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# Anti-implantation activity of different extract of the peels of Citrus medica,Linn

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**Abstract:** Three extracts of the peels of *Citrus medica* linn. (Family: Rutaceae) including oil, ethanolic and chloroform extract were investigated for antifertility activity. The extractive values of the oil, ethanolic and chloroform extracts of the peel of Citrus medica were found to be 0.45, 22.2, and 20.0 % respectively. The total ash, water soluble ash, acid insoluble ash, alcohol soluble extractive value and water soluble extractive values were found to be 4.25, 0.87, 2.11, 3.41 and 26.05% respectively. The alcoholic extract at the dose of 2.5gm/kg and the chloroform extract at dose of 1.0 gm/kg on female wistar rats on days 1-7 *post-coital,* exhibited significant mean anti-implantation activity. The oil extract at the dose of 100mg/kg on days 1-7 did not exhibit significant anti-implantation activity.
**Keywords:** Citrus medica linn; Anti-implantation activity.

## Introduction

In fertility regulation, the ancient literature has mentioned the use of a number of plants/preparations as abortifacients and local contraceptives.. WHO and ICMR provide systemic guidelines for the evaluation of anti-fertility plants to generate reproducible results, i.e., proper authentication and systemic screening methods [1].

Many plant preparations are reported to possess anti-fertility properties in ancient Indian literature[2]. Many plants have been tested for their anti-fertility activity in laboratory animals[3], but so far no single plant is available which can be used on humans as a potent anti-fertility agent. Hence, the search needs to be continued.

In the *Citrus* species, *Citrus hystrix* (Chinese species) belonging to the family Rutaceae, the fruit peels were investigated for anti-fertility activity in pregnant rats, results indicated that both alcohol and chloroform extract possesses *post-coital* anti-fertility activity[4]. The ethyl acetate fraction of alcoholic extract of another species *Citrus limonum* seeds showed reversible anti-fertility effect in mice by virtue of its anti-zygotic action[5]. Another species *Citrus aurantium* has also been reported to be once used as a contraceptive[6]. *Citrus medica* Linn (peel) was used as the traditional/folkloric medicine for anti-fertility activity[7,8]. The petroleum ether of *Citrus medica* Linn seeds has been reported to exhibit estrogenic activity[9].

*Citrus medica* linn. (Family: Rutaceae) is raised through seeds and stem cuttings. The genus Citrus belongs to the Rutaceae or Rue family, sub-family Aurantoideae. This family contains 140 genera and 1300 species distributed throughout the world.

The peel contains citroflavonoids consisting of a mixture of hesperidoside (rhamnoglucoside of hesperetol), naringoside and ecryodietyoside (flavonones). Essential oils and Vitamin C are also found. Glucosides hesperidin (Vitamin P) and rutin are also present. Some varieties contain the flavonoid naringin. The triterpenoids e.g. limonin gives the

intensely bitter taste to some citrus species[10]. Hesperidine is the major flavone derivative in the peel of *C.medica* Linn[11]. The essential oil of the peel is regarded as an antibiotic[12].

Since no scientific work with respect antifertility to has been reported in the peels of *Citrus medica* Linn (Turanj), the different extracts of the fruit peel was subjected to anti-implantation activity.

## Experimental

## Materials and Methods

### Plant material

*Citrus medica* Linn also known as Turanj in Hindi and Citron in English, mostly found in Sitakund Hill, Khasi and Garo hills of northern India were undertaken.

Peels of *Citrus medica linn* , collected from Shamsi Dawakhana, Khari Bhawli, Delhi. Peels were dried in shade at the temperature 28±2°C. The peels of *Citrus medica linn* were authenticated at NISCAIR (National Institute of Science Communication and Information Resources), CSIR, New Delhi.

### Extraction of oil from Citrus medica linn peels

Peels of *Citrus medica linn* were dried at room temperature and crushed to small pieces in a mortar pestle. The oil was obtained by hydro-distillation of the peel (500mg) with a modified Clevenger apparatus [13].

### Ethanolic extraction of Citrus medica Linn peels

The dried powder (1 kg) of the peels of *Citrus medica linn* was extracted with 95% (v/v) alcohol in Soxhlet apparatus. The extract was filtered and distilled under reduced pressure at 55 ± 5°C

### Chloroform extraction of Citrus medica Linn peels

The dried powder (1 kg) of the peel of *Citrus medica* Linn was extracted with chloroform in Soxhlet apparatus.The extract was filtered and distilled under reduced pressure

### Pharmacognostical evaluation

Sample of dried powder of *Citrus medica* Linn peel was subjected to quantitative standards like moisture content, total ash value, acid insoluble ash value, and water soluble ash value[14].

### Pharmacological studies

### Experimental Animals

All the animal experiments were conducted on animals collected from Animal House of DIPSAR(Delhi Institute of Pharmaceutical Sciences and Research) in accordance with CPCSEA (Committee for the purpose of control and supervision of experiments on animals) guidelines and were approved by the Institutional Animal Ethical Committee.

Female *Wistar* rats (150-275 gm body weight)

Male *Wistar* rats (200-300 gm body weight)

All the animals were kept under identical conditions (12:12 dark: light cycle). The room temperature was maintained at 25± $3^0$ C throughout the studies. They were housed in plastic cages with proper bedding (rice husk) and were fed with "standard laboratory feed pellets" diet (manufactured by Gold Feed, 894/8, Main Bazaar, Mehrauli, New Delhi-110030) and water *ad libitum.*

### Anti-Fertility Studies of *Citrus medica* Linn extracts

Female rats of established fertility in proestrus or estrus stage were housed with mature male rats of established fertility (in the ratio of 3 female: 1 male). Each female was then examined for the presence of spermatozoa in the early morning vaginal smear. The day, on which the spermatozoa were present in the vaginal smear, was taken as the day 1 of pregnancy. The female was then separated and caged singly. After the dosing schedule got completed, the animals were laparotomized on day 10 of pregnancy; the number of implants in the uterine horn and the number of corpora lutea on the ovary were counted. The animal was observed till delivery (i.e. 21 to 23 days) and numbers of litters delivered were counted[15].

The animals were divided into six groups consisting of six rats in each group.

### Anti-fertility activity of Citrus medica Linn peel oil, when administered orally on days 1-7 post coital in female albino rats.

Twelve female albino rats were divided into two groups of six animals each.

Group I served as control: propylene glycol (1 ml each)

Group II received the drug at the dose of 100 mg/kg body weight, orally, on days 1-7 *post-coital* with the help of an oral catheter.

### Anti-fertility activity of alcoholic extract of Citrus medica Linn peel when administered orally on days 1-7 post coital in female albino rats.

Twelve female albino rats were divided into two groups of six animals each.

Group I served as control and received 2% gum acacia (1 ml each),

Group II received the drug at the dose of 2.5 g/kg body weight, orally, on days 1-7 *post-coital* with the help of an oral catheter.

**Anti-fertility activity of chloroform extract of Citrus medica Linn peel, when administered orally on days 1-7 post coital in female albino rats.**

Twelve female albino rats were divided into two groups of six animals each.

Group I served as control and received 2% gum acacia (1 ml each) while,

Group II received the drug at the dose of 1.0 g/kg body weight, orally, on days 1-7 *post-coital* with the help of an oral catheter.

On day 10 of pregnancy, the animals were laparotomized and the numbers of implants present in both the uterine horns as well as the number of *corpora lutea* on each ovary were counted. The animals were allowed to complete the gestation period (usually 21-23 days) and the number of litters delivered if any, were counted.

Pre-implantation loss and Post-implantation loss were calculated using following formulas: -

Pre-implantation loss = No. of *corpora lutea* on Day 10 - No. of implants on Day 10.

Post-implantation loss = No. of implants on Day 10 - No. of litters delivered.

% Pre-implantation loss =
$$\frac{\text{No. of C.L} - \text{No. of Implants}}{\text{No. of C.L}} \times 100$$

% Post-implantation loss =
$$\frac{\text{No. of implants} - \text{No. of litters}}{\text{No. of implants}} \times 100$$

% Anti-implantation activity=
$$\frac{\text{No. of C.L.} - \text{No. of litters}}{\text{No. of C.L.}} \times 100$$

**Statistical Analysis**

All anti-implantation results are expressed as mean ±standard error. The ANOVA calculations were done using XLSTAT GraphPad Insat software for the determination of variation in the pre-implantation and post-implantation values between the control and all the treated group.One way ANOVA was followed using unpaired t-test where the treated group was compared with control [16].

**Table 1. Pharmacognostical evaluation of the powder of the peel of *Citrus medica* Linn (Turanj)**

| FOREIGN MATTER | Total ash % | Water soluble ash % | Acid-insoluble ash % | Alcohol soluble Extractive value % | Water soluble Extractive value % |
|---|---|---|---|---|---|
| Not more than 2% | 4.25 | 0.87 | 2.10 | 3.34 | 26.05 |

**Table 2. Percentage yield of the different extracts from the *Citrus medica* Linn peel**

| S.No | Extracts | % Yield in gm |
|---|---|---|
| 1 | Oil | 0.45 |
| 2 | Ethanolic | 22.2 |
| 3 | Chloroform | 20.0 |

**Table 3. Comparative anti-implantation activity of different extracts of Citrus medica peels**

| Treatment | Mean number of corpora lutea | Mean number of implants | Mean number of litters delivered | Mean Percentage anti-implantation activity |
|---|---|---|---|---|
| **Isolated Oil** | | | | |
| Control (1% propylene glycol) | 12.8 ± 2.2 | 9.7 ± 1.63 | 8.3 ± 1.9 | 35.07% |
| 100mg/kg | 14.7 ± 2.7 | 10.2 ± 1.60 | 7.5 ± 2.3 | 48.84% * |
| **Ethanolic extract** | | | | |
| Control (2% gum acacia | 12.7 ± 1.96 | 10.7 ± 1.63 | 9.3 ± 10.81 | 26.36% |
| 2.5g/kg | 13.7 ± 13.14 | 7.6 ± 1.9 | 3.8 ± 1.6 | 71.96%** |
| **Chloroform extract** | | | | |
| Control (2% gum acacia | 12.8 ± 1.83 | 10.0 ± 1.54 | 9.3 ± 10.81 | 27.28% |
| 1.0g/kg | 13.7 ± 13.14 | 7.6 ± 1.9 | 3.8 ± 1.6 | 77.19%** |

* $p > 0.05$ when compared with control

** $p < 0.05$ when compared with control

## Results

The total ash value, water soluble ash value, acid-insoluble ash value and alcohol soluble extractive value, water soluble extractive values of the *Citrus medica* Linn peel powder were calculated (Table 1).

The percentage yield of the tree extracts was calculated as,

Oil: The yield was 0.45% (on a dry weight basis),

Ethanolic extract: a yellowish green colored substance was obtained (crude extract, 22.2%), Chloroform extract: a dark green colored substance was obtained (crude extract, 20%). (Table 2)

The mean anti-implantation activity of the oil isolated from the peel of *Citrus medica* Linn was calculated to be 48.84% as compared to the control (35.07%) when administered orally on days 1-7 *post-coital*. The percent pre-implantation and post-implantation loss for treated groups analyzed statistically using paired t-test was not found to be significant ($p>0.05$).

The mean anti-implantation activity of the ethanolic extract from the peel of *Citrus medica* Linn was calculated to be 71.96% as compared to the control (26.36%) when administered orally on days 1-7 *post-coital* at the dose of 2.5g/kg. The mean anti-implantation activity of the chloroform extract when administered orally on days 1-7 *post-coital* at the dose of 1.0 g/kg was calculated to be 77.19% as compared to the control (27.28%).

The percent pre-implantation and post-implantation loss for treated groups was analyzed statistically with respect to control and was found to be significant in both extracts ($p<0.05$).(Table 3.)

## Discussion

Present study reveals significant values of anti-fertility activity of the alcoholic and chloroform extract as compared to oil extract on administration from days 1-7 of the pregnancy which suggests that alcoholic and chloroform extract might be interfering in the preparation of uterus for implantation to occur. This loss of implantation caused may be due to antizygotic, blastocytotoxic or antiimplantation activity as described by Hafez[17]. However, further experiments including oestrogenic evaluation are required to elucidate its exact mechanism of action.

No teratogenic effects were seen and the drug showed reversibility of action.

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