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Evaluation of *in vitro* antispermogenic activity of isolated glycoside and methanolic extract of *Mangifera indica* L. roots

BIPLAB DE[1]*, ZERRYL CH. MOMIN[1], SAIKAT SEN[2], RAJA CHAKRABORTY[2] and BINOY BEHARI GOSWAMI[1]

[1]Regional Institute of Pharmaceutical Science and Technology, Abhoynagar, Agartala, Tripura - 799 005 (India).
[2]C.E.S. College of Pharmacy, Chinnatekur, Kurnol, Andra Pradesh - 518 218 (India).

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ABSTRACT

Metahnolic extract of *Mengifera indica* L. root produces *in vitro* antispermogenic activity. *In vitro* antispermogenic activity of six chromatographic fractions obtained from metahnolic extract of *Mengifera indica* L. root also performed. Three (M1, M2, M6) out of six fractions showed antispermogenic activity. Among them fraction M6 showed most potent effect observed by percentage decrease in motility. Structure elucidation of M6 showed presence of a glycoside named 1-heptyl-(1-phenyl)-3-xylose benzoate, thus isolated glycoside may responsible for its antispermogenic activity.

Key words: *Mengifera indica,* antispermogenic activity, methanolic extract, glycoside.

INTRODUCTION

Mangifera indica L. (Anacardiaceae) commonly known as mango grows in the tropical and subtropical region. Frits of this plant widely appreciated worldwide. Different parts of the plant are commonly used as folk medicine for a wide variety of remedies like treatment of bleeding hemorrhoids, jaundice, cough, asthma, bronchitis, fever, piles, tooth ach, anemia, skin disease, leprosy, anthelmintic, wounds, diabetes, urinary tract infection, rheumatism, gastric disorder, syphilis and as carminative[1-3]. Wide range of therapeutic activity of *Mangifera indica* has been explored like analgesic, anti-inflammatory[4], antioxidant[5,6], immunomodulatory[7,8], antidiarrheal[9], dyslipidemic[10], antidiabetic[11,12], antiamebic[13], antiulcer[14], antimicrobial[15,16], anthelminthic and antiallergic[17]. Phytochemical investigation showed presence of different phenolic constituents like triterpenes, flavonoids, phytosterols and polyphenols in different parts of *Mangifera indica*[18-21].

Though some of the synthetic spermicidal agents are available but most of them produces severe side effect. Hence, use of the drug from herbal source with spermicidal property is absolute need in modern era. Therefore aim of the study is to evaluate antispemogenic activity of methanolic extract of *Mengifera indica* root (MEMI) and to isolate active compound which may play key role in its therapeutic effect.

MATERIALS AND METHODS

Plant material

Roots of *Mangifera indica* were collected from Agartala, Tripura in November 2007 and dried under shed. Plant parts were authenticated from Department of Pharmacognosy, RIPSAT and a

voucher specimen (No: 128/08) is deposited at the Regional Institute of Pharmaceutical Sciences and Technology, Tripura, India.

Extraction and fractionation of the extract

Air dried roots of *Mangifera indica* (400 g) were powdered and exhaustively extracted (Soxhlet) with methanol (b.p. 64–66°C). MEMI (22% w/w) was concentrated to dryness under reduced pressure and residue of MEMI (88 g) thus obtained used for further studies. The density of the extract was found to have 0.7 g/ml. The extract fractionates using column chromatography. MEMI (100 ml) was chromatographed on a glass made column (55 cm × 1.6 cm) using silica gel (60-120 mesh) as stationary phase and ethyl acetate (% purity ≥ 99% GC) was used as mobile phase. Total six fractions were collected separately by observing the colors band on the chromatographic column. The fractions (M1, M2, M3, M4, M5, M6) were concentrated and dried under reduced pressure, weight of the dried fractions were found 60 mg, 55 mg, 42 mg, 33 mg, 11 mg, 25 mg respectively.

Physicochemical and phytochemical screening of methanolic extract

Physicochemical parameters like density of MEMI were determined using density bottle and specific gravity was calculated accordingly as described by Bhal[22]. The pH of MEMI was determined using a digital pH meter. R_f value was determined by TLC. Butanol:water:dioxane (4:2:1), butanol:acetic acid:water (4:1:1) and benzene were used as solvent system. MEMI was analyzed for the presence of alkaloid, protein, carbohydrate, starch, tannin, phenolic compound, saponin, fixed oil, fat, steroid, gum and mucilage using the standard method[23,24].

Spermicidal activity

Spermicidal activity of the MEMI and its different fractions (M1, M2, M3, M4, M5 and M6) were carried out adopting the standard procedure as described by Debnath *et al.*[25]. Briefly, sperm were collected from the healthy adult male volunteer. Only those considered normal heading 100-150 million spermatozoa/ml, ≥ 80% motility, 2.1 ml/ejaculate, pH 7.9 and with minimum contamination of debris or cells other than spermatozoa were used for the assay. Sperm count motility was assessed microscopically. Extract and various fraction were dissolve separately in dimethyl sulfoxide (DMSO) to make the concentration of the solution 1 mg/ml. Sperm volume (1.0 ml) were mixed with MEMI and different fractions. The sperm volume and extract or fractions volume was 10:1 for each case. Each experiment is repeated for six times. DMSO is used as a control. After the treatment of sperm with the extract or fractions the sperm motility were observed after 10, 20, 30 min. The percentage of inhibition of sperm motility is the indicator of spermicidal activity.

Purification and isolation of most active principle

MEMI and fractions thus obtained screened for spermicidal activity. Most active fraction M6 was purified by recrystallization using acetone. The purity of the recrystallized compound was tested by single spot in TLC plate using benzene as solvent system. Structure elucidation of the isolated, purified most active compound (M6) was performed using IR, Mass, ^{1}H NMR, ^{13}C NMR spectral data.

Statistical analysis

Values are calculated using statistical package for social science (SPSS) version 10 and percentage decrease in motility were calculated comparing with the normal motility.

RESULT AND DISCUSSION

Physicochemical and phytochemical observation of MEMI

Physicochemical properties of a compound provides important database to develop a new pharmacological active compound and also important for mechanism of action, possible

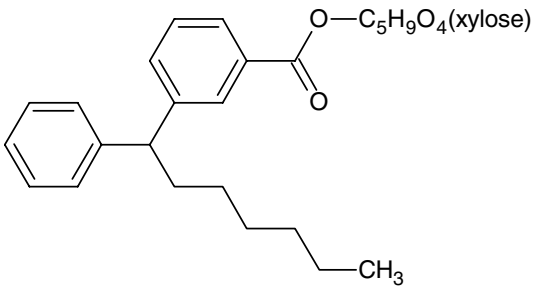

Fig. 1: 1-heptyl-(1-phenyl)-3-xylose benzoate

biological activity of metabolites and drug design[26]. Different physicochemical parameters of the MEMI were screened and density, specific gravity, pH were found 07 gm/ml, 1.4, 6 respectively. TLC was carried out using three solvent system i.e. butanol: water: dioxin (4:2:1), butanol: acetic acid: water (4:1:1) and benzene and found 5, 5, 6 spots respectively. Preliminary phytochemical screening of the MEMI showed the presence of alkaloids, saponin, phenolic compound, tannin and steroids but starch, protein, carbohydrate, fixed oil, fat, mucilage and gum found absent.

Spermicidal activity of MEMI and its fractions

In the present study, i*n vitro* spermicidal activity of MEMI and its chromatographic fractions were carried out and results were shown in Table 1. Percentage decrease in motility is the indicator of spermicidal activity which was observed after 10, 20, 30 min. MEMI and three fractions (M1, M2, M6) out of six shows spermicidal activity. Fraction M3, M4 and M5 does not produce any activity. MEMI, M1, M2 produces 34%, 14%, 29% spermicidal activity after 30 min. Fraction M6 produces highest activity (36%) after 30 min.

Isolation of most active compound

M6 produces highest activity therefore structure elucidation of most active compound (M6) was performed using IR, Mass, ^{1}H NMR, ^{13}C NMR spectral data. Spectral data of the compound are given below.

Table 1: Spermicidal activity of methanolic extract and fractions of *M. indica* root

Components	Percentage decrease in motility		
	10 min	20 min	30 min
MEMI	15	25	34
M1	5	10	14
M2	12	21	29
M3	0	0	0
M4	0	0	0
M5	0	0	0
M6	15	27	36
control	0	0	0

MEMI – methanolic extract of *Mangifera indica* root, M1-M6 are different fractions of MEMI, DMSO served as control.

IR analysis

C-OH, C=O and C=C banding at 3400, 1712, 1604 cm^{-1}. EI-MS analysis: $[M+H]^+$ at m/z 429, $[M+Na]^+$ at 451. ^{1}HNMR analysis: aliphatic C-H at δ0.79-2.10(m), sugar moiety at δ3-19-3-70(couple of singlet), C-H attached to two aromatic ring at δ5-21(s), Aromatic proton at δ6.72-7.50. ^{13}CNMR analysis: carbonyl ether at δ157.10, two aromatic ring carbons at δ136.39, δ123.69, δ123.81, δ124.90, δ119.90, δ125.78, δ136.67, δ137.27, δ152.92, δ145.03, δ143.39, δ140.34.

Spectral analysis confirmed the presence of a glycoside named 1-heptyl-(1-phenyl)-3-xylose benzoate in M6 (Fig. 1) in which, xylose present as a glycon which is linked with aglycon part by ester linkage. It was found to have a long aliphatic carbon chain linked with two phenyl groups through same carbon atom.

Antispermogenic activity of various glycosides already reported[27]. Therefore, antispermogenic activity of MEMI may be due to presence of this glycoside. Different type of synthetic antispermogenic drug presently available in the market but repeated use of those product may cause some serious adverse effect like inflammation, genital ulceration, HIV-1 infection[28,29].

Therefore, MEMI and its fractions and isolated glycoside from *Mangifera indica* root may serve as a new drug having antispermogenic activity with fewer side effects.

CONCLUSION

In conclusion, this study suggests that methanolic extract of *Mangifera indica* and isolated glycoside have spermicidal activity and it might be a better alternative source of anti-fertility agents that could overcome the problem of already existing products in the market. Further a details study need to be carried out to explore exact mechanism of action of isolated spermicidal compound.

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