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# PREGNANE - A PARENT OF PROGESTERONE FROM *TRIGONELLA FOENUM - GRAECUM* LINN.

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**ABSTRACT:** There are some herbal contraceptives that have the property to avoid the embryo implantation. *Trigonella foenum-graecum* Linn. is used in folk medicine as alcoholic and aqueous preparations for prevention of pregnancy. Pregnane- progesterone is one of the important steroid has direct connection with contraceptive activity. Pregnane is a $C_{21}$ steroid and indirectly, a parent of progesterone. The present work was carried out to find out the responsible phytochemical for contraceptive activity. The objective of this study was to provide an alternative contraception and thus evaluate the pregnane progesterone. The present work was carried out in *Trigonella foenum-graecum* Linn. with the presence of compound "Pregnane" and its importance in contraceptive activity. 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 with retention time 36.23, peak area 5.335% ($C_{28}H_{43}NO_6$ Mol wt- 489). This pregnane was found first time in this plant. Pregnane progestin contraception is effective and well tolerated, thus providing an excellent contraceptive alternative to the currently used methods. Pregnane can be isolated from *Trigonella foenum-graecum* Linn. for the preparation of Depo-Provera as a contraceptive measure.

**INTRODUCTION:** There are some herbal contraceptives that have the property to avoid the embryo implantation. *Trigonella foenum-graecum* Linn. is used in folk medicine as alcoholic and aqueous preparations for prevention of pregnancy [1, 2]. The contraceptive activity may be due to various types of phytochemicals present in *Trigonella foenum-graecum* Linn. It is an annual crop belonging to the family fabaceae. It is about 30-60cm tall and cultivated throughout the country. It is native to Western Asia where it has covered Europe, the Mediterranean region, and the rest of Asia. It is used as anti-diabetic, anti-fertility, anti-microbial, anti-parasitic and hypocholesterolaemic, antileptic, antibronchitis, carminative, aphrodisiac, analgesic, antipyretic, anticancer [3], antioxidant, immunomodulator in phlegm disorders, and recently reported in balancing the blood sugar level. In India, fenugreek powder is also used as a lactation stimulant and protective against ethanol toxicity [4]. Progesterone is sometimes called the "hormone of pregnancy". Progesterone, the name of the hormone reflects this understanding ('pro': in favour; '-gest': gestation; '-one': ketone chemical structure).

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Progesterone was discovered as a hormone produced by the corpus luteum, essential for pregnancy maintenance [5-7]. It regulates cyclical changes in the endometrium of the uterus and maintains a pregnancy. Progesterone possesses an essential role in regulating female fertility, with prominent actions throughout the female reproductive axis [8]. Pregnane- progesterone is one of the important steroid has direct connection with contraceptive activity. Pregnane is a $C_{21}$ steroid and indirectly, a parent of progesterone. Various synthetic hormones are used in contraceptive pills, whose adverse health effects have been well documented over the past 30 years [9].

The present work was carried out to find out the responsible phytochemical for contraceptive activity. Combined oral contraceptives can worsen the course and increase the risk of thrombosis [10]. The pregnane is the parent of contraceptive progesterone so, the objectives of this study were to provide an alternative contraception and thus evaluate the pregnane progesterone. The present work was carried out in *Trigonella foenum-graecum* Linn. with the presence of compound "Pregnane" and its importance in contraceptive activity.

## MATERIAL AND METHODS:

**Collection of Plant Material:** Seed samples of *Trigonella foenum-graecum* Linn. (Fabaceae) were collected from various areas of Ahmednagar District, India in their natural habitat. It was identified and authenticated from Botanical Survey of India, Western Circle, Pune. The voucher specimens were deposited in the Herbarium, BSI, Pune as well as Herbarium of Department of Botany, New Arts, Commerce and Science College, Ahmednagar (ABK 004).

**Extraction of the Plant Materials:** The seed were air dried at room temperature followed by pulverization to powder from using a 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. Solvent was removed by vacuum rotary evaporator at room temperature. The remaining residue was 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 6890 series) 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 1.0µm. 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 (1mg 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.

**RESULTS:** Identified compounds are listed in **Table 1** with their Retention time, chemical formula, molecular weight and the peak area. The seed extract of *T. foenum-graecum* was analyzed for the phytochemical composition. The phyto-chemicals were screened by GC-MS technique. GC–MS analysis of the Petroleum ether seeds extract led to the identification of 6 different compounds from total 16 different peaks. The major compounds detected were 2,4-Decadenal (E β 2E, 4E) Deca-2,4-dienal (Aldehyde-1.325%); 6-(3- acetyl- 2- methyl cycloprope- 1- enyl)-6-methyl

heptanes-2-one (Ketones-0.679%); n-Hexadecanoic acid (Carboxylic acid-7.878%); 9, 12 Octadecanoic acid (Carboxylic acid 40.400%); Propanoic acid 2 (3-acetoxy-4-4) 4-trimethyl androsta-8-en (Steroid-3.299%) and [5β] Pregnane-3,20,β-diol,[4α] 8α-[4-methyl-3 oxa]-4-azabutane-diyl-diacetate (Steroid-5.335%) (**Fig. 1**). The highest peak area was given by Octadecoinic acid which is the major constituent. Propanoic acid and Pregnane are two steroids were found where Pregnane is the parent of progesterone [12]. In the present work the compound pregnane was focussed because it is one of the important compounds with respect to contraceptive activity.

**TABLE 1: GC-MS CHEMICAL COMPOSITION OF PETROLEUM ETHER EXTRACT OF *T. FOENUM-GRAECUM***

| Sr. no | Retention time | Name of component | Molecular formula | Molecular weight | Peak area in % |
|---|---|---|---|---|---|
| 1 | 13.573 | 2,4-Decadenol (E.B.) | $C_{10}H_{16}O$ | 152 | 1.325 |
| 2 | 21.59 | 6-(3-acetyl-2-methyl cyclopropane-1-enyl)-6-methyl heptane-2-one | $C_{14}H_{22}O_2$ | 222 | 0.679 |
| 3 | 28.98 | n-Hexodecanoic acid | $C_{18}H_{32}O_2$ | 256 | 7.878 |
| 4 | 30.98 | 9,12 Octodecadenoic acid | $C_{18}H_{32}O_2$ | 286 | 40.400 |
| 5 | 34.51 | Propanoic acid 2(3-acetoxy-4-4)4-trimethyl androsta-8-en-17 YL | $C_2H_{42}O_4$ | 430 | 3.299 |
| 6 | 36.23 | [5 Beta]-Pregnane-3,20,beta-diol,[4 alpha]8-alpha-[4-methyl-3-oxa]oxa-4-ozabutane-1,4 diyl-diacetate | $C_{28}H_{43}N\ O_6$ | 489 | 5.335 |

**DISCUSSION:** Pregnane is a $C_{21}$ 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 ($C_{21}$). Progesterone is an endogenous steroid and progestogen sex hormone involved in the menstrual cycle, pregnancy, and embryogenesis of humans and other species [13]. The progestogens are named for their function in maintaining pregnancy, although they are also present at other phases of the oestrous and menstrual cycles [14, 15]. 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 [16].

There is coordination amongst Pregnane, corpora lutea, and progesterone. It was reported that Homogenates obtained from bovine corpora luteal tissue were found to catalyze the synthesis of 3 beta- hydroxy- 5 alpha- pregnan- 20- one (allopregnanolone) from progesterone but not from pregnenolone [17]. 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.

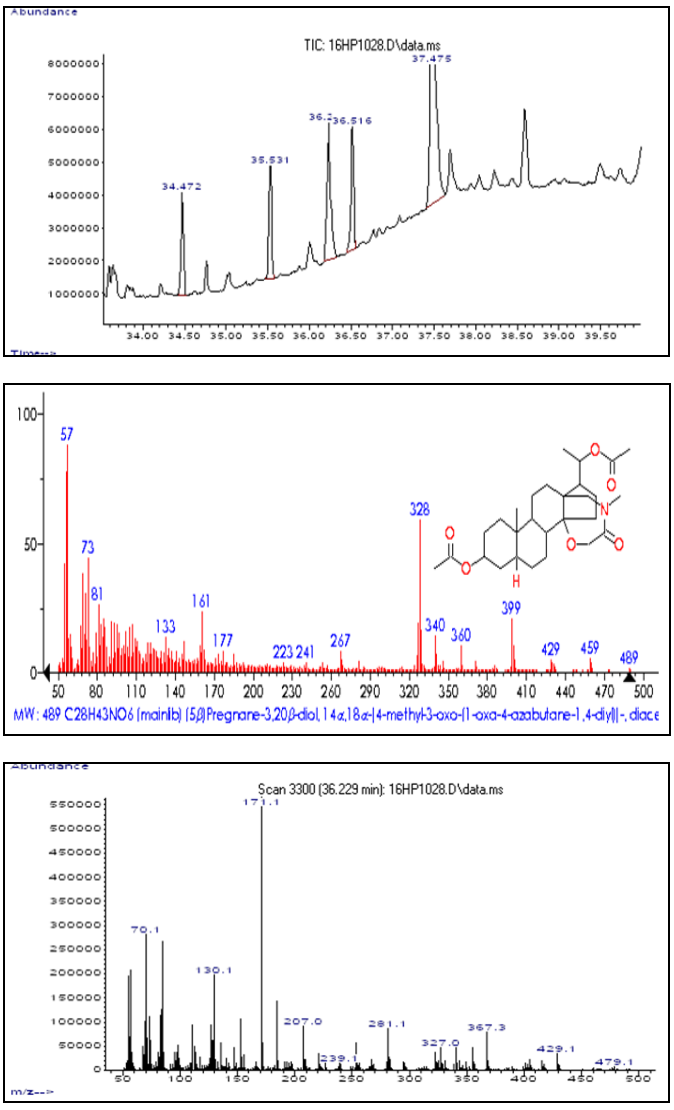


**FIG. 1: GC MS PROFILES OF *T. FOENUM-GRAECUM SEEDS* SHOWING PREGNANE**

In the VTA, the formation and actions of one progestogen, 5α-pregnan-3α-ol-20-one (3α, 5α-THP), are necessary and sufficient to facilitate sexual responding of female rodents [18]. The roles of progesterone, and other progestogens (referring here to progesterone and its neuroactive products, including 5α-pregnan-3α-ol-20-one; 3α, 5α-THP), beyond their pro-gestational effects are of interest.

Progestogens are involved in facilitating successful mating before gestation, have organising effects on the nervous system during gestation / perinatal, and can alter adult behaviours that ultimately are adaptive (reducing stress / anxiety, enhancing cognition and conferring protection to neural insults / ageing [19] of interest are the novel sources and targets of progestogens for such effects.

Further it was reported that synthesis for the derivatives of estrane, androstane, and pregnane, the contraceptives estradiol, testosterone, and progesterone, respectively are involved [20]. 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 with retention time 36.23, peak area 5.335% ($C_{28}H_{43}NO_6$ Mol wt - 489). It is a steroid and metabolite of Progesteron. It was revealed that Pregnane-3, 20á-diol with retention time 42.94 and peak area 0.61 ($C_{28}H_{43}NO_6$, Mol Wt- 489) and Pregn-5-ene-3, 20-dione with retention time 44.30, peak area 0.47 ($C_{21}H_{30}O_2$, Mol Wt - 314) from marine red seaweeds [21]. Phytochemical study of *Hoodia gordonii* aerial parts led to isolation of seven pregnane glycosides [22]. The bioassay-guided phytochemical investigation of *Sarcococca hookeriana* with respect to cholinesterase inhibitory properties has yielded two new 5alpha-pregnane-type steroidal alkaloids [23]. The acetylated pregnane, 17α- acetoxy- 6α- methylpregn- 4- ene-3, 20-dione [medroxyprogesterone acetate (MPA)], is used extensively in hormone replacement therapy and contraception [24]. There are different kinds of contraceptive measures. Depo-Provera is one the contraceptive measures containing Medroxy progesterone acetate (MPA) which is known as pregnane [25]. MPA is a manufactured hormone of the progestin type. It is used as birth control and as part of hormone replacement therapy for menopausal symptoms. It is also used to treat endometriosis, abnormal uterine bleeding, abnormal sexuality in males, and certain types of cancer. It is used by mouth or injection into a muscle or under the skin. MPA is a pregnane ($C_{21}$) steroid and a derivative of 17α- hydroxy-progesterone. Specifically, it is the 17α-acetate ester of medroxy-progesterone or the 6α-ethylated analogue of hydroxyprogesterone acetate. Medroxy progesterone acetate (MPA) is known chemically as 6α-methyl-17α-acetoxyprogesterone or as 6α-methyl-17α-acetoxypregn-4-en-3, 20-dione, and its generic name is a contraction of 6α-methyl-17α-hydroxyprogesterone acetate. Another class of pregnane found in *Trigonella foenum-graecum* Linn. seeds extract was $C_{28}H_{43}NO_6$ Mol wt- 489 ($C_{28}$). This pregnane was found first time in this plant.

**CONCLUSION:** Pregnane found in *Trigonella foenum-graecum* Linn. seeds extract was $C_{28}H_{43}NO_6$ Mol wt- 489 ($C_{28}$). This pregnane was found first time in this plant. Pregnane progestin contraception is effective and well tolerated, thus providing an excellent contraceptive alternative to the currently used methods. Pregnane can be isolated from *Trigonella foenum-graecum* Linn. for the preparation of Depo-Provera as a contraceptive measure.

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