INTERNATIONAL RESEARCH JOURNAL OF PHARMACY

www.irjponline.com

ISSN 2230 – 8407

Research Article

A STUDY ON PHYTOCHEMICAL ANALYSIS OF *PHYLLANTHUS TENELLUS* ROXB.

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Article Received on: 02/06/17 Approved for publication: 28/06/17

DOI: 10.7897/2230-8407.086104

ABSTRACT

Phyllanthus tenellus is a dominant plant in herbaceous family to appear frequently in various humid environments in Warangal region in Telangana. This plant is used to treat different disease like urolithiasis, inflammatory bowel disease, diabetes and hepatitis B. The present study developed phylogenetic relationship with secondary metabolites and other related compounds of 2 other species of *Phyllanthus* belongs to family Euphorbiaceae. This investigation was to establish the phylogenetic relationship with secondary metabolites and other related compounds of *Phyllanthus tenellus* of Euphorbiaceae family. Among them, *Phyllanthus tenellus* shows unique relationship with secondary metabolites, protein related compound.

Keywords: Phytochemical analysis, *Phyllanthus amarus, Phyllanthus emblica, Phyllanthus tenellus*

INTRODUCTION

The Indian medicinal plants of the native flora are frenzied with little or no knowledge of their pharmacological properties. These plants are generally used for medical purposes other than those of home-grown and local communities. Furthermore, it is estimated that there is nearly 25,000 plant species are used for the production of worldwide medicines, including those synthesized from natural products and those are commercialized herbal medicines[1]. The toxicity of medicinal plants and the resultant medicines from them may seem to be a little importance compared to that of conventional treatments. However, this is not the case for public health; the toxicity of medicinal plants is a serious problem[2]. The unfavorable effects of plant medicines, of individual products or synergistic action i.e. interaction with other drugs, are currently unknown[3].

Research on the plant medicines and derived drugs is still at an early stage, and the control of their commercialization by official agencies is not well maintained in free fairs, stores or public markets for natural products. Now a days, there are approximately 120 clinical products are used in allopathic medicine that are originated from plants used by indigenous groups. These plant drugs are extensively commercialized in various countries like France, Italy, United Kingdom, Asian countries and in the USA[4].

Phyllanthus family is one of the largest and commonest plant families belong to Euphorbiaceae. *Phyllanthus* are used traditionally to treat different diseases like diabetes, hepatitis B and urolithiasis inflammatory bowel disease. One of the species of *Phyllanthus* is a "stone breaker" (quebra-pedra) is Phyllanthus *tenellus* Roxb. which is inhabitant to a common weed in humid environments and Brazilian tropical and sub-tropical regions[5]. This species is found in cultivated areas, sidewalk edges land strips and garden seed beds. Various literature reported by scientists P*hyllanthus tenellus* Roxb. possesses immune modulatory, analgesic and anti-hepatitis activity[6-9]. Regarding the chemical composition, different authors examined different aerial parts of *Phyllanthus tenellus Roxb*[10-11]. Hence the present investigation was to establish the phylogenetic relationship with secondary metabolites and other related compounds of *Phyllanthus tenellus* of Euphorbiaceae family with 2 other *Phyllanthus* species i.e. *Phyllanthus amarus* and *Phyllanthus emblica* of the same family

MATERIAL AND METHODS

Plant materials

Three species of *Phyllanthus* plant materials were used (Table 1) of the family Euphorbiaceae were collected different areas of Telangana, India. Samples were deposited in Herbarium of Botany Department, Kakatiya University. The whole plants were oven dried at 60°C for one week, and powdered and stored in airtight containers. 10 g of each of the powdered plant materials were extracted in a soxhlet extractor containing 40 ml of 80% methanol. The resulting extracts were evaporated under reduced pressure. The plant specimens were collected with flower and fruit for the purpose of determination of their identity. The specimens were identified with the help of volume II of Flora of the Presidency of Madras[12] and the current global taxonomic literature and e-floras on Euphorbiaceae. A brief description of each and every taxon studied is provided using the standard, universally accepted terminology for comparison and assessment of their morphological relationships. It is intended to provide a contrast to the chemical relationships currently revealed.

Amino Acids

The shade-dried plant material of each species was made into powder, which was extracted with 80% methanol. The extract was then treated with chloroform in order to remove the chlorophyll pigment. The mixture was shaken thoroughly and was allowed to stand for a day in a separating funnel. The supernatant liquid was removed and it was used for the detection

of the amino acids. The chromatographic separation was carried out on Whatman No.1 chromatographic paper (28 x 23 cm). A fraction (0.01ml) of the extract was used for separation. The solvent system used in the first direction was BAW (n-butanol: Acetic acid: Water; 4:1:1 v/v). In the second direction, the paper was placed perpendicular to the first in the solvent containing phenol and water (4:1).

The paper was rolled into a vertical cylinder and the chromatogram was run in a chromatographic chamber (cylindrical glass jar). The paper after development was dried and sprayed with 0.2% ninhydrin in acetone. It was allowed to air dry. Then it was kept in hot air oven for 10 minutes, maintained at 60ºC, comparing the R_F values with authentic samples (**Loba-chemei Indoaustranal Co, Bombay)**, the amino acids were identified. The R_F (mobility relative to front) values (0.01-0.99), which did not tally with those of the standards, were designated as A-W.

The R_F values were calculated as:

$$R_F = \frac{\text{The distance travelled by compound}}{\text{The distance travelled by solvent}}$$

Phenolic Acids

The phenolic acids are ubiquitous in almost all the plant groups. Therefore, an attempt has been made to survey the phenolic acids and secondary metabolites, both known and unknown, present in the 3 taxa of South Indian Euphorbiaceae (Table 2). The shade dried plant material of various species of *Phyllanthus* are first made free from dust and then boiled in 2N HCl for about 20 minutes on water both. It was then filtered and cooled. The filtrate was then extracted with diethyl ether. This was repeated twice or thrice. The extract was then concentrated and it was used for two dimensional ascending paper chromatography. Whatman No. 1 chromatographic paper of 20 x 20 cm dimensions was used. A vertical line leaving a margin of 3 cm from the left and a horizontal line, 3 cm above the lower margin of the paper, were drawn. With a micropipette, the concentrated ether extract was applied on the point of interaction of these two lines. After it is dried up, the same was repeated. The solvent system used in the first direction was 1% HCl propanol: ammonia: water (10:1:4 v/v) were used in the second direction. The procedure regarding the in-rolling of the filter paper and its lowering into the chromatographic chamber containing the solvent system was the same adopted for the amino acids. The solvent system was allowed to run for about 2 hr in the second direction.Every time the chromatographic paper was removed from the chamber, the solvent front was marked before it was allowed to dry while hanging to a rope with plastic clips. Then, the paper was sprayed with ninhydrin (prepared in acetone) for the development of spots. The chromatographic paper was again allowed to dry in the air. A number of spots of different colours developed on the chromatographic paper. With a pencil, the spots were ringed and their central points were noted to calculate the R_f values in both the directions. The spots were identified by comparing the position, colour and R_f values of these spots with those of the authentic samples of phenolic acids run earlier under identical conditions. Apart from the spots identified, a number of other spots with different colours and hRf values (100 x R_f) which did not tally with those of authentic samples were also noted. These were treated as 'unknown' phenolic acids and designated as A-Z, AA-AZ and BB-BR with their hR_f values.

Secondary Metabolites

Eight secondary metabolites, namely alkaloids, ellagic acid, iridoids, lignans, methylene-dioxy compounds, steroids, tannins and terpenoids were tested for their presence using the standard phytochemical tests[13-17]. The following chemical tests were employed to identify the secondary metabolites (Table 2).

RESULTS AND DISCUSSION

Amino acids

21 amino acids are detected in the 3 species of Phyllanthus investigated (Table.2). Besides, there are 24 non-protein amino acids present in them. The commonly found amino acid is L-Proline. It is present in all 3 species of Phyllanthus (*P.amarus, P.emblica, P.tenellus)*. D.L Alanine was observed in *P.amarus, P.tenellus.* DL.Isoleucine and DL-Methionine are common in *P.amarus*, L-Hydroxy Proline is present in *P.emblica* whereas DL-Dopa, Glycine, L-Histidine HCl L-Lysine HCl. Were found in *P.tenellus*. Were observed that maximum amino acids are found in *P.tenellus* among the *Phyllanthus* species are taken. T(hRf 44.4) occurs in *P.amarus, P.emblica, P.tenellus* whereas F(hRf 9.7) is found in *P.tenellus* J(hRf 20.7) is observed in *P.emblica, P.tenellus*. The non-protein amino acids C(hRf 5.1), K(hRf 26.4), R(hRf 37.9) are found in *P.amarus* and the non-protein amino acids S(hRf 40.3) present *P.tenellus* and Q(hRf 35.9) observed in *P.emblica* given in table 2.

Phenolic acids

There are 10 Known and 4 unknown phenolic acids detected in the 3 species of *Phyllanthus.* Of the known phenolic acids Gentisic acid present in all species of *Phyllanthus*. Protocatechuic acid is present *P.amarus* and *P.tenellus.* Vanillic acid are present in *P.tenellus.* Salicylic acid is present in *P.amarus.*The unknown phenolic acid AB(hRf 70/55) is present in *P.tenellus* only other secondary metabolites. 3 species of *Phyllanthus* were tested for 8 secondary metabolites alkaloids are present in all species. While ellagic acid iridoids and steroids are found in both the species of *P.amarus* and *P.emblica.* Lignins, Tannins are characteristics of two species of *P.amarus* and *P.tenellus.* Tri, tetraphenols are found in *P.emblica* methylenedioxy compound is absent in all species of *Phyllanthus.*

Table 1: The species of Euphorbiaceae examined for phytochemical study

S. No.	Name of the Taxa	Source
1	*Phyllanthus amarus* Schum. & Thonn.	*A.Komuraiah*1812,Hanamkonda,TS
2	*Phyllanthus emblica* L.	*V.S.Raju* & *A. Ragan* 1815, Hanamkonda, TS
3	*Phyllanthus tenellus* Roxb.	*A. Komuraiah* 1828, Hanamkonda, TS

TS = Telangana State

Table 2: Distribution of chemical constituents in Phyllanthus

S. No.	Chemical Constituents	1	2	3
	AMINO ACIDS			
1	DL-Alanine	1	0	0
2	DL-2-Amino-n butyric acid	0	0	0
3	L-Arginine-HCl	0	0	0
4	L-Cysteine-HCl	0	0	0
5	DL-Dopa	0	0	0
6	L-Glutamic acid	0	0	0
7	Glycine	0	0	0
8	L-Histidine-HCl	0	0	0
9	L-Hydroxy praline	0	1	0
10	DL-Iso-Leucine	1	0	0
11	DL-Nor- Leucine	0	0	0
12	L- Leucine	0	0	0
13	L-Lysine-HCl	0	0	0
14	DL-Methionine	1	0	0
15	L-Ornithine-HCl	0	0	0
16	DL-β-Phenyl alanine	1	0	0
17	L-Proline	0	0	0
18	DL-Threonine	0	0	0
19	DL-Tryptophan	0	0	0
20	L-Tyrosine	0	0	0
21	DL-Valine	0	0	0
	UNKOWN AMINO ACIDS			
22	A (hRf 03.1)	0	0	0
23	B (hRf 04.2)	0	0	0
24	C (hRf 05.1)	0	0	0
25	D (hRf 06.5)	1	0	0
26	E (hRf 08.3)	0	0	0
27	F (hRf 09.7)	0	0	1
28	G (hRf 12.5)	0	0	0
29	H (hRf 14.6)	0	0	0
30	I (hRf 18.3)	0	0	0
31	J (hRf 20.7)	0	1	1
32	K (hRf 26.4)	1	0	0
33	L (hRf 27.4)	0	0	0
34	M (hRf 28.2)	0	0	0
35	N (hRf 30.0)	0	0	0
36	O (hRf 32.5)	0	0	0
37	P (hRf 34.3)	0	0	0
38	Q (hRf 35.9)	0	1	0
39	R (hRf 37.9)	1	0	0
40	S (hRf 40.3)	0	0	1
41	T (hRf 44.4)	1	1	0
42	U (hRf 48.0)	0	0	0
43	V (hRf 49.4)	0	0	0
44	W (hRf 58.6)	0	0	0
45	X (hRf 63.4)	0	0	0
	PHENOLIC ACIDS			
46	Benzoic acid	0	0	0
47	Caffeic acid	0	0	0
48	Cinnamic acid	0	0	0
49	Gentisic acid	1	1	1
50	Oxalic acid	0	0	0
51	Protocatachuic acid	1	0	0
52	P-OH-Benzoic acid	0	0	0
53	Salicylic acid	1	0	0
54	Vanillin	0	0	1
55	Vanilic acid	0	0	1
	UNKWON PHENOLIC ACIDS			
56	Y (hRf 75/13)	0	0	0
57	Z (hRf 33/6)	0	0	0
58	AA (hRf 47/5)	0	0	0
59	AB (hRf 70/55)	0	1	0
	SECONDARY METABOLITES			
60	Alkaloids	1	1	1
61	Ellagic acid	1	1	0
62	Iridoids	1	1	0
63	Lignans	0	1	1
64	Methylenedioxy compounds	0	0	0

65	Tannins	0	1	1
66	Steroids	1	1	0
67	Triterpenoids	0	1	0

Table 3: *Phyllanthus* plant secondary metabolites and chemical test

S.No	Secondary metabolites	Chemical Test
1.	Alkaloids	Alkaloids test
2.	Ellagic acid	Ellagic acid test
3.	Iridoids	Trim-Hill test
4.	Lignans	Badouni test
5.	Methylene-dioxy compounds	Labat test
6.	Steroids	Salkowski reaction
7.	Tannins	Tannin test
8.	Triterpenoids	Libermann-Burchard test

Table 4: Paired, Group Affinity and Isolation Values of genera of Phyllanthus

Taxa	Taxa				IV	
	1	2	3	GA	IVi	IVn
1	100	50	36	186	40	8.95
2		100	45	195	23.07	4.47
3			100	181	50	13.43

1.Phyllanthus amarus;* 2. *Phyllanthus emblica;* 3. *Phyllanthus tenellus

GA = Group Affinity; IV = isolation Values; Ivi = Individual isolation value, Ivn = The percentage of unique characters with in a taxon with respect to the grand total of all different characters in all taxa

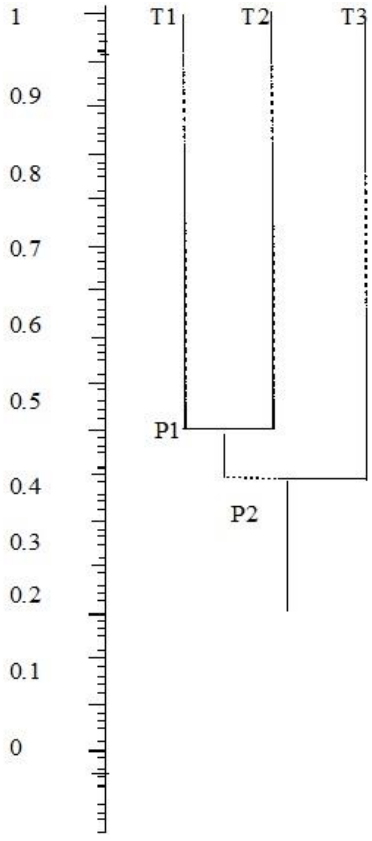

Figure 1: Phenogram of Phyllanthus

T1. *Phyllanthus amarus;* T2. *Phyllanthus emblica ;* T3. *Phyllanthus tenellus*.

Phytochemical diversity

The micromolecules found in the plants are innumerable in the present study, only the amino acids, phenolic acids and select secondary metabolites were chosen of these, as is expected, the greater number of species studies, the higher is the number or diversity of the compounds detected, therefore, the discussion is a comparison within the group (Genus Phyllanthus) as regards to the category of the micromolecules present in number (not quantity).In *Phyllanthus*, there are 21 known, and unknown protein amino acids, 10 known and 4 unknown phenolic acids besides all the 8 other secondary metabolites tested. It is very obvious the interrelationships are the percentage similarity is ranged between 50-19.5 naturally the group affinity value is more for *P.emblica* (19.5) less for *P.tenellus* (181). However, the individual isolation value is high (50) for *P.tenellus* and

unique character are high (Ivi) (40) and (Ivn) 8.95, Ivi (23.07) and Ivn (4.47) as such because of unique in nature

Phenetic relationship

The numerical assessment of the micromolecules in *Phyllanthus* are examined through the construction of dendrograms for the species of *Phyllanthus* the pointers are justified phenons between 50% and 45.5%, therefore, *P.amarus* T1 and *P.emblica* T2 join at 0.5 to form a group P1 (Figure 1) are allied pair than each other to *P. tenellus* (T3) with which they join at 0.40. The geographic evidence and economic value also reflect this point.The profile of three medicinal plants used in this study is shown in (Table 1). Tests were conducted in the presence of phytochemicals in all of these methanolic extracts (Table 2). Lignans, tannins and phenols were detected in all the 3 tested plants. These results are in parallel to the earlier studies conducted on terpenes, alkaloids, lignans, flavonoids and tannins in *Phyllanthus* species[18-21]. The difference in the findings might be due to the nature of the solvent used for extraction, which determines the presence or absence of a metabolite in the extract.

CONCLUSION

The present study of phylogenetic relationship based on secondary metabolites and other related compounds of 3 species of *Phyllanthus*. Among them, *Phyllanthus tenellus* shows the unique relationship with secondary metabolites, protein related compound and their antimicrobial activities.

ACKNOWLEDGMENT

Dr. A. Komuraiah, acknowledges the financial assistance from the University Grants Commission (UGC), New Delhi as the Minor research grant

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Cite this article as:

Ananthagiri Komuraiah and Ajmeera Ragan. A study on phytochemical analysis of *Phyllanthus tenellus* Roxb. Int. Res. J. Pharm. 2017;8(6):95-99 http://dx.doi.org/10.7897/2230-8407.086104

Source of support: Nil, Conflict of interest: None Declared