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EVALUATION OF THE POST-COITAL ANTI-FERTILITY ACTIVITY OF MICHELIA CHAMPACA LINN. AERIAL EXTRACT IN FEMALE WISTAR RAT

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ABSTRACT

***Background*:** The plant *M. champaca L.*, commonly known as Champa, has traditionally been utilized for its medicinal properties, particularly in women's health, for managing sterility and birth control. Previous literature primarily focuses on the anti-fertility activity of leaf extracts, while limited research has explored the potential of other aerial parts, such as the bark and flowers. Therefore, this study aims to investigate the female anti-fertility actions of various aerial parts (including leaves, branches, bark, and flowers) of the *M. champaca* plant, expanding our understanding beyond the previously studied leaf extracts. **Material and Method**: The petroleum ether (PEAEMC), ethyl alcohol (EAEMC), and chloroform water (AAEMC) extracts of aerial parts of *M. champaca* at doses of 100 mg/kg and 200 mg/kg were administered to female Wistar rats by using an experimental model, i.e., anti-implantation and estrogenic/Anti-estrogenic activity. **Result**: All three extracts showed significant anti-implantation activity ($p<0.01$). Among all, only EAEMC Showed activity corresponding to the standard. EAEME caused an increase in the vaginal opening size and increased height and width of the endometrium in immature ovariectomized female rats; it showed an estrogen-like action when given alone, however when given along with Ethinylestradiol, it showed anti-estrogenic action. **Conclusion:** It was observed that EAEMC (the Ethyl alcohol extract of *M. champaca*) showed dose dependent anti-fertility activity. The chemical constituents like steroids, alkaloids, and flavonoids identified from the photochemical screening may be responsible for the anti-fertility activity of the aerial parts of *M. champaca L.*

INTRODUCTION

The population is at an alarming stage in developing countries, which indicates the necessity for effective birth control measures [1]. In decreasing the fertility rate, synthetic contraceptive agents have played a significant role, but they have some undesirable side effects [2]. The adverse and undesired effects of hormonal contraceptives include High blood pressure, cervical cancer, stroke, breast cancer, endocrine gland dysfunction, weight gain, depression, headaches, hypermenorrhea, and fatigue [3]. The

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activity of natural hormones is affected by synthetic or chemical compounds that disturb the equilibrium of normal hormone levels by fluctuating their production and metabolism or blocking hormones [4,5]. As per the World Health Organization (WHO), for the production of synthetic drugs in India, only locally available plants are to be used. As per the literature, Medicinal plants have traditionally been used for contraceptive potential and anti-fertility effects since ancient times [6]. The medicinal plants may induce infertility in females in distinct ways, such as inhibiting hormonal action on the uterus and ovary, inhibiting hormone production, interfering with implantation and sperm penetration, and preventing fertilization by generating a protective layer around an egg. Therefore, the mechanism of action of the plants can be divided into categories such as anti-fertility plants, anti-implantation plants, contraceptives, and Abortifacients [7].

Michelia champaca L. (Magnoliaceae), commonly known as yellow Champa in sub-Himalayan tracts up to 3,000 ft, is found in Assam, Burma, South India [8]. It is reported to have significant wound healing, antimicrobial, antidiabetic, antitumor, anti-inflammatory, antioxidant, and anti-infective properties [9].

The aerial parts of the plant contain parthenolide, β-sitosterol, michampanolide, liriodenine, 8-acetoxy parthenolide, Magno grandiose, costunolide, dihydro parthenolide, ushinsunine, magnoflorine and micheliolide [10]. Traditionally, *M. champaca* leaves are used by women for sterility in Chhattisgarh state, India [11]. The leaf extract of *M. champaca* L. showed anti-fertility action [12].

As per the literature, the bark of *M. champaca* L. has been used in fertility regulation in the western Ghat area of Maharashtra state, India [13]. Therefore, scientists need to scientifically explore other parts of the plant for their anti-fertility actions. The present study was performed to explore the aerial parts of the *M. champaca* plant for post-coital anti-fertility activity by using different extracts prepared using various extraction methods to identify the most suitable extract.

MATERIAL AND METHODS

Collection of Plant and Authentication

The aerial parts of *the M. champaca Linn* plant (flowers, leaves, and branches) were collected. The plant was collected from the Herbal Garden, Panjab University, Chandigarh. A voucher sample of plant vide letter No. RBIPH/17/169 Dated 23rd May 2017, submitted to the Raw Material Herbarium and Museum, Delhi (RHMD), authenticated by Dr. Sunita Garg, Emeritus scientist, CSIR–NISCAIR with Ref. No. NISCAIR/RHMD/ Consult/2017/3078-27 for future reference.

Preparation of extract

The aerial parts (flowers, leaves, and branches) were shade-dried for two weeks and coarsely powdered. At room temperature, the plant material was macerated with petroleum ether (60-80) %. Then filtered, pet ether Extract (I) was obtained. The marc was again subjected to continuous hot Soxhlet extraction with ethyl Alcohol (70-80) % at 40-50° temperature. Then, filtered ethanol Extract (II) was obtained. Then, the marc left behind was macerated with chloroform water at 40-50° temperature. Then filtered, aqueous extract (III) was obtained, and Marc was discarded. The extract was evaporated under reduced pressure, and % the age of yield was found to be 3.5%, 5.3%, and 2.6%. Then it was stored in the refrigerator for further use.

Phytochemical Screening

For the phytochemical screening, petroleum ether, ethyl alcohol, and aqueous extracts of M. *champaca* were subjected to preliminary phytochemical screening as per reported methods [14].

Biological Activity

The extract obtained above was subjected to female anti-implantation activity

Experimental Animals

All the male and female Wistar rats weighing 150-200 g were selected for the anti-implantation study. Maharishi Markandeshwar University (Mullana) (Regn. No. 1355/PO/RE/ L/10/CPCSEA) approved by the Institutional Animal Ethical Committee. The study was according to the guidelines of CPCSEA, Ministry of Environment, Govt. of India. The animals were obtained from the animal house of Maharishi Markandeshwar University (Mullana). The laboratory conditions were maintained under temperature (21.5±22°C), humidity (60±1%), and 12-hour light and dark cycle. The experimental animals were allowed free access to feed and water.

Dose selection and preparation

As per the literature, *M. champaca* plant extract is safe up to 2000 mg/kg; therefore, the 100 mg and 200 mg/kg extracts were selected as the maximum dose for the study [13]. The extract dose was constituted by suspending the required quantity of extract in CMC (0.5% v/v in saline) freshly prepared and given by oral route (p.o.). Vehicle control groups were given an equal volume of CMC (0.5% v/v in saline), and Chlomiphen citrate, 1mg/day) was given to the standard group.

Estrous cycle

Smears were taken every morning between 08:00 and 09:00 by placing a dropper containing 1 to 2 ml of normal saline (0.9% NaCl) into the vagina. Place the genital fluid on a slide. Use on e slide for each animal. Observation of colorless objects in light without intensifiers using a microscope [15].

Anti-implantation activity

The female rats were caged with the male rats of proven fertility in the ratio of 2:1 in the evening of proestrus and examined the following day for evidence of copulation.

The female rats having copulation plugs or thick clumps of spermatozoa in their vaginal smear were selected as the experiment models, and that was designated as 1st day of pregnancy, and each group constituted 5-6 animals as given below:

Group I: Vehicle only (CMC 0.5%) and served as control. Group **II:** Chlomiphen citrate, 1mg/day and served as Standard
Group III (PEAEMC): 100mg/kg;
Group IV (PEAEMC): 200 mg/kg;
Group V (EAEMC): 100 mg/kg;
Group VI (EAEMC): 200 mg/kg,
Group VII (AAEMC): 100 mg/kg;
Group VIII (AAEMC): 200 mg/kg

The above treatment was given from 1 to 7 days of pregnancy, and on the 10th day, laparotomy was performed under anesthesia using sterile conditions. The number of implants was counted by examining the uterus. Weight gained by each group of rats was recorded, and anti-implantation activity was calculated [16].

***Estrogenic and anti-estrogenic activity*:**

Immature ovariectomised female rats (21–23 days) between 25 and 30 gm were used during the experiment. During the process, animals were divided into experimental and control groups, with five animals in each group. CMC 0.5% was administered to the control animals by oral route. Ethinyl estradiol in olive oil 1 µg/rat/day was injected subcutaneously for 7 days to induce estrous as a standard. The extracts were suspended in 0.5% CMC and, for 7 days, were administered orally at the dose level of 200 mg/kg body weight and given with or without ethinyl estradiol.

On the 8th day of the experiment, all the animals were sacrificed by decapitation under light ether anesthesia, and the uteri were dissected out, surrounding tissues removed, blotted on filter paper, and weighed. A portion of the uterine tissues and adrenal glands from the control were fixed in Bouin's fluid for 24 hours, dehydrated in alcohol, and then embedded in paraffin. The paraffin blocks were sectioned at 6 mm intervals and stained with hematoxylin-eosin for histological examinations [17].

Statistical analysis

The data was analyzed by using one-way Anova Dunnett's multiple comparison test, and $p<0.01$ was considered to be statistically significant for control and standard.

RESULTS

Phytochemical Screening

The preliminary phytochemical screening of the plant extracts showed the presence of alkaloids, flavonoids, tannins, and steroids.

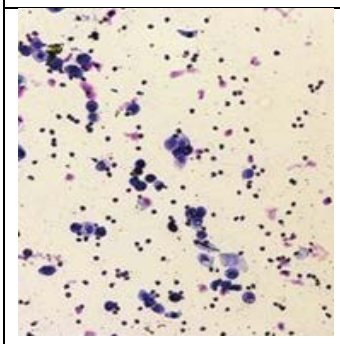
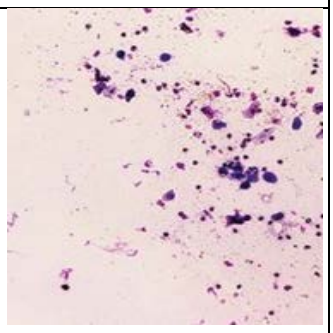
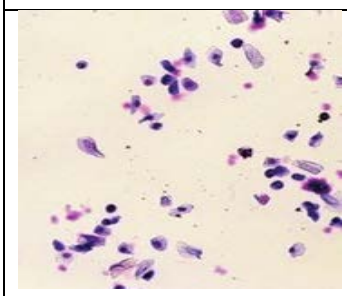
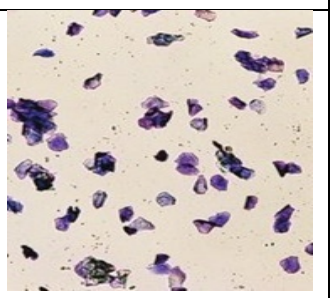
Observation of Estrus Cycle

4-day estrous cycle seen in selected female rats by using a magnifying microscope. Diestrus – 1st day, Proestrus - 2nd day, Estrus – 3rd day, Metaestrus – 4th day. From vaginal smear investigation estrus cycle of female rats is identified. Female rats with regular estrus cycles were selected for the study and placed in a separate cage with 3:1. (3 female and 1 male rat). Different stages of the estrus cycle in selected female rats are shown in Table 1

Anti-Implantation Activity

As per table no. 2, All the extracts of *M.champaca* showed the significant anti-implantation activity ($p<0.01$) at dose 100 mg/kg and 200 mg/kg. A Dose-dependent anti-implantation activity was observed. At dose 200 mg/kg, EAEMC (Ethanol extract) of found to be most active anti-implanation extract.

Table No. 1 Estrous cycle

Estrous cycle stages observation	
Diestrus	**Proestrus**
 Lots of epithelial cells appear in clusters	 Vaginal smear looks much clear as diestrus. Cornified epithelial cells appear without a nucleus
Estrus	**Metaestrus**
 smear consists nearly entirely of keratinized superficial cells. Leucocyte cell is found.	 Smear became darker as compare to estrus cycle. more giant intermediary cells and leucocytes are also present.

Body weight gain:

As shown in Table 3, the mean body weight of all the treated groups was less than that of the control group. The body weight of rats in the 200 mg/kg group is less than that of the group that received 100 mg/kg. The change in body weight of the treated group was significantly less, corresponding to the control group. Change in body weight was also observed. The body weight of female rats with EAEMC extract 200mg/kg corresponds to the standard. Therefore, only EAEMC at 200mg/kg will be used in further estrogenic/anti-estrogenic activity.

Estrogenic/Anti-Estrogenic activity:

The effect of EAEMC on body weight, uterine weight, and vaginal cornification has been shown in Table no. 4. Administration at the dose (200 mg/kg) p.o either alone and or with ethinyl estradiol significantly ($p<0.01$) increased the uterine weight of ovariectomized in comparison with control. EAEMC Extract evidenced the vaginal opening and presence of cornified cells in a vaginal smear of all female rats at 200 mg/kg doses either alone or along with EE.

However, the number of cornified cells in the EAEMC-treated groups is more than the control but less than EAEMC along with the ethinyl estradiol group. It significantly increases the uterine diameter, thickness of diameter, and height of the endometrium when compared with the control. When EAEMC extract is given with ethinyl estradiol, it decreases the endometrium's diameter, thickness, and height compared to the EE group.

Table 2: % inhibition of implantation sites in female rats in different groups

Group		**Dose/extract**	**Number of implantation sites**	**Average of implantation sites**
Group I	Control	Vehicle only (CMC 0.5%)	9, 6, 8, 8, 5	7.3±0.31
Group II	Standard	Chlomiphen citrate,1mg/day)	1, 3,0, 0,1	1.0±0.12
Group III	PEAEMC	100mg/kg	6, 5, 3, 7, 5	5.2±0.38
Group IV	PEAEMC	200 mg/kg	5, 2, 2, 3, 3	3.1±0.23
Group V	EAEMC	100 mg/kg	5, 6, 4, 5, 5	5.0±0.22
Group VI	EAEMC	200 mg/kg	1, 2, 2, 2, 0	1.8±0.19
Group VII	AAEMC	100 mg/kg	6, 5, 4, 4, 5	4.9±0.28*
Group VIII	AAEMC	200 mg/kg	4, 2, 2, 2, 3	2.7±0.18^

Dunnett's Multiple Comparison Test and $p<0.05$ was considered statistically significant; a test vs normal control; $p< 0.05$ = *; $p< 0.01$= ^; $p< 0.001$= #.

Table 3: % Change in body weight of female rats in different groups

Group		**Initial body weight**	**Average**	**Final body weight**	**Average**	**Change**	**%Change**
Group I	Control	202, 209, 207, 198, 211	205.4±1.92	229, 227, 235, 231, 238	232.0±1.55	26.6±1.22	12.9±0.90
Group II	Standard	204, 218, 207, 211, 205	203±1.65	212, 230, 223, 221, 219	218.8±1.96	14.8±1.28	6.40±0.82^
Group III	PEAEMC 100 mg/kg	211, 218, 200, 211, 219	211.8±1.28	241, 237, 211, 224, 235	229.6±2.11	17.8±1.33	8.4±0.70
Group IV	PEAEMC 200 mg/kg	214, 210, 217, 201, 200	21.4±1.44	221, 229, 221, 210, 224	221.0±2.02	12.6±1.29	6.07±0.84^
Group V	EAEMC 100 mg/kg	222, 210, 217, 202, 209	212±1.52	248, 238, 232, 217, 225	232.0±1.95	20.0±1.35	9.43±0.91
Group VI	EAEMC 200 mg/kg	204, 210, 206, 211, 215	209.2±1.68	220, 231, 219, 220, 222	222.4±1.59	13.2±1.40	6.3±0.73^
Group VII	AAEMC 100mg/kg	196, 208, 204, 215, 209	206.4±1.73	208, 229, 225, 231, 225	223.6±2.16	17.2±1.38	8.33±0.62
Group VIII	AAEMC 200 mg/kg	200, 212, 203, 210, 211	207.2±1.92	209, 228, 217, 231, 227	222.4±2.12	15.2±1.25	7.33±0.78*

Dunnett's Multiple Comparison Test and $p<0.05$ was considered to be statistically significant; test vs normal control $p< 0.05$ = *; $p< 0.01$= ^; $p< 0.001$= #.

Table No. 4 Estrogenic/Anti-Estrogenic activity of the EAEMC

Group	Dose	Ethinyl estradiol µg/rat	Body weight gain	Uterine weight	Vaginal cornification
Control Vehicle only	(CMC 0.5% v/v)	-	23.2±2.3	0.80±0.002	Nil
Ethinyl Estradiol *s.c*	-	1	42.6±4.4	1.70±0.006**	+++
EAEMC	200mg/kg	-	54.3±2.5ns#	1.50±0.024**	++
EAEMC +EE	200 mg/kg	1	62.1±1.5**	1.62±0.002**#	+++

n = 5 in each group; Ethinyl Estradiol as standard; ** $p < 0.01$ compared to control; ns non-significant compared to control; # $p < 0.05$ compared to standard

DISCUSSION

The anti-fertility potential of *M. champaca L.* aerial parts was investigated through extraction with petroleum ether, followed by sequential extraction with ethyl alcohol and chloroform water. Phytochemical analysis revealed the presence of flavonoids, steroids, and alkaloids in all extracts. Anti-implantation activity was evaluated in female Wistar rats using 100 mg/kg and 200 mg/kg of each extract. Significantly higher activity was observed with the 200 mg/kg dose, indicating a dose-dependent effect. Furthermore, a decrease in body weight was noted, consistent with established standards. Of particular interest was the ethyl alcohol extract of *M. champaca L.* (EAEMC) at 200 mg/kg, which exhibited notable anti-implantation activity ($p<0.01$). This finding suggests a potential for this extract as a contraceptive agent. Implantation equilibrium, governed by estrogen and progesterone levels, is critical for successful pregnancy [18]. Disturbances in these hormonal levels can lead to implantation failure [19].

The observed anti-implantation activity may be attributed to the extract's disruption of this hormonal equilibrium.Further investigation into the estrogenic activity of EAEMC revealed an increase in vaginal opening, vaginal cornification, and endometrial dimensions in immature ovariectomized rats when administered alone. This suggests that EAEMC possesses

estrogenic properties, likely due to its influence on estrogen levels. However, when administered concurrently with ethinylestradiol (EE), a decrease in endometrial dimensions and vaginal opening was observed. This indicates an anti-estrogenic effect, possibly mediated by negative feedback inhibition on the anterior pituitary. These results underscore the potential of *M. champaca L.* aerial parts, particularly the ethyl alcohol extract, as a source of compounds with anti-fertility properties. Further studies are warranted to elucidate the mechanisms underlying these effects and explore the potential for therapeutic applications.

CONCLUSION

The study highlights the anti-fertility potential of *M. champaca L.* aerial parts, mainly through the ethyl alcohol extract (EAEMC). Phytochemical analysis revealed the presence of bioactive compounds such as flavonoids, steroids, and alkaloids, which likely contribute to their pharmacological activities. The anti-implantation experiments demonstrated dose-dependent efficacy, with the 200 mg/kg dose exhibiting significant activity. This suggests a promising avenue for further exploration as a contraceptive agent. These findings underscore the multifaceted pharmacological potential of *M. champaca L.* aerial parts, highlighting its ability to modulate hormonal balance critical for fertility. Further research is warranted to elucidate the specific bioactive compounds responsible for these effects and to explore their potential for contraceptives and other therapeutic applications.

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Nil

CONFLICT OF INTEREST

The authors declare no conflict of interest

AUTHOR CONTRIBUTION

All the authors contributed to designing the manuscript, conceptualizing it, doing the literature review, and doing the final draft. Seema Devi worked under the supervision of Chander Mohan.

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