Vitaceae is selected for the current study. It usually spread on rocks, grasslands and climb on large trees. It may badly affect the supporting tree by completely covering its canopy finally leading to the death of host tree [5].
The present work aims in determining the phytochemical constituents present in *P. renukae* by HPTLC and GC-MS technique.

2. Materials and Methods
2.1 Plant Exploration
During the field trip conducted for taxonomical collection the plant *P. renukae* was collected from hilly regions of Mangad, Thrissur. And its identity was confirmed by referring relevant literature.

2.2 Plant extraction method
Shade dried stem powder weighing 10g *P. renukae* were extracted with 300ml of ethanol using soxhlet apparatus. The extract was filtered & concentrated using vacuum distillation, under high pressure at 110°C. It is then subjected to screen the phyto chemicals.

2.3 HPTLC analysis of ethanol stem extract of plant sample for alkaloid profile
The plant sample was centrifuged at 3000rpm for 5min and this solution was used as test solution for HPTLC analysis. 2 µl of test solution and 2 µl of standard solution were loaded as 5mm band length in the 3x 10 Silica gel $60F_{254}$ TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument. The plates loaded with samples was kept in TLC twin trough developing chamber (after saturated with Solvent vapour) with respective mobile phase

(Ethyl acetate-Methanol-Water [10:1.35:1]) and the plate was developed up to 90mm. The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in Photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images at visible light, UV 254nm and UV 366nm.The developed plate was sprayed with respective spray reagent (Alkaloid) and dried at 100°C in Hot air oven. The plate was photo-documented in Visible light and UV 366nm mode using Photo-documentation (CAMAG REPROSTAR 3) chamber. Before derivatization, the plate was fixed in scanner stage (CAMAG TLC SCANNER 3) and scanning was done at UV 366nm. The Peak table, Peak Chromatogram and Peak densitogram were noted. The software used was win CATS 1.3.4 version.

2.4 GC-MS analysis

In the present study the ethanol stem extract of *P. renukae* was subjected to GC-MS analysis using Thermo GC-Trace Ultra Ver: 5.O, Thermo MS DSQ II and gas chromatograph interfaced to a mass Spectrometer (GC-MS) instrument. The following conditions were maintained while running GC-MS, column DB 5- MS Capillary standard non- polar column with the dimension of 30 MTS, ID: 0.25 mm Film: 0.25µM Helium was used as carrier gas at a constant flow of 1.0 ml/min, The oven temperature was programmed from 70°C to 260°C at increasing rate of 6°C/min. Injection volume of the sample was 1micro liter. Interpretation on mass spectrum of GC-MS was done by comparing the mass spectral data with Wiley and NIST data base. From this the compound name, probability, molecular formula, molecular weight, & structure of the components of the test materials were ascertained.

3. Results and Discussion

3.1 HPTLC analysis

Table 1: Peak table

Track	Peak	Rf	Height	Area	Assigned substance
Sample S1	1	0.35	80.7	1696.3	Alkaloid 1
Sample S1	2	0.38	64.2	3276	Alkaloid 2
Sample S1	3	0.55	21.6	749.4	Unknown
Sample S1	4	0.64	17.5	758.2	Unknown
Sample S1	5	0.80	133.6	3421.3	Unknown
Sample S1	6	0.85	30.2	819.2	Unknown
Sample S1	7	0.95	215.4	8047	Unknown
STD	1	0.31	477.3	34186.2	Alkaloid standard

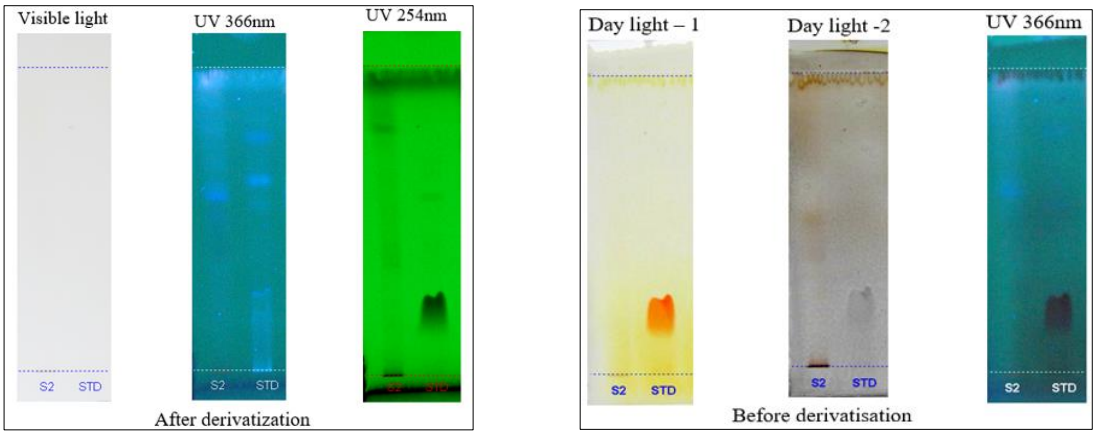

Fig 1: HPTLC Chromatogram of stem ethanol extracts of *P. renukae* and standard alcohol

HPTLC analysis showed the presence of alkaloid in the stem ethanol extract. Alkaloid standard was used as a reference marker. The Rf (Retention factor) value of standard is 0.31 and the rf value of first 2 peaks are 0.35 and 0.38. They are comparable with the standard and thus identified as alkaloids (Table- 1).

After derivatization an orange-brown coloured zone at Day light mode 1 was observed in the standard track and sample track (disappeared soon after appeared), which confirmed the presence of Alkaloid in the sample. Also a brownish yellow coloured zone at Day light mode 2 was observed in the sample track, after derivatization with 10% Ethanolicsulphuric acid reagent which may be due the presence of Alkaloid/Nitrogen containing compounds in the given sample (Fig 1).

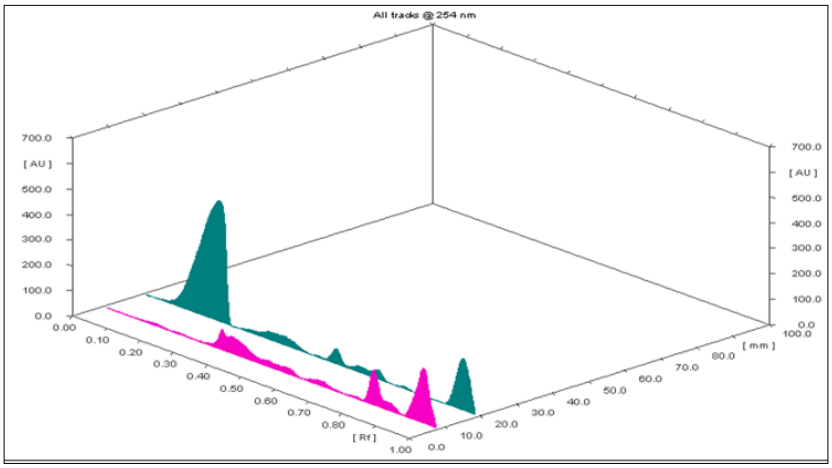

Fig 2: 3D densitogram of stem ethanol extract of *P. renukae* and alcohol standard tracks at 254nm.

The 3-D display of all tracks at 254nm was prepared and observed. It also shows similarity at certain regions. So the similar regions shows the presence of alkaloid in the sample (Fig 2).

3.2 GC-MS analysis

The GC – spectrum of stem ethanol extract of *P. renukae* plant exhibited seven major peaks at retention time 9.24, 12.96, 16.18, 20.36, 26.25, 31.34 and 35.36.

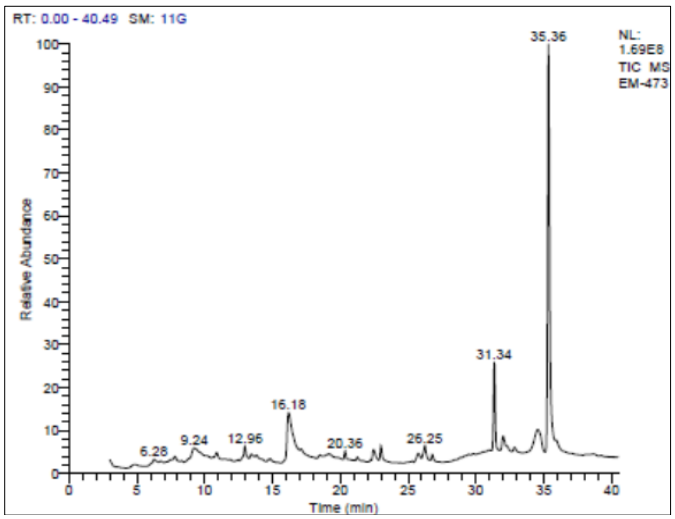

Fig 3: GC-MS chromatogram for stem ethanol extract of *P. renukae*

The major findings are listed in the below table (Table 2)

Table 2: Major findings of GC-MS Analysis based on the loss of functional group

RT	M+	m/z	Fuctional group lost	Compound
9.24	50	335	$-C_4H_2$	Straight chain alcohol
	41	294	$-C_3H_5$	
	29	265	$-C_2H_5$	
	54	211	$-C_4H_6$	
	27	184	$-C_2H_3$	
	39	145	$-C_3H_3$	
	29	97	$-C_2H_5$	
RT	**M+**	**m/z**	**Fuctional group lost**	**Compound**
12.96	72	343	Acetyl	Fatty acid ester
	29	274	CHO	
	31	243	$-OCH_3$	
RT	**M+**	**m/z**	**Fuctional group lost**	**Compound**
16.18	29	289,212	–CHO	Flavonoid
	18	129,81	H2O	
	17	195	–OH	
RT	**M+**	**m/z**	**Fuctional group lost**	**Compound**
20.36	19	223	H_3O^+	Straight chain alcohol.
	18	149	H_2O	
	17	132	-OH	
	29	328	C_2H_5	
	56	167,76	C4H8	
RT	**M+**	**m/z**	**Fuctional group lost**	**Compound**
26.25	71	335	$C_3H_7CHNH_2$	Alkaloid
	41	181	CH_2CN	
	30	151	CH_2NH_2	
	54	97,223	C_2H_4CN	
	28	69	CH=NH	

Similar patterns were observed for the spectrum at retention time 31.34 and 35.36. So these compounds may also be alkaloids. Hence the extract contains fatty acid esters, flavonoids, alcohols and alkaloids.

Since the major compounds includes alkaloids, alcohols, flavonoids and fatty acid esters they can be incorporated in medicines. Many alkaloids are obtained from plants and that have medicinal properties, like nicotine, papaverine, vindesine, vincristine etc. Alcohols are prescribed for all sorts of ailments from snake bite to disease control. Flavonoids shows anti-allergic, anti-oxidant, anti-microbial and anti-cancer activities.

Certain compounds having probability above 70% are obtained at minor peaks with retention time 21.26, 22.44, 22.99, 26.78 and 35.36. The compounds are 7, 9-Di-tert-butyl-1-oxaspiro (4, 5) deca-6, 9-diene-2, 8-dione, Hexadecanoic acid, Hexadecanoic acid ethyl ester, Octadecanoic acid ethyl ester and Docosenamide respectively. At retention time 21.26 a compound is obtained. It shows 78.18% similarity with 7, 9-Di-tert-butyl-1-oxaspiro (4, 5) deca-6, 9-diene-2, 8-dione ($C_{17}H_{24}O_3$). The molecular mass of this compound is 276. The molecular mass of resultant may be 261.2 or 281. At retention time 22.44 a compound is obtained which shows 68.17% similarity with Hexadecanoic acid (Palmiticacid) ($C_{16}H_{32}O_2$). Molecular mass of this compound is 256 and the molecular mass of the resultant is 256.3. Consumption of palmitic acid increases risk of developing cardiovascular diseases. At retention time 22.99 compound showing similarity of 72.43% with Hexadecanoic acid, ethyl ester ($C_{18}H_{36}O_2$) is obtained. Its molecular mass is 284 and that of resultant is 284.3. At retention time 26.78 a compound is obtained which shows 72.49% with Octadecanoic acid, ethyl ester (stearic acid ethyl ester). Its molecular mass is 312 and that of resultant is 312.3. At retention time 35.36 a compound is obtained which shows 79.57% similarity with 13-Docosenamide (Erucylamide) ($C_{22}H_{43}NO$). Its molecular mass 337 and the molecular mass of resultant is 337.4. All these compounds are used in medicines as well as for other purposes. The difference of compounds from the library search results may be due to the presence or absence of any functional groups. For this kindly send the corresponding MS spectrum for modification.

4. Conclusion

This plant shows the presence of many secondary metabolites and bio active compounds that are medicinally and industrially important. Further research work is required to find out bioactive molecules and their mechanism of action to explore their therapeutic potential before it can be recommended for clinical use.

5. References

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