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A REVIEW OF DIFFERENT EXTRACTION METHODS OF SAPONIN GLYCOSIDES FROM *SAPINDUS EMARGINATUS*

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ABSTRACT: *Sapindus emarginatus Vahl*. is extensively used in Indian traditional and folklore medicines to cure various human ailments. The preliminary phytochemical screening of the leaves revealed the presence of saponins, terpenoids, tannins, acids, flavonoids, cardiac glycosides, and sugars. Saponin applications were observed due to various biological, medicinal, and pharmaceutical actions. Several other uses for *Sapindus* have also been reported, such as making arrows from wood and decorative objects from seeds. The presence of saponin glycosides in the plant parts produces many beneficial pharmacological actions. These Sapindus drugs contain different active constituents, which various methods of extraction and separation methods can isolate. The choice of extraction procedure depends on the nature of the plant material and the components to be isolated. Maceration extraction, Soxhlet extraction, and microwave-assisted extractions give the most effective extraction of saponins from Sapindus species. Hence, this present review article provides detailed information about the different isolation procedures of saponin glycosides.

INTRODUCTION: *Sapindus emarginatus* is an evergreen tree with a dense, broad crown; it can grow up to 18 meters tall. *Sapindus* is a genus of about five to twelve species of shrubs and small trees in the lychee and belongs to the family Sapindaceae. Members of the genus are commonly known as soapberries or soapnuts because the fruit pulp is used to make soap [1]. The leaves are alternate, 15–40 cm (5.9–15.7 in) long, pinnate, with 14-30 leaflets, the terminal leaflet often absent. The flowers form in large panicles, each flower small, creamy white. The fruit is a small leathery-skinned drupe 1–2 cm (0.39–0.79 in) in diameter, yellow ripening blackish, containing one to three seeds [2].

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The drupes (soapnuts) contain saponins, which have surfactant properties, having been used for washing by ancient Asian and American peoples. Several other uses for *Sapindus* have also been reported, such as making arrows from wood and decorative objects from seeds [6]. The preliminary phytochemical screening of the leaves revealed the presence of saponins, terpenoids, tannins, acids, flavonoids, cardiac glycosides, and sugars. Traditionally, it is used as an anti-inflammatory and antipyretic. It is used to purify the blood. The seed is an intoxicant, and the fruit rind has oxytocic action. Its powder is used in nasal insufflation. The fruits possess several medicinal properties and are widely used, for example, in treating asthma, colic, dysentery, and during childbirth [11].

Extraction of Secondary Metabolites from Plants: Crude drugs contain different active constituents, which various extraction and separation methods can isolate. Extraction is defined as the process of isolation of soluble material from an insoluble residue, which may be liquid or solid, by treatment with a solvent based on the physical nature of the crude drug to be extracted, *i.e.,* liquid or solid, the extraction process may be liquid-liquid or solid-liquid extraction. There are different methods for the extraction of crude drugs. Maceration and Percolation are generally slow and time-consuming extraction methods and are used for small-scale extraction procedures. Supercritical fluid extraction is one of the methods of the large-scale extraction process [1, 2].

All plant material used for extraction should be properly authenticated. The choice of extraction procedure depends on the nature of the plant material and the components to be isolated. Dried materials are usually powdered before extraction, whereas fresh plants can be homogenized or macerated with a solvent such as alcohol. The latter also stabilizes fresh leaves by dropping them into the boiling solvent. Alcohol is a general solvent for many plant constituents (most fixed oils excepted) and, as such, may give problems in the subsequent elimination of pigments, resins, *etc*. Water-immiscible solvents are widely used-light petroleum (essential and fixed oils. steroids), ether, and chloroform (alkaloids, quinones) [3]. The extraction of organic bases (*e.g.,* alkaloids) usually necessitates basifying the plant material if a water-immiscible solvent is used. For aromatic acids and phenols, acidification may be required. The extraction may be performed by repeated maceration with agitation, Percolation, or continuous extraction (*e.g.,* in a Soxhlet extractor). Special methods for volatile oils, such as the enfleurage process, are used. Ultrasound may enhance the extraction process for some plant materials and the BP uses this in the preparation of a 50% ethanolic solution of opium to assay alkaloids [4, 5].

Saponin Glycosides: Saponins are naturally occurring glycosides described by the soap-like foaming, and consequently, they produce foams when shaken in aqueous solutions. Structurally saponins have one or more hydrophilic glycoside sugar moieties combined with a lipophilic triterpene molecule. Literature shows that saponins exhibit a biological role and medicinal properties such as hemolytic factor anti-inflammatory, antibacterial, antifungal, antiviral, insecticidal, anticancer, cytotoxic, and molluscicide action. They are commonly available in dicot plants belonging to the family Rubiaceae, Compositae, Rutaceae, Umbelliferae, *etc* [6]. Saponins are rarely crystalline and generally amorphous powders with high molecular weight. They carry many asymmetric centers and are optically active. They are generally soluble in water and form colloidal solutions.

These are also soluble in ethyl and methyl alcohol and are usually insoluble in organic solvents like petroleum ether, chloroform, acetone, *etc*. They are bitter in taste and non-alkaline in nature, produce sneezing, and have the property of lowering surface tension. Acids hydrolyze them and alkalis to yield an aglycone called sapogenin and one or more molecules of the same or different sugars or their oxidation products. Enzymes, soil bacteria, and photolysis can also hydrolyze them. In mild conditions using very dilute acids (0.01–0.1 N), organic acids give rise to partially hydrolyzed saponins called pro sapogenin [7]. Saponins are naturally occurring bioorganic compounds having at least one glycosidic linkage (C-O-sugar bond) at C-3 between an aglycone and a sugar chain. Hydrolysis of saponin molecule produces two portions, an aglycone and a sugar moiety. Isolated amorphous solid saponins have a high molecular weight and contain 27 to 30 carbon atoms in the non-saccharide portion [8, 9].

General Methods of Extraction of Saponin Glycosides: Different extraction methods are available for the extraction of saponins from crude drugs. Ordinary extraction and Soxhlet extraction methods are well-known extraction methods for saponins. Some factors affecting the efficiency of extraction of saponins from plant material are temperature, pH, solvent, composition, and particle size of the plant material. To increase the efficiency of extraction, some pre-treatment steps should be followed. Pre-treatment steps include drying, particle size reduction, and defatting. Particle size reduction is carried out to increase the mass transfer efficiency of the extraction. Most of the saponin extractions are performed on powdered plant material using methanol, ethanol, and water as extracting solvents [10].

Maceration Extraction: The extraction of saponins by maceration technique is famous for using ordinary solvent-like alcohols and n-butanol. It is asolid–liquid interface extraction where saponin compounds inside the Sapindus plant material can easily extract by immersing or soaking the plant materials in a suitable specific solvent for a specific period of time with or without stirring or shaking [11]. Some operational variables affect the efficiency of the ordinary extraction process, including the solvent's polarity, temperature, maceration time, solubility of saponins, and its effective diffusion in the liquid phase. Normally, the polar saponins dissolve in polar solvents, and the nonpolar compounds dissolve in nonpolar solvents [12].

The concentration gradient between the solid and liquid phases is the driving force of the diffusion of saponins into the solvent. The ordinary maceration technique is simple and does not need a sophisticated experimental setup. Ethanol C_2H_5OH, methanol CH_3OH, acetone CH_3COCH_3, ethyl acetate $CH_3COOC_2H_5$, dichloro methane CH_2Cl_2, and a mixture of solvents are the ordinary solvents used for the extraction of saponins from plant material, but ethanol (50-98 %) and n-butanol CH_3 (CH_2) $2CH_2OH$ are the commonly used solvent. Maceration of plant materials by organic solvents may be accelerated or facilitated by heat, shaking, and/or magnetic stirring.

After maceration, the alcoholic crude extract of plant materials then evaporated to obtain a more concentrated saponin-containing solution. This solution may dilute with water and be directly subjected to a solvent extraction process using n-butanol and a separating funnel. Finally, n-butanol is easily removed using a rotatory evaporator under a vacuum, and the remaining saponin residue stays in the round-bottomed flask. The dry residual saponin material can be fractionated and identified using one or more techniques *e.g.*, column chromatography, Sephadex, thin layer chromatography (TLC), and/ or high-performance liquid chromatography (HPLC). The most commonly employed solvent system for TLC is chloroform - glacial acetic acid – methanol-water (60:32:12:8) and ethyl acetate - formic acid - glacial acetic acid - water (100:11:11:26).27 Methanol water system (MeOH - H_2O) is the common solvent system used with HPLC.28 The extraction and identification of saponin from the plant material are not easy and are described as a tedious process [13].

Extraction using Soxhlet Apparatus: The Soxhlet extraction process of plant material by distillation method is faster than the ordinary maceration process because it involves heating the organic solvent to its boiling point and then returning the condensed vapors to the original flask after passing through the plant tissue in the condenser. And the extraction process occurs *via* direct contact between the plant tissue and the hot fumes of the solvent. After a considerable extraction time, the colorless solvent becomes a dark green solution due to the mass transfer into the solvent [5].

Then the solution was dried by a rotary evaporator to dryness to obtain the dry crude extract of the plant, which was suspended in water, extracted by n-butanol, and fractionated as mentioned above. Still, Soxhlet extraction is affected greatly by the polarity of the solvent, extraction time, and extraction temperature. The ordinary maceration process is static extraction, but Soxhlet is dynamic extraction due to solvent circulation during extraction. The plant powder may initially be defatted with 60- 80˚C petroleum ether or n-hexane statically or dynamically until the fatty components had been removed before the final extraction [14].

Microwave-assisted Extraction (MAE): Microwaves are non-ionizing electromagnetic waves with a frequency range from 0.3 to 300 GHz. MAE has recently drawn attention to bioactive compound extraction from plant material due to its short extraction time, minimal solvent usage, and special heating mechanism [15]. The recent applications of MAE of plant secondary metabolites such as flavonoids, quinones, phenylpropanoids, terpenoids, alkaloids, and saponins have been reviewed [16]. Microwaves can penetrate biomaterials and generate heat by interacting with polar molecules such as water inside the materials. The penetration depth of microwaves into plant matrix depends on the dielectric constant, moisture content, temperature, and the frequency of the electrical field. The water contained in plant material is responsible for the absorption of microwave energy, which led to internal superheating and cell structure disruption, and, consequently, facilitates the diffusion of bioactive compounds from the plant matrix. The efficacy of MAE has relied on the effect of microwaves on extraction solvent and plant matrix cell structure 2009 [17, 18, 19].

CONCLUSION: Saponins are important secondary metabolites derived from various plant sources because of their valuable pharmaceutical properties. The extraction process of saponin glycosides is difficult if the proper procedure is applied. This may provide an overview and quick reference for future lab-scale experimental design. The knowledge of extraction techniques employed for an objective is vital and can be extended to its uses in drug discovery and various pharmacological studies.

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