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RESEARCH PAPER

Anti-Fertility Activity of *Butea Monosperma* Linn in Albino Rats

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Abstract

In recent years, there has been a considerable interest in plants with possible antifertility effect. Only a few plants, reported to possess antifertility activity, have reached the stage of scientific clinical evaluation. In the present study, anti-fertility activity of Butea monosperma Linn. were characterized using different methods. Qualitative phytochemical screening of all the extracts was also conducted using qualitative tests to identify presence of alkaloids, phenolic compounds, tannins, flavonoids, saponins, glycosides, carbohydrates, proteins in the extracts. In vivo antiovulatory activity and in vitro spermicidal activity were also performed for pharmacological evaluation. The physical parameters such as total ash value, methanolic soluble extractive value, water soluble extractive value and loss on drying were found to be 12.67%, 18.54%, 9.59% and 6.97% respectively. The qualitative phytochemical test of the methanolic extracts showed the presence of carbohydrates, steroids, alkaloids, terpenoids, fatty acids, flavonoids and glycosides. The toxicity study showed that all the extracts tested were safe at all dose levels tested. By the result of the study it was concluded that, stem bark of Butea monosperma can be considered as a potential source for developing an effective and safe herbal contraceptive formulation that could deliver action comparable to the currently available hormonal contraceptive formulations or spermicidal preparations, which are effective but have several limitations.

Keywords

Antifertility activity, Antiovulatory activity, Spermicidal, HPTLC

INTRODUCTION

Many methods of contraception have been developed and advocated for use from time to time. Only a few plants, reported to possess antifertility activity, have reached the stage of scientific clinical evaluation.[1] These plants or plant materials are Gossypol from *Gossypium herbaceum*, *Montanoa tomentosa*, *Embelia ribes*, *Hibiscus rosasinensis* and *Vicoa indica*. Compounds that are being sought in particular are those, which are orally active, non-steroidal, non-estrogenic, safe and effective for prevention or disruption of implantation in women and those that will inhibit spermatogenesis or interfere with sperm maturation in man.[2]

Butea monosperma is an ethno plant, which is known to be used by the population of India to prevent pregnancy. The objective of the research work was to scientifically evaluate the antifertility activity of *Butea monosperma*, so as to validate the traditional claim and also investigate the phytochemistry of the plant, to understand whether the plant can be a potential source for bioactive phytoconstituents.

MATERIAL AND METHODS

Plant material collection

Fresh stem bark of *Butea monosperma* were collected from local region. Barks were dried and authentified by botanist from a horticulture college. The specimen was submitted in department of pharmacognosy for future reference.

Extraction Methodology

Stem barks were dried in shades and subjected to coarse powder and extracted by using methanol as solvent. The solvent was evaporated to obtain dried residue. The powder of the leaves and roots was then macerated in distilled water and 90% (v/v) ethanol in a ratio of 1 to 6 (w/v) and 1 to 4 (w/v) respectively. The suspensions of the samples were rotated on a shaker for 24 h at room temperature. Then each sample was filtered using cotton wool and Whatman filter paper No 1. The water extracts were lyophilized while the ethanol extracts were evaporated to dryness using Rotavapour at 40o C. The resulting residues were stored at – 200 C until used for the experiments. The produced extract samples were named as butea bark water extract and buteae bark ethanol extract.

Qualitative Phytochemical Evaluation of the Crude Drug

Qualitative phytochemical screening of all the extracts was conducted using qualitative tests to identify presence of alkaloids, phenolic compounds, tannins, flavonoids, saponins, glycosides, carbohydrates, proteins etc in the extracts.[41-43]

Experimental animals

Laboratory bred Swiss albino female mice in the weight range of 20-35 g were selected for the study. Animals were maintained under standard environmental condition of temperature (21 ± 2°C), humidity (51 ± 10%) and 12:12 h light–d ark cycles (0800-2000 h/light) with standard pellet diet and water *ad libitum*. The experiments were carried out between 0900 and 1800 h.

Toxicity Study

Acute toxicity study of extracts of *Butea monosperma* was carried out in mice as per OECD guidelines 423. [44-45]

PHARMACOLOGICAL EVALUATION

1. *In vivo* antiovulatory activity
2. *In vitro* spermicidal activity [46-49]

***In vivo* antiovulatory activity**

Healthy female Swiss albino mice weighing between 20-35 grams with 3 normal and regular estrous cycles were selected for the study. The 3 normal and regular estrous cycles of the mice were observed, by microscopic examination of vaginal smears daily for 15 days [i.e. 3 estrous cycles] for presence of 3 types of cells, observed at the same time daily [10am -12 am]. The vaginal smears were observed microscopically for the presence of three cell types, (a) leukocytes, (b) epithelial cells, which are round with easily distinguishable nuclei, and (c) cornified cells in which nuclei are difficult to discern or are absent and the cell shape is irregular. According to the presence or absence of the cell types and the relative proportion of each cell type, the phases of the estrous cycle of each mouse were determined.

The extract was administered to the test animals in 2 different dose levels [100,150 mg/kg body weight of the mouse]. Methanolic extract was prepared in distilled water. All the treatment and control groups received the respective extracts for 30 days [6 estrous cycles] orally. The dosing was started in the diestrus phase or the proestrus phase of the estrous cycle that is before the estrus phase. Dosing the female animals before estrus phase would target on development of the ova/egg within the ovaries and on release of the ova from the ovaries [antiovulation effect]. After 30 days when the dosing was discontinued, mating of mice was still not allowed and vaginal smears were further observed for 15 more days [3 more estrous cycles] to see if the antiovulatory effect caused by the test extract is reversible after the dose is discontinued.

***In vitro* spermicidal activity**

In vitro spermicidal activity was carried out to evaluate the spermicidal effect of extracts using Sander-Crammer's method.

The extract of *Butea monosperma* [i.e. methanolic extract) was evaluated at the concentration range of 10mg/ml, 25mg/ml, 50mg/ml and 100mg/ml. All the test samples were prepared by dissolving the extracts in 0.9% saline solution. The experiments were conducted on human semen samples. According to the Sander-Crammer's method, human semen samples with an average sperm counts ranging from 90-120million/ml, normal morphology of more than 70% and grade IV motility [rapid, linear and progressive] of 70 80% with more than 75% sperm viability were used for this study.

The spermicidal activity was performed using a Sander and Cramer's test, which measures the minimum time required by the spermicidal agent to kill 100% sperms in the semen samples. Test extracts of various concentrations were mixed with sperm suspension in 1:1 ratio. i.e. 10µl of test extract was mixed with 10µl of semen sample on a glass slide, covered with a cover slip and at least five fields were examined microscopically to record sperm motility. The effect of different concentrations of extracts on percentage motility of sperms was studied.[50-51]

RESULT & DISCUSSION

Percentage Yield of extract of *Butea monosperma*

The percentage yield (w/w) of extract was determined with respect to the air dried seed powder used for extraction and yield was found to be 14 %.

Physical Evaluation of the Crude Drug

The physical evaluation was carried out which involved determination of the ash value, extractive value and loss on drying of the powdered crude drug as per the standard procedures. Results are mentioned in Table no. 1

Table 1: Physical Evaluation of the Crude Drug

S. No.	Parameters	Result
1	**Ash Value**	
A	Total Ash Value	12.67%
B	Water Soluble value	5.23%
C	Acid insoluble value	6.43%
2	**Extractive Value**	
A	Methanolic soluble value	18.54%
B	Water soluble value	9.59%
3	**Loss on Drying**	6.97%

Antiovulatory effects

After 30 days, when the dosing was discontinued, mating of mice was still not allowed and vaginal smears were further observed for 15 more days [i.e. 3 more estrous cycles] to see if the antiovulatory effect caused by the test extracts was reversible after the dose was discontinued. The indication for antiovulatory effect in mice was reduction in estrus phases [ovulatory phase] and prolongation of diestrus phases of estrous cycle of mice. [54]

Methanolic extract showed antiovulatory effect. In case of methanolic extract treated mice, the diestrus phases were prolonged while the estrus phases were reduced, indicating an antiovulatory effect. In case of the treatment group, that received extract in a dose of 100mg/kg body weight of the mice, the diestrus prolongation was observed for a shorter duration that is 5-6 days. This diestrus prolongation was statistically non-significant as compared to the control. The diestrus arrest was reversed before the dosing was discontinued, showing a weak antiovulatory effect in mice. For an ideal contraceptive effect, the antiovulatory effect should continue till the test extract is administered. [54]

Table No 2: Antiovulatory effects of methanolic extract

S. No.	Data	Control	Methanolic extract	
			100 mg/kg	**150 mg/kg**
1	No. of estrous cycles studied while giving the test extract	6	6	6
2	Reduction of estrus phases in 6 cycles	6	4.2 ± 0.311***	2.11 ± 0. 209***

3	Prolongation of diestrus phases in 6 cycles	6	17.33 ± 0.508***	26.19 ±0.407***

Values are expressed as mean ± SEM. The results were evaluated by application of One way ANOVA. ***p<0.001 statistically significant when compared to the control group.

***In vitro* spermicidal activity**

An ideal spermicidal agent should kill or immobilize the sperms immediately as soon as it is in contact with the semen sample. Methanolic extract was tested for *in vitro* spermicidal activity using *Sander-Crammer's* method.

Methanolic extract immobilized sperms in 6.29 ± 0.312 minutes at 100mg/ml concentration. The extract had the ability to immobilize the sperms, but the time required to immobilize the sperms was long as compared to the marketed spermicidal formulation containing nonoxynol-9 (spermicidal agents), which showed 100% immobilization of human sperms within 20 secs at 2% concentration.

By *in vitro* spermicidal activity, using Sander-Cramer's method, 100% immobilization of sperms was observed microscopically. But immobilization of sperms, did not confirm that the sperms were dead. So, to confirm that the test extracts treated sperms that were immobilized were also dead, 2 tests were performed sperm viability and sperm revival test. From these test it was confirmed that the spermicidal effect of methanolic extract was spermicidal. In case of sperm viability test, all the bioactive extracts immobilized sperms were stained, when treated with eosin nigrosin stain and so were considered to be dead. In case of sperm revival test, the bioactive extracts immobilized sperms were suspended in phosphate buffered glucose solution (10.2g of Na2HPO4. 12H2O, 0.4g KH2PO4 and 16g.

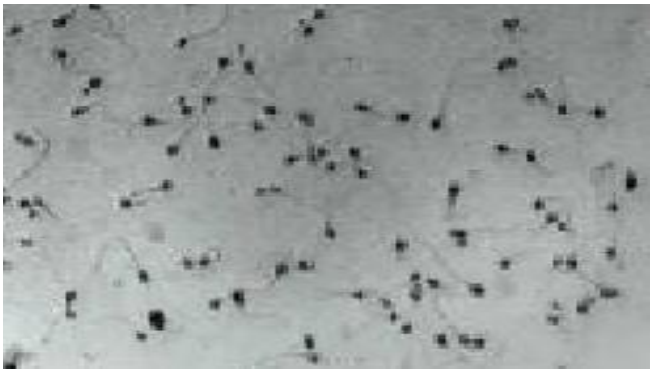

Figure No 1: Human sperm treated with 0.9% sodium chloride

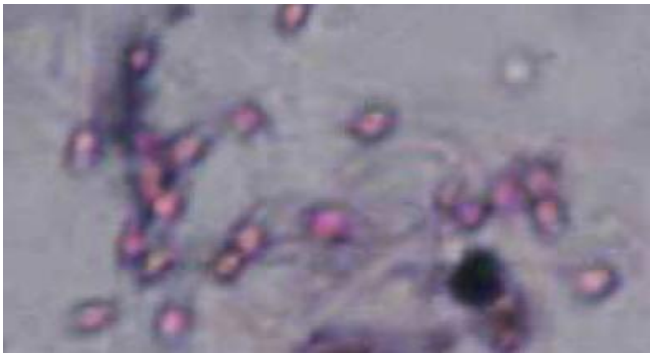

Figure No 2: Semen treated with test extract and then stained with eosin and nigrosin which confirmed that all sperms were dead

Phytochemical Evaluation of Methanolic Extract of *Butea monosperma*

Before proceeding for isolation of phytoconstituents from methanolic extract, some physical characteristics were studied. The dark brown and shiny crystalline flakes were obtained which was soluble in methanol, ethanol and water.

Phytochemical evaluation of methanolic extract includes phytochemical test, HPTLC and isolation of active compounds.

HPTLC Fingerprint of Methanolic Extract

For HPTLC fingerprinting, 5mg/ml solution of extract was spotted on the precoated silica gel TLC plate. The plate was developed using mobile phase [Toluene: Ethyl acetate: Formic acid (4:7:2)] which gave the best resolution of spots for the extract. The developed plate was scanned at 254 nm. At 254nm, Quenching zones were observed on HPTLC plate. The plate showed 7-8 prominent quenching bands. Quenching bands indicated that extract may contain compounds with conjugated double bonds, such as anthraglycosides, arbutin, coumarins, flavonoids, and propylphenols in essential oils or some alkaloids such as indole, isoquinoline or quinoline alkaloids.

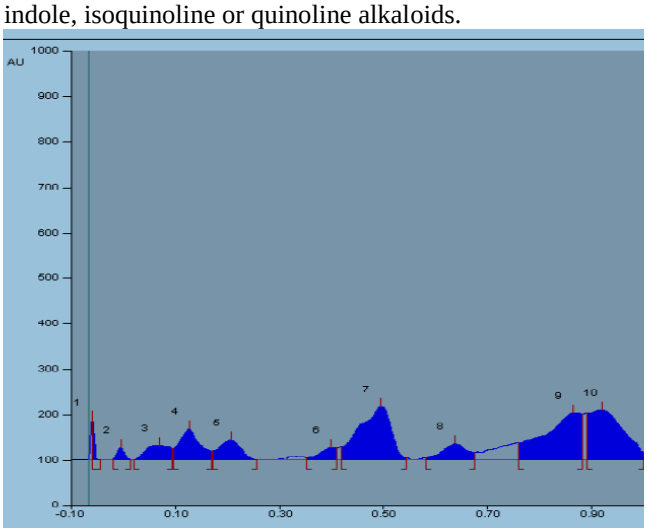

Figure No 3: HPTLC chromatogram of methanolic extract at 254nm

Characterization of Isolated Compounds

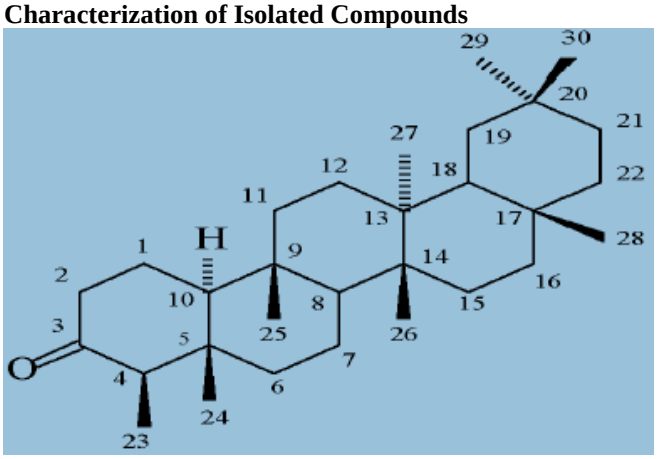

Figure No 4. Structure of Friedeline

IR Spectroscopy

The IR spectrum of IEE-FI-iso4 displayed absorption peaks at 3334 cm-1 , 2931 cm-1 , 1745 cm-1, 1663 cm-1,1452 cm-1, 1389 cm-1, 1284 cm-1, 1171 cm-1, 1135 cm-1, 1058 cm-1 , 973 cm-1, 837cm-1, 794 cm-1 , 779 cm-1 and 701 cm-1. Intense band at 1741 cm-1 in the IR spectrum is suggestive of a six member ring ketone.**166**

Mass Spectra

In ESI-MS data was recorded using ESI method conducted in the positive mode. It showed a molecular ion peak [M+H] + at m/z 427, which confirmed that the mass of the isolate IEE-FI-iso4 is 426. Other fragment ion peaks were observed at m/z 412, 397, 342, 303, 274, 218, 205, 150, 124, 95, and 82. The fragment ion peak m/z 274 [(M+H)-153] +, m/z 303 [(M+H)-124] + and m/z 342 [(M+H)-85] + were suggestive of a friedelane derivative with 3-keto substituent**.**

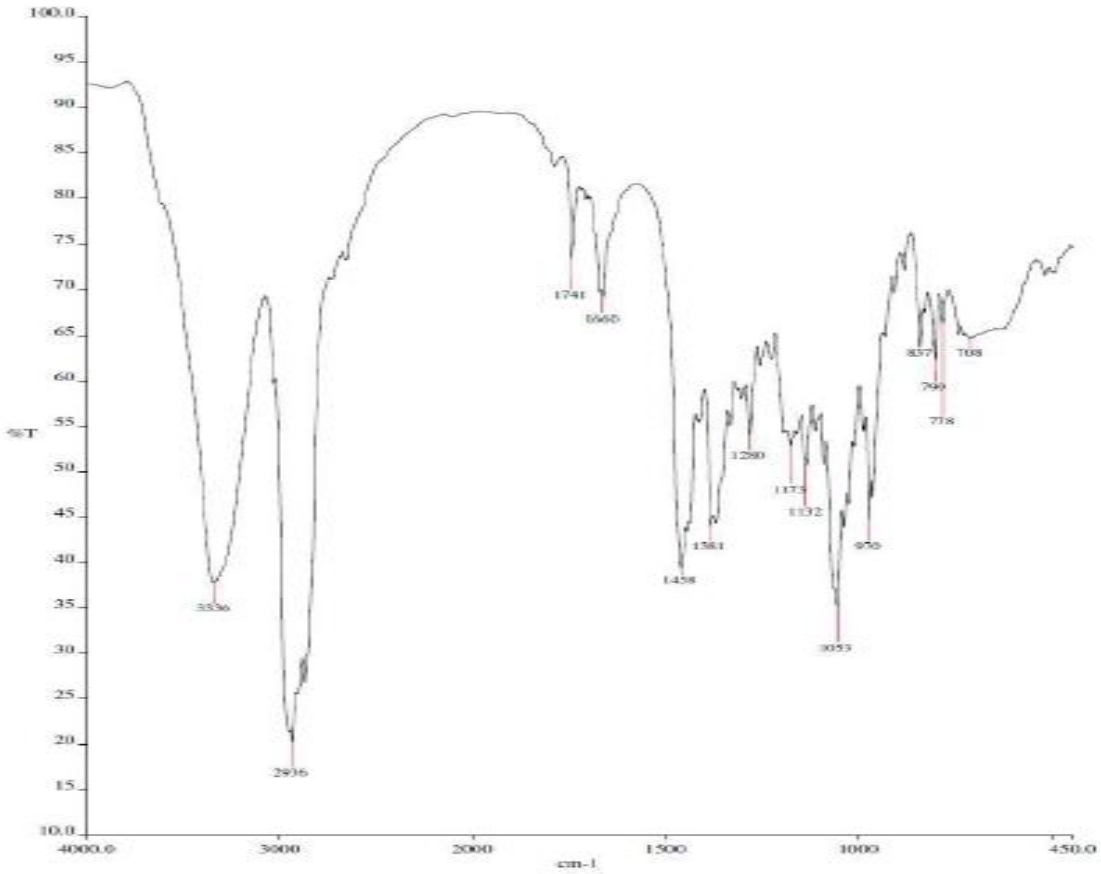

Figure No 5: IR spectra of isolated compound

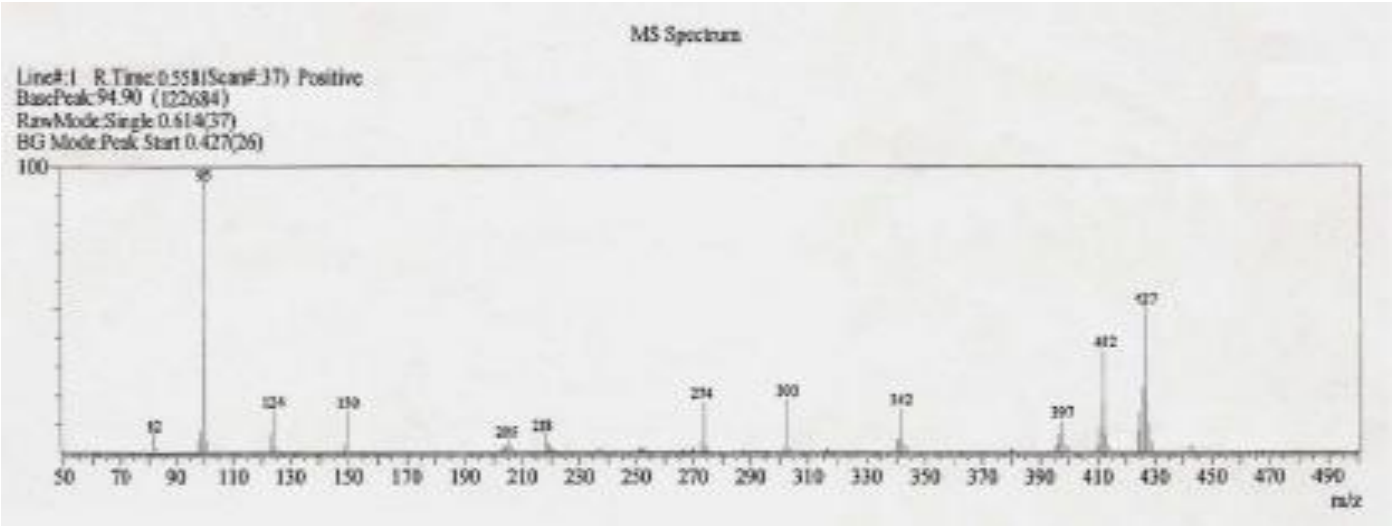

Figure No 6: Mass spectrum of isolated compounds

The stem bark of *Butea monosperma* was collected from local region and authentified at Survey of medicinal plants unit by botanist and authentic herbarium specimen was submitted in department of pharmacognosy. The physicochemical evaluation of the crude drug of *Butea monosperma* was performed. These studies conducted will serve as a valuable source of information and provide suitable standards to determine the quality of *Butea monosperma* in future investigations or applications. It will also help in differentiating the plant material of *Butea monosperma* from other closely related species of *Butea monosperma*.

The coarse powder of stem bark of *Butea monosperma* was extracted successively by *soxhlet* extraction method using solvents methanol.

Acute oral toxicity studies in mice for methanolic extract of *Butea monosperma* was carried out as per OECD guidelines 423. It suggested that the highest dose that was found to be safe in mice was 2000 mg/Kg body weight for extracts. The study also suggested a broad range for LD50 for all the extract, which was between 2000- 5000 mg/Kg body weight of the test animal or it may be above 5000 mg/Kg body weight for extracts of stem bark of *Butea monosperma*.

In pharmacological investigation, the methanolic extract of *Butea monosperma* was tested for two antifertility activities, *in vivo* antiovulatory activity & *in vitro* spermicidal activity.

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