



Received on 21 February 2025; received in revised form, 06 March 2025; accepted, 23 March 2025; published 01 July 2025

EVALUATION OF ANTI-IMPLANTATION ACTIVITY OF *LUFFA ACUTANGULA* FRUIT EXTRACT ON FEMALE ALBINO RATS

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Keywords:

Female albino rats, Anti-implantation, Resorption, *Luffa acutangula* Fruit, Yavatmal district

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ABSTRACT: The study investigates the anti-implantation and early abortifacient effects of *Luffa acutangula* fruit, traditionally claimed by tribals in the Pandharkawada region of Maharashtra, on female albino rats. To evaluate its efficacy, fertile male and female albino rats were paired, and the females were divided into 13 groups, including a control group receiving distilled water and 12 experimental groups administered varying doses (100, 200, 400 mg/kg body weight) of *L. acutangula* extracts for 1-7 days. On the 10th day of gestation, laparotomy was performed to assess implantation sites and corpora lutea counts. Phytochemical analysis revealed the presence of several bioactive compounds, including alkaloids, flavonoids, and steroids, while no toxicity symptoms were observed in the treated animals. Results indicated significant post-coital antifertility activity, with a reduction in implantation rates ranging from 12.61% to 72% across different extracts. Notably, the chloroform and ethyl acetate extracts yielded higher litter sizes compared to the control group. However, other reproductive metrics, such as litter body weight, total body length, gestation period, and sex ratios, did not show significant differences when compared with controls. This research underscores the potential of *Luffa acutangula* as a natural anti-fertility agent, warranting further exploration into its mechanisms and applications.

INTRODUCTION: The development of safe, orally effective, fertility-regulating agents from higher plants for use in humans is not a new idea. In the ancient literature, fertility regulation with plant preparation in indigenous systems of medicine has been reported¹. For centuries, virtually every indigenous culture has utilized plants in one form or another in an attempt to limit the population of culture. A number of plant species were tested for fertility regulation years ago and were subsequently fortified by national and international agencies^{2,3}.

Medical abortion has emerged as a valuable alternative to surgical abortion and will contribute to safe reproductive control worldwide^{4,5}. At present, a global attempt has been made to search out the effect of herbal products for contraceptive purposes⁶. Few herbal contraceptives have been developed, but the potential of these contraceptives is very minimal and the mode of action is beyond our knowledge, till now. However, the search for an orally active, safe and effective plant preparation or its compound is yet to be needed for fertility regulation due to incomplete inhibition of fertility or side effects⁷.

Therefore, research on systematic investigation of medicinal plants for their efficacy, including antifertility properties, is necessary. *Luffa acutangula* (Family: Cucurbitaceae), commonly known as Ridge gourd and dodaki. The fruit of the *Luffa acutangula* plant is significantly used as an

<p>QUICK RESPONSE CODE</p> 	<p>DOI: 10.13040/IJPSR.0975-8232.16(7).2027-33</p> <hr/> <p>This article can be accessed online on www.ijpsr.com</p> <hr/> <p>DOI link: https://doi.org/10.13040/IJPSR.0975-8232.16(7).2027-33</p>
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anti-implantation, antifungal agent, and anti-diabetic activity^{8, 9, 10}. Therefore, the present study deals with the anti-implantation or early abortifacient activity of *Luffa acutangula* fruit as claimed by tribals from the Pandharkawada region, Yavatmal district of Maharashtra.

This study may focus the researcher's attention on the phytochemical and pharmacological investigation of the *Luffa acutangula*, an antifertility regulating plant. In the present study, the aim is to identify a potent antifertility agent with minimal side effects from an herbal source that could serve as an alternative remedy for available synthetic medicine and to formulate it into a suitable contraceptive formulation.

MATERIALS AND METHODS:

Collection of Plant Material: The fruits of the *Luffa acutangula* plant were collected from the Pandharkawada region of Yavatmal district during the flowering period of February to March or June to July. Identified and authenticated by experts from the Botanical Survey of India, Pune (Accession No. DD- 4).

Procurement and Rearing of Experimental Animal: Healthy wistar strain female albino rats of about two months old and weighing 150–250 gm were procured from Sudhakar Rao Naik Institute of Pharmacy, Pusad (Maharashtra). The rats were housed in polypropylene cages and maintained in an environmentally controlled room provided with a 12:12- hour light and dark cycle at approximately 25°C. They were fed on pellets (Trimurti Lab Feeds, Nagpur) and tap water *ad libitum*. The rats were allowed to acclimatize to the laboratory environment for 15 days before experimentation.

All experimental protocols were subjected to the scrutinization and approval of the Institutional Animal Ethics Committee [registration number 1060/ac/07/CPCSEA (IAEC/7/2009)]

Preparation of Extract: The fruits of *Luffa acutangula* were collected, shade dried, powdered, and subjected to Soxhlet extraction successively with distilled water, ethanol, chloroform, and ethyl acetate. The extract was evaporated to near dryness on a water bath, weighed, and kept at 4°C in the refrigerator until further use.

Phytochemical Screening: The presence of various plant constituents in the plant extract of *Luffa acutangula* was determined by preliminary phytochemical screening as per Thimmaiah¹¹.

Acute Toxicity Study: Healthy female albino rats were starved for 3-4 hours and subjected to acute toxicity studies as per Organization of Economic Cooperation and Development (OECD)¹² guidelines No. 423. They were divided into 5 groups of 6 animals each and kept singly in separate cages during the experiment. Group 1 represented the control group, which received 10 ml/kg of distilled water orally. Groups 2-5 received suspension of different extracts (aqueous, alcohol, chloroform, and ethyl acetate) of *Luffa acutangula* fruit orally at doses of 1000, 2000, and 4000 mg/kg daily for 7 days, respectively. The rats were observed continuously for 2 hours for behavioral, neurological, and autonomic profiles and for the next 24 and 72 hours for any lethality or death.

Anti-implantation Activity: The anti-implantation activity was determined according to the method of Savadi and Alagawadi¹³; Khanna and Choudhary¹⁴. An estrus cycle study was done for fixing the date of pregnancy by the vaginal smear method. Female rats of established fertility in the proestrous or estrous stage were mated with matured male rats of established fertility (in the ratio female 2:1 male). The female albino rats of regular estrus cycle were observed for vaginal smear analysis every morning microscopically¹⁵. Females in an early estrous stage were selected for the study and were left overnight with proven fertile male albino rats in 1:2 ratios.

The next morning, vaginal smears of females were observed for the presence of sperm and the formation of vaginal plugs. The day on which the spermatozoa were found in the smear was considered the first day of pregnancy (Day 1). These rats were randomly distributed into 13 groups, 1 control group and 12 experimental groups of 6 animals each. The first group served as a control group and received distilled water orally, and the remaining 12 groups received different extracts of *L. acutangula* fruit at doses of 100, 200, and 400 mg/kg body weight for 1-7 days.

On the 10th day of pregnancy, rats of all groups were laparotomized under anesthesia to know the presence of implantation sites in the uterine horns and corpora lutea counted, and the anti-implantation activity of the extract was evaluated. After delivery, the pups were counted. The following parameters were computed:

Pre-implantation loss = (Number of corpora lutea on the 10th day – Number of implantations on the 10th day)

% pre-implantation loss = (Number of corpora lutea – Number of implantations / Number of corpora lutea) × 100

% survival ratio = (Number of live fetuses / Number of live + dead fetuses) × 100

% resorption index = (Total number of resorption sites / Total number of implantation sites) × 100

The anogenital distance (AGD) and crown-rump length (CRL) of litters were measured by using a measuring tape. The variations in birth weight of litters and gestation period between control and experimental animals were also determined to check the early abortive effect of plants¹⁶.

Statistical Analysis: All the data are expressed as mean ± SEM (standard error). Statistical analysis was done by Student's t-test and one-way ANOVA¹⁷.

RESULTS:

Phytochemical Screening: Preliminary phytochemical screening of the fruit extract of *L. acutangula* revealed the presence of alkaloids, flavonoids, steroids, tannins, phenols, and saponins, whereas anthraquinones were not detected.

Acute Toxicity Study: Clinical toxicity symptoms such as respiratory distress, salivation, weight loss, and change in appearance of hair as well as maternal mortality were not observed at any period of the experiment.

Similarly, no mortality and changes in the behavioral, neurological, and autonomic profile were observed in treated groups of the rats up to the highest dose of 4000 mg/kg body weight. Hence, one-tenth of the treated dose, 400 mg/kg, was selected for the present investigation.

Anti-implantation Activity: The anti-implantation activity is expressed as the percentage of animals

showing absence of implantations in uteri when laparotomized on day 10 of pregnancy. The aqueous, alcohol, chloroform, and ethyl acetate extracts of *Luffa acutangula* fruit at the doses of 100, 200, and 400 mg/kg body weight exhibited significant anti-implantation activity (12.61% to 72%). All the extract-treated groups reduce the number of litters born significantly, confirming the antifertility activity of the plant used.

This may be due to the resorption of the fetuses from the implantation sites after day 10 or due to abortion. Administration of 400 mg/kg body weight of the alcohol extract resulted in 72% anti-implantation, while the 100 and 200 mg/kg body weight of the alcohol extract resulted in 28.55% and 39.33% anti-implantation activity. However, the extract treatment did not alter the number of corpus lutea, which were similar to those of the control **Table 1** and **2, Fig. 1**.

No vaginal bleeding was observed, indicating that resorption has taken place in the uterus. The litters born to the experiment and control animals showed no morphological defects. Hence, the treatment does not exhibit any teratogenic effects.

There was a decrease in the litter size with an increase in the dose of the extract of the plant *Luffa acutangula* in all the treatment groups. The litter size of chloroform and ethyl acetate group rats was the highest when compared to control.

The litter body weight recorded in animals administered with alcoholic, aqueous, and ethyl acetate extracts of *Luffa acutangula* was not much significant as compared with the control. The AGD to CRL ratio of the litter of rats dosed with plant extract at doses of 100, 200, and 400 mg/kg body weight was similar to that of the control group. Similarly, the total body length of litters at day 1 of birth also did not vary significantly from that of the control.

When the sex ratios of the litter were determined, it was found that there was no significant difference between the number of female and male sexes compared to the control group. The gestation period did not show any variation in the extract-treated group of animals as compared to the control group **Table 3**.

TABLE 1: ANTI- IMPLANTATION ACTIVITY OF THE AQUEOUS, ALCOHOL, CHLOROFORM AND ETHYL ACETATE EXTRACTS OF *LUFFA ACUTANGULAIN* FEMALE ALBINO RATS

Treatment groups	Dose (mg/kg body wt.)	No. of corpora lutea	No. of implantation sites	No. of resorptions in individual rats	No. of resorption (mean± S.E)	Anti-implantation activity (%) (Pre- implantation loss)
(Group- 1) Control	Vehicle	12,10,9,10,11, 10	12,10,9,10,11,10	0,0,0,0,0,0	0	Nil
(Group- 2 to 4) Aqueous extract	100	11,10,14,9,13,11	9,7,12,9,10,8	2,3,2,0,3,3	2.16±0.47**	19.94
	200	10,9,8,10,7,9	8,8,6,7,4,7	2,1,2,3,3,2	2.16±0.30***	26.48
	400	7,10,9,11,9,13	3,6,4,5,7,8	4,6,5,6,2,5	4.66±0.74***	46.23
(Group- 5 to 7) Ethanolic extract	100	10,14,15,11,13,9	7,10,12,7,10,6	3,4,3,4,3,3	3.33±0.21***	28.55
	200	8,9,8,9,7,10	5,4,4,6,3,5	3,5,4,3,4,5	4±0.36***	39.33
	400	9,10,8,10,9,8	2,3,3,8,2,3	5,7,5,2,7,5	5.16±0.97**	72
(Group- 8 to 10) Chloroform extract	100	13,12,11,9,10,9	12,10,10,9,8,7	1,2,1,0,2,2	1.33±0.06***	12.61
	200	10,9,10,9,8,11	7,7,7,7,6,10	3,2,3,2,2,1	2.16±0.30	23.64
	400	8,9,11,8,10,12	5,4,5,5,6,7	3,5,6,3,4,5	4.33±0.09***	42
(Group- 11 to 13) Ethyl acetate extract	100	8,10,9,7,12,10	7,10,9,6,11,10	1,0,0,1,1,0	0.5±0.02 ^{ns}	5.85
	200	9,12,11,10,9,9	8,10,10,9,8,7	1,2,1,1,1,2	1.33±0.10***	13.36
	400	8,11,9,12,10,11	6,9,6,9,6,8	2,2,3,3,4,3	2.83±0.06***	30.19

Values are expressed as mean ± S.E. for six animals in each group. P values: *<0.05, **<0.01, ***<0.001, When compared between group, ns= non-significant.

TABLE 2: ANOVA (SINGLE FACTOR) TABLE FOR THE ANTI-IMPLANTATION EFFECT OF AQUEOUS, ETHANOL, ETHYL ACETATE AND CHLOROFORM EXTRACT OF *LUFFA ACUTANGULA* (FRUIT) ON FEMALE RATS

F- value	Aqueous extract	Alcohol extract	Chloroform extract	Ethyl acetate extract	Fcrit
	7.45633	13.52673	10.68900	31.5670	4.387374

Ho: There was no significant difference in the means of the three samples *Ho*: Rejected

TABLE 3: EFFECT OF AQUEOUS, ETHANOL, ETHYL ACETATE AND CHLOROFORM EXTRACT OF *LUFFA ACUTANGULA* (FRUIT) ON THE GESTATION PERIOD AND LITTER PARAMETERS

Treatment groups	Dose (mg/kg body wt)	Gestation period (days)	Litter size (No.)	Litter body weight (g)	AGD/CRL (mm)	Total body length of litter at 1 st day of birth (mm)	Sex ratio of live fetuses (male/female)	Viable fetuses (%)	Fetus resorptions (Resorption index) (%)
(Group- 1) Control	Vehicle	22.16±0.30	7.5±0.50	4.46±0.06	1.33±0.03	62.3±0.05	24/21	100	0
(Group- 2 to 4) Aqueous extract	50	22.5±0.16 ^{ns}	7±0.18 ^{ns}	4.57±0.04 ^{ns}	1.12±0.03 ^b	63.2±0.01 ^a	20/22	80.89	19.11
	100	22.23±0.50 ^{ns}	2.16±0.06 ^c	4.48±0.40 ^{ns}	1.29±0.02 ^{ns}	63.6±0.03 ^b	08/05	75.48	24.52
	200	22.60±0.33 ^a	1.16±0.13 ^c	4.9±0.10 ^a	1.20±0.01 ^a	63.4±0.03 ^{ns}	03/04	52.55	47.45
(Group- 5 to 7) Ethanolic extract	50	22.7±0.42 ^b	5.33±0.20	4.7±0.22 ^a	1.20±0.05 ^b	64.2±0.04 ^c	19/13	72.23	27.77
	100	22.46±0.70 ^{ns}	0.83±0.10 ^c	5.69±0.20 ^b	1.35±0.07 ^a	64.0±0.60 ^c	02/03	57.9	42.10
	200	23.2±0.80 ^a	1±0.1 ^c	5.3±0.33 ^a	1.42±0.03 ^b	63.5±0.11 ^b	03/03	42.6	57.40
(Group- 8 to 10) Chloroform extract	50	23±0.28 ^b	8±0.19 ^a	5.58±0.11 ^c	1.20±0.01 ^a	60.5±0.18 ^a	23/27	87.5	12.5
	100	22.8±0.60 ^a	5.16±0.13 ^b	6±0.28 ^c	1.45±0.03 ^b	60±0.40 ^b	25/19	77.2	22.80
	200	22.75±0.43 ^a	1.33±0.42 ^c	5.68±0.33 ^a	1.28±0.01 ^{ns}	62.7±0.60 ^{ns}	15/12	55.18	44.82
(Group- 11 to 13) Ethyl acetate extract	50	22.16±0.90 ^{ns}	8.33±0.32 ^b	5.30±0.78 ^b	1.50±0.02 ^b	61.3±0.20 ^{ns}	20/24	98.22	1.78
	100	22.3±0.06 ^{ns}	7.33±0.11 ^{ns}	5.86±0.10 ^b	1.19±0.01 ^a	62.4±0.90 ^b	13/18	86.67	13.33
	200	22.5±0.32 ^a	4.5±0.15 ^c	6.2±0.02 ^c	1.60±0.8 ^c	63.7±0.50 ^b	12/16	72.14	27.86

Values are expressed as mean ± S.E. for six animals in each group. P values: ^a<0.05, ^b<0.01, ^c<0.001, When compared with control, ns= non-significant.

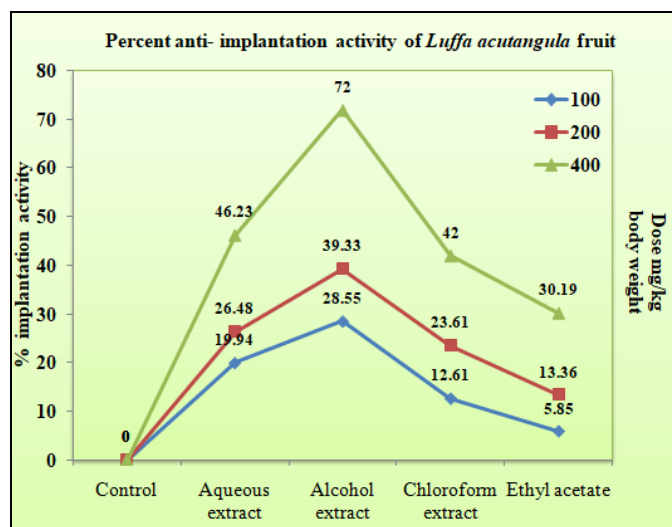


FIG. 1: GRAPHICAL REPRESENTATION OF ANTI-IMPLANTATION EFFECT OF LUFFA ACUTANGULA FRUIT EXTRACT ON FEMALE ALBINO RATS

DISCUSSION: Preliminary phytochemical studies of the *Luffa acutangula* fruit extract indicated the presence of tannin, flavonoids, saponins, and steroids, which are reported to have contraceptive activity^{15, 18}. The phytoestrogenic compounds of plants have been found to reduce fertility in animals upon continuous administration¹⁹. The presence of steroids and alkaloids in the extract of *Ricinus communis* exerts both positive and negative feedback effects on the release of gonadotrophins from the pituitary glands²⁰. In the luteal phase of the menstrual cycle, the combined effect of estrogen and progesterone will be to block the release of LH and FSH from the pituitary. This situation helps inhibit maturation of the follicle in the ovary and prevent ovulation²⁰. Since, in the present study, also extracts gave positive tests for steroids and alkaloids and sex hormones being steroidal compounds, the plant's sterols (phytosterols) may be suspected to be responsible for the anti-implantation effects of the fruit of *Luffa acutangula*. Our study also coincides with the findings of Ahmad *et al.*,²¹ while working on the aqueous extract of seeds of *Linum usitatissimum* and with the study of Padmashali *et al.*,²² on *Balanites roxburghii*, which shows antifertility activity due to the presence of saponin, glycosides, and flavonoids in female rats. Preliminary phytochemical analysis of *Passiflora foetida* leaves indicated the presence of sterols, polyterpenes, polyphenols, flavonoids, and alkaloids, which are known for their estrogenic effects on the uterus and vagina of rats²³.

The absence of clinical toxicity symptoms in the treated female rats, such as tremors, weakness, refusal of feeds, diarrhea, weight loss, hair loss, coma, and death, suggests that the extract was not clinically toxic to female rats²⁴. Our study also corroborates the finding of Padmashali *et al.*,²² on *Balanites roxburghii* and Mustapha *et al.*,²⁵ on *Rhynchosia sublobata* in female rats.

Luffa acutangula is popularly used by women who aim to interrupt gestation²⁶. Several studies have identified proteins from *Luffa acutangula* and other species that have abortifacient action^{27, 28, 29}. The loss of implantation caused by ethanol extract may be due to antizygotic, blastocytotoxic, or anti-implantation activity³⁰. These results are in agreement with the earlier finding of Bhardwaj and Mathur³¹.

Bhargava and Prakash³² observed the anti-implantation effect of herbal preparation from neem bark extract in rats, in which the control group showed a good number of corpus lutea and implantation sites; however, when treated with the extract of the bark of *Azadirachta indica*, the rats showed anti-implantation as well as fetal resorption. Administration of all the crude extract of *Luffa acutangula* fruit caused a significant change in the number of live fetuses and fetal survival percent, indicating the possible anti-implantation activity of the extract. This result is in agreement with previous findings by Oluyemi *et al.*,³³ and Awe *et al.*,³⁴ who showed that the methanol extract of the fresh leaves of *V. amygdalina*, when administered to pregnant mice, caused anti-implantation.

The reduction in weight of fetuses of pregnant rats as observed in the present study had also been reported when *Acanthus montanus* leaf extract was administered to pregnant rats during gestation³⁵. The AGD to CRL ratio of the litter of rats dosed with plant extract at doses of 100, 200, and 400 mg/kg body weight was similar to that of the control group. Similarly, the total body length of litters at day 1 of birth also did not vary significantly from that of the control. When the sex ratios of the litter were determined, it was found that there was no significant difference between the number of female and male sexes compared to the control group.

The gestation period did not show any variation in the extract-treated group of animals as compared to the control group. Our results are in line with the plant extract of *Labisia pumila* and *Solanum lycocarpum* in rats^{36, 37}. Similar results were observed in the present study, demonstrating the possible therapeutic role of *Luffa acutangula* as an antifertility agent. However, further study on the pharmacokinetic profile and active principle of the plant needs to be done.

CONCLUSION: The aqueous, alcohol, chloroform, and ethyl acetate extract of *Luffa acutangula* fruit possesses significant anti-implantation activity (12.61% to 72%). All the extract-treated groups significantly reduce the number of litter born, confirming the anti-implantation activity of the plant used. Further studies to identify the bioactive principles responsible for the anti-implantation activity of the extract are in progress.

ACKNOWLEDGEMENT: The author is thankful to the Department of Science and Technology, Government of India, for funding the present work as a part of the Ph. D. program in the form of INSPIRE Fellowship. The authors are grateful to CPCSEA, Chennai, Ministry of Justice and Empowerment, Government of India and IAEC, Government Vidarbha Institute of Science and Humanities, Amravati (M.S) for giving the permission for doing the experimental work on rat.

CONFLICTS OF INTEREST: The author declares they do not have any conflict of interest.

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How to cite this article:

Dabhadkar DK: Evaluation of anti-implantation activity of *Luffa acutangula* fruit extract on female albino rats. Int J Pharm Sci & Res 2025; 16(7): 2027-33. doi: 10.13040/IJPSR.0975-8232.16(7).2027-33.

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