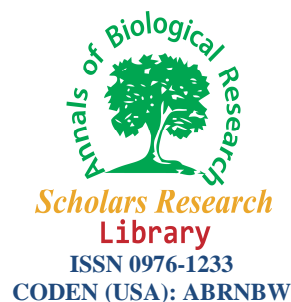




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Evaluation of spermicidal property of aqueous ethanolic extract of *Lawsonia inermis* linn. leaves

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ABSTRACT

This study was designed to assess the spermicidal property of aqueous ethanolic extract of *Lawsonia inermis* leaves. We applied different concentrations of the extract and determined the effect on sperm motility using the in vitro immobilization assay. The result showed that at 160 mg/ml, there was an instant immobilization of all the spermatozoa on application of the extract. At a concentration of 80 mg/ml, the sperm motility were 9.20 ± 0.00 , 9.80 ± 0.50 , 9.73 ± 0.35 , 9.60 ± 0.28 , 9.34 ± 0.30 and 9.17 ± 0.33 at 0, 15, 30, 60, 120 and 180 seconds respectively. Similarly, at 40 mg/ml, 20 mg/ml and 10 mg/ml, reduced sperm motility in a concentration- and time-dependent manner was observed. In conclusion, this study shows that the crude extract of *Lawsonia inermis* leaves possesses spermicidal activity.

Key words: contraceptive, herbal medicine, *Lawsonia inermis*, spermicidal.

INTRODUCTION

Herbal medicine is a major component of all indigenous people's traditional medicine and is so important that World Health Organization [1] in assessing the health care systems in developing countries suggested that common medicinal plants could be utilized as substitutes for drugs to reduce overdependence on importation of allopathic drugs. However, there is need for proper scientific verification of their efficacy and systemic effects, particularly on reproduction. Herbal contraceptives are in popular demand because they are cost effective, readily available from local sources and have fewer side effects. However, herbal medicines may impair fertility in male and female animals or humans. Whilst some medicinal plants tested for their antifertility properties caused reduction in sperm counts and altered the motility of the sperm cells, others altered hormone levels and the histoarchitecture of the testis. Many plants used as contraceptives or sterility agents decrease spermatogenesis [2], impair implantation [3] or are spermicidal [4]. Some research findings have confirmed the spermicidal properties of *Cestrum parqui* [5], *Carica papaya* [6] and *Hymenocardia acida* [7]. Global search for antifertility agents as an alternative to resolve population explosion has continued to receive attention especially in developing countries. For decades, efforts have been made to develop safe and effective contraceptives from natural sources. Plants having folkloric reputation have been identified and evaluated for their contraceptive efficacy. In recent years, there is a renewed interest in the control of fertility by using plants as male contraceptives [8].

Although *Lawsonia inermis*, a popular medicinal and cosmetic plant of the family Lythraceae widely cultivated in tropical and subtropical regions of Africa, has been evaluated for its antifertility effect in female [9], its spermicidal

property has not yet been reported. The aim of the present study was to evaluate the spermicidal property of aqueous ethanolic extract of *Lawsonia inermis* leaves.

MATERIALS AND METHODS

The leaves of *Lawsonia inermis* were collected within Makurdi metropolis, Benue state, Nigeria and authenticated at the College of Forestry, University of Agriculture, Makurdi. Voucher specimen was deposited at the College herbarium. The leaves were washed, air dried at room temperature, pulverized and stored in airtight container until required. One hundred grams of powdered material was soaked in 500 ml of 70% ethanol and stirred intermittently for 48 hours at room temperature. The material was filtered using sterile cotton wool and Whatman (No. 1) filter paper; the residue was re-suspended in the same amount of solvent and then filtered three more times. The filtrates obtained were dried at room temperature under the electric fan to obtain a crude extract. The extracts were stored in airtight containers at 4 °C until needed.

Animals

White albino rats (Wistar strain) were kept in polypropylene cages under room temperature, with 12-hour light and 12-hour dark cycle and were allowed to acclimatize for two weeks. The animals were provided commercial feed (Grand Cereals and Oil Mills Ltd, Bukuru, Jos, Nigeria) and clean water freely. Protocols for this experiment were in accordance with the guidelines on the care and well-being of research animals [10] and were approved by the Ethics Committee.

Experimental design

The rats were anaesthetized using diethyl ether. A scrotal incision was made to exteriorize the testis and epididymides. The epididymides were carefully dissected out of the testes and blotted free of blood. To prepare sperm suspension, epididymal sperm were obtained by teasing the cauda epididymides placed in prewarmed beaker containing 2 ml of physiological saline (maintained at 37 °C). Sperm suspension obtained from each rat was used for the *in vitro* immobilization activity as previously described [7]. Briefly, ten micro litres of the plant extract dissolved in physiological saline solution at varying concentrations (1%, 2%, 4%, 8% and 16 %) were mixed with epididymal sperm suspension (1:1 v/v) and tested for their effects on sperm motility. A drop of the evenly mixed sample was immediately placed on a clean and dry glass slide covered with cover slip and mounted on a prewarmed stage. This slide was then examined under the binocular microscope (Olympus, Japan) at magnifications of x10, x40. At least five fields were rapidly examined and 100 spermatozoa were counted. For the control, 10 µl of physiological saline was used instead of plant extract. The motility of spermatozoa was observed at various time intervals (15, 30, 60, 90, 120 and 180 seconds).

Statistical Analysis

The results were analyzed and expressed as mean \pm S.E.M using Graph Pad Prism Version 3.0 for Windows (Graph Pad Software, San Diego, California).

RESULTS

The effect of aqueous ethanolic extract of *Lawsonia inermis* leaves on sperm motility at different times (duration in seconds) is shown in Table 1. The extract caused significant decreases ($P < 0.05$) in spermatozoa motility in a concentration-dependent manner. An instant immobilization of spermatozoa was observed when 16% concentration was applied.

DISCUSSION

The present study evaluated spermicidal properties of aqueous ethanolic extract of *Lawsonia inermis* leaves and revealed a concentration-dependent reduction ($P < 0.05$) in the motility of sperm cells (Table 1). A similar study on the extract of *Hymenocardia acida* stem bark caused instant immobilization of the rat epididymal spermatozoa at 10 % concentration [7].

However, the extract of *Lawsonia inermis* immobilized sperm cells immediately on application at 16% concentration. The result of the present study is in agreement with the findings of Lohiya and others [6] who showed that partially purified compounds of ethyl acetate sub-fractions of *Carica papaya* seeds when administered at 2% concentration reduced motility of spermatozoa. An impaired motility was reported as index of spermicidal activity of *Achyranthes aspera* and *Stephania hernandifolia* [4]. As was observed with *Cestrum parqui* [5], aqueous ethanolic extract of *Lawsonia inermis* leaves possesses *in vitro* spermicidal effect on rat sperm cells.

Different phytochemicals in plants screened for their spermicidal or sperm immobilization property were reported to contain saponins, flavonoids and phenol compounds [11]. Most plant derived spermicides which caused sperm immobilization in animals and humans were confirmed to contain saponins [12]. The extract of *Lawsonia inermis* leaves revealed the presence of saponins and other phytoconstituents (data not shown). The result of this study showed that aqueous ethanolic extract of *Lawsonia inermis* leaves has spermicidal effect. However, the efficacy of *Lawsonia inermis* as spermicidal agent need to be further investigated.

Table 1: Effect of *Lawsonia inermis* leaves extract on spermatozoa motility (expressed as percentage).

Data are expressed as mean \pm S.E.M.

Concentrations	0 seconds	15 seconds	30 seconds	60 Seconds	120 seconds	180 Seconds
Normal saline	75.70 \pm 1.56	74.70 \pm 1.90	73.70 \pm 1.25	70.08 \pm 2.34	66.21 \pm 2.75	61.30 \pm 2.90
10 mg/ml	26.90 \pm 0.00	26.90 \pm 0.00	26.53 \pm 0.37	25.68 \pm 0.90	25.00 \pm 0.97	23.85 \pm 1.40
20 mg/ml	20.10 \pm 0.30	20.50 \pm 0.40	20.23 \pm 0.35	19.58 \pm 0.70	17.58 \pm 2.07	16.05 \pm 2.28
40 mg/ml	12.70 \pm 0.00	13.10 \pm 0.40	12.43 \pm 0.71	12.40 \pm 0.55	11.90 \pm 0.52	11.43 \pm 0.63
80 mg/ml	9.20 \pm 0.00	9.80 \pm 0.50	9.73 \pm 0.35	9.60 \pm 0.28	9.34 \pm 0.34	9.17 \pm 0.33
160 mg/ml	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00

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