



Development of Molecular Condom by Using Anti-Fertility Phytochemical

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ABSTRACT: There are some herbal contraceptives that have the property to avoid the embryo implantation. *Trigonella foenum-graecum* L. is used in folk medicine for prevention of pregnancy. Pregnane- progesterone is one of the important steroid found in *Trigonella* has direct connection with contraceptive activity. Pregnane is a C₂₁ steroid and indirectly, a parent of progesterone. The present study showed the presence of [5 Beta]-Pregnane-3, 20, beta-diol, [4 alpha] 8-alpha-[4-methyl-3-oxa] oxa-4-ozabutane-1, 4 diyl-diacetate. This pregnane was found first time in this plant. Pregnane progestin contraception is effective and well tolerated, thus providing an excellent Molecular contraceptive alternative to the currently used methods. Pregnane can be isolated from *Trigonella foenum-graecum* L. for the preparation of Depo-Provera as a contraceptive measure. Ethynyl-estradiol is an estrogen that is active when taken by mouth and is used in almost all formulation of combined birth control pills, and is nearly the exclusive estrogen used for this purpose. Estrogen contributes to ovulation suppression. The ethynyl estradiol has high estrogenic potency when administered orally, and is often used as the estrogenic component in oral contraceptive. Diosgenin in is the most widely used precursor in the preparation of many steroid drugs, sex hormones and oral contraceptives pills. In the present study the concentration of Diosgenin was estimated in the seeds of *Trigonella foenum-graecum* L. The above mentioned anti fertility phytochemical can be used as to develop molecular condoms. © 2019 iGlobal Research and Publishing Foundation. All rights reserved.

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INTRODUCTION

Phytochemical found in plants are progressively gaining popularity over conventional artificial drugs to prevent or treat chronic diseases. During the recent past the interest has developed again in study and use of traditional medicinal plants. More people are realizing that the natural is better. Most of the traditional plant based remedies are back in use and find increasing as therapeutic agent, raw material for new synthetic compound and a major molecule for the discovery of molecule. The use of herbal crude drugs, extracts and their

remedies have significantly increased throughout the world and the green revolution in terms of herbal medicine has now achieved astonishing popularity. Natural drugs are being used as antimicrobial [1, 2, 3], antifungal [4], antiviral and anti-fertility [5].

Since many years workers are trying to find out innovative plant based drugs containing medicinally valuable photochemical. These are alkaloids, glycosides, phenolic compounds, steroids, and terpenoid derivatives. The

assessment of the drugs is based on phytochemical and pharmacological approaches proceed to the drug discovery referred as natural product screening [6]. The plant parts contain active components [7]. People have been trying to reduce and treat diseases with different plant extracts and formulations [8]. It was reported that more than 119 phytochemical were isolated and identified from higher plants that were being used worldwide as drugs [9]. However, the phytochemical composition of the aqueous and petroleum ether extracts of *Trigonella foenum-graecum* L. and *Moringa oleifera* Lam. used for medicinal purposes has not been sufficiently studied which may be further evaluated and used for the development of molecular condom. The specific compounds or molecules have not been precisely defined at certain level.

Hence, it becomes essential to evaluate the anti-fertility phytochemical of pharmaceutical importance in relation to development of molecular condom. The phytochemical study was carried out from *Trigonella foenum-graecum* L. and *Moringa oleifera* Lam. The presence of active compounds was further correlated with contraceptive activity and evaluation of authenticity of plants.

MATERIALS & METHODS

Collection of plant material

On the basis of literature and survey of tribal people [10] following plants were selected for the scientific study of spermicidal activity. The selected plants were *Trigonella foenum-graecum* L. (Fabaceae) and *Moringa oleifera* Lam. (Moringaceae). Seed samples were collected from various areas of Ahmednagar District, India in their natural habitat. They were identified from Botanical Survey of India, Western Circle Pune. The voucher specimens were deposited in the Herbarium, BSI Pune as well as in Herbarium of Department of Botany, New Arts, Commerce and Science College, Ahmednagar.

Extraction of the plant materials

The seed were air dried at room temperature followed by pulverization to powder form using mortar and pestle. The powdered seeds were subjected to aqueous extraction as well as extraction of active components from seed powder was performed with petroleum ether by using Soxhlet. Polar and non-polar solvent were taken into consideration for extraction. The residues were collected and preserved at 4°C for further experiment. The non-polar Petroleum ether was used which being more effective than methanol extracts [11], so the extracts were made in this non polar solvent.

Gas Chromatography-Mass Spectrometry Analysis

The GC-MS analysis was carried out using a Hewlett packed gas chromatography (model 6890series) equipped with a flame ionization detector and Hewlett Packard 7633 series indicator, MS transfer line temperature of 250 °C. The GC was equipped with a fused silica capillary column HP -5MS (30x0.25 mm), film thickness 1.0 µm. The oven temperature was held at 50°C for 5 min holding time and raised from 50 to 250°C at a rate of 2°C/min, employing helium gas (99.99%) as a carrier gas at a constant flow rate of 22 cm/s. 1.0 micron of extract (1 mg dissolved in 1ml absolute alcohol), at a split ratio of 1:30 was injected. MS analysis was carried out on Agilent. Technology network Mass spectrometer (model 5973 series) coupled to a Hewlett Packard Gas chromatography Model 6890 series) equipped with NIST08 Library software database. Mass spectra were taken at 70 eV/200°C scanning rate of 1 scan/s.

Identification of compounds

Interpretation of mass spectrum of the unknown component was conducted by comparing the mass spectra with the spectrum of the known components stored in the data system National Institute Standard and Technique library (NIST-2008, Turbo mass Ver. 5.4.2). The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The name, molecular weight, structure and mass fragmentation of the components of the test materials were given.

HPLC analysis method

10 mg of standard diosgenin was weighed and dissolved in 5 ml of methanol by means of sonicated for 15 min. The solution was diluted up to 10 ml with methanol (1 mg/ml). 1 ml solution was taken out from stock solution and diluted up to 10 ml with methanol (100µg/ml). The ethinylestradiol and saponin were extracted effectively by alkaline hydrolysis followed by HPLC-UV determination.

The details of the instrument used were Shimadzu Prominence HPLC modular system, Binary Gradient with PDA detector with Shim-pack solar 18C column (Chromolith Chromatography, A Japan Company). It was a reverse phase (stationary phase was non-polar and mobile phase was polar). The column temperature was 40°C maintained for all samples. The mobile phases were composed of acetonitrile and water in the proportion of 90:10. The adjustable experimental variables were the conditions of gradient modes and mobile phase compositions. The flowrate was kept at 1 ml/minute. The injection volume was 25 µl. Ethinylestradiol was detected using absorbance at PDA multilevel 225 nm, 4 nm, while diosgenin and saponins were detected using absorbance at

PDA multilevel 203 nm, 4 nm. Optimization of HPLC conditions as a standard procedure was carried out prior to analysis.

RESULTS AND DISCUSSION

The selected plants for the study were used by the ethnic and tribal people as traditional contraceptives in Ahmednagar District [12]. So these plants were analyzed to find out the novel phytochemical which may be responsible and correlate with contraception. Phytochemical were screened by GC-MS and HPLC technique. The results are represented in Tables, Graphs and Figures.

Major phytochemical of contraceptive plants:

The major compounds found in traditional contraceptive plants were further analyzed by HPLC technique (Ethinylestradiol and Diosgenin) and GC-MS technique (Pregnane). High pressure liquid chromatography (HPLC) has been found to be an efficient and sensitive method for identification and quantification of different classes of Ethinylestradiol, Diosgenin and Saponins along with standards. The proposed method is found to be a rapid, efficient method which requires a simple sample preparation for the quantification of phytoestrogens. It was reported that these compounds are directly involved in contraceptive [13, 14, and 15]. The present research investigated the properties of Ethinylestradiol, Diosgenin and Saponins in *Moringa oleifera* Lam., *Trigonella foenum-graecum* L. and *Sapindus emarginatus* Vahl., respectively. HPLC studies showed that the peaks of phytoestrogens obtained from plant extracts were similar with the peaks obtained by standards.

Pregnane- Progesteron by GC-MS:

Pregnane is a C₂₁ steroid and indirectly a parent of progesterone. The progestogens are one of the five major classes of steroid hormones. All endogenous progestogens are characterized by their basic 21-carbon skeleton, called a pregnane skeleton (C₂₁). Progesterone is an endogenous steroid and progestogen sex hormone involved in the menstrual cycle, pregnancy and embryogenesis of humans and other species [16]. The progestogens are named for their function in maintaining pregnancy, although they are also present at other phases of the estrous and menstrual cycles [17, 18]. Progesterone affects the vaginal epithelium and cervical mucus, making it thick and impenetrable to sperm. Progesterone is anti-mitogenic in endometrial epithelial cells and as such mitigates the tropic effects of estrogen [4]. There is coordination amongst Pregnane, corpora lutea and progesterone. It was reported that homogenates obtained from bovine corpora lutea tissue were found to catalyze the synthesis of 3 beta-hydroxy-5 alpha-

pregnan-20-one (allopregnanolone) from progesterone but not from pregnenolone [19]. The major metabolites of progesterone included allopregnanolone, 5 alpha-pregnane-3,20-dione and fatty acid esters of allopregnanolone. Progestogens have actions in the midbrain ventral tegmental area to mediate motivated behaviours such as those involved in reproductive processes and mating among female rodents. In VTA the formation and actions of one progestogen, 5alpha-pregnan-3alpha-ol-20-one (3alpha, 5alpha-THP) is necessary and sufficient to facilitate sexual responding of female rodents [20]. The roles of progesterone and other progestogens (referring here to progesterone and its neuroactive products including 5alpha-pregnan-3alpha-ol-20-one; 3alpha, 5alpha-THP) beyond their pro-gestational effects are of interest.

Ethinylestradiol by HPLC

Ethinylestradiol is an estrogen that is active when taken by mouth and is used in almost all formulation of combined birth control pills and is nearly the exclusive estrogen used for this purpose. Estrogen contributes to ovulation suppression [21]. High-performance liquid chromatography (HPLC) analysis was developed and validated for the determination of the contents of Ethinylestradiol compounds in *Moringa oleifera* Lam. seeds. Analysis of Ethinylestradiol derivatives extracted in petroleum ether and aqueous seed extracts of *Moringa oleifera* Lam. revealed significantly high levels. Three peaks were elucidated in the chromatogram developed in petroleum ether extract. The highest peak area was obtained by the peak having retention time 3.092 minute with 82.43% peak area. Second higher peak was obtained at retention time 6.206 minutes which has covered 11.20% peak area. It was observed that the third peak of the Moringa sample has showed about same retention time 6.206 minutes for Ethinylestradiol with less peak area 11.20%. Simultaneously, the standard Ethinylestradiol peak area and retention time was compared with the Sample peak. The peak obtained in standard was at retention time 6.161 minute with 62.77% peak area. The spectra 6.206 obtained in petroleum ether extract of Moringa indicate that Ethinylestradiol may be present in more amounts and may be associated with its derivatives. So retention time of standard and observed spectra of sample may be different on chromatogram. The peaks obtained in aqueous extract were totally different with respect to petroleum ether extract due to the concentration.

In *Moringa oleifera* Lam. the calculated value of ethinylestradiol in petroleum extract was 108.199µg/ml and 655.26µg/ml in aqueous extract. As the concentration of the samples increased the peak area was also increased. Hence the above values for the amount of ethinylestradiol in petroleum ether and aqueous extract are variable. From these

results it was confirmed that *Moringa oleifera* Lam. contains ethinylestradiol. It is a steroidal estrogen and a derivative of estradiol [22]. It is very essential to evaluate observed (Retention Time 6.206 min) compound by more sensitive technique for characterization and quantification of Ethinylestradiol. It has high estrogenic potency when administered orally, and is often used as the estrogenic component in oral contraceptive.

Diosgenin by HPLC

Diosgenin is a major bioactive constituent of some of the plants. It is the most widely used precursor in the preparation of many steroid drugs, sex hormones and oral contraceptives pills [23, 24]. Presence of diosgenin was reported in *Helicteres isora* L. [25], *Dioscoria* [26, 27], *Trigonella foenum-graecum* L. [28]. It was reported that *Trigonella foenum-graecum* L. seeds contain considerable amount phytoestrogens such as isoflavones, lignans and coumestrol [29]. In the present study the concentration of Diosgenin was estimated in the seeds of *Trigonella foenum-graecum* L. by using HPLC technique.

The concentrations of diosgenin in aqueous and petroleum ether extract of *Trigonella foenum-graecum* L. were calculated

by measuring the peak area of samples comparing with peak area of standard diosgenin. In petroleum ether extract of *Trigonella foenum-graecum* L. multiple peaks of compounds were obtained but one of them was having major contribution with higher peak area. It was 6762643 peak areas at 3.131 retention time. At this peak concentration of diosgenin was 1.280 mg/ml. In the aqueous extract also various peaks were obtained. Two of them were major with higher peak areas. The first peak was with retention time 2.779 and the area was 26667760 while second peak was with retention time 2.893 and the area was 13150414. These areas were calculated and compared with the peak area of standard. The concentration of diosgenin in aqueous extract was 5.098 mg/ml in the first peak with retention time 2.779 while it was 2.506 mg/ml in the second peak with retention time 2.893. The present HPLC analysis showed that *Trigonella foenum-graecum* L. contains measurable amount of diosgenin. In the present study the highest diosgenin levels were observed in seeds of *T. foenum-graecum* L. which was in agreement and comparable with the report [28, 30, 31].

Figure 1 GC MS Profile of *T. foenum-graecum* seeds showing Pregnane

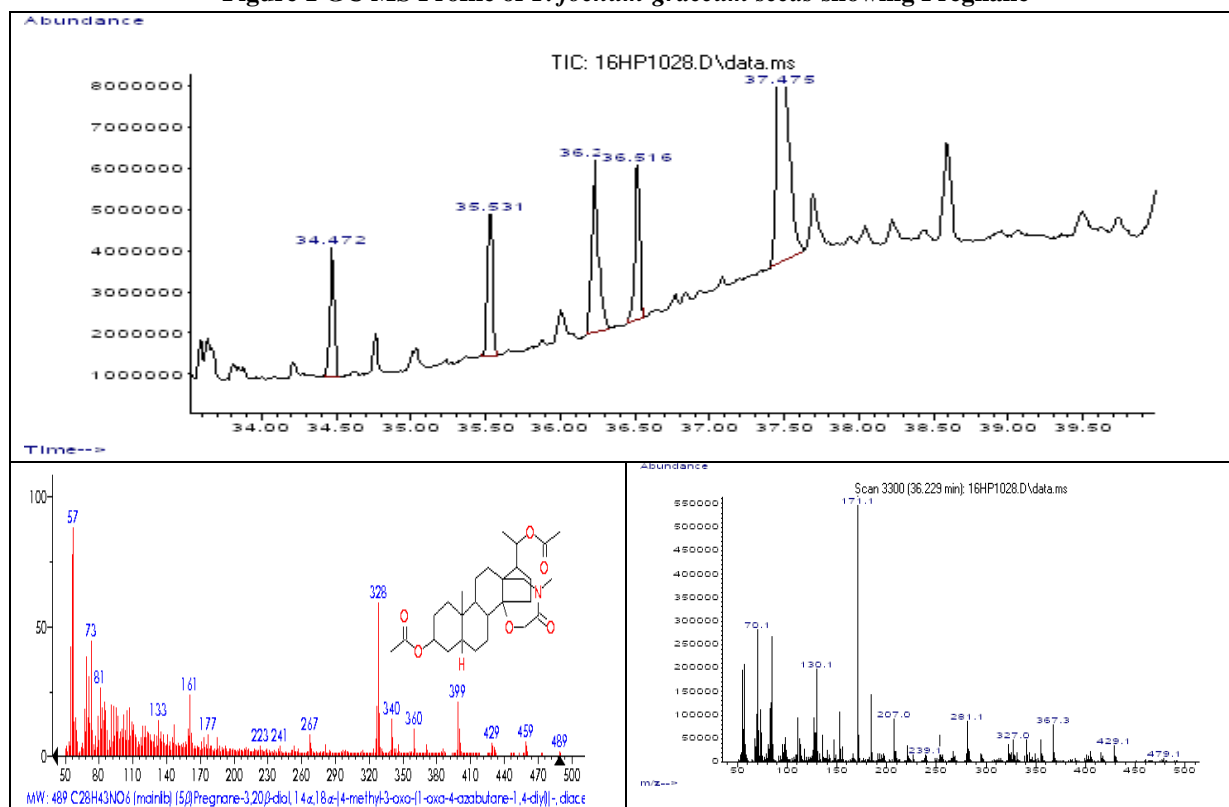


Figure 2: HPLC Chromatogram of Ethynylestradiol standard and extracts of *Moringa oleifera* Lam.

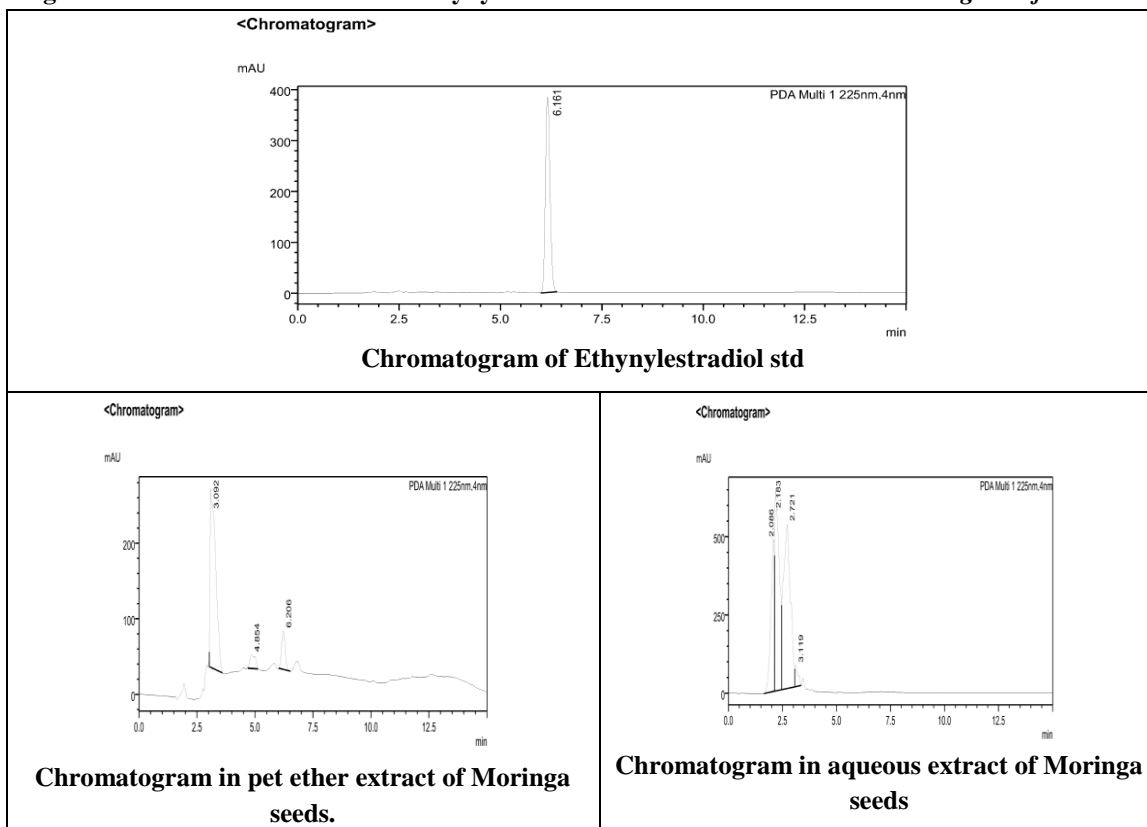
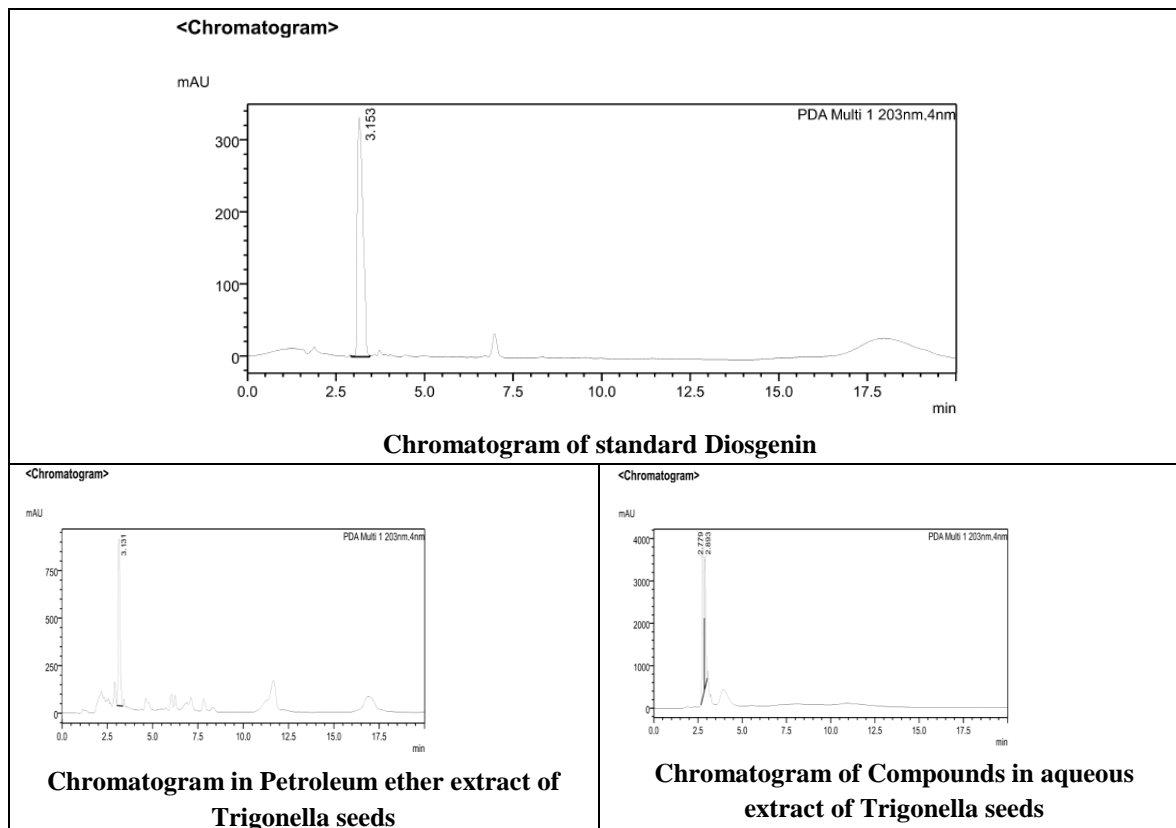


Figure 3: HPLC Chromatogram of standard Diosgenin and extract of Trigonella seeds



CONCLUSION

Pregnane, Ethynylestradiol and Diosgenin are the phytochemicals which showing antifertility activity. In future, these may be used for preparation of molecular condom. It may be used for developing a non-hormonal form of contraception that could be used after sex to prevent sperm from reaching their goal. This molecular condom could be useful for those who are opposed to using existing options. There is also the possibility that the molecular condom which is channel blockers could work their magic from the man's side before they set off on their journey delivered through a pill or patch.

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