ISSN 2394 - 7837

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Qualitative Analysis of Phytochemicals in Leaf Extracts of Tree Phyllanthus

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Received 08/01/2015 Accepted 16/04/2015

Abstract

Preliminary qualitative analysis to identify metabolites by characteristic color changes revealed the presence of alkaloids, tannins, saponins, steroids, coumarins, flavanoids, terpenoids and cardiac glycosides. Presence of compounds varied with species as well as with the solvent fractions. Phlobatannin was absent in all fractions of leaf extracts of both the Phyllanthus species studied. Flavanoids were found in ethyl acetate, methanol and ethanol fractions of *P. emblica*. They were found in petroleum ether and chloroform fractions of *P. acidus* in addition to its presence in ethyl acetate fraction. Tests for alkaloids yielded positive results for all the fractions except the ethyl acetate and methanol extract of *P. acidus*. Terpenoids were absent in petroleum ether extracts of *P. emblica* but was present in that of *P. acidus*. The presence of such phytoconstituents in the leaf extracts of both the plants suggest the possibility of utilizing leaf extracts for isolation of active compounds which could be a potential source of hepatoprotective and antimicrobial compounds.

Keywords: Phytochemicals, *Phyllanthus acidus, Phyllanthus emblica*

Introduction

Man has always depended on plant diversity for his many needs including food, clothing, shelter and medicine. The widespread use of herbal remedies and healthcare preparations, as those described in ancient texts such as the Vedas and the Bible, and obtained from commonly used traditional herbs and medicinal plants, has been traced to the occurrence of natural products with medicinal properties (Hoareau and DaSilva, 1999). The unscrupulous use of plants for extraction of metabolites especially those found in roots and bark has become a threat to the existence of many medicinal plants. But the ever increasing demand for plant based drugs emphasizes the need for discovering alternative sources for these metabolites.

Plants of genus Phyllanthus (Euphorbiaceae) have long been used in traditional medicine for the treatment of liver disorders, fever, smallpox, vomiting, and bronchitis among many others. *Phyllanthus acidus* and *Phyllanthus emblica* are two important tree species of the genus well known for medicinal properties of their fruits (Md. Mominur Rahman *et al.*, 2011; Habib *et al.*, 2011), bark (Biswas *et al.*, 2011) and roots. But reports on the active principle from leaves are very few. Since leaf biomass of *P. acidus* and *P. emblica* are considerably good it is imperative to look for metabolites from them. This study is an attempt to this end and highlights the potential of using leaves for detection of active metabolites from these species for subsequent drug development.

Materials and Methods

The shade dried powered leaves of *Phyllanthus emblica* and *Phyllanthus acidus* were subjected to soxhlet extraction procedure using five solvents (petroleum ether-PTE, chloroform-CHL, ethyl acetate-EA, ethanol-ET and methanol-MT) of different polarity. Qualitative analysis of phytochemicals was done using standard procedures (Siddiqui and Ali, 1997; Harborne, 1998). Presence of flavanoids was tested with the use of NaOH and H_2SO_4 test; tannins with ferric chloride solution (Iyengar, 1995), coumarins with $FeCl_3$ and Fluorescence test and saponins with its ability to produce stable foam and steroids with Libermann Burchard reagent. The test for alkaloids was carried out using Mayer's reagent, Wagner's reagent (Shanmugam *et al.*, 2010), Dragendroff's reagent as well as Marquis Test (Siddiqui, and Ali, 1997). For cardiac glycosides, the Killer-Kiliani test (Shanmugam *et al.*, 2010) was adopted. Salkowski test revealed the presence of terpenoids. Phenols tested with $FeCl_3$ and Phlobatanins using HCl (Shanmugam *et al.*, 2010). The presence of metabolites was identified by characteristic color changes. These tests were carried out in triplicate using various concentrations of sample.

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Results

The phytochemical screening of leaf extracts of *P. emblica* and *P. acidus* showed the presence of secondary metabolites like alkaloids, cardiac glycosides, flavanoids, terpenoids, tannins, steroids, saponin and coumarins. Different fractions of the extracts contained different metabolites. Considerable variability was observed in the chemical constituents of both the species studied (Table 1). Tests for alkaloids yielded positive results for all the fractions except the EA and MT extract of *P. acidus* (Fig. 1). Froth test of saponin was positive for PTE, CHL and ET fractions of *P. emblica*. The saponin level was comparatively higher in PTE and EA fractions of *P. acidus* leaves. While flavanoids were found in EA, MT and ET fractions of *P. emblica*, they were found in PTE and EA fractions of *P. acidus* (Table 1). Terpenoids were absent in PTE extracts of *P. emblica* but was present in that of *P. acidus*. In *P. acidus* leaf extracts, except for EA fraction, positive test result was obtained for tannins (Table 1).

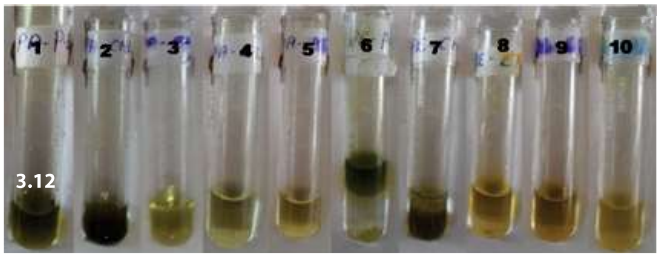

Figure 1. Qualitative Test (Mayer's test) Result for Alkaloids of Leaf Extracts of Phyllanthus Species: (1-5) *P. acidus* extracts (6-10) *P. emblica* extracts (In the order Petroleum ether, Chloroform, Ethyl Acetate, Ethanol, Methanol)

Table 1. Results of phytochemical analysis of *Phyllanthus acidus* and *Phyllanthus emblica* leaf extracts

Metabolite	*Phyllanthus emblica*					*Phyllanthus acidus*				
	PTE	CHL	EA	ET	MT	PTE	CHL	EA	ET	MT
Alkaloids	+	+	+	+	+	+	+	+	+	-
Flavanoids	-	-	+	+	+	+	-	+	+	+
Steroids	+	+	-	+	+	+	+	-	-	-
Terpenoids	-	-	+	+	+	+	-	+	-	-
Saponins	+	+	+	-	-	+	-	+	-	-
Phenols	+	+	+	+	+	+	+	+	+	+
Coumarins	-	-	+	+	+	+	+	+	+	+
Cardiac glycosides	-	+	+	-	-	+	+	+	-	-
Tannin	-	-	+	+	+	+	+	-	+	+
Phlobatanin	-	-	-	-	-	-	-	-	-	-

'+' indicate- Presence; '-' indicate-Absence; PTE-petroleum ether; CHL-chloroform; EA- ethyl acetate; ET- ethanol; MT- methanol

Discussion

A number of the Phyllanthus species have been reported to have extensive history in medical systems (Unander *et al.*, 1990; Mdlolo *et al.*, 2008) and are known for its numerous antimicrobial and antiviral activities. The antimicrobial effect of plant extracts could be due to the presence of phytochemicals like alkaloids, flavanoids, saponins and phenolic compounds (Cushnie and Lamb, 2005). Flavanoids, tannins and phenolic compounds from fruits of both *P. acidus* (Amadi *et al.*, 2010) and *P. emblica* (Gulati *et al.*, 1995) have been reported to have high antioxidant and hepatoprotective property. Alcoholic extracts of *P. emblica* leaves yielded tannins which are in agreement with the previous reports (Dhale and Mogle, 2011). The presence of such phytoconstituents in the leaf extracts of both the plants analyzed in the present study suggests the possibility of using leaves as an alternative source of hepatoprotective compounds as well as antimicrobial agents.

Absence of flavanoids in chloroform fractions of *P. emblica* is supported by earlier studies of Dhale and Mogle (2011). In *P. acidus* leaf extracts, except for EA fraction, positive test result was obtained for tannins (Table 1). Con-

versely, previous phytochemical analysis of extracts of bark (Biswas *et al.*, 2011) and fruits (Habib *et al.*, 2011) of the species reported absence of tannins. This clearly indicates that different classes of secondary compounds accumulate in different plant parts. The relative amount of phytochemical substances from plant extraction depends on the solubility of the phytochemical in the solvent used for extraction (Olowosulu and Ibrahim 2006). Hence it is necessary to evaluate each plant part to understand the presence of phytochemicals in order to isolate and purify different phytochemicals for pharmaceutical purposes.

Conclusion

Present study revealed that like the fruits and bark of P. *acidus* and *P. emblica*, leaf extracts are also rich in chemical constituents which may be tapped for use in pharmaceuticals without causing much damage to the plants as such. Further studies are in progress to quantify and assess the biological properties of the active principles from the leaf extracts of these plants.

Acknowledgement

The authors thank University Grants Commission (UGC), New Delhi, India for the financial support in form of Minor Research Project to JF and NEA.

References

1. Amadi, J.E, Salami, S.O and Eze, C.S. 2010. Antifungal properties and phytochemical screening of extracts of African Basil (*Ocimum gratissimum* L.). Agric. Biol. J. N. Am., 1(2): 163-166.
2. Biswas SK, Anusua Chowdhury, Joysree Das, Utpal Kumar Karmakar, Manik Chandra Shill and Sheikh Zahir Raihan. 2011. Assessment of cytotoxicity, antibacterial activity and phytochemical screening of ethanol extract of *Phyllanthus acidus* L. (family: Euphorbiacceae) bark. J. Appl. Pharma. Sci. 01 (06): 112-114
3. Cushnie TPT and Andrew J Lamb. 2005. Antimicrobial activity of flavonoids. Int.J. Antimicrobial Agents, 26: 343-356
4. Dhale and Mogle 2011. Phytochemical Screening and Antibacterial Activity of *Phyllanthus emblica* (L.) Science Research Reporter 1(3): 138 -142
5. Gulati, R.K., S. Agarwal and S.S. Agarwal. 1995. Hepatoprotective studies on *Phyllanthus emblica* Linn and quercetin. Ind. J. Exp. Biol., 33: 261-268.
6. Habib MR, Sayeed MA, Rahman MM, Hasan MR and Saha A. 2011. In Vitro Evaluation of Cytotoxic, Antibacterial, Antioxidant and Phytochemical Screening of Petroleum Ether Extract of *Phyllanthus acidus*. Int. J. Pharma. Sci. Res. 2(4):875-881
7. Harborne JB. 1998. Phytochemical methods: a guide to modern techniques of plant analysis. (3rd ed.) London: Chapman & Hall. ISBN: 0-412-57270-2, 302 p
8. Hoareau L, DaSilva EJ. 1999. Medicinal plants: A reemerging health aid. Electronic J Biotechnol 2: 56–70.
9. Iyengar, M.A. 1995. Study of Crude Drugs.8th ed., Manipal Power Press, Manipal, India.pp. 2.
10. Md. Mominur Rahman, Md. Razibul Habiba, S. M. Raquibul Hasanb, Mohammed Aktar Sayeeda, Md. Sohel Ranac. 2011. Antibacterial, Cytotoxic and Antioxidant Potential of Methanolic Extract of *Phyllanthus acidus* L. Int. J. Drug Dev. & Res., 3 (2): 154-161
11. Mdlolo CM., Shandu J S and Oyedeji OA. 2008. Phytochemical constituents and antimicrobial studies of two South African Phyllanthus species. Afr. J. Biotechnol.Vol. 7 (5): 639-643
12. Olowosulu, A.K and Ibrahim, Y.K.E. 2006. Studies on the antimicrobial screening of Aqueous extracts of five plants used in Folk medicine in Nigeria. West Afr. J. Biol. Sci., 3(5): 21-26.
13. Shanmugam S, Sathish T. Kumar and K. Panneer Selvam. 2010. Laboratory handbook on Biochemistry. Published by Asoke K Ghosh, PHL learning PVT LTD, New Delhi.
14. Siddiqui, A.A. and Ali, M. 1997: Practical Pharmaceutical Chemistry. First ed., CBS Publishers and Distributors, New Delhi, pp. 126-131.
15. Unander D.W., Webster D.W., Blumberg B.S. 1990. Record of usage or assays in Phyllanthus (Euphorbiaceae) I. Subgenera Isocladus, Kirganelia, Cicca and Emblica. J Ethnopharmacol. 30: 233- 264.