

EXTRACTION OF NATURAL DRUG DIOSGENIN FROM HERBAL PLANT TRIBULUS TERRISTRIS

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ABSTRACT

Diosgenin is a steroidal saponin present in various medicinal plants. one of the novel bioactive compound as 'Diosgenin', because of its versatility, and it grabs the attention of researchers to explore its wide pharmacological activities. Tribulus terrestris is a natural herb which is at a high rate of medicinal activity according to the present novel research works. Accordingly, Tribulus terrestris have a major content of saponin which results in positive treatment of various disorders. Between the saponin content of tribulus herb, diosgenin is one of the major saponin present in it. In the present work, saponin is extracted from the T.Terristris with Ethanol and in turn leads to acid hydrolysis for diosgenin separation with 5 % HCl. The diosgenin extraction is performed by direct crude acid hydrolysis and Ethanolic saponin acid hydrolysis for better yield of diosgenin as 92.57 % and 59% respectively. The estimation of Diosgenin extracted is compared to the standard (0.75) and Rf values of the samples are 0.74,0.61. The diosgenin sample extracted from Tribulus is rich of medicinal effects, so as natural drug can be used in our daily lives with minute side effects.

INTRODUCTION:

Diosgenin (25R-spirost-en-3b-ol), (Fig 1) an , isospirostane derivative, a compound that leads to a tremendous interest over last decade[1]. Diosgenin is a biologically active phytochemical responsible for different type of pharmacological activities including functional food. Biomedical studies of diosgenin is a promising focus of interest at various research investigating works in and around the world. The richest source of diosgenin is among *Dioscorea* [2] and *tribulus* species such as *T.territis*, *D. nipponoca* , *D .tokora*, *D .maxicana*, *D .spiculiflora*, etc. The fruits of *T. terrestris* as long used in Ayurveda and the different traditional system like chinese, indian medicine for the treatment of arthritis, eye based infections, edema, abdominal distention, sexual dysfunction, and kidney disorders[3].

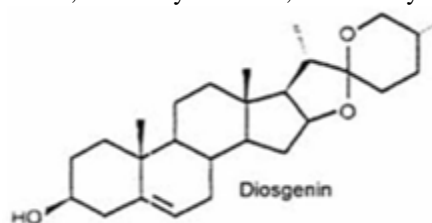


Fig 1 Diosgenin structure

MATERIALS AND METHOD:

Collection of Plant:

Tribulus terrestris (Fig 2) fruits and roots are collected and dried. After drying, the callus are powdered and sieved with 120 mesh size[5]



Fig 2 Tribulus terrestris collected

SAPONIN CRUDE EXTRACTION:

a. Aqueous Extraction:

1 gm of *T. terrestris* powder is taken and 100 ml of water is added to it. The sample is thoroughly mixed with solvent and kept for boiling on water bath at 70°C for 1 hr (Fig 3). The aqueous extract obtained is collected, cooled and filtered[6]. The supernatant is preserved for further use.



Fig 3 Aqueous Extraction of *T. Terrestris*

b. Soxhlet Extraction:

The soxhlet apparatus[7,8] is the best and highly accurate extraction method mainly for the alcoholic extraction. For the ethanolic/methanolic extraction soxhlet apparatus is used. 8 gm of *T. terrestris* powder is weighed and poured in an soxhlet apparatus and 250 ml ethanol is measured and taken in round bottom flask (Fig 4). The extraction is performed for 6-8 hrs and the sample is then collected. Diosgenin concentration is estimated by UV-spectrophotometer of λ_{max} at 530 nm



Fig 4 Ethanolic extraction through soxhlet apparatus

DISOGENIN EXTRACTION FROM CRUDE ETHANOLIC EXTRACT:

The crude ethanolic extract is prepared and then the further extract is performed for the further compound extraction as diosgenin. Diosgenin can be evidenced from the saponin content by there methods[9,10]:

Procedure:

The extract (500 mg) obtained from the Soxhlet extraction method was hydrolyzed with 100mL 3-M aqueous hydrochloric acid under reflux for 0.5 to 5 hr at 100°C in water bath. The solutions are allowed to cool and neutralized with 10% sodium carbonate solution. The afore mentioned solution are filtered and marc is washed with water and dried overnight in a hot air oven at 50°C. After drying the marc is extracted with diethyl ether (50mL*3) using liquid-liquid extraction. The combined diethyl ether extract is dried under vacuum; the residue was dissolved in 25mL methanol for TLC the percentage of diosgenin in the plant sample can be determined by the analysis of the TLC rf values.

RESULTS AND DISCUSIION:

Extraction of diosgenin includes extraction of saponin from the Tribulus terrestris and followed by acid hydrolysis to obtain diosgenin. The direct hydrolysis can also been performed and the estimation of diosgenin is further performed by Thin Layer Chromatography [11,2].

Extraction of saponins from crude sample:

The effect of extraction solvents for Soxhlet extraction is investigated to get a high rate of saponin level. The saponins extracted with different polar solvents like ethanol, methanol, water, and their combinations were generally employed for their extraction as in table 1. In Present study, it was reported that the polar solvents are better for the extraction of steroidal saponins than non polar ones(Fig 6).

s.no	Types of solvents	Saponin concentration (gm/ml)	% extraction of the saponins
1	Ethanol	1.95	51.75
2	methanol	2.72	51
3	Water	3.95	37.625

Table 1Effect of % yield of saponin with different solvents

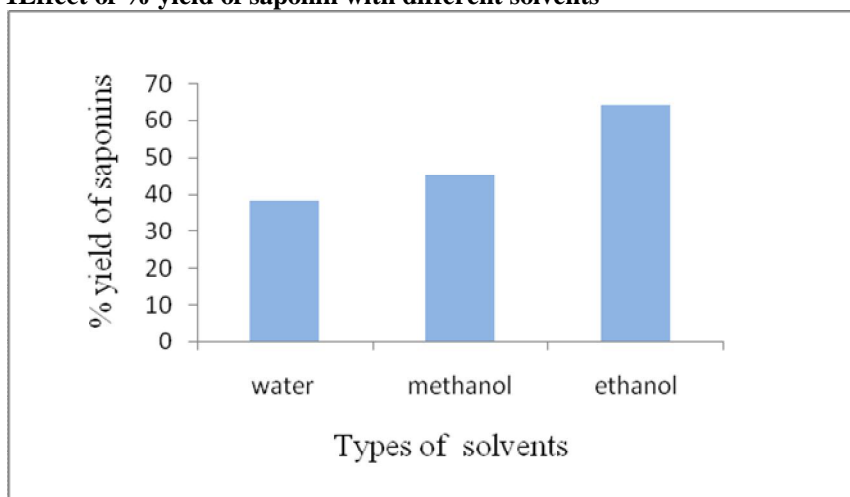


Fig 5 Extraction of saponin with different polar solvents

2. ACIDIC HYDROLYSIS OF THE EXTRACTED SAMPLE:

The extract (500 mg) obtained from the Soxhlet extraction method was hydrolyzed with 100mL 3-M aqueous hydrochloric acid under reflux for 0.5 to 5 hr at 100°C in a water bath as in Fig 6. The hydrolysate obtained was treated in the same manner as mentioned under the Optimization of Acid Strength section. As shown in Table-2 and Fig 7, the

percentage of diosgenin in plant samples was highest at 1 hr of hydrolysis time for hydrochloric acid, and was chosen for further optimization of acid hydrolysis conditions.



Fig 6 Acidic hydrolysis of Saponin extract to yield diosgenin

s.no	Hydrolysis time (min)	Concentration of diosgenin precipitated (% w/v)	% yield of diosgenin
1	30	3.65	52.94
2	60	4.49	61.56
3	90	5.46	71.49
4	120	6.19	80.91
5	150	6.82	88.34
6	180	7.51	92.57

Table 2 Acid hydrolysis of saponin sample

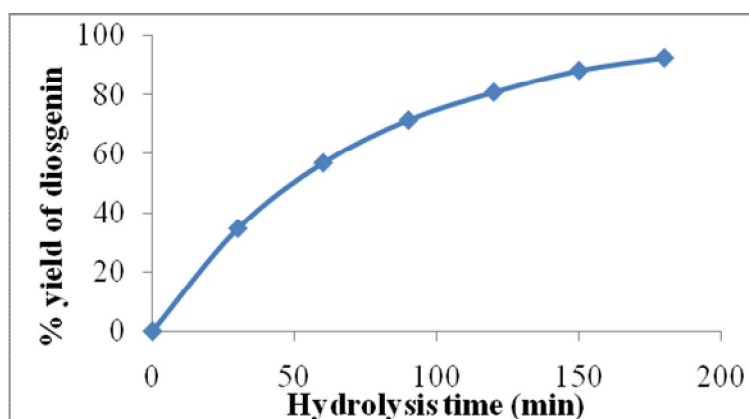


Fig 7 Acid hydrolysis of saponin sample

According to the above acid hydrolysis, concentration of diosgenin is found to be increased from 30 mins to 180 mins. And the precipitate is filtered and further the sample is extracted with hexane for 8 hrs and finally sample is centrifuged for the final precipitate of crude diosgenin.

3. DIRECT HYDROLYSIS OF CRUDE SAMPLE:

10 gms of T.territis powder is weighed and collected. To it, 250 ml of 5% hydrochloric acid is added. The sample mixture is taken into reflux condenser and kept it on water bath at 100°C for 2 hr and in fig 9. The sample mixture

after 2 hr leads to the formation of saponin precipitation. After, the saponin precipitation, the sample is filtered and the marc is then extracted with toluene for 8 hrs. Once after the extraction is complete then filtered and the sample is extracted with hexane at 40-60°C for 2 hrs as in Table 3 and Fig 9 .The final samples are centrifuged and precipitate is then dried in hot air oven. Finally white color sample are formed.



Fig 8 Extraction of diosgenin from crude sample

s.no	Hydrolysis time (min)	Concentration of diosgenin precipitated (% w/v)	% yield of diosgenin
1	0	0	0
2	30	2.43	24.59
3	60	3.25	39.46
4	90	4.49	46.29
5	120	5.26	53.36
6	150	5.51	57.95
7	180	5.76	59.16

Table 3 Acid hydrolysis of crude sample

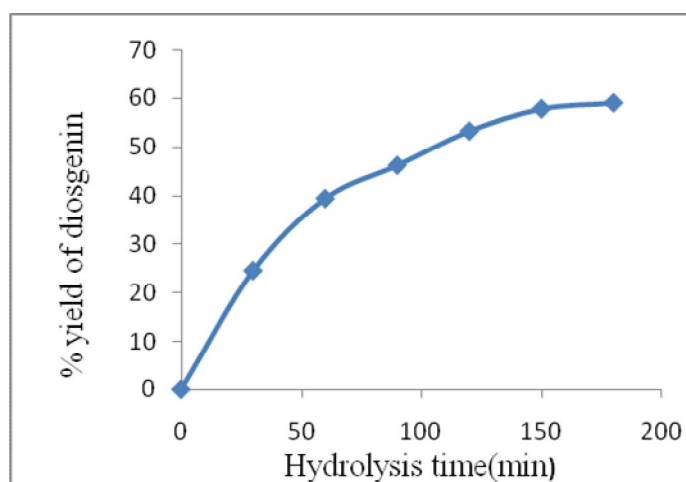


Fig 9 Acid hydrolysis of crude sample

ESTIMATION OF DIOSGENIN-TLC:

Preparation of samples:

The diosgenin in each process are collected and dissolved in relevant solvent to form a clear solution. The samples of hydrolysis and biotransformation are clearly separated. The standard sample of the diosgenin is weighed at an accurate amount and dissolved in ethanol. The three solutions as in Tab 4 and Fig 10 .

Samples	Alocoholic-acid hydrolysis	Direct Hydrochloric acid
R _F Value	0.73	0.61
R _F value of standard diosgenin	0.751	

Table 4 Rf values of diosgenin in various extracts in TLC method

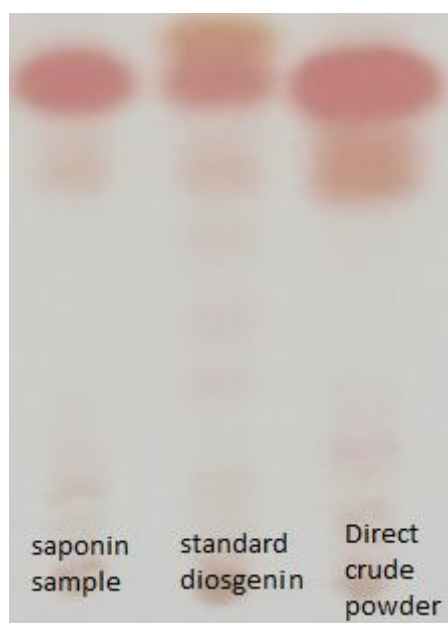


Fig 10. TLC plate containing diosgenin

CONCLUSION:

Diosgenin is one of the novel steroidal saponins present in *T. terrestris* at a high percentage of saponins. Diosgenin promotes increasing attention due of its effectiveness in inflammation, arthritis, hyperlipidemia, cancer therapy, and skin aging. Diosgenin (25R-spirost-en-3b-ol), a derivative of isospirostane, isospirostane derivative, a compound that leads to a tremendous interest over last decade. As of which, saponin are extracted from the tribulus using different solvents like ethanol, methanol and aqueous and of different concentrations of the solvent. The acid hydrolyzed of the saponin compounds through soxhlet extractor with ethanol leads to yield of 92.57% diosgenin. The Rf values are compared to the standard value as 0.751 and can be concluded as the nearest value is found to be the best yield of diosgenin extraction. The Rf values of alcohol-acid extraction, direct hydrolysis and biotransformation is found to be 0.73, 0.61 respectively. *T. terrestris* is a natural herb with high medicinal drugs which are natural and minimal side effects. So from above work, it is concluded that the Tribulus have a saponin helps in various treatments. Diosgenin contain extracted from the medicinal plants are a pure and natural drug which can be used in natural treatment then other drugs with a high rate of side effects.

REFERENCE:

- [1.] V. K. Ghosh, S. G. Bhope, V. V. Kuber, and A. D. Sagulale, An Improved Method For The Extraction And Quantitation Of Diosgenin In *Tribulus Terrestris* L., *Journal Of Liquid Chromatography & Related Technologies*, 1141–1155, 35(9),2012.
- [2.] Shan-Shan Qi, Yue-Sheng Dong&, Yang-Kun Zhao, Zhi-Long Xiu, Qualitative and Quantitative Analysis of Microbial Transformation of Steroidal Saponins in *Dioscorea zingiberensis*, *Chromatographia*, 865–870, 69(10),2009.
- [3.] J. W. Rothrock, P. A. Hammes, And W. J. Mcaleer, Isolation of Diosgenin by Acid Hydrolysis of Saponin, *Industrial And Engineering Chemistry*, 185-188, 49(2) ,1957.
- [4.] Yuesheng Dong, Hu Teng, Shanshan Qi, Lin Liu, Hui Wang, Yangkun Zhao, Zhilong Xiu, pathway and kinetic analysis of biotransformation of *dioscorea zingibirenes y aspergillus orygae*, *Biochemical Engineering Journal*, 123–130, 52(1),2010.
- [5.] Harshal A. Deshpande & Sanjivani R. Bhalsing, Isolation and characterization of diosgenin from in vitro cultured tissues of *Helicteres isora* L, *Physiol Mol Biol Plants* 89–94, 20(1),2014.
- [6.] B. Jasim, Roshmi Thomas, Jyothis Mathew, E.K. Radhakrishnan, Plant growth and diosgenin enhancement effect of silver nanoparticles in Fenugreek (*Trigonella foenum-graecum* L.), *Saudi Pharmaceutical Journal*, 443–447, 25(3),2017.
- [7.] Sardar A. Farooq, Talat T. Farook and Salim H. Al-Rawahy, Bioactive Compounds From *Tribulus Terrestris* L.(Zygophyllaceae), *Nova Science Publishers, Inc.*, 246-268, 4(1), 2012.
- [8.] Jingzhou Dong , Can Lei, Dayan Lu, Ying Wang, Direct Biotransformation of Dioscin into Diosgenin in Rhizome of *Dioscorea zingiberensis* by *Penicillium dioscin*, *Indian J Microbiol*, 26-37,36(1), 2014.
- [9.] Bertrand Liagre, Pascale Vergne-Salle, Cecile Corbiere, Jean L Charissoux, Diosgenin, a plant steroid, induces apoptosis in human rheumatoid arthritis synoviocytes with cyclooxygenase 2 overexpression, 373-383, 6(4),2004.
- [10.] Deshpande HA, Bhalsing SR, Plant Derived Novel Biomedicinal: Diosgenin, *International Journal of Pharmacognosy and Phytochemical Research* 780-784,6(4);2015.
- [11.] Suresh Reddy Yanala, D. Sathyanarayana, K. Kannan, A Recent Phytochemical Review – Fruits of *Tribulus terrestris* Linn, *J. Pharm. Sci. & Res.* 132-140,8(3) ,2016.
- [12.]Lubna Fatima Ms , Arshiya Sultana Md , Saad Ahmed, Md And Shabiya Sultana Md .Pharmacological Activities Of *Tribulus Terrestris* Linn: A Systemic Review *The World Journal of Pharmacy And Pharmaceutical Sciences*, 136 -150, 4 (2),2012.