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Full Length Research Paper

Anti-fertility activity of aqueous root bark extracts of *Asparagus africanus* Lam and *Annona senegalensis* Pers combination on female Sprague Dawley rats

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Many rural and poor women have now resorted to use of potential medicinal plants as a means of fertility control. *Asparagus africanus* Lam and *Annona senegalensis* Pers are some of the plants used for this purpose. The efficacy and safety of many of such plants, however, have not been verified. Therefore, screening for anti-fertility activity of potential medicinal plants, would thus provide alternative safe and affordable contraceptive, if effective and less toxic. This study aims to carry out phytochemical screening, acute toxic effects and antifertility activity in female rats of the aqueous extracts of *A. africanus* Lam and *A. senegalensis* Pers combination. Acute toxicity test was done according to Lorke's methods and antifertility activity of the extracts by use of the method described by Khanna and Chaudhary, with modification for our local use. The percentage inhibition of conception of the extract was compared with those of the controls. Phytochemical screening revealed the presence of known anti-fertility principles such as saponins, alkaloids and phenolic compounds. Acute toxicity studies indicated that the extract was non-toxic up to the highest dose of 12.8 g and the antifertility activity of the aqueous crude extract was found to be dose dependent. This study therefore demonstrated that the aqueous root extract of *A. africanus* Lam and *A. senegalensis* Pers combination has antifertility activity and is safe at the doses employed in this study.

Key words: Anti-fertility, rats, phytochemical screening, acute toxicity, *Asparagus africanus*, *Annona senegalensis*.

INTRODUCTION

Uganda has one of the fastest growing populations in the world with a fertility rate of 6.7 and an annual population growth rate of 3.2%. This is due in part to low contraceptive use as a result of lack of access to safe and affordable contraceptives *(UDHS 2011)*. The capacity to address the world wide incidence of

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unintended pregnancy and abortions lies in the ability to discover an alternative effective, safe and affordable contraceptive (WHO). The current contraceptive methods are associated with serious adverse effects such as: thromboembolic events, hepatic tumors, breast cancers, cardiovascular diseases, uterine cervical cancer, breakthrough bleeding, spotting, amenorrhea, diabetes mellitus, gall bladder disease, continuous change in healthy body metabolism (that is, causes migraine headaches, weight increase, moodiness, and loss of libido), hypertension, depression, abortion (Kahlenborn, 2006; Giannitrapani, 2006) which are inconveniencing and less satisfactory probing in adherence and acceptability problems. Despite plants are extremely exploited in traditional healing systems, only in some cases their therapeutic potential in human has been substantiated (Rad et al., 2013; Sharifi-Rad et al., 2013, 2014a, b). Screenings for anti-fertility activity of potential medicinal plants provided alternative safe and affordable contraceptive (Farnsworth, 1975). There are several plants that have been known to possess contraceptive activity (Mukund et al., 2012). *Asparagus africanus* Lam and *Annona senegalensis* Pers, locally known as "Ogwaro" and "Obwolo" respectively are some of the traditionally used anti-fertility plants in Abim, Uganda. However, there were no reports on both ethno botanical and pharmacological profile of these plants. *A. africanus* Lam (Liliaceae) is an erect armed herb that grows up to 5 ft high. The plant is widely distributed in tropical Africa. In traditional medicine, the plant is used for the treatment of headache, backache, stomach pain and as an aid in childbirth (Msonthi and Magombo, 1983), heamaturia, heamorrhoids (Desta, 1993), malaria, lishmaniansis, bilharziasis, syphilis and gonorrhoea (Oketch-Rabah et al., 1992). The root extract is applied externally for the relief of pain, rheumatism and chronic gout (Watt and Breyer-Brandurijk, 1962). It is also used as a diuretic, for sore throat and otitis (Oliver, 1960). Three steroidal saponins have been isolated from the roots of *A. africanus* (Debella et al., 1999). *A. senegalensis* takes the form of a small tree, growing between two and six meters tall. Occasionally, it may become as tall as 11 m. The plant is widely distributed in tropical Africa. It is among medicinal plants that have been documented to possess antibacterial effects (Muanza et al., 1994). It is also used in the treatment of wounds and infectious diseases such as diarrhea (Suleiman et al., 2008), periodontal and other oral infections (More et al., 2008). Furthermore, the anticonvulsant, sedative and muscle relaxant (Okoye et al., 2010) as well as anti-inflammatory (Okoye et al., 2011) effects of the root bark extract and fractions of *A. senegalensis* have been reported. This study was carried out to determine the antifertility activity of the crude extract of *A. africanus* Lam and *A. senegalensis* Pers root combination, with particular emphasis on phytochemical screening, determining the toxicity profile and evaluating the anti-fertility effects using experimental models in rats.

MATERIALS AND METHODS

Study site

The study was carried out in the Pharmaceutical Chemistry Laboratory, Biochemistry Laboratory and Animal Research Facility of Mbarara University of Science and Technology.

Study design

This was an experimental short term prospective research study.

Plant collection and extract preparation

The roots of *A. africanus* Lam and *A. senegalensis* Pers were collected from Abim district, North Eastern Uganda in January 2014. The plants were identified by a botanist at the Department of Plant Biology, the Herbarium section at Makerere University, Kampala main campus and authenticated and assigned the voucher numbers by a botanist at the Department of Plant Biology, Faculty of Science, Mbarara University of Science and Technology (Oscar P'Okello Okidi 001 and 002, respectively). The roots of the plants were washed and shade dried at room temperature for a period of 21 days, crushed using mortar and pestle to reduce the size and then blended using a blender to powder of which 400 g of the combined plant material in the ratio of 1:1 was cold macerated in distilled water for 24 h, filtered with filter paper (Whatman No.1) and the solvent evaporated using an oven at 65°C for 4 days resulting in the recovery of a brownish dark solid.

Experimental animals

Cyclic virgin female Sprague Dawley rats, 3 months old (200±10 g) were used for the acute toxicity testing and anti-fertility activity while the male rats were used for mating in the anti-fertility assay. All animals were housed in standard cages with uniform conditions of lighting (12 h dark: 12 h light cycle) and at room temperature. Animals were fed on pellet and tap water. Animals were handled in this study as per the National Institute of Health guidelines (1978) for the care and use of laboratory animals.

Phytochemical screening

Identification of the chemical constituents of the aqueous roots bark extract of *A. africanus* Lam and *A. senegalensis* Pers combination was carried out as per the method described by Trease and Evans (2009).

Determination of oral median lethal dose (LD_{50})

The acute toxicity (LD_{50}) was determined in female rats by the method of Lorke (1983) using the oral route. The test was performed in one phase. Animals in the three different groups received oral administration of one of 5000, 8000, and 12,800 mg/kg (n=3) and observed for 24 h for mortality (number of deaths) and general behavior. The rats were further observed for fourteen more days for delayed toxicities and deaths.

Table 1. Phytochemical screening results of the aqueous root extracts of *Asparagus africanus* Lam and *Annona senegalensis* Pers combination.

Phytochemical	Test method	Deduction
Free amino acids	Amino acid test	Present
Alkaloids	Dragendorrf's test	Present
Terpenoids	Liebermann-Burchard test	Present
Reducing sugars	Fehling's test	Present
Tannins	Ferric chloride test	Present
Phenolic compounds	Ferric chloride test	Present
Flavonoids	Ammonia test	Absent
Saponins	Frothing test	Present
Proteins	Million's test	Absent

Anti-fertility assay

Anti-fertility activity was carried out using the method described by Khanna and Chaudhary (1968) with minor modifications. Five groups of 3 months old virgin female rats (n=5) were used. Three experimental groups received the aqueous extract (test sample) in doses of 125, 250 and 500 mg/kg body weight every 12 h. While the positive control received injectaplan (0.33 ml, that is, 50 mg) intramuscularly once, the negative control group received distilled water. Rats receiving the aqueous extract in each of the experimental groups were continuously treated as in Day 1 up to Day 5. All animals were allowed to mate with proven fertility male rats (n=3) on Day 6 for five (5) days, that is, up to Day 10 while maintaining daily treatment of the rats as per the previous days. The male rats were withdrawn from each group on Day 11 and administration of the aqueous extract continued to Day 13. The rats were then anaesthized, dissected and the uterus observed for the presence of fetus within the uterus to confirm for pregnancy on Day 21. The number of pregnant and non-pregnant animals was recorded and the antifertility activity determined (Kong et al., 1989).

Data analysis

The number of non-pregnant rats between the three extract treated groups (125, 250 and 500 mg/kg), distilled water and Depo-Provera (50 mg) were entered into Excel sheet, exported into Graphpad prism version 6 software and analyzed using Kruskal Wallis test followed by Dunn's multiple comparison test at 95% Confidence Interval. Results have been presented in table format. Those results with p-value ($P \leq 0.05$) were considered statistically significant.

Ethical consideration

This work was approved by the Faculty Research and Ethics Committee of the Faculty of Medicine, Mbarara University of Science and Technology.

Deep anesthesia was achieved during operation to determine whether the rats were pregnant or not using chloroform. The NIH guidelines for handling animals in teaching and research were clearly followed.

RESULTS

Phytochemical screening

Phytochemical screening of aqueous extract indicated the presence of free amino acids, terpenoids and saponins, alkaloids, reducing sugars, phenolic compounds and tannins. However flavonoids and proteins were not detected (Table 1).

Determination of oral median lethal dose (LD_{50})

The oral LD_{50} of the aqueous extract was not calculated as the extract did not cause any mortality unto a dose of 12,800 mg/kg body weight. However rats showed signs of clinical toxicity such as poor appetite, inactivity and drowsiness.

Acute toxicity test results

At the highest dose used of 12800 mg/kg body weight, inactivity, drowsiness and poor appetite were noted but no death during the 14 days of observation.

Anti-fertility assay

Treatment of animals with aqueous extract resulted in a significant dose-dependent inhibition of conception (Table 2). Table 2 shows that there was a dose dependent increase in antifertility activity of the extract, with 500 mg/kg of extract giving the same effects as the standard drug Depo-provera.

DISCUSSION

The current study has indicated that the combined

Table 2. Comparison of the antifertility effects of water (Control), extract and Depo-Provera.

Test	Number	Mean Rank Diff	Adjusted P-value at 95% CI
Control vs. Extract (125 mg/kg)	5	5	0.7868
Control vs. Extract (250 mg/kg)	5	10	0.0393
Control vs. Extract (500 mg/kg)	5	12.5	0.0050
Control vs. Depo-Provera (50 mg)	5	12.5	0.0050

aqueous extracts of *A. africanus* Lam and *A. senegalensis* Pers has significant antifertility effects. In the phytochemical screening, the presence of saponins, alkaloids and phenolic compounds which have been reported to possess anti-fertility activity, may support the claimed anti-fertility effects of the aqueous extract as reported to have contraceptive activities (Shibeshi et al., 2006; Padmashali et al., 2006; Ramya et al., 2011).

The combined aqueous extract of the roots of *A. africanus* and *A. senegalensis* did not cause any mortality up to a dose of 12,800 mg/kg orally and was thus considered to be non-toxic. However, milder signs of toxicity such as poor appetite, inactivity, and drowsiness were observed.

Administration of aqueous crude roots extract to the three experimental groups of female virgin rats at three dose levels of 125, 250 and 500 mg/kg body weight twice a day for 13 days showed significant dose-dependent anti-fertility effect (Table 2) by inhibition of conception suggesting that the extract has anti-fertility effect. This finding is consistent with those of Shibeshi et al. (2006) and Ramya et al. (2011) that showed similar results in female virgin rats following treatment with *Achyranthes aspera* and *Dodonea viscose* Linn, respectively. Based on the results of the present study with animal models, it can be concluded that the combined aqueous crude roots extract of *A. africanus* Lam and *A. senegalensis* Pers when administered orally has effective dose-dependent anti-fertility activity and reasonable safety. However, the study has limitations, in that it was conducted using a combined crude extract of the two plants that contains many anti-fertility components and quantitative determination of principal active ingredient responsible for observed effects was not done. It is also not possible at this point to tell if this effect was due to components from one plant or a synergistic effect of the two plants. Further studies should therefore be done using the same method to determine the reproducibility of this result, which will confirm this activity. Also, study on the possible mechanism as well as investigation on the fractionated isolates should be pursued. There is also need for standardization of the preparations being used by the local people in the community.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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