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MINI REVIEW

Analysis of Diospyrin: A Short Review

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Various analytical techniques are developed for detection, separation and quantification of a specific phytochemical or a component in a biological sample, particularly in relation to their therapeutic aspects. Diospyrin, a *bis* hydroxy naphthoquinonoid, is an important bioactive constituent of several *Diospyros* and *Euclea* spp. This compound has been identified as a prospective 'lead molecule' for novel chemotherapy for its significant *in vivo* and *in vitro* pharmacological activities. In this short review, some chromatographic and potentiometric methods of detection and quantitative estimation of diospyrin have been discussed.

Keywords: Diospyrin, HPTLC, HPLC, Differential pulse voltammetry.

INTRODUCTION

Plants are considered as a huge reservoir of structurally diverse compounds with a variety of pharmacological activities [1,2]. Out of nearly 98,000 species of higher plants around the globe only a small proportion has been investigated phytochemically as well as pharmacologically, leaving a large number of them still waiting to be studied in detail [3,4]. Thus, a multidisciplinary approach should be adopted for rapid chemical analysis and also simultaneously for biological screening of the plant extracts, so that we will get an information not only about their chemical constituents, but also to accomplish the development of interesting 'lead molecules' into important pharmacophore for maintenance of human health [5,6]. For metabolic profiling of a crude plant extracts, various analytical techniques are available in practice for distinguishing between structurally known compounds and novel molecules [7-10]. Some of them, such as thin layer chromatography (TLC), column chromatography, *etc.* are simple method, which is easy to run, reproducible and inexpensive. To eliminate long and tedious separation process, different sophisticated chromatographic and electrochemical technologies have been introduced for the simultaneous analysis of phytochemicals as well as screening against various pharmacological targets. High performance liquid chromatography (HPLC) coupled to a UV photodiode array detector (LC-PDA) or mass spectrometry (LC-MS) or nuclear magnetic resonance (LC-NMR) methods have been most commonly used for the analysis and structural identification of the compounds present in the crude plant extracts [11-18]. Now a days, high speed counter current chromatography (HSCCC), supercritical fluid extraction (SFE), capillary electrophoresis (CE) are also employed for this purpose [19-27].

Diospyrin, a dimeric hydroxy quinonoid is abundantly present in the stem bark of *Diospyros montana* Roxb. and also in the root and stem bark of several other *Diospyros and Euclea* spp [28-31]. Almost all the parts (stem, root, leaf, flower, seed, heartwood, twig, *etc.*) of these plants have been reported in traditional herbal medicines for the treatment of various ailments *viz.* diarrhoea, menor, high fever, pleuracy, pneumonia, wounds, ulcers, whooping cough, leprosy, jaundice. The fruits are applied externally to boils to heal sore skin. Crushed leaves and fruits are being used by the tribal people to stupefy and poison fishes [28,32]. Different parts of these plants also exhibit remarkable pharmacological activities and some of them reported that diospyrin was identified as the main bioactive principle responsible for those activities [33-46]. The stem bark extract of *Diospyros montana* inhibited the growth of *Ehrlich ascites* carcinoma in mice [47] and also it was found

to have antiprotozoal activity against *Leishmania donovani* promastigotes [48]. The root extract of *Euclea natelensis* was found to have antitubercular activity against both drug-sensitive and drug-resistant strains of *Mycobacterium tuberculosis* [49,50].

Several analytical methodologies for extraction, separation and standardization were adopted to identify the diospyrin and some other analogues quinonoids from tropical plant sources. Some aspects of the analytical techniques for the detection and estimation of diospyrin in different plant sources are described as follows:

Isolation and structural elucidation of diospyrin: Diospyrin was first isolated from the carbon tetrachloride and chloroform extracts of defatted stem bark of *Diospyros montana* Roxb. by solvent extraction followed by repeated crystallization [51]. Subsequently, isolation of diospyrin was reported by various groups of workers from other *Diospyros* and *Euclea* spp. [28-31] by using solvent extraction, preparative thin layer chromatography and soxhlation methods (Table-1). After several trials, Hazra *et al*. [52] described a modified soxhlation procedure to get a better yield of diospyrin.

The structure of diospyrin (Fig. 1) were elucidated through elaborate spectroscopic analysis in which, it was reported that it is a *bis*-naphthoquinone with a quinone-benzene linkage between C-2 and C-6′ [53,54]. Diospyrin is optically inactive and thus there is no restricted rotation around the connecting bond between C-2 and C-6′ [55]. However, the unequivocal confirmation of the structure was obtained through its total synthesis by Yoshida & Mori [56] and more recently by Pullella *et al*. [57]. Yoshida & Mori [56] successfully synthesized diospyrin by employing the Suzuki-Miyaura cross coupling reaction between two 7-methyljuglone units and established the structure as 2,6′-*bis*(5-hydroxy-7-methyl-1,4-naphthoquinone). Later on, it was reconfirmed by X-ray crystallographic analysis [58].

Analysis of diospyrin

High performance liquid chromatography (HPTLC) method: The quantitative estimation of diospyrin in the stem bark of *Diospyros montana* was first achieved by Ravishankara *et al.* [59] by using HPTLC technique. The method was very

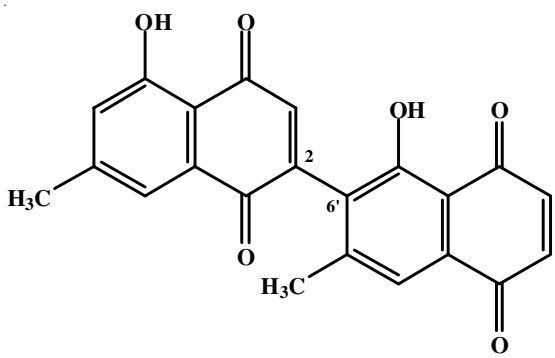

Fig. 1. Structure of diospyrin

simple, specific and sensitive, in which the plant samples were collected from different locations of Karnataka state of India. state, India. The analysis was carried out on a pre-coated silica gel HPTLC plate using an automatic sample spotter. The crude sample (10 µL) was applied triplicate on the TLC plate, developed in a solvent system of toluene:ethyl acetate:cyclohexane: glacial acetic acid (6:1:1:0.1, v/v/v/v) for 30 min at 25 ± 2 ºC and then scanned at 445 nm (R_f value of diospyrin = 0.64). By recording the peak areas, amount of diospyrin in the sample was estimated. By employing this technique, diospyrin was well resolved in the presence of other compounds in the crude extract of stem bark of *D. montanta* (LOD = 30 ng/spot, LOQ = 100 ng/spot in the range 100-500 ng/spot).

LC-UV method: Sanyal *et al.* [52] developed a reversed phase liquid chromatographic method coupled with PDA detector for rapid detection and quantitative estimation of diospyrin in a semi purified stem bark sample of *Diospyros montana*, collected from different climatic zones in eastern and north-eastern parts of India. An isocratic elution was done with a mobile phase of various concentrations of acetonitrile and water at three different pH range (pH = 4.9, 7.0 and 9.0; acidic and basic pH values of eluent were obtained by addition of 0.5% acetic acid and trimethyl amine, respectively) followed by UV detection at 255 nm. At ambient temperature with a flow rate of 1 mL/min, diospyrin was estimated in the crude sample at 14 min (pH = 4) by using acetonitrile-water (50:50, v/v) in isocratic mode. The R_f values of diospyrin in different

TABLE-1
DISTRIBUTION OF DIOSPYRIN IN VARIOUS *Diospyros* AND *Euclea spp*.

Diospyros sp. [28,29]				*Euclea sp*. [29-31]	
Plants	Part	Plants	Part	Plants	Part
D. abyssinica Hiern	Stem bark	*D. lycioides* Desf.	Root, stem	*E. crispa* Gürke	Root, fruit
D. assimilis Bedd.	Root	*D. mannii* Hiern	Stem bark	*E. divinorum* Hiern	Root
D. austro-africana De Win.	Root, stem	*D. mespiliformis* Hochst	Stem bark	*E. lanceolata* E.Mey.	Root bark
D. batocana Hiern	Root	*D. moonii* Thw.	Stem bark	*E. natalensis* A. DC.	Root, stem
D. chloroxylon Roxb.	Stem bark	*D. montana* Roxb.	Root, stem	*E. pseudebenus* E.Mey.	Root
D. cinnabarina Gürke	Stem bark	*D. natalensis* Brenan	Root, stem	*E. schimperi* A. DC.	Root
D. discolor Willd.	Root	*D. obliquifolia* Hiern	Stem bark	*E. undulata* Thunb	Root
D. fragrans Gürke	Wood	*D. piscatoria* Gürke	Root		
D. hirsuta L.f.	Stem bark	*D. quaesita* Thw.	Stem bark		
D. inhacaensis White	Root, stem	*D. rotundifolia* Hiern	Stem bark		
D. kaki Thunb	Root	*D. spinescens* Kosterm	Stem bark		
D. kamerunensis Gürke	Stem bark	*D. sylvatica* Roxb.	Root		
D. lotus Linn.	Root	*D. thwaitesii* Bedd.	Stem bark		
D. longiflora Let.& F.White	Stem bark	*D. walkeri* Gürke	Stem bark		

mobile phase compositions of acetonitrile and water is summarized in Table-2.

TABLE-2
LC-UV ANALYSIS OF DIOSPYRIN IN CRUDE CHLOROFORM EXTRACT OF *Diospyros montana* USING DIFFERENT COMPOSITION OF ACETONITRILE AND WATER IN ISOCRATIC MODE, FLOW RATE = 1 mL/min AT AMBIENT TEMPERATURE [52]

Isocratic eluent $CH_3CN:H_2O$	pH	Retention time (min) of diospyrin
60:40	7.0	7.1
50:50	4.0[a]	14.0
	9.0[b]	12.4
40:60	7.0	31.0

[a]Addition of 0.5% acetic acid; [b]By addition of triethyl amine

This method was found to be more sensitive than HPTLC technique [59], with ~ 4-5 times lower LOD (8 ng) and LOQ (20 ng) values over the concentration range of 1-1000 µg/mL of the sample. Jobert *et al.* [31] also reported an analytical HPLC method to quantify the concentration of diospyrin along with three analogous quinonoid compounds *viz.* 7-methyl juglone, isodiospyrin and neodiospyrin in the root extracts of eight different South African *Euclea* species. The chloroform extracts of root samples were analyzed on a C_{18} reversed phase column, using a mobile phase with a linear gradient of acetonitrile-water-acetic acid (62.5:32:0.5, v/v/v) at a flow rate of 1 mL/min at 25 ºC. The UV diode array detector was operated at 430, 325 and 254 nm. Simultaneous quantification of four quinonoids was achieved in a single HPLC run within 14 min. Out of eight tested samples, diospyrin was detected in only five *Euclea* species *viz*. *E. crispa*, *E. divinorum*, *E. natalensis*, *E. pseudebenus* and *E. undulata* and eluted isocratically at 10.3 min. The assay was linear in the range from 0.2-1000 µg/mL with low LOD and LOQ values of diospyrin.

DPV method: A simple and cost-effective differential pulse voltammetric technique was developed by Goulart *et al*. [60] for the determination of diospyrin in the crude chloroform extract of the stem bark of *Diospyros montana*. They designed a sensor, based on glassy carbon electrode coated with cobalt tetrasulfonated phthalocyanine (CoTSPc) and poly-L-lysine (PLL) film for rapid electron transfer and thereby increasing the sensitivity of the system.

The voltametric measurements were performed in a mixture of acetate buffer and DMSO (1:1, v/v) at pH 5.4 by using a saturated solution of Ag|AgCl|Cl^- as reference electrode, a Pt wire as auxiliary electrode and modified glassy carbon with CoTSPc and PLL as working electrode. The crude chloroform extract of *D. montana* was accurately weighed, dissolved in DMSO (200 µg/mL) and diluted with a mixture of acetate buffer:DMSO (1:1, v/v, pH = 5.4; 5 mL). An aliquot (50 µL) was added into the measurement cell and the voltammogram was obtained by differential pulse voltammetry. The method showed good electrochemical activity with high sensitivity (220.46 nA l $nmol^{-1}$ cm^{-2}) and repeatability (SD 4.4%) achieving both LOD (0.3 $nmol^{-1}$) and LOQ (1.0 $nmol^{-1}$) for diospyrin in nanomolar concentrations. The process was less time consuming without any prior step of preliminary separation of crude plant extract and also sensitive enough as compared to the HPTLC and LC-UV methods described earlier (Table-3).

TABLE-3
COMPARATIVE STUDY OF DIFFERENT ESTIMATION METHODS FOR THE QUANTIFICATION OF DIOSPYRIN IN CRUDE CHLOROFORM EXTRACT *Diospyros Montana*

Analytical technique	LOD	LOQ	Ref.
HPTLC	30 ng/spot	100 ng/spot	[59]
LC-UV	8 ng	20 ng	[52]
DPV	0.3 $nmol^{-1}$	1 $nmol^{-1}$	[60]

Conclusion

Diospyrin and some of its analogous compounds have been found to possess significant pharmacological activities and are useful as 'taxonomic markers' for the respective plants having various commercial importance. Several analytical techniques are being employed for detection and quantitative estimation of diospyrin. It is very much useful for pharmacognostic study of the plants in terms of proper identification, authentication, standardization and suitable quality control measures. For further development in this field, some modern technologies should be adopted to achieve more rapid and efficient analysis and also more precise quantification of this bioactive compound, which will be very helpful for the preparation of herbal monographs in future research on these plants.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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