



Preliminary Phytochemical screening of the Ethanolic extract of *Dioscorea villosa* Tubers and Estimation of Diosgenin by HPTLC Technique.

Anitha Roy^{1*}

R V Geetha²

1. Faculty of Pharmacology,
Saveetha Dental College and
Hospitals, Chennai, India

2. Faculty of Microbiology,
Saveetha Dental College and
Hospitals, Chennai, India

Corresponding Authors:

Anitha Roy

Email id:

anitharoypeeter@yahoo.co.in

Abstract: In the present study, simple phytochemical screening procedures were carried out to find the various constituents present in the ethanolic extract of *Dioscorea villosa* tubers. Further, estimation of Diosgenin in *Dioscorea villosa* tubers, was carried out by HPTLC technique. The preliminary screening showed presence of proteins, flavonoids, tannins, alkaloids, phenolic compounds, saponins and glycosides. The extract was chromatographed on silica gel GF254 plates with Toluene: Ethyl Acetate: Acetic Acid: Formic Acid (4: 3: 1:1) as mobile phase. Detection and quantification were performed by densitometric scanning, at 366 nm. The average recovery of diosgenin was found to be 0.48 %. The HPTLC technique has provided a good resolution of diosgenin from other constituents present in the ethanolic extract.

Keywords: Phytochemical, HPTLC, Diosgenin, *Dioscorea villosa*, Extract.

INTRODUCTION:

Dioscorea villosa (wild yam) family *Dioscoreaceae* is a perennial, tuberous, twining vine with pale-brown, knotty, woody and cylindrical tubers. The tubers are crooked and bear horizontal branches.^[1] Many species of *Dioscorea* have many organic acids and poly phenols present in them and have antioxidant property.^[2] Diosgenin, obtained after hydrolysis of yam saponins is used as industrial starting material for the partial synthesis of steroidal drugs.^[3] Commercial diosgenin is reported to have different pharmacological activities like hypoglycemic, antioxidant, hypolipidaemic, neuroprotective, vasodilating activity etc; and has role in melanogenesis and cholesterol metabolism.^[4] Diosgenin has been isolated from many other plants like Fenugreek^[5,6] and other species of *Dioscorea*.^[7] So, in the present study an

effort was made for the preliminary phytochemical screening and estimation of Diosgenin content, as the literature survey revealed that no scientific study has been carried out for the estimation of diosgenin in this particular variety of wild yam.

MATERIALS AND METHOD:

Plant material

The ethanolic extract of *Dioscorea villosa* tubers was obtained from Green Chem Herbal Extract & Formulations, Bangalore.

Preliminary phytochemical analysis^[8-12]

The extract was subjected to the preliminary phytochemical analysis following standard methods. This was to screen the presence of the various active principles in a qualitative way and the result is given in table 1.

1. Phenolic compound

To the extract, few drops of alcoholic ferric chloride solution are added. Bluish green or Bluish black colour indicates the presence of phenol.

2. Reducing sugars

The extract is mixed with Fehling's solution I and II; formation of red coloration indicates the presence of sugars.

3. Flavones**a) Shinoda test**

To the extract, a few magnesium turnings and few drops of concentrated hydrochloric acid are added and boiled for five minutes. Red coloration shows the presence of flavones.

b) To the extract 10% sodium hydroxide solution or ammonia is added. Dark yellow color indicates the presence of flavones

4. Glycosides

The Extract is mixed with a little anthrone on a watch glass; one drop of concentrated sulphuric acid is added and warmed gently over water bath. Dark green coloration indicates the presence of glycosides.

5. Saponins

Extract is shaken with water; copious lather formation indicates the presence saponins.

6. Alkaloids

To the extract, add a few drops of acetic acid followed by Dragendroff's reagent and shaken well. Formation of orange red precipitate indicates the presence of alkaloids

7. Anthraquinones**Borntrager's Test**

Extract is macerated with ether and after filtration; aqueous ammonia or caustic soda is added. Pink, red, or violet color in the aqueous layer after shaking indicates the presence of anthraquinones.

8. Quinones

To the extract, sodium hydroxide is added. Blue, green, or red color indicates the presence of quinones.

9. Proteins

To the extract, few drops of Biuret reagent is added. Formation of blue color indicates the presence of protein.

10. Tannins

The extract is mixed with basic lead acetate solution. Formation of orange red precipitate indicates the presence of tannins.

HPTLC FINGERPRINTING [13,14]

The finger printing has been done using the following chromatographic conditions. Chromatography was performed on a 10x10 cm pre-activated HPTLC silica gel 60F 254 plate. Samples were applied to the plate as 6mm wide band with an automatic TLC applicator Linomat 5 with N₂ flow (CAMAG, Switzerland), 8mm from the bottom. Densitometric scanning was performed on CAMAG scanner III. The plates were pre-washed by methanol and activated at 60^o C for 5 minutes prior to chromatography. The slit dimension was kept at 5 minutes x 0.45 minutes and 20 minutes scanning speed was employed. The mobile phase was chosen after running extract in different mobile phases of varying polarity (Toluene, Toluene: Ethyl acetate and Ethyl acetate: Methanol etc ;). Linear ascending development was carried out in 20cm x 10cm twin glass chamber saturated with the mobile phase and 10 ml of mobile phase was used per chromatography

CHROMATOGRAPHIC ANALYSIS

The plant extract and standard of required concentration have been prepared in methanol and were spotted using CAMAG Linomat 5

applicator. The method was optimized by selecting appropriate mobile phase for the plant extract and respective compounds and developed in a twin trough chamber, 10 x 10cm at 25°C. The plates were dried by hair dryer. The developed plates were scanned at appropriate wavelength using CAMAG TLC scanner 3 and photo-documented using CAMAG REPROSTAR 3.

Preparation of stock solutions:

Preparation of diosgenin standard solution:

Quantification of Diosgenin

The standard diosgenin (100 mcg/ml) and the sample (50mg / ml) were prepared in methanol and estimated with the help of Toluene: Ethyl Acetate: Acetic Acid: Formic Acid (4: 3: 1:1) as the mobile phase . Silica gel GF254 was used as the stationary phase. 2 µl, 4 µl, 6 µl and 8 µl of the sample were run in track 1-4 respectively and standard diosgenin in track 5 using ascending development mode and the developed plates were scanned at 366 nm using CAMAG TLC scanner 3 and photo-documented using CAMAG REPROSTAR 3 and the result is given in the figure 1 and 2 and the peak area obtained from different track is reported in figure 3-7

PHOTODOCUMENT

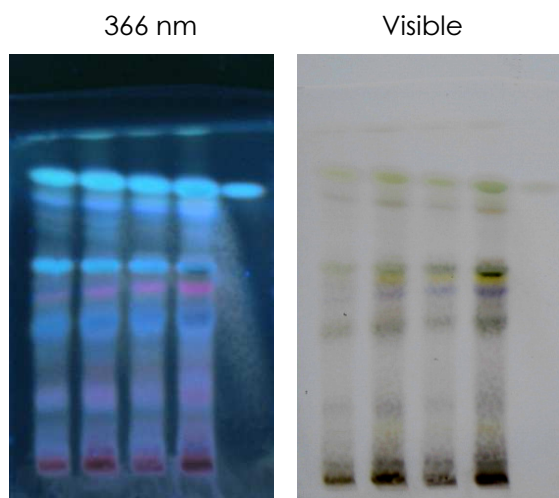


Figure 1: Fingerprint of the *D.villosa* extract in 366nm

Figure 2: Fingerprint of the *D.villosa* Extract visible wavelength

HPTLC Densitogram of *D.villosa* Extract observed in track 1-5

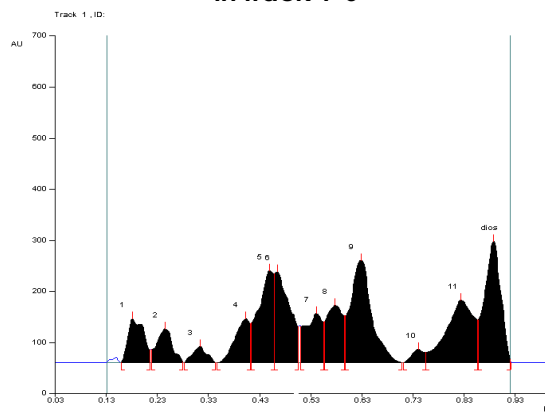


Figure 3: Densitogram of *D.villosa* extract in track 1

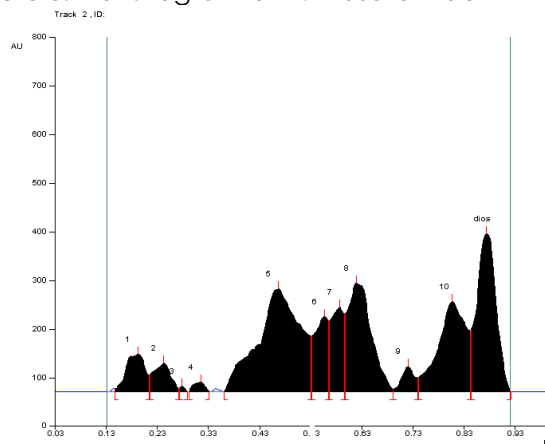


Figure 4: Densitogram of *D.villosa* extract in track 2

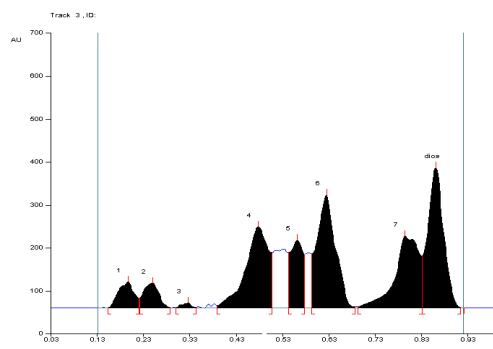


Figure 5: Densitogram of *D.villosa* extract in track 3

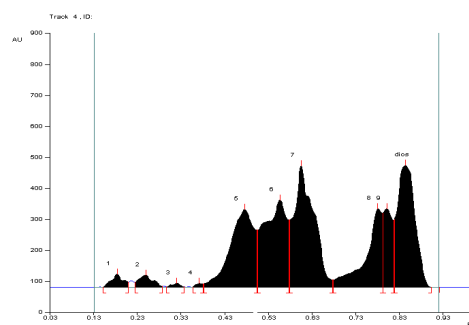


Figure 6: Densitogram of *D.villosa* extract in track 4

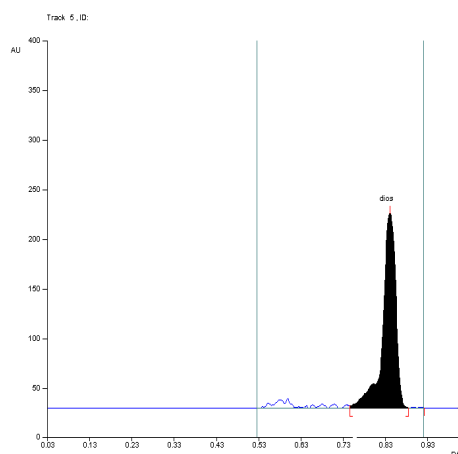


Figure 7: Densitogram of standard Diosgenin in track 5

Phytochemicals	<i>Dioscorea villosa</i> extract
Protein	+
Reducing Sugar	-
Phenol	++
Tannin	+
Flavonoid	+
Saponin	++
Alkaloid	++
Anthraquinone	-
Quinone	-
Glycosides	+++

Table 1: Phytochemical Analysis of ethanolic extract *Dioscorea villosa*

+ - mild; ++ - moderate; +++ - high; - - absent

Sample	mcg/mg	%
Diosgenin		
<i>Dioscorea villosa</i>	4.76	0.48

Table 2: Quantification of Diosgenin in *D. villosa* extract

RESULT AND DISCUSSION:

The medicinal properties or the therapeutic effect of many plants are attributed to the presence of alkaloids, flavonoids, coumarins, terpenoids, tannins, phenolic acids and antioxidants.^[15,16] Here the preliminary screening showed presence of proteins, flavonoids and tannins in mild levels and moderate levels alkaloids, phenolic compounds

and saponins. The extract also showed high levels of glycosides. The preliminary phytochemical analysis results obtained are reported in the Table.1

The high performance thin layer chromatography seems to be a highly reliable technique for isolation and estimation of plant components.^[13,14] The finger printing following the chromatographic condition showed a comparable peak of diosgenin in the extract as that of the standard diosgenin in every track 1-4 (Figure 3-6). The diosgenin percentage was calculated from the peak area and was found to be 0.48 % (Table 2)

CONCLUSION

The preliminary phytochemical analysis as well as the estimation of the diosgenin content in the ethanolic extract of *D. villosa* is promising, as diosgenin has many pharmacological actions. Further *in-vitro* and *in-vivo* studies in the unexplored areas of research with *D.villosa* may bring new mile stones in the field of diabetes and other conditions.

ACKNOWLEDGMENT:

The authors take opportunity to thank R Rajendren,CEO, Green Chem, Bangalore for providing gift sample of the extract for the research work. Authors are thankful to the Principal, Dr.N.D Jayakumar, Saveetha Dental College and Hospital, Chennai, for the constant motivation to carryout research work. Finally authors extend due thanks to R.P. Parameswari, Senior Research Fellow, Herbal and Indian Medicine Research Laboratory, Sri Ramachandra University, Chennai, for her timely help and interpretation.

REFERENCES

- 1) Saurabh Satija, Meenu Mehta, Vandna Kalsi, wild yam : a review to *Dioscorea villosa*, *IJPRD*, 2011, Vol 3(5), 117 - 121 .
- 2) Tn suk S, Kim JH, Sohn HY. Antioxidative and hypolipidemic effects of diosgenin, a steroidal saponin of Yam, on High-Cholesterol Fed Rats. *Biosci Biotechnol Biochem* 2007; 71:3063-3071.
- 3) Chao-chin hu, jau-tien lin, shih-chuan liu and deng-jye yang., a spirostanol glycoside from wild yam (*dioscorea villosa*) extract and its cytostatic activity on three cancer cells, *J. Food and Drug Analysis*, Vol. 15(3) , 310-315(2007)
- 4) Mirunalini and Shahira, Novel effects of Diosgenin –a plant derived steroid-A review *Pharmacologyonline* ,2011, Newsletter 1, 726-736
- 5) Taylor WG, Elder JL. Microdetermination of diosgenin from fenugreek (*Trigonella foenum-graecum*) seeds. *J. Agric Food chem.*;(2000) 48, 5206 -5210.
- 6) Jayadev Raju and Chinthalapally V. Rao (2012). Diosgenin, a Steroid Saponin Constituent of Yams and Fenugreek: Emerging Evidence for Applications in Medicine, *Bioactive Compounds in Phytomedicine*, Prof. Iraj Rasooli (Ed.), ISBN: 978-953-307-805-2, InTech, Available from:<http://www.intechopen.com/books/bioactivecompounds-in-phytomedicine>.
- 7) Sougata Ghosh, Mehul Ahire, Sumersing Patil, et al., "Antidiabetic Activity of *Gnidia glauca* and *Dioscorea bulbifera*: Potent Amylase and Glucosidase Inhibitors," *Evidence-Based Complementary and Alternative Medicine*, (2012) Vol. 2012, Article ID 929051.10 pages .
- 8) Harborne, J.B., 1998. *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. 3rd Edn., Chapman and Hall, London, Pages: 302.
- 9) Trease GE and Evans WC., *Pharmacognosy* ,2002, 15th Ed. London: Saunders Publishers; pp. 42–44. 221–229, 246–249, 304–306, 331–332, 391–393.
- 10) Kokate, C. K. *Practical Pharmacognosy*, 4th edn. VallabhPrakan, New Delhi. 1994, 179-181.
- 11) Metwally AM, Omar AA, Harraz FM, El Sohafy SM. Phytochemical investigation and antimicrobial activity of *Psidium guajava* L. leaves. *Phcog Mag.* 2010; 6:212-8.
- 12) Panda SK, Padhi LP, Mohanty G. Antibacterial activities and phytochemical analysis of *Cassia fistula* (Linn.) leaf. *J Adv Pharm Technol Res.* 2011; 2(1):62-7.
- 13) Dr. S. M. Arif Zaidi et al: Current strategy for research on quality identification of *Rheum emodi* Wall. Rhizome *Int. J. Drug Dev. & Res.*, 2013, 5(1): 297-304.
- 14) Bairwa NK, Sethiya NK, Mishra SH , Protective effect of stem bark of *Ceiba pentandra* linn. against paracetamol-induced hepatotoxicity in rats, - *Pharmacognosy Res* (2010) Jan-Feb; 2(1): 26–30.
- 15) Czinner E ,Hagymazi k, Blazovics A, Kery A, Szoke E, Lemberkovics E, The *in vitro* effect of *Helichysi flos* on microzomal lipid peroxidation, *J ethanopharmacol*, 2001; 77: 31-5
- 16) Manach C, Morand C, Demigne C, Texier O, Regeat F, Remesy C, bioavailability of rutin and quercetin in rat, *FEBS Lett*, 1999; 409:12-6.

Article History:-----

Date of Submission: 20-06-2013

Date of Acceptance: 09-07-2013

Conflict of Interest: NIL

Source of Support: NONE

