Several methods of contraception are effectively been used and highly accepted worldwide to control the rapidly growing world population. Despite a huge increase in contraception globally, 51 million unintended pregnancies in developing countries occur every year, to women not using contraception. Another 25 million pregnancies occur because the contraception method fails during use or because the contraceptive is used incorrectly. Contraceptive technology has undergone a tremendous revolution with the expansion of method choice for millions throughout the globe. Continuous studies are conducted on the safety and effectiveness of the modern contraceptives, but still there is a quest for alternative means. Traditional system of medicines which has a holistic approach of treating with safety and long term benefits can be considered as great source for development of newer contraceptive method.

*Bridelia crenulata* is an ethnoplant, which is known to be used by the inhabitants of Orissa to prevent pregnancy. The objective of the research work was to scientifically evaluate the antifertility activity of *Bridelia crenulata*, 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.

The stem bark of *Bridelia crenulata* was procured from Tamil Nadu and authentified at Survey of medicinal plants unit - Siddha Govt., Tirunelveli, Tamil Nadu using an authentic herbarium specimen [SMPU Spec.No.8324 February 2004]. The pharmacognostic study & physicochemical evaluation of the crude drug of *B. crenulata* were performed. These studies conducted will serve as a valuable source of information and provide suitable standards to determine the quality of *Bridelia crenulata* in future investigations or applications. It will also help in differentiating the plant material of *Bridelia crenulata* from other closely related species of *Bridelia*.
The coarse powder of stem bark of *Bridelia crenulata* was extracted successively by *soxhlet* extraction method using solvents with increasing polarity, such as petroleum ether, toluene, chloroform, ethyl acetate, methanol and water. Two individual extracts were also prepared, individual ethanolic extract prepared by *soxhlet* extraction method and individual water extract prepared by reflux method.

Acute oral toxicity studies in mice for all eight extracts of *Bridelia crenulata* 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 all the extracts. The study also suggested a broad range for LD$_{50}$ 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 all extracts of stem bark of *Bridelia crenulata*.

In pharmacological investigation, all the 8 extracts of *Bridelia crenulata* were tested for two antifertility activities, *in vivo* antiovulatory activity & *in vitro* spermicidal activity. In case of *in vivo* antiovulatory activity, it was observed that out of all the eight extracts tested, successive petroleum ether extract [SPEE] and individual water extracts showed antiovulatory activity. Successive petroleum ether extract [SPEE] showed a dose dependant, statistically significant, antiovulatory activity as compared to the control at dose levels 100 and 150 mg/Kg body weight of the mice. Also, the antiovulatory effect caused by SPEE in mice was reversible, as soon as the dosing was discontinued. Thus, it did not produce a permanent infertility in mice.

The extracts of stem bark of *Bridelia crenulata* also showed good *in vitro* spermicidal action. Out of all the 8 extracts, SPEE, successive ethyl acetate extract and individual ethanolic extract [IEE] showed 100% immobilization of human sperms. SPEE immobilized sperms in 7.37 ± 0.214 minutes [mean ± SEM (n=6)] at 100mg/ml concentration and
successive ethyl acetate extract immobilized sperms in $1.29 \pm 0.486$ minutes at 100mg/ml concentration. IEE immobilized sperms within $0.44 \pm 0.030$ minutes at a concentration of 100mg/ml. IEE showed a gradual decrease in human sperm motility with time and the decrease was statistically significant with increasing concentrations as compared to the control. Thus, IEE exhibited a dose dependant *in vitro* spermicidal activity. It was also confirmed that the spermicidal effect of all the three bioactive extracts of *B. crenulata* were spermicidal and not spermiostatic.

Phytochemical investigation of the bioactive extracts, SPEE and IEE were carried out.

1. **SPEE**:

   Significant antiovulatory activity was exhibited by SPEE in mice. Phytochemical investigation of SPEE included preliminary qualitative phytochemical tests, HPTLC fingerprinting profile, qualitative estimation of marker compounds in the extract and isolation of phytoconstituents from the extract and their characterization and structural elucidation. Preliminary qualitative phytochemical tests suggested that steroids / triterpenoids may be present. Qualitative estimation of marker compounds in the extract using HPTLC method, indicated presence of quercetin, kaempferol and β-sitosterol in SPEE of stem bark of *B. crenulata*. Presence of quercetin and kaempferol is reported for the first time for stem bark of *Bridelia crenulata*. As per literature, Quercetin has estrogenic activity, Kaempferol has anti-implantation activity and β-sitosterol has spermicidal activity. So, it can be assumed that, these three compounds may be contributing to the excellent antiovulatory activity and weak spermicidal activity of SPEE.

   Using column chromatography, isolate F5_iso1 was successfully isolated in pure form from SPEE of *Bridelia crenulata*. Its spectral characterization and structural elucidation confirmed that the compound was stigmasterol. Isolation of stigmasterol from stem bark of *B.crenulata* is
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not reported earlier. Stigmasterol is a precursor of progestin, so it can be assumed that stigmasterol present in Bridelia crenulata is capable of producing progestin like contraceptive action. Thus, stigmasterol may be one of the important phytoconstituents, contributing to the antiovulatory activity exhibited by SPEE of stem bark of Bridelia crenulata.

2. IEE

Individual ethanolic extract [IEE] showed significant in vitro spermicidal activity as compared to the control. Qualitative phytochemical test showed that constituents such as steroids, triterpenoids, flavonoids and tannins may be present in IEE. Qualitative estimation of marker compounds in the extract using HPTLC method indicated, presence of β-sitosterol in IEE. As per the literature, β-sitosterol has spermicidal property. So, it can be assumed that, β-sitosterol may be contributing to the spermicidal action of IEE of stem bark of Bridelia crenulata.

Tannin fraction was separated from IEE. The fraction showed good spermicidal effect. 100mg/ml concentration of tannin fraction showed zero sperm motility in 0.34 ± 0.049 minutes. Column chromatography of IEE was performed and fractions were tested for spermicidal activity. Fraction I and fraction IX showed significant spermicidal activity in 0.44 ± 0.017 & 0.39 ± 0.03 minutes respectively. From bioactive fraction I, isolate IEE-FI-iso4 was successfully isolated in pure form using column chromatography method. Its spectral characterization and structural elucidation confirmed that the compound was Friedelin.

Thus, to conclude, in future stem bark of B.crenulata 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. From the phytochemical investigation, the phytoconstituents found to be present in Phytochemical and pharmacological investigation of an ethnoplant with antifertility activity.
stem bark of *B.crenulata* were quercetin, kaempferol and β-sitosterol. Also, systematic column chromatographic methods were successfully developed to isolate stigmasterol and friedelin in pure form, from the stem bark of *B.crenulata*.