Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2016, 8(11):76-82
(http://derpharmachemica.com/archive.html)

Antispermogenic activity of triterpenoids isolated from *Dioscorea glabra* tuber

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ABSTRACT

Dioscorea glabra is a folk medicinal plant of India. The plant is used by the ethnic community of Tripura, India to treat different diseases. Present study was undertaken to determine the antispermogenic activity of Dioscorea glabra tuber and isolate few bioactive constituent. Dried tuber power was extracted using methanol and extract was fractionated to get pure compound. Antispermogenic activity of methanol extract and isolated fractions (1 mg/mL) were evaluated using in vitro assay method. Purification and characterization of fractions produced potent effect was carried out to identify the bioactive molecules. Extract and its two chromatographic fractions (T1 & T2) showed in vitro spermicidal activity as 30% and 33%, 37% sperm immobilization after 30 min respectively. Structural elucidation of these two fractions confirmed the constituents as triterpenoids. Present study proved that D. glabra tuber contains several spermicidal bioactive constituents. Future investigations could be useful to establish them as potent spermicidal molecule for human.

Keywords: *Dioscorea glabra*, tuber, spermicidal activity, triterpenoids

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INTRODUCTION

Dioscorea glabra (family Dioscoreaceae) is a traditional medicinal plant used in the treatment of rheumatic arthritis, bilious colic, painful abdominal neuroses, gastro intestinal irritation, indigestion problem with hepatic derangement, nausea, vomiting and also possesses antispasmodic action.[1-3] Tuberous roots of the plant found useful as appetizer, aperient, vulnerary, tonic, and exert beneficial effect in anorexia, dyspepsia, constipation, wounds, foul ulcers and general debility.[2] A number of plants from *Dioscorea* also investigated for antifertility activity. Ethanol extract of *Dioscorea esculenta* tuber produced antifertility effects observed in male albino rat.[4,5]

In addition, the information collected from the local tribal people and folk practitioner of Tripura, India revealed that tubers of *Dioscorea glabra* are being used as antispermogenic agent. However, in best of our knowledge the scientific evaluation on antispermogenic effect of *Dioscorea glabra* tuber have not been carried out. Therefore, the present investigation was undertaken to assess the possible antispermogenic activity of *Dioscorea glabra* tuber and to isolate bioactive molecule responsible for such activity if any.

MATERIALS AND METHODS

Plant material and extraction: Tuber of *Dioscorea glabra* was first collected from Agartala, Tripura, India and authenticated by the Dept. of Pharmacognosy, RIPSAT, Agartala, Tripura. The clean dried sample was ground to powder by a mechanical grinder and and stored in airtight container. Tuber power was extracted with methanol in soxhlet apparatus. The extract was collected and solvent was evaporated to dryness under reduced pressure. Fractionation of methanol extract was carried out by column chromatography (Borosil glass column, 50 cm in length and 2 cm in diameter), where ethyl acetate was used as mobile phase and silica gel as stationary phase. The separated components were dried in room temperature.

Physicochemical and phytochemical evaluation: Density, specific gravity and p^H of methanol extract were determined using standard protocol.[6] Color of the extract was observed by naked eyes. R_f value of methanol extract was recorded by thin layer chromatography (TLC), where silica gel G was used as stationary phase. Several solvent system like butanol: water: dioxane (4:2:1), butanol: acetic acid: water (4:1:1), and benzene were used. The spot in TLC was identified in iodine vapor chamber. Qualitative phytochemical test of methanol extract to find the presence of different phytochemicals were carried out.[7]

Spermicidal activity: Spermicidal activity of the crude methanol extract and separated components (T1, T2, T3, T4 and T5) were carried out by adopting the standard procedure.[8,9] Briefly, sperm were collected from the healthy adult male volunteer. Only those considered normal heading 100-150 million spermatozoa/mL, ≥80% motility, 2.1mL/ejaculate, p^H 7.9 and with minimum contamination of debris or cells other than spermatozoa were used for the assay. Sperm count motility was assessed microscopically. Extract and various fractions were dissolved separately in dimethyl sulfoxid to make the concentration of the solution 1 mg/mL. Sperm volume (1.0 mL) was mixed with methanolic extract of *D. glabra* and different fractions. Volume of sperm and test component was in 10:1 ration for each case. Each experiment is repeated for six times. After the treatment of sperm with extract/fractions, the sperm motility were observed after 10, 20, 30 min. Percentage of inhibition of sperm motility was considered as an indicator of spermicidal activity.

Isolation, purification and characterization: In order to identify the active constituents most active fractions (T1 and T2, described in section 3.4) were purified by recrystallization process using acetone. Melting point of both the compounds was determined by open capillary method (by using INDO M-AB-92 melting point apparatus) and expressed in ºC were uncorrected. Purity of the recrystallized compounds was confirmed by single spot in TLC plate using benzene as solvent system. Structure elucidation of the isolated purified compounds were carried out using IR in KBr pellet (FTIR Jasco 5300), LC-Mass (Shimadzu 2010A), ^1H & ^{13}C –NMR (Bruker AC – F 300).

Statistical analysis: Results obtained from spermicidal activity were calculated using statistical package for social science (SPSS) version 10 and percentage decrease in motility were calculated comparing with the normal motility.

RESULTS AND DISCUSSION

Physicochemical investigation: Physicochemical properties of the compounds provided important database to develop new pharmacologically active compounds and also important for mechanism of action, possible biological activity of metabolites and drug design.[10] In this study, different physicochemical parameters of the methanolic extract of *Dioscorea glabra* were screened. Yield of methanol extract was 7.4% w/w. Density, specific gravity and p^H of the extract were found as 0.8 g/mL, 1.2 and 6.1 respectively. Color of the extract was light orange. TLC was carried out using three solvent system i.e. butanol: water: dioxane (4:2:1), butanol: acetic acid: water (4:1:1) and benzene, where 6 spot (R_f values: 0.15, 0.27, 0.49, 0.70, 0.85, 0.95), 5 spot (R_f values: 0.17, 0.34, 0.55, 0.85, 0.98), 5 spot (R_f values: 0.11, 0.32, 0.40, 0.72, 0.89) were found respectively.

Preliminary phytochemical screening: Preliminary phytochemical screening revealed that methanol extract of *Dioscorea glabra* contain alkaloids, saponin, phenolic & tannin and steroids, but starch, protein, carbohydrate, fixed oil, fat, mucilage and gum were absent. An investigation reported that the extracts of tubers of different Discorea species contain phytochemicals like flavonoids, saponins, steroids, cardiac glycosides and terpenoids.[11] Presence of different phytochemicals may responsible for diverse biological activity of the plant.

Fractionation of methanol extract of *D. glabra:* Total five fractions (T1-T5) were obtained through fractionation. Quantity of fraction volume collected was 200, 150, 120, 95 and 82 mL for T1, T2, T3, T4, T5 respectively. Fractions were dried to get five solid compounds (T1, 56 mg; T2, 35 mg; T3, 22 mg; T4, 15 mg; T5, 11mg). Fractionation and evaluation of biological activity of the fraction is necessary to identify effective fraction and isolate a potent compound.

Spermicidal activity: In the present study, *in vitro* spermicidal activity of methanolic extract of *Dioscorea glabra* and its chromatographic fractions were carried out, where percentage decrease in motility is the indicator of spermicidal activity recorded after 10, 20, 30 min. Methanol extract, and isolated compounds, T1 & T2 had shown a sperm immobilizing effect on human spermatozoa *in vitro*. Methanolic extract of *Dioscorea glabra* and fraction T1 and faction T2 showed spermicidal activity as 30, 33 and 37% respectively after 30 min; whereas, T3, T4 and T5 failed to produced any effect (Table 1).

Fertilizing capacity of sperm depends on number of parameter including sperm motility (ability of sperm to travel properly towards an egg). Sperm motility also considered as vital factor for semen quality. If sperm not able to swim properly then it will not reach the egg, and fertilization will hamper. Insufficient sperm motility is a usual cause of subfertility or infertility. Loss of membrane integrity results excessive leakage of ATP and other metabolites which may alter sperm motility.[12,13] Methanol extract of *D. glabra* and its two fractions (T1 and T2) showed potent activity, and these sperm immobilization activity may be due to surface action activity, disrupting the plasma membrane of the spermatozoa of tested compounds

Table 1. Effect of the extract and the separated components of *Dioscorea glabra* on sperm

Sl No	Components (1mg/mL)	% Decrease in motility		
		10 minutes	20 minutes	30 minutes
1.	Methanol extract	12	22	30
2.	T_1	12	24	33
3.	T_2	15	28	37
4.	T_3	00	00	00
5.	T_4	00	00	00
6.	T_5	00	00	00

Characterization of bioactive molecule: T_1 and T_2 showed potent spermicidal activities, therefore purification and structure elucidation of these two fractions were performed using FTIR, LC-Mass, ^{1}H-NMR, ^{13}C-NMR spectral data. Spectral data of T_1 were recorded, as IR in KBr pellet showed absorption at cm^{-1}: 3400 for OH, 2928 for CH_2, 2854 for CH_3, 1720 for C=O, 1640 for C=C, 1601 for aromatic skeletal vibration, 1417 for OCH_3, 1053 for C-O attached with 6 membered ring; ^{1}H-NMR (δ ppm) recorded at 0.64, 0.68,0.78, 0.82: (s) for –CH_3 of 04 nos., 1.16: (s) for 02 nos. CH_3 in same position, 1.57: (s) for 02 nos. CH_3 in same position nearer to –O-, 1.42-1.45: (m) for –CH_2- of six membered ring, 1.85: (m) for –CH- of six membered ring, 2.07: (d) for -CH attached with –C=C-, 2.50: (d) for –CH- attached with –C-O-, 2.98-3.01: (m) for the presence of sugar moiety, 3.27: (s) for –CH_3- attached with -O-, 5.20: (d) for anomeric proton, 7.45: (s) for C_6H_4; ^{13}C-NMR (δ ppm) showed peak at 38.65 for C1, 30.72 for C2, 119.26 for C3, 38.25 for C4, 56.51 for C5, 22.00 for C6, 33.27 for C7, 39.00 for C8, 48.52 for C9, 39.33 for C10, 26.40 for C11, 148.58 for C12, 159.25 for C13, 40.15 for C14, 29.68 for C15, 23.22 for C16, 37.70 for C17, 55.00 for C18, 39.73 for C19, 41.77 for C20, 29.29 for C21, 50.50 for C22, 16.45 for C23, 21.88 for C24, 15.80 for C25, 24.33 for C26, 29.68 for C27, 29.98 for C28, 13.41 for C29, 13.26 for C30, 70.44 for C1´, 76.99 for C2´, 77.63 for C3´, 78.26 for C4´, 93.03 for C5´, 174.41 for C=O, 165.38 for CH_3 of OCH_3, 132.69 for C1´´, 132.81 for C4´´, 129.8 for C2´´, C3´´, C5´´, C6´´. Mass Spectral fragmentation (m/z) given in Figure 1.

Spectral data of T_2 were also recorded, as IR in KBr pellet showed absorption at cm^{-1}: 3429 for OH, 2926 for CH_2, 2852 for CH_3, 1743 for C=O, 1651 for C=C, 1379 for $COCH_3$, 1074 for C-O attached with 6 membered ring; ^{1}H-NMR (δ ppm) observed at 0.52, 0.69, 0.72, 0.84: (s) for –CH_3 of 04 nos., 1.10: (s) for 02 nos. CH_3 in same position, 1.38: (s) for 02 nos. CH_3 in same position nearer to -O-, 1.41-1.44: (m) for –CH_2- of six membered ring, 1.84-1.86: (m) for –CH- of six membered ring, 2.11: (s) for CH_3 attached to –C=O, 2.14: (d) for –CH- attached with –C=C-, 2.44: (d) for –CH- attached with –C-O-, 2.71-2.82: (m) for the presence of sugar moiety, 5.18: (d) for anomeric proton; ^{13}C-NMR (δ ppm) recorded at 38.54 for C1, 26.87 for C2, 77.00 for C3, 37.50 for C4, 55.61 for C5, 19.00 for C6, 33.72 for C7, 37.00 for C8, 47.00 for C9, 38.90 for C10, 24.60 for C11, 129.00 for C12, 142.00 for C13, 42.00 for C14, 28.96 for C15, 22.32 for C16, 35.50 for C17, 53.00 for C18, 40.24 for C19, 40.67 for C20, 29.29 for C21, 49.85 for C22, 14.65 for C23, 20.77 for C24, 13.95 for C25, 22.32 for C26, 28.96 for C27, 29.39 for C28,

11.83 for C29, 11.71 for C30, 108.00 for C1´, 73.00 for C2´, 75.00 for C3´, 66.00 for C4´, 63.00 for C5´, 172.00 for C=O, 78.26 for CH_3, and Mass Spectral Fragmentation (m/z) interpretation given in Figure 2.

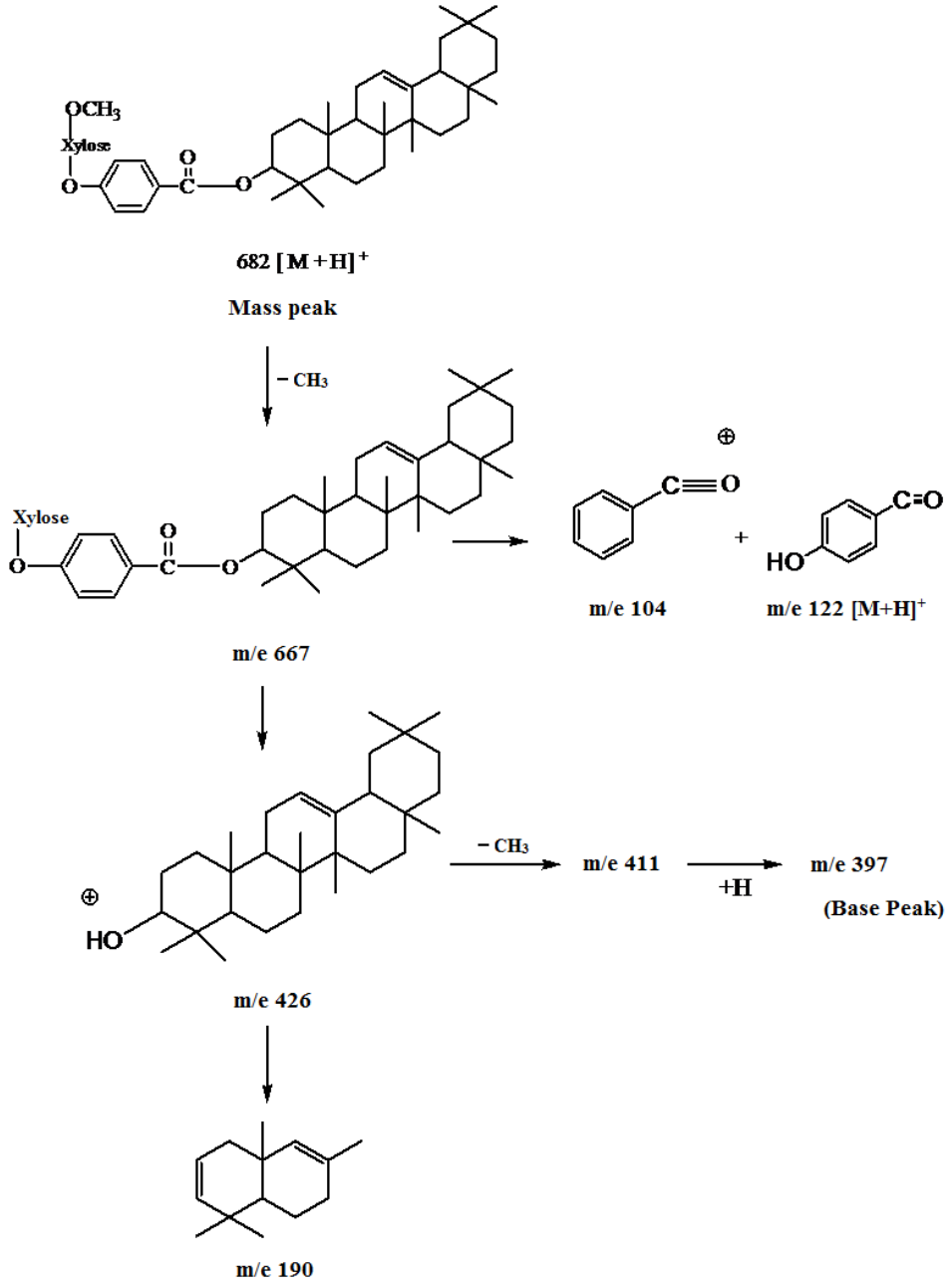


Fig 1. Mass Spectral fragmentation (m/z) of T1

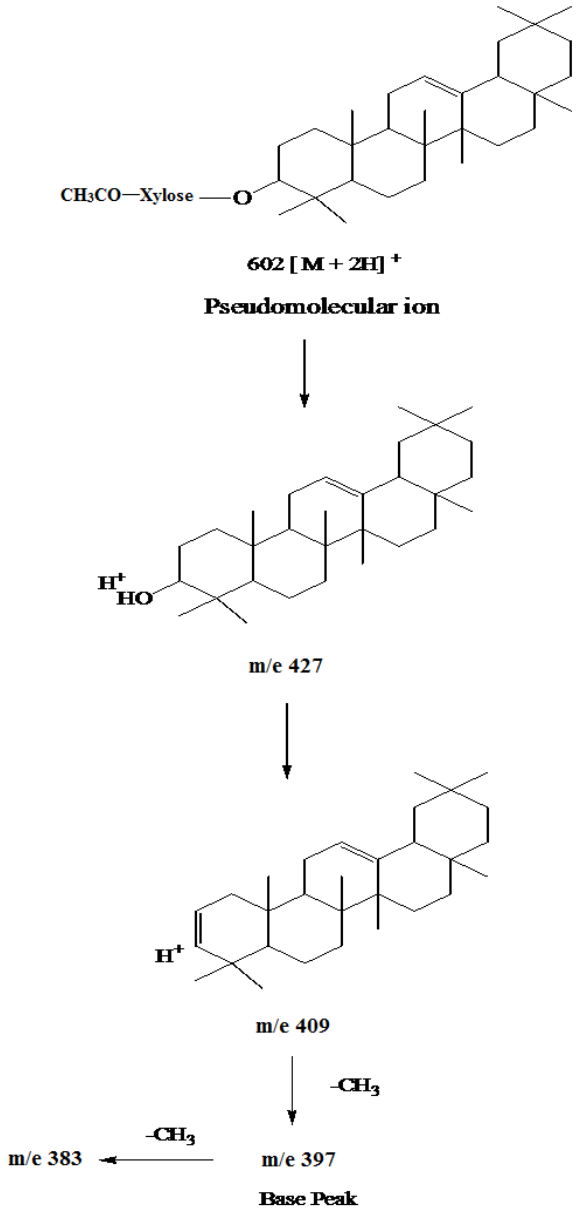
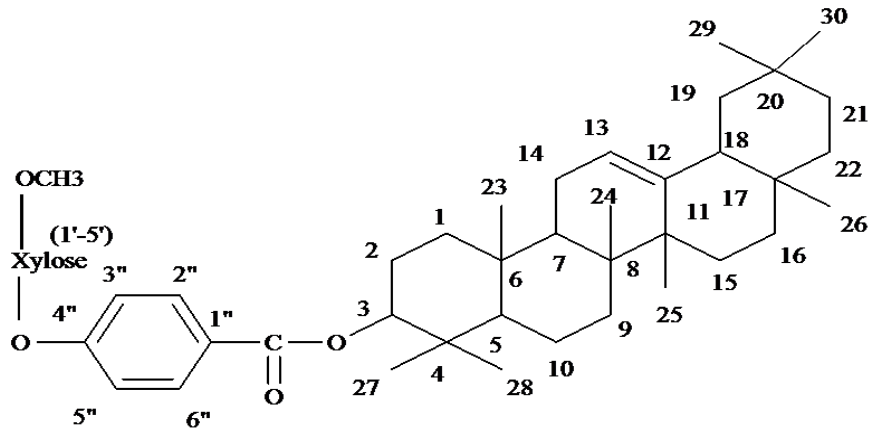
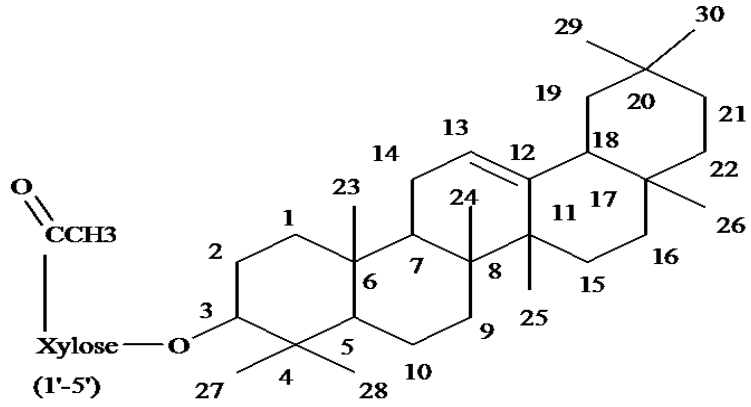


Fig 2. Mass Spectral fragmentation (m/z) of T2

The single peak of both the compounds in LC (0.565 and 0.553 at 254 nm respectively for T_1 and T_2) were indicating the purity of the compounds. Melting point of T1 and T2 were recorded as 99ºC and 160 ºC respectively. Structures of T_1 and T_2 were thus characterized as 4-(2-Hydroxy-1-hydroxymethyl-3-methoxy-4-oxo-butoxy)-benzoicacid-4,4,6a,6b,8a,11,11,14b-octamethyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12, 12a,14,14a,14b-icosahydro-picen-3-yl-ester and 2-Acetyl-3,5-dihydroxy-4-(4,4,6a,6b,8a,11,11,14b-octamethyl-1,2,3,4,4a,5,6,6a,6b, 7,8,8a,9, 10,11,12, 12a,14,14a,14b-icosahydro-picen-3-yloxy)-pentanal (Fig. 3).



Structure of T1



Structure of T2

Fig 3. Biomolecule isolated from *D. glabra*

Dioscorea glabra is a favorite therapeutic agent among eclectic physicians, who have advantageously used it for more than 60 years.[7] The decisive objective of the present research is to search for spermicidal agents from *D. glabra* that may efficiently serve as a valuable male contraceptive in future through further researches. The increase in population all over the globe is overwhelming and this situation intensifies the necessity for effective birth control measures. Consequently, rigorous efforts have been carried out to control the birth rate by different means and to find different natural contraceptives.[14] Contraceptives in female stop fertilization and ovulation, obliterate the zygote or induce abortion, on the other hand, in male it averts spermatogenesis, stops testosterone, or alters gonadotrophin of the organs or cause mortality of sperm.[15] Previously a large numbers of phytochemicals such as sesquiterpenes, tarpenes, flavanoid, phenols, terpenoid, saponins, and phenolic acid isolated from different plants have showed spermicidal properties.[16] Currently, search for natural spermicides of natural are in rise. Spermicidal compounds can be used in antispermogenic preparation, by develop them as gel or used in condom coat.[17] A number of antispermogenic compounds are isolated from plants, several bioactive compounds including terpenoids isolated from plant and marine sources exerted antispermogenic effect.[18,19] This study suggested that the isolated terpenoids confer good antispermogenic activity and can be possessed further for development of new male contraceptive.

CONCLUSION

The present study scientifically proved the spermicidal activity of the plant tuber. Isolated triterpenoids from the methanolic extract of the tuber of *Dioscorea glabra* possess antispermogenic activity *in vitro*. Further research is essential to better ascertain the bioactivity of these compounds through *in vivo* study, and also to understand their underlying cellular mechanism of action.

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