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EXTRACTION, ANALYSIS AND APPLICATIONS OF DIOSGENIN: A SYSTEMATIC REVIEW

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ABSTRACT

Diosgenin (DG), a well-known steroidal sapogenin, is present abundantly in medicinal herbs such as Dioscorea rhizome, Dioscorea villosa, Trigonella foenum-graecum, Smilax China, and Rhizoma polgonati. DG is utilized as a major starting material for the production of steroidal drugs in the pharmaceutical industry. It exhibits anticancer, cardiovascular protective, anti-diabetes, neuroprotective, immunomodulatory, estrogenic, and skin protective effects, mainly by inducing apoptosis, suppressing malignant transformation, decreasing oxidative stress, preventing inflammatory events, promoting cellular differentiation/proliferation, and regulating T-cell immune response, etc. The present review work mainly focused on compilation of the data related to extraction, analysis and biopotential of Diosgenin from the previously published literatures.

Keywords *– Diosgenin, HPTLC, Isolation, Identification, HPLC Analysis.*

1. INTRODUCTION

Diosgenin (DG) is a naturally occurring steroidal sapogenin and most found in the plant species belonging to family Agavaceae, Dioscoreaceae, Liliaceae, Solanaceae, Scrophulariaceae, Amaryllidaceae, Leguminosae, and Rhamnaceae [1-6]. Diosgenin is present abundantly in medicinal plants such as *Dioscorea rhizome*, *Dioscorea villosa*, *Trigonella foenum-graecum*, *Smilax China*, and *Rhizoma polgonati*. It has been widely investigated for the management and treatment of different types of cancer, osteoporosis, cardiovascular diseases, atherosclerosis, diabetes mellitus, and skin diseases [7-12]. DG is being extensively studied in the treatment of neurological diseases [13]. Several studies have confirmed that DG and its derivatives have preventive and therapeutic effects against various neurological disorders. Animal experiments have revealed that DG is active in the treatment of nervous system diseases such as Parkinson's disease and Alzheimer's disease [14-16].

Regardless of its pharmacological activities in the treatment of numerous diseases, the clinical application of DG is sternly hindered due to its low aqueous solubility, poor bioavailability and pharmacokinetics, and rapid biotransformation under physiological conditions [17]. Numerous current reviews have provided an inclusive explanation of its pharmacological effects in cancer [7], diabetes mellitus, metabolic syndrome [18], and others [19]. The present review was aimed at providing collective information related to extraction, analysis, and biopotential / applications of Diosgenin reported in the previously published literatures.

2. PHYSICOCHEMICAL PROPERTIES OF DIOSGENIN

Diosgenin (25*R*-spirost-en-3*β*-ol) is a C27 spiroketal steroid sapogenin. The chemical structure of Diosgenin and its glycoside, Dioscin, is represented in Fig. 1. DG is a white needle crystal or light amorphous powder with a proven thermal and chemical stability under various physical conditions. Its molecular formula is $C_{27}H_{42}O_3$ with a relative molecular mass of 414.62 and melting point in the range 204-207°C. DG is relatively stable against temperature and light exposure but destabilized when exposed to hydrochloric acid [20]. DG is strongly hydrophobic (with Log), and it is insoluble in water with aqueous solubility around 0.7 ng/mL [21-23]. However, it is highly soluble in most nonpolar organic solvents (such as chloroform, dichloroethane, propanol, ethyl acetate, and propylacetate) and in partially polar solvents (such as acetone, methanol, and anhydrous ethanol).

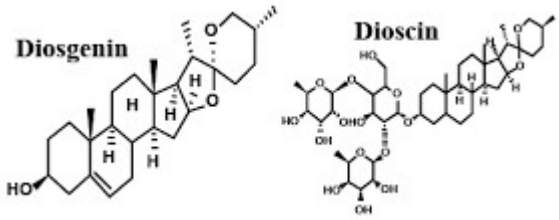

Fig. 1: Diosgenin and its glycoside (Dioscin)

3. EXTRACTION OF DIOSGENIN

DG is found in high concentration in tubers of various wild yams (*Dioscorea villosa* Linn). A total of 137 types of Dioscorea species contains DG. Of them, 41 contain DG at more than 1%. The seeds of fenugreek (*T. foenum graecum* Linn) [24] and the rhizomes of *Dioscorea zingiberensis* are also important sources of DG. In addition, *Trillium govanianum* and *Costus speciosus* contain around 2.5% and more than 2.12% of DG, respectively. DG is mainly generated by the hydrolysis of steroidal saponins in the presence of a strong acid, base, or enzyme catalyst. Currently, microbial transformation is a promising method for the production of DG because of its environmentally friendly, highly specific, and mild reaction conditions at a low cost [25-29].

Several studies have been reported in the previously published literature concerning the isolation and purification of diosgenin from different plant materials. Normally, direct acid hydrolysis of dioscin and spontaneous fermentation or enzymatic catalysis followed by liquid-liquid extraction (LLE) or solid phase extraction (SPE) are the most commonly used techniques to obtain diosgenin.

Nevertheless, these procedures can have disadvantages such as low efficiency, need of high volumes of solvents, and contamination of the extract with potentially toxic solvents and sometimes long extraction times are needed [30]. For these reasons, other methods have been developed to extract diosgenin, namely, from *Rhizoma dioscoreae*, involving supercritical fluid extraction (SFE) (using supercritical CO_2) after acid hydrolysis, followed by high-speed counter-current chromatography (HSCCC) with evaporative light scattering detection (ELSD) [31]. In addition, the previously referred to conventional techniques were optimized using multienzymatic catalysis in combination with acid hydrolysis, allowing obtaining high purity diosgenin (>96%) from *Dioscorea zingiberensis* [30]. Moreover, a focused microwave-assisted extraction (MAE) followed by acid hydrolysis was developed by *Kaufmann et al.* [32] to extract diosgenin from fenugreek (*Trigonella foenum* graecum) leaves and roots. The preparation of plasma samples for further analysis can be achieved through a single-step procedure of protein precipitation [33].

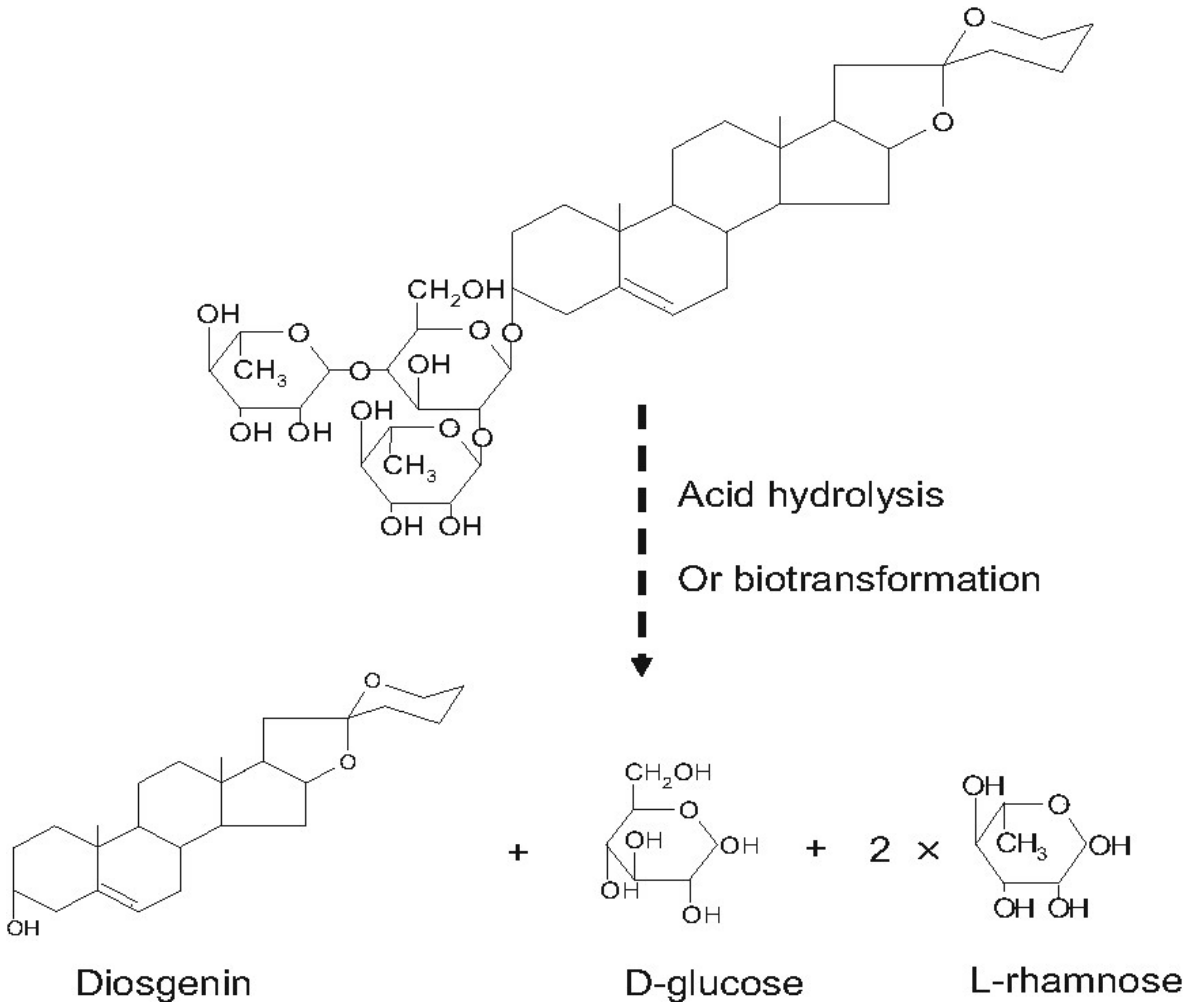

Fig. 2: Hydrolysis of Dioscin to Diosgenin

4. ANALYSIS OF DIOSGENIN

Various analytical methods have been reported in previously published literatures for qualitative and quantitative analysis of Diosgenin. In addition, as classical techniques presented some drawbacks, other methods have emerged, in particular, more advanced TLC methods (e.g., HPTLC), immunoenzymatic assays (ELISA), GC, LC, UPLC, UHPLC, and HPLC, coupled to different detectors. Nuclear magnetic resonance spectroscopy is another analytical technique which can be very useful in the detection and characterization of diosgenin and other sapogenins isolated from plants [34-35].

As a relevant example, an optimized and validated method involving TLC that overcomes the background interference problems in post derivatization was described by *Trivedi et al.* [36]. This was achieved through the use of a modified anisaldehyde-sulfuric acid reagent which allowed visualizing the spots and the quantification of diosgenin was performed by densitometry. Later, a validated TLC method for the simultaneous detection and quantification of diosgenin and sarsasapogenin in *Asparagus officinalis* L. was developed. In this method the plant extract was acid-hydrolyzed and after a liquid-liquid extraction a densitometric-TLC was performed. The results were verified by HPLC-UV and HPLC-MS [37].

The summary of the chromatographic conditions used for detection and estimation of diosgenin using modern analytical tools like HPLC and UPLC is given in Table-1[38].

Table 1: Analytical methodologies in different specimens for diosgenin determinations by means of HPLC and UHPLC

Matrix	Sample amount	Extraction process	Chromatographic conditions	Instrumental analysis
Dioscorea species and related medicinal plants (*Smilax* and *Heterosmilax* species)	0.5 g	15 mL of methanol at room temperature for 0.5 h Hydrolysis with HCl 10% under vacuum at 60°C LLE (10 mL of chloroform)	Mobile phase: 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) using an isocratic elution of 82% (B) in 0–10 min. Flow rate at 0.3 mL/min Column temperature: 40°C Stationary phase: Waters BEH C_{18} column (2.1 × 100 mm, 1.7 μm)	HPLC-DAD at 203 nm UPLC-MicroToFQ (ESI+)
Root extracts and polyherbal formulations containing *Smilax China*	10 g	SPE (Soxhlet apparatus with petroleum ether, chloroform, and methanol)	HPTLC Mobile phase: toluene: ethyl acetate (7: 3% v/v) HPLC Mobile phase: acetonitrile : water 90 : 10 (% v/v)	HPTLC and HPLC with densitometry: 425 nm
Berries extracts and formulations containing *Solanum nigrum*	20 g	LLE with 20% of H_2SO_4 in 70% IPA and hexane for 8 h	Mobile phase: acetonitrile: water 92: 08 (% v/v). Flow rate at 1.0 mL/min Column temperature: 25°C Stationary phase:	HPLC-DAD at 203 nm

			C_{18} Thermo Hypersil column (250 mm × 4.6 mm, 5 μm)	
Cultured cells of *Dioscorea zingiberensis*	0.1 g	20 mL of 95% ethanol, for 2 h. Hydrolysis with 20 mL H_2SO_4 1 M at 121°C for 2 h. LLE with petroleum ether. The combined petroleum and NaOH 1 M.	Mobile phase: acetonitrile: water 90: 10 (% v/v) Flow rate at 1.0 mL/min Column temperature: 30°C Stationary phase: reversed-phase Agilent TC-C_{18} column (250 × 4.6 mm, 5 μm)	HPLC-DAD at 203 and 410 nm
Cosmeceutical formulations	2.5 g	LLE with 10 mL of methanol mixed with 50% of tetrahydrofuran	Mobile phase: water: acetonitrile 15: 85 (% v/v) Column temperature: room temperature Stationary phase: Phenomenex Luna-C_{18} column (150 × 4.6 mm, 5 μm)	HPLC-DAD at 210 nm
Seed extract of *Trigonella foenum graecum*	1 g	SPE (Soxhlet with water and ethanol mixture (1: 1) for 72 h at 70°C). 80 mL of HCl 3 N for 1 h at 100°C. LLE with diethyl ether	Mobile phase: acetonitrile: water (10: 90 v/v) gradient mode. Flow rate at 1.0 mL/min. Column temperature: 30°C Stationary phase: reversed-phase Symmetry C_8 column (250 ×	HPTLC and HPLC-DAD at 205 nm

			4.6 mm, 5 μm)	
Pharmaceutical forms containing *Trigonella foenum graecum*	0.01 g	25 mL of methanol for 15 min	Mobile phase: acetonitrile: water 90: 10 (% v/v). Flow rate of 1.0 mL/min Column temperature: room temperature. Stationary phase: Phenomenex RP-C_{18} column (150 × 4.6 mm, 5 μm)	HPLC-UV at 203 nm
Polyherbal formulation containing *Tribulus terrestres* Linn.extract	1 g	90 mL HCl 3 N for 1 h 30 at 100°C LLE with 75 mL diethyl ether 75 mL	Mobile phase: methanol: water 15: 85 (% v/v), gradient mode. Flow rate at 1.0 mL/min Column temperature: 30°C Stationary phase: Symmetry RP-C_{18} column (250 × 4.6 mm, 5 μm)	HPLC-DAD at 205 nm
Rhizomes or tubers of various *Dioscorea* species and dietary supplements	0.5 g for solids and 1 mL for liquids	9 to 25 mL of methanol	Mobile phase: acetonitrile: water 75: 25 (v/v%) containing 0.05% formic acid. Flow rate at 0.27 mL/min. Column temperature: 40°C Stationary phase: Acquity UPLC™ BEH	UHPLC-ELSD and DAD

			Shield RP_{18} (100 × 2.1 mm, 1.7 μm)	

GC-MS methods are also described in the literature. An important work in this context was performed by *Taylor et al.* [39] which described the analysis of steroidal sapogenins from Amber Fenugreek *(Trigonella foenum graecum)* by capillary GC and combined GC-MS. As another relevant example, *Kaufmann et al*. [32] described an analytical method for the detection of diosgenin in different plant parts (seeds, air-dried roots, and both air-dried and fresh leaves) of fenugreek *(Trigonella foenum graecum)* using a microwave-assisted extraction and capillary GC-MS.

5. BIOPOTENTIAL / APPLICATIONS OF DIOSGENIN

Diosgenin, biologically active phytochemicals have been used for the treatment of various types of disorder such as leukemia, inflammation, hypercholesterolemia, and cancer. It is also able to prevent bone loss to the same extent as that of oestrogen[40].

Diosgenin has shown a vast range of pharmacological activities in preclinical studies. It exhibits anticancer, cardiovascular protective, anti-diabetes, neuroprotective, immunomodulatory, estrogenic, and skin protective effects, mainly by inducing apoptosis, suppressing malignant transformation, decreasing oxidative stress, preventing inflammatory events, promoting cellular differentiation/proliferation, and regulating T-cell immune response, etc. It interferes with cell death pathways and their regulators to induce apoptosis. Diosgenin antagonizes tumor metastasis by modulating epithelial-mesenchymal transition and actin cytoskeleton to change cellular motility, suppressing degradation of matrix barrier, and inhibiting angiogenesis. Additionally, diosgenin improves antioxidant status and inhibits lipid peroxidation. Its anti-inflammatory activity is through inhibiting production of pro-inflammatory cytokines, enzymes, and adhesion molecules. Furthermore, diosgenin drives cellular growth/differentiation through the estrogen receptor (ER) cascade and transcriptional factor PPAR [41]. Diosgenin is a precursor for several hormones, starting with the Marker degradation process, which includes synthesis of progesterone [42]. The process was used in the early manufacturing of combined oral contraceptive pills [43].

6. CONCLUSION

Diosgenin and its derivatives have attracted considerable attention from researchers worldwide. Several studies have described the pharmacological effects of DG and its derivatives against a variety of diseases such as cancer, diabetes, osteoporosis, and stroke. Due to its pharmacological relevance, several analytical assays have been reported in the literature over the last years to detect and quantify diosgenin in different matrices, including natural sources and pharmaceutical compositions containing it, and also in animal matrices in pharmacological studies. The compiled information will be useful to the scientists / researchers and manufacturers for extraction, isolation, identification, and quantitation of Diosgenin in the plant raw material, crude extracts, and their formulations.

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