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Spermicidal activity of marine sponge *Xestospongia officinalis* var. *Ceylonensis* Dendy

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ABSTRACT

Back ground: With a view to explore the possibilities of finding new molecules with proven therapeutic efficacy for human use with potent sperm attenuating activity and lower side effect profile than nonoxynol-9 (N-9). the present study was designed to investigate spermicidal activity in *Xestospongia officinalis.*

Material and Methods: The extract, fractions followed by isolation and characterization of bioactive molecules were done from freshly collected animals.

Results:The methanol extract of the *Xestospongia officinalis* caused 100% mortality of human sperms at 0.01% concentration *in vitro* while N-9 (reference control) exhibited an equivalent activity at 0.05%. On further fractionation, the activity was localized in n-butanol soluble fraction from which the major compounds purified was mixture of alkaloids which was found to be equipotent to N-9 and killed 100% human sperm at the concentration of 0.05% in ~20 s, *in vitro*. Conclusions:The mixture of alkaloids were much safer than N-9 towards normal vaginal flora (Lactobacillus) in vitro though it affected the viability of HeLa cells like other surfactants. Thus alkaloids from *Xestospongia officinalis* can suitably replace N-9 in vaginal contraceptives to make them more vaginal eco-friendly.

KEYWORDS

Spermicidal activity;
Marine sponge *Xestospongia officinalis;*
Alkaloids.

INTRODUCTION

Marine sponges are incredible source of novel pharmacologically active compounds which have earlier shown potent efficacy against various diseases[1]. Marine sponges are known to possess diverse pharmacological activity in several diseases such as cancer, neurodegeneration, type-2 diabetes, fungal and microbial infections[2-7]. These biological activities have been attributed to the presence of novel sterols, metabolites including steroids, terpenoids, alkaloids, cyclic peptides and unsaturated fatty acids[8-10]. With a view to explore the possibilities of finding new molecules with proven therapeutic efficacy for human use, a programme is operational at the Central Drug Research Institute,Lucknow, India for screening of extracts of marine organisms for a wide range of biological activities. The programme consists of collection, identification and extraction of marine flora and

fauna along the Indian coasts for biological screening. *Xestospongia officinalis* was selected for detailed chemical and biological investigations with a view to isolate bioactive compounds.

X. officinalis var. *Ceylonensis* Dendy belongs to the Phylum Porifera, Class Demospongiae and Family Spongiidae.. Sponges are utilized as therapeutic agents. A literature search revealed that few compounds have been reported from *X. officinalis* are alkaloids and steroids[11-17].

The high rates of unintended pregnancies and STDs (including HIV) call for the development of novel strategies to help individuals avoid these risks[18]. Contraceptive microbicides are intended to interact with sperm and pathogens in the vagina to irreversibly inactivate them. Surface active molecules, acid buffers, receptor blockers, antimicrobial substances, enzyme inhibitors, etc. are currently under development for dual protection. Although functionally different, the basic plasma membrane structure of sperm and of lymphocytes and monocytes (from which HIV takes its envelope) is highly susceptible to the disruptive action of surface-active molecules[18]. Nonoxynol-9 (N-9), the active ingredient used in the majority of over the counter local contraceptives, has been evaluated as a dully active contraceptive microbicide. However, in spite of potent microbicidal activity *in vitro*, N-9 not only failed to protect against HIV but also increased the incidence of this infection in users[19,20]. This anomaly was largely attributed to the strong detergent nature of N-9 that caused pro inflammatory reaction in the vagina resulting in recruitment of HIV host (immune) cells to the site of viral entry and increased susceptibility to HIV. On the other hand, saponins are weaker surfactants that partially permeabilize the cell membrane but are not classified as detergents, although some are equipotent to N-9 in killing human sperm[21-23]. Lipid rafts are cholesterol rich cell membrane micro domains which play an important role in functional survival of both sperm and HIV[24].

MATERIALS AND METHODS

Collection of marine animals

Xestospongia officinalis was collected from the Ramnad of the Tamil Nadu coast of the India. in the month of november. A specimen sample has been preserved in the herbarium of the Botany Division of the Institute as specimen number 412.

Extraction, fractionation, and isolation of pure compounds

The freshly collected marine animals (2.0 kg) were thoroughly washed with distilled water to remove extraneous materials and then filled in steel containers and soaked in methanol. This was then transported to the laboratory of Central Drug Research Institute, Lucknow, India. The meth-anol extract was drained off and the animals were chopped into small pieces and filled in a glass percolator soaked in 80% aqueous methanol and left for 24 h at room temperature. The process of extraction was repeated four times and the combined percolates were concentrated under reduced pressure 50^0 C to a green viscous mass. It was further dried under high vacuum to remove last traces of the solvent (weight 40.0 g). A portion of the crude extract (25.0 g) was fractionated into four fractions by macerating with hexane, chloroform, and n-butanol successively. The hexane soluble (0.45 g), chloroform soluble (2.25 g), n-butanol soluble (4.25 g), and n-butanol insoluble (18.0 g) fractions (Figure 1) which were evaluated for spermicidal activity. The maximum activity was localized in the n-butanol soluble fraction (TABLE-1). It was analyzed by LCMS and few known compounds (Xestospongins and araguspongins) were identified as a major compounds. The work on pure compounds is in progress and will be reported separately.

Biological evaluations human semen samples

Fresh human semen samples obtained by masturbation into a sterile vial from healthy, young, fertile donors were liquefied for 45 min at 37^0 C and used for in vitro spermicidal assays. The semen characteristics, viz., volume, pH, viscosity and morphology, were determined according to World Health Organization guidelines. Sperm count and motility analysis were also performed in a CASA system (HTMIVOS; Hamilton Thorn Research, Beverly, MA) using a small drop of liquefied semen placed on a ''Makler''counting chamber (Sefy´ Medica,

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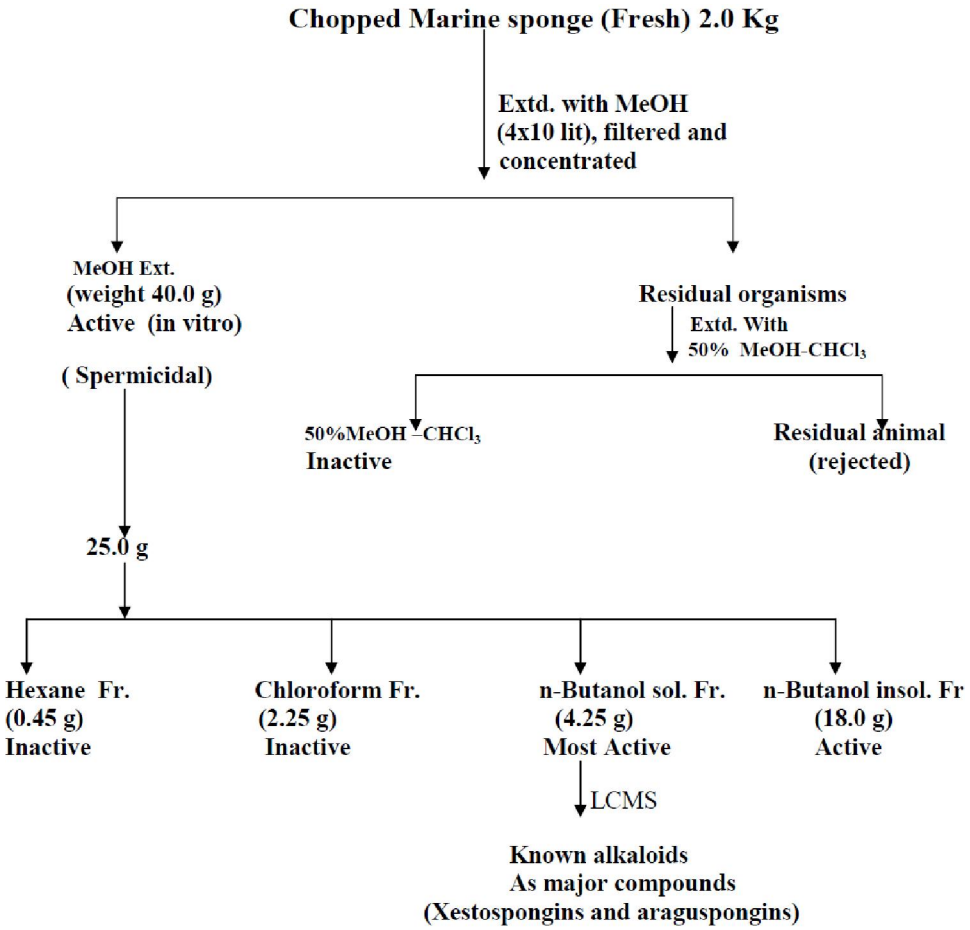

Figure 1 : Extraction, fractionation and isolation procedure

TABLE 1 : Spermicidal activity of the extract and fractions of *X. officinalis* against human spermatozoa *in vitro*

Exts./frs./compds.	Solubility	MEC(%w/v)	Remarks
Mthanol extract	Aqueous	0.1	Active
Methanol–Chloroform Extract	Aqueous	1.0	Inactive
Hexane fraction	Aqueous	1.0	Inactive
Chloroform fraction	Aqueous	1.0	Inactive
n-Butanol soluble fraction	Aqueous	0.25	Active
n-Butanol insoluble fraction	Aqueous	0.05	Active
Nonoxynol-9 (standard)	Aqueous	0.05	Active
Sapindus saponins(As standard)	Aqueous	0.05	Active

Haifa, Israel) pre warmed to 37^0 C. Semen samples with normal sperm count (>60 million/ml), motility (>75% motile), sperm morphology (>70% normal), pH (7.4 - 8.0), viscosity, and volume were used. Ethical approval for this study was obtained from the Institute's ethics committee.

Minimum effective concentration (MEC) determination

The test compounds were dissolved in physiological saline to make a 1.0% (10 mg/ml) solution and diluted serially up to 0.01%. A spermicidal test was performed with each dilution starting from 1.0% until the MEC was arrived at, following the modified method of Sander and Cramer[25]. Briefly, 0.05 ml of human semen was added to 0.25 ml of spermicidal compound solution and vortexed for 10 sec. A drop was immediately placed on a microscope slide, covered with a cover glass and examined under a phase-contrast microscope. The result was scored

positive if 100% spermatozoa became completely immotile within 20 sec. The weakest dilution that completely immobilized all the spermatozoa in 20 sec was recorded as MEC in gram % (w/v). MEC was determined in three individual semen samples from different donors.

Sperm motility parameters

Effect of extract and fractions of *Xestospongia officinalis* on human sperm motility parameters [%Mot. = percent motile; rapid = percent sperm with velocity >VAP; VAP = average path velocity (m/s); ALH = amplitude of lateral head displacement (m); BCF = beat cross frequency (Hz)] was assessed at ED_{50} concentration *in vitro* using Computer Assisted Sperm Analyzer (CASA) system, as detailed earlier[26].

RESULTS

Spermicidal Potential of the n-butanol fraction (TABLE1) N-9, and Sapindus saponins exhibited a minimum effective spermicidal concentration (MEC or ED_{100}) of 0.025% that irreversibly immobilized 100% human sperm in 20 sec *in vitro*. TABLE 1 shows the spermicidal MEC of the extract and fractions of the sponge, N-9 and Sapindus saponins that killed 100% human sperm in Sander Cramer Assay.

DISCUSSION

However, this gentle action was adequate to functionally attenuate 100% human sperm irreversibly in 20 sec for contraception. CASA analysis indicates that at ED_{50} concentration (concentration immobilizing 50% sperm in vitro) 50% sperm became immotile and the remaining 50% sperm showed major changes in motility pattern, which was characterized by significantly reduced sperm velocity and visibly reduced lateral head displacement and beat frequency. However, these changes were very similar to those seen in case of treatment with Sapindus saponins.

CONCLUSIONS

The mixture of alkaloids were much safer than N-9 towards normal vaginal flora (Lactobacillus) in vitro though it affected the viability of He La cells like other surfactants. Thus alkaloids from *Xestospongia officinalis* can suitably replace N-9 in vaginal contraceptives to make them more vaginal eco-friendly.

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