Middle-East Journal of Scientific Research 10 (1): 78-81, 2011
ISSN 1990-9233

Preliminary Comparative Phytochemical Screening of *Diospyros lotus* Stewart

[1]*Ghias Uddin,* [1]*Abdur Rauf,* [2]*Bina Shaheen Siddiqui and* [3]*Syed Qaiser Shah*

[1]Centre for Phytomedicine and Medicinal Organic Chemistry,
Institute of Chemical Sciences, University of Peshawar, Peshawar, Pakistan
[2] HEJ research Institute of Chemistry, University of Karachi, Karachi, Pakistan
[3]Centre for Nuclear and Molecular Studies, University of Peshawar, Peshawar, Pakistan

Abstract: Phytochemical screening is one of the necessary steps to find out the chemical constituents which lead the isolation of compounds. Diospyros lotus extract (leaves, bark, hard wood and roots) was performed for the biologically active secondary metabolites: alkaloids, flavonoids, steroids, terpenoids, saponins, anthraquinones, tannins, phlobatanins, glycoside, reducing sugars. Phytochemical screening revealed the presence of anthraquinones, terpenoids, steroids and tannins in the extracts. The roots extract which is generally used as a folk medicine due to the presence of anthraquinones, terpenoids and steroids. The leaves extracts contain steroids, terpenoids and tannins while the bark extracts showed the presence of anthraquinones, terpenoids and steroids. The hard wood extracts showed the presence of anthraquinones and terpenoids.

Key words: Phytochemical screening · *Diospyros lotus* · Anthraquinones · Terpenoids · Steroids · Tannins

INTRODUCTION

Plants are natural source of producing wide number of phytoconstituents in a most efficient way and with precise selectivity. Since the middle of the 19th century, different bioactive phytoconsitutants have been isolated and characterized. Many of these are used as the active ingredients of the modern medicine, or as the lead compounds for new drugs discovery. Several plant derived medicines, are rich in phenolic compounds [1], such as those used in protection against coronary heart diseases and carcinogenesis [2]. *Diospyros lotus* which belongs to family Ebenaceae consists of about 500 species is distributed in tropical and subtropical regions of the world. The genus *Diospyros* is important one containing more than 350 species. The characteristic features of the *Diospyros* species are tree. The *Diospyros lotus* tree growing to size ranging about 9 m in height by 6 m in width in medium rate. The plant grows well in acidic, neutral and alkaline components containing soil. It can also grow in semi-shade or no shade area. It requires moist soil [3].This plant is rarely found in Britain and native of the Himalayas. From old days the different *Diospyros* species are known for their medicinal uses. In many traditional medicinal systems of the world, a number of *Diospyros* plants are used as medicinal agents against various diseases. All parts of these plants have been used for medicinal purposes such as the leaves are used for lumbago; the fruits are carminative, astringent and cure biliousness the seeds are sedative and the bark is bitter, astringent and febrifuge. Leaf extract of Japanese persimmon *D. khaki* in combination with jasmine is useful in Japan for making anti-tobacco smoking candies since leaves of some of the *Diospyros* species e.g. *D. melanoxylon* and *D. tomentosa* are also used for wrapping bidis. The triterpenoids belonging to the lupane, oleanane and ursane series have anti-inflammatory activity [4-5]. *Diospyros* species in traditional medicinal system of the world are used as antifungal and (a) for internal hemorrhage and bedwetting in children (b) woman's medicine, for insomnia and hiccough (c) antihypertentsive (d) dysponea (e) vermicide and vermifuge (f) sedative (g) antifebrile (h) promotes secretions (I) astringent, and bactericidal [6-7].

Corresponding Author: Dr. Ghias Uddin, Institute of Chemical Sciences, Centre for Phytomedicine and Medicinal Organic Chemistry, University of Peshawar, Peshawar, Pakistan.
Fax: +92-91-9216652.

Triterpenoids have been isolated from *Diospyros lotus*, showing anti-inflammatory activity [4, 8]. Phytochemical studies have been previously carried out on many *Diospyros* species and have revealed the widespread presence of naphthoquinones and naphthalene derivatives, dimeric naphthoquinones and lupane triterpenes [9].

METERIALS AND METHODS

Plant Material: *Diospyros lotus* roots were collected from Razagram, Toormang district Dir Khyber Pakhtunkhwa (KPK) province of Pakistan during the month of August, 2009. The plant was identified by Prof. Dr. Abdur Rashid, Department of Botany, University of Peshawar, Peshawar, Pakistan and a Voucher specimen (Rauf-6645) was deposited at the Herbarium, Department of Botany, University of Peshawar, Peshawar, Pakistan.

Extraction and Fractionation: The shaded dried and crushed roots of *Diospyros lotus* (875 g) was subjected to cold extraction with MeOH (3 x 10 L) at room temperature. The extract was then concentrated by Rota-vapor under reduced pressure at temperature below 50°C. The final residue obtained was weighed 37.5 g. This methanolic extract was suspended in water and successively partitioned.

Phytochemical Screening: The chemical tests were performed on the n-hexane, chloroform, ethyl acetate and methanolic extracts of *Diospyros lotus* whole plant using standard procedure to identify the constituents as described by Sofowora [10], Trease and Evans [11] and Harborne [12].

Alkaloids: About 0.2 g of each of extract was warmed with 2% H_2SO_4 for two minutes. Then they were filtered and a few drops of Dragendrof's reagent were added to each filtrate. No orange red precipitate indicated the absence of alkaloids.

Tannins: A small quantity of each extract was mixed with water and heated on water bath and then filtered. A few drops of ferric chloride were added to each of the filtrates. A dark green solution indicated the presence of tannins.

Anthraquinones: About 0.5 g of each extract was boiled with 10 % HCl for few minutes in water bath, filtered and allowed to cool. Equal volume of $CHCl_3$ was added to the filtrates. Few drops of 10% ammonia was added to the mixtures and heated. Formation of rose-pink color indicated the presence of anthraquinones.

Glycosides: The extracts (0.5 g) were hydrolyzed with HCl and neutralized with NaOH solution. A few drops of Fehling's solution A and B were added. No red precipitate indicated the absence of glycosides.

Reducing Sugars: The extracts were shaken with distilled water, filtered and boiled with few drops of Fehling's solution A and B for few minutes. No orange/red color indicates the absence of reducing sugars.

Saponins: About 0.2 g of the extract was shaken with 5ml of distilled water and then heated to boiling. No frothing (appearance of creamy miss of small bubbles) shows the absence of saponins.

Flavonides: Extracts of about 0.2 g were dissolved in diluted NaOH and HCl was added. No yellow solution that turns colorless indicates the absence of flavonides.

Phlobatanins: The extracts (0.5 g) were dissolved in distilled water and filtered. The filtrate was boiled with 2% HCl solution. Nored precipitate shows the absence of phlobatanins.

Steroids: Each extract (0.5 g) was dissolved in 2 ml of acetic anhydride and added 2 ml of conc. sulphuric acid. The color change from violet to blue or green in some samples indicated the presence of steroids.

Terpenoids Test: 0.2 g of each extract was mixed with 2 ml of chloroform and concentrated H_2SO_4 (3 ml) was carefully added to form a layer. The formation of a reddish brown coloration at the interface indicated positive results for the presence of terpenoids.

RESULTS

The total weight percentage yield of the crude extracts of methanol, hexane, chloroform and ethyl acetate of *Diospyros lotus* are shown in Table 1 while the preliminary phytochemical screening results of the whole plant is shown in Table 2 to Table 5.

Table 1: Percents yield of roots, leaves, hard wood and bark of *Diospyros lotus*

Parts	Methanol	n-Hexane	Chloroform	Ethyl acetate
Roots	4.285%	7.6%	10.67%	26.66%
Leaves	1.7%	6.33%	8.11.6%	20.22%
Bark	1.99%	6.80%	9.83%	21.16
Roots	5.53%	8.32%	12.13%	28.37%

Table 2: Phytochemical screening of the crude extracts *Diospyros lotus* hard wood.

Chemical components	n-Hexane ext.	Chloroform ext.	Ethyl acetate ext.	Methanol ext.
Alkaloids	-	-	-	-
Steroids	-	-	-	
Terpenoids	-	+	+	+
Flavonides	-	-	-	-
Anthraquinones	-	+	+	+
Tannins	-	-	-	-
Phlobatanins	-	-	-	-
Saponins	-	-	-	-
Glycoside	-	-	-	-
Reducing sugars	-	-	-	-

Table 3: Phytochemical screening of the crude extracts of *Diospyros lotus* leaves.

Chemical components	n-Hexane ext.	Chloroform ext.	Ethyl acetate ext.	Methanol ext.
Alkaloids	-	-	-	-
Steroids	+	+	-	+
Terpenoids	-	-	+	+
Flavonides	-	-	-	-
Anthraquinones	-	-	-	-
Tannins	-	-	+	+
Phlobatanins	-	-	-	-
Saponins	-	-	-	-
Glycoside	-	-	-	-
Reducing sugars	-	-	-	-

Table 4: Phytochemical screening of the crude extracts of *Diospyros lotus* bark.

Chemical components	n-Hexane ext.	Chloroform ext.	Ethyl acetate ext.	Methanol ext.
Alkaloids	-	-	-	-
Steroids	+	+	-	+
Terpenoids	-	+	+	+
Flavonides	-	-	-	-
Anthraquinones	-	+	+	+
Tannins	-	-	-	-
Phlobatanins	-	-	-	-
Saponins	-	-	-	-
Glycoside	-	-	-	-
Reducing sugars	-	+	-	-

Table 5: Phytochemical screening of the crude extracts of *Diospyros lotus* Roots.

Chemical components	n-Hexane ext	Chloroform ext	Ethyl acetate ext	Methanol ext
Alkaloids	-	-	-	-
Steroids	+	+	-	+
Terpenoids	-	+	+	+
Flavonides	-	-	-	-
Anthraqunones	-	+	+	+
Tannins	-	-	-	-
Phlobatanins	-	-	-	-
Saponins	-	-	-	-
Glycoside	-	-	-	-
Reducing sugars	-	-	-	-

Key word: ext.: extracts

DISCUSSION

Phytochemical screening of various extracts of the *Diospyros lotus* (roots, leaves, bark and hard wood) fractions showed the presence of secondary metabolites, anthraquinones, terpenoids, steroids and tannins. The medicinal value of the title plant can be correlated due to the presence of various bioactive chemical constituents. Crude methanolic extract of the plant showed the presence of polar and non polar phytoconstituents. The plant is used as a folk medicine is due to the presence of bio-active phytoconstituents which need further research. In the present study crude methanolic extract of *Diospyros lotus* contains all the polar and non polar components present in the roots. The presence of anthraquinones, terpenoids and steroids in the roots has not been reported before. The literature revealed that the genus *Diospyros* have pesticide and biological activities [4-8, 13]. The specific activity of the *Diospyros* may be attributed to the presence of anthraquinones, terpenoids and steroids which need further investigation. The roots are used as a folk medicine which related to the presence of anthraquinones, terpenoids and steroids in the roots extract. Small amount of extract was obtained from leaves as compared to roots. The crude methanolic, ethyl acetate and chloroform extracts obtained from leaves showed the presence of terpenoids and tannins, while bark extracts showed the presence of terpenoids, steroids and anthraquinones. The presence of such components in bark may have good antinfalmatory activities but no work have been reported so far. Similarly the hard wood extracts also showed the presence of anthraquinones, terpenoids and steroids, which need further investigation.

ACKNOWLEDGEMENT

The authors greatly acknowledge the Higher Education Commission for financial support.

REFERENCES

1. Scalbert, A., 1993. Polyphenolic Phenomena, INRA Editions. Versailles Cedex, France, pp: 15-16.
2. Hertog, I., M.G. Kromhout, D. Aravanis, C. Blackburn, H. Buzina, R. Fidanza, F. Giampaoli, S. A. Jansen, Menotti and A.S. Nedeljkvoic, 1995. Archintern medicine, 155: 381-386.
3. Chittendon, F., 1956. Dictionary of Plants plus Supplement, Oxford University Press, Oxford, UK.
4. Watt, J.M. and M.G. Breyer-Brandwijk, 1932. The Medicinal and Poisonous Plants of South Africa, E. and S. Livingstone: Edinburgh, pp: 92-95.
5. Chopra, R.N., S.L. Nayar and I.C. Chopra, 1956. Glossary of Indian Medicinal Plants; CSIR: New Delhi, India.
6. Tezuka, M., C. Takahashi, M. Kuroyanagi, M. Satake, K. Yoshihira and S. Natori, 1973. Phytochemistry, (12): 175-183.
7. Thomas, P.S., G. Karagianis and R. Brun, 2006. Phytochemistry, 67: 1950.
8. Chopra, R.N., S.L. Nayar and I.C. Chopra, 1956. In Glossary of Indian Medicinal Plants, CSIR: New Delhi, India.
9. Hegnauer, R., 1989. Ebenaceae, Chemotaxonomie der Pflanzen, Birkhauser Verlag, (8): 402-411.
10. Sofowora, A., 1993. Medicinal plants and Traditional Medicine in Africa. John Wiley and son Ltd., pp: 150-153.
11. Treasem, G.E. and W.C. Evans, 1989. Pharmacology, 11th Edn. Brailliar Tiridel and Macmillian Publishers. London.
12. Herborne. J.B., 1973. Phytochemical Methods 3rd Edn. Chapman and Hall Ltd., London, pp: 135-203.
13. Kirtikar, K.R. and B.D. Basu, 1933. Indian Medicinal Plants 2nd Edn. Allahabad Press: Allahabad, India, pp: 274.