Increasing population is one of the major problems which India is facing today. In the last century, India’s population has increased from 250 million to 1000 million, an increase of about 400%. Nearly 22 million people are born every year in India. Such increasing population is leading to illiteracy, increased percentage of people below poverty line and instability in country’s economy and peace. Efficient methods of contraception are the only solution to check this growing population. Many methods such as condoms, oral contraceptives, spermicides and intrauterine devices are available since long, but there is still a quest for alternative means. The reason for this is the shortcomings of these methods. For instance, synthetic oral contraceptives available are effective, but produce severe side effects such as hormonal imbalance, hypertension, increased risk of cancer and weight gain.\textsuperscript{1} Thus, there is a need to replace these methods with alternative methods such as plant based contraceptives which would be effective as well as safe.

India is one of the world’s twelve leading biodiversity centers having over 45,000 different plant species.\textsuperscript{2} Many of these plant species have high beneficial values and are used for several years by our ancestor’s as effective home remedies or remedies suggested by the Vaidya’s or Hakim’s. Such medicinal plants are called as Ethnoplants.\textsuperscript{3,4} These ethnoplants can be considered as a great source of therapeutic agents. However, the biggest constrain is that they have not been systematically studied to understand their pharmacological and phytochemical potentials.
Thus, an understanding of the pharmacology and phytochemistry of these ethnomedicines would provide an authentic data which would enable such precious source of medicinal plants to fulfill demands of the global market.

*Bridelia crenulata* is one such ethnoplant, which is known to be used by the inhabitants of Orissa to prevent pregnancy. The objective of the present study is to systematically evaluate the antifertility activity and phytochemistry of this ethnoplant, so that a potential herbal contraceptive can be developed.

*Bridelia crenulata* Roxb, family : Euphorbiaceae is distributed in Tamilnadu, Karnataka and Orissa. The only pharmacological activity reported is, *in vitro* antimicrobial activity against 10 human pathogenic bacteria and 4 fungal strains exhibited by aqueous and methanolic extracts of stem bark and their fractions. Also, methanolic extract of *Bridelia crenulata* stem bark (1.5625 - 50mg/ml) and its isolated luteoforol (0.25 - 2mg/ml) showed concentration-dependent antimicrobial activity.

The stem bark of *Bridelia crenulata* was collected from forest area of Tirunelveli, Tamilnadu in the month of July and August. The plant was authentified at Survey of medicinal plants unit- Siddha, Govt. Siddha medical college campus, Palayamkottai, Tirunelveli-627 002, Tamilnadu [SMPU spec.No.8324 February 2004].

*Phytochemical and pharmacological investigation of an ethnoplant with antifertility activity.*
Pharmacognostic study of Bridelia crenulata was carried out in which macroscopic and microscopic studies of the fresh stem bark was carried out. Further the stem bark was separated, washed, air-dried and powdered by hammer mill to obtain a coarse powder. The coarse powder was extracted successively by soxhlet extraction method using different solvents with increasing polarity such as petroleum ether, toluene, chloroform, ethyl acetate, methanol and water. Individual extracts were also prepared such as individual ethanolic extract prepared by soxhlet extraction method and individual water extract prepared by reflux method.

All the extracts were first subjected to preliminary phytochemical tests which indicated presence of steroids in the successive petroleum ether (60-80 °C), toluene and chloroform extracts. The successive ethyl acetate, methanol, water extracts & individual ethanolic and water extracts were found to contain carbohydrates, steroids, phenolic compounds/tannins, and alkaloids.

As mentioned earlier, the inhabitants of Orissa used this plant to prevent pregnancy; hence the several extracts prepared were tested for two different antifertility activities, in vivo antiovulatory and in vitro spermicidal activity.

All the extracts prepared were tested for in vivo antiovulatory activity. The activity was carried out in healthy female albino mice having normal estrous cycle. All the groups of mice were administered with
respective doses, orally, for 30 days [6 estrous cycles]. Also the stages of estrous cycles were checked by observing vaginal smears daily of all the groups. The vaginal smears were observed for the presence of leucocytes, epithelial cells and cornified cells. According to the proportion of these cells the respective phase was recorded. Antiovulatory activity is reported, if the estrus phase of the cycle is reduced and the diestrous phase is prolonged.\(^1\)

Diestrous phase is a non-ovulatory phase, hence its prolongation indicates antiovulatory effect. Even after 30 days of administration, vaginal smears were further observed for 15 days more [3 estrous cycles] to see if the antiovulatory effect caused by the test extracts is reversible after the dose is discontinued.

Daily observation of the estrous cycle showed that estrus phase and diestrous phase of the control group animals were found to be normal. As compared to that in successive petroleum ether extract [SPEE] treated groups, the estrus phase was significantly reduced and the diestrous phase was prolonged, but in case of other extracts the diestrous prolongation is not very significant. Further, the vaginal smears were checked of all the groups for three more cycles after 6 cycles of dosing period. It showed that the diestrous arrest reverse back to proestrus phase, which indicated that the antiovulatory activity is reversible. Thus, SPEE showed excellent antiovulatory activity which is reversible on discontinuation of the treatment.
All the extracts were further subjected to another type of antifertility activity, *in vitro* spermicidal activity. The activity was carried out using Sander-Crammer’s method. Semen samples (n=6) from healthy fertile males were subjected to routine semen analysis following liquefaction at 37°C. According to the Sander-Crammer’s method, human semen samples with an average sperm counts ranging from 90-120 million/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. Semen samples were collected after 72-96 hours of sexual abstinence. All semen samples [n=6] used for the study had 80-85% motility. Ten µl of human semen and ten µl of the extract [vehicle used was 0.9% saline] was taken on the glass slide, mixed with a glass rod and observed under a microscope (40x). The time at which the sperms lost their motility completely [i.e. it showed 0% motility] was recorded.

The three controls used for comparison with the test extracts were, blank-semen sample, olive oil [vehicle used for petroleum ether, toluene and chloroform extracts] and 0.9% saline [vehicle used for ethyl acetate, methanol, water, individual ethanolic and individual water extract]. In case of all the three controls used, the sperms were motile for more than an hour.

Successive petroleum ether extract immobilized sperms in less than 8 minutes at 100mg/ml concentration and successive ethyl acetate extract immobilized sperms in less than one and a half minutes at 100mg/ml concentrations.
concentration. These two extracts 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, which showed 100% immobilization of human sperms within 20 secs at 2% concentration.

In case of individual ethanolic extract [IEE], at a concentration of 100mg/ml the sperms were immobilized in less than 50 seconds. Further, when the concentration was increased to 150 and 200 mg/ml, the time required for 100% sperm immobilisation was nearly the same. In case of IEE, a gradual decrease in human sperm motility with time was observed and the decrease was statistically significant with increasing concentrations as compared to the control. Thus, IEE exhibited a dose dependant in vitro spermicidal activity.

By performing in vitro spermicidal activity using Sander-Cramer’s method, it could only be observed microscopically whether the sperms were immobilized or not. But immobilization of sperms, did not confirm that the sperms were dead. So, to confirm that the test extract treated sperms that were 100% immobilized were also 100% dead, 2 tests were performed, (1) Sperm viability test and (2) Sperm revival test. From these two test it was confirmed that the spermicidal effect of all the three bioactive extracts of *B.crenulata* were spermicidal and not spermiostatic.
Individual ethanolic extract [IEE] which showed the best spermicidal effect was selected for further phytochemical evaluation. It was assumed that tannins/phenolic compounds are definitely contributing to the spermicidal effect shown by ethanolic extracts as the extracts showed presence of tannins in preliminary phytochemical tests. Ethanolic extract was subjected to fractionation by using 10% lead acetate. The separated tannin fraction from ethanolic extract was also capable of immobilizing the human sperms in less than 40 seconds. Preliminary TLC studies were carried out on tannin fraction of IEE using precoated TLC plates to develop a suitable mobile phase. The tannin fraction did not show good TLC resolution of components. Therefore, further isolation of phytoconstituents from tannin fraction was not considered.

HPTLC fingerprinting\textsuperscript{13} was conducted for the ethanolic extract. Mobile phase used was toluene: ethylacetate: formic acid [3:6:1.5]. Derivatisation of the extract with various spray reagents like anisaldehyde-sulphuric acid showed violet-blue bands confirming presence of steroidal components and ferric chloride showing blue bands confirming presence of condensed tannins/phenolic components.

Considering the results of HPTLC fingerprinting and preliminary phytochemical screening, ethanolic extract was subjected to purification using preparative TLC. The amount of isolate obtained was very less for carrying out characterization. So column chromatographic technique was
used to purify IEE. Silica gel column containing ethanolic extract was run with different proportions of petroleum ether, ethyl acetate, methanol and water. A few phytoconstituents have been isolated from the extract and their characterization using various spectroscopic techniques viz. UV, IR, $^1$H-NMR, $^{13}$C-NMR and MS is under progress.

Petroleum ether extract which showed the best *in vivo* antiovulatory activity and weak spermicidal effect was selected for further phytochemical evaluation including isolation of phytoconstituents and their characterization. HPTLC fingerprinting was done for the extract. Mobile phase used was toluene: ethylacetate [9.3:0.7]. HPTLC plate was derivatised with anisaldehyde-sulphuric acid spray reagent giving violet-blue bands confirming the presence of steroidal components. SPEE was subjected to purification using column chromatography. The extract was run through the silica gel column using different proportions of hexane, ethylacetate and methanol. A few phytoconstituents have been isolated and their characterization using various spectroscopic techniques viz. UV, IR, $^1$H-NMR, $^{13}$C-NMR and MS is under progress.

REFERENCES


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