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Original Article

In vitro Antitrichomonal Activity of *Acacia nilotica* L different extracts

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ABSTRACT

Background: *Trichomonas vaginalis* is the major worldwide health problem of non viral sexual from all transmitted diseases (STD) especially in the third world.
Methods: In the present work barks and fruits of *Acacia nolotica* were extracted by methanol, chloroform and water, with different concentrations to be investigated *in vitro* against *T. vaginalis*.
Results: Methanolic extracts of both of *A. nilotica* fruits and bark exhibit 100% mortality at concentration 250 µg µml after 192 hours, this was compared with metrondizole powder which gave 100% mortality at concentration312.5 µg/ml after 216 hours, while the fruits chloroform extracts gave mortality 83.2% at 1000 µg/ml after 216 hours, mean while, the bark chloroform extracts gave mortality 100% at 1000 µg ml after 192 hours. Water extract of both of *A. nilotica* fruits and bark exhibit 85.5% and 97.1% respectively at concentration 250 µg/ml after 192 hours.
Keywords: *Trichomonas vaginalis, Acacia nilotica,* Metronidazole

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INTRODUCTION

Trichomonas vaginalis infection should be considered as one of the major vaginal infection that serves markers for others sexually transmitted diseases in recent years (Seema and Arti , 2008). Moreover, *T. vaginalis* infection have been associated with several adverse outcomes including adverse pregnancy, pelvic inflammatory premature delivery, infertility, postoperative infections, low birth weight , cervical neoplasia and increased susceptibility to HIV infection (Schwebke *et al*., 1997).

Medicinal plants are still invaluable source of safe, less toxic, lower price, available and reliable natural resources of drugs all over the world. People in Sudan and in other developing countries have relied on traditional herbal preparations to treat themselves. Therefore, it is useful to investigate the potential of local plants against these disabling diseases (Koko *et al*., 2008).

Thus the need of alternative drugs to reduce their burden of purchasing the synthetic drugs especially after the problem of getting resistant to many clinical patients against metronidazole (Iran *et al*, 2006; Pratibha *et al*, 2008). In addition, metronidazole sometimes causes adverse effects, e.g., myopia, neuralgia, and allergic dermatitis (Upcrof *et al*, 2006), and thus new anti-trichomonal drugs are probably required.

Sudan is rich in medicinal flora with antiprotozoal, antimicrobial and antifungal activities e.g. *Acacia nilotica* (EL-Kamali *et al*, 1996). With the

purpose of searching for new antitrichomonal agents Acacia nilitica which were used traditionally for treatment of clinical signs associated with trichomoniasis such as venereal diseases were selected to evaluate the activity of their chloroform, methanol and aqueous crude extracts against *T. vaginalis* trophozoites *in vitro*.

MATERIALS AND METHODS

Plant materials

The fruits and barks of *A. nilotica* were collected between January and February 2008 from Alsunut Jungle in the middle of Khartoum State central of Sudan. The plant was identified and authenticated by the taxonomists of Medicinal and Aromatic Plants Research Institute (MAPRI). All plant parts were air-dried, under the shadow with good ventilation and then ground finely in a mill to be used for extracts preparation.

Preparation of Crude extracts

Extraction was carried out according to the method described by Harbone, 1984. To prepare extracts for screening of antitrichomonal activity, briefly 50 g were macerated in 250 ml of Chloroform for 3 hours at room temperature with occasional shaking for 24 hours at room temperature, the supernatant was decanted and clarity field by filtration through a filter paper, after filtration, the solvent was then removed by rotary evaporator at 55 ˚C. Each residue was weighed and the yield percentage was determined (% of dry weigh) and stored at -20 ˚C for further analysis in tightly sealed glass vial. The remaining extracts which not soluble by chloroform successively extracted by methanol using the previous technique.

For aqueous extract 50 g of each plant sample was covered with 250 ml hot distilled water for 4 hours then filtered with Whattman filter paper. Extracts kept in deep freezer for 48 hours, then induced in freeze dryer (Virtis, USA) till completely dried. The residue was weighed and the yield percentage was determined. The extracts were kept in refrigerator until the time of their use.

Parasite isolate

T. vaginalis used in all experiments were taken from patient with pelvic inflammatory diseases or vaginal discharge complaints at Ombadda and Ibrahim Malik Hospitals. All positive samples were examined by wet mount preparation. Then the positive sample was transported to MAPRI in nutrient broth medium. Trophozoites of *T. vaginalis* were maintained in CPLM medium. Sub culturing of the parasite was performed at 37 ± 1°C in RPMI 1640 medium containing 5% bovine serum. The trophozoites were maintained for the assays and were employed in the log phase of growth.

Wet mount preparations

The samples were placed into a tube and centrifuge deposit of urine (Ackers *et al*, 1978). A drop was put on a slide and cover slip applied and the deposit examined under high power field 40X of light microscope for parasite viability.

CPLM cultivation method

The culture was done according to the technique previously described by Oyerinde (1999) with slight modification. Mid stream urine samples were collected from indoor hospital patients, the urine samples were centrifuged and the deposits were collected in CPLM medium. The cultivated materials were incubated at 37 ± 1°C in anaerobic condition and their microscopic examination was done after 24 and 48 hours by taking a drop from the bottom of the culture using sterile Pasteur pipette, transferring to a slide and examined under the high power objective.

Inoculums

T. vaginalis was inoculated in the RPMI 1640 medium and incubated at 37 ± 1˚C for 48 hours. Parasites were counted under the microscope by haemocytometer chamber.

In vitro susceptibility assays

In vitro susceptibility assays used the sub- culture method of Cedilla *et al*., (2002). This is highly stringent and sensitive method for assessing the anti-protozoal effects (gold standard) particularly in *Entamoba histolytica, Gairdia intestinalis* and *T. vaginalis* (Arguello *et al*., 2004).

Five mg from each extract was dissolved in 50µl of dimethyl sulfoxide (DMSO) at eppendorf tube containing 950 µl D.W in order to reach concentration of 5 mg/ml (5000ppm). The concentrates were stored at -20^{0}C for further

analysis. Sterile 96-well microtite plate was used for different plant extracts, positive control and negative control.

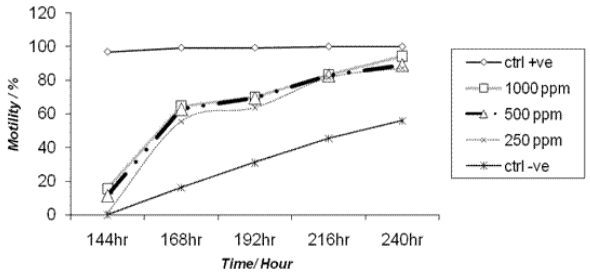

Fig.1. The activity of *Acacia nilotica* fruit chloroform extract against Trichomonas vaginalis

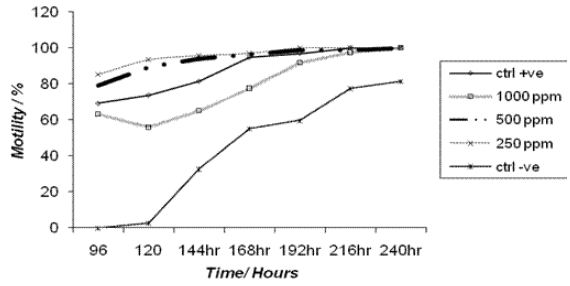

Fig.2. The activity of *Acacia nilotica* fruit methanol extract against Trichomonas vaginalis

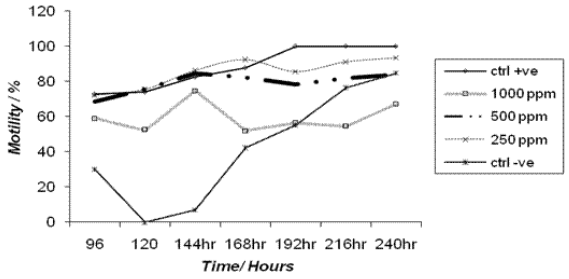

Fig.3. The activity of *Acacia nilotica* fruit aqueous extract against Trichomonas vaginalis

Twenty µl of complete RPMI medium were placed in the wells-except the first three wells C-1 (which 40 µl of an extract solution of 5 mgµml were added in the first three wells and the final concentrations were 1000 µg/ml). 20 µl of complete RPMI medium were placed in the wells in the following (C-2 which was 500 µg/ml and C-3 which was 250 µg/ml). 80 µl of culture medium was complemented with parasite and added to all wells. The final volume in each well was 100 µl.

Each test included metronidazole pure compound [(1-(2-hydroxyethl)-2-methyl-5nitroimidazole], a trichomonocide, was used as positive control in concentration 312.5 µg/ml, whereas untreated cells used as a negative controls (culture medium plus trophozoites). Samples were taken for counting at 0, 24, 48, 72, 96, 120, 144, 168, 192, 219 and 240 hours. For counting the samples were mixed with Trypan blue in equal volume. The final number of parasites was determined with haemocytometer in triplicate.

The mortality % of parasite for each extracts activity was carried out according to the following formula:

$$\text{Mortality of parasite (\%)} = \frac{(\text{Control negative} - \text{tested sample with extract}) \times 100\%}{\text{Control negative}}$$

Only 100% inhibition of the parasite considered, when there was no motile parasite observed.

Statistical analysis

All data were presented as means ± S.D. Statistical analysis for all the assays results were done using Microsoft excel program. Student t.test was used to determine significant difference between control and plant extracts at level of $P < 0.05$.

RESULTS

The yield % of *A. nilotica* fruit chloroform, methanol and water extract was 1.3, 29.4 and 5.9 respectively. While, the yield % of *A. nilotica* bark in chloroform, methanol and water extract were 1.0, 14.2 and 1.5 respectively.

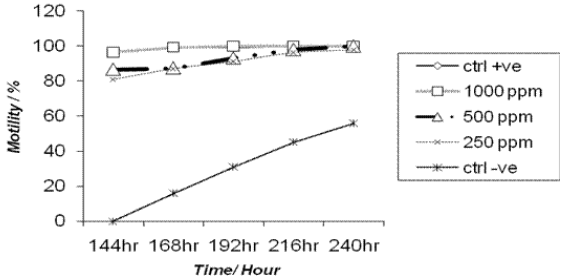

Fig.4. The activity of *Acacia nlotica* bark chloroform extract against *Trichomonas vaginalis*

The highest effective concentration of *A. nilotica* Fruit chloroform extract against *T. vaginalis* was 1000 µg/ml with mortality of 83.2% after 216 hours Fig (1). While 312.5 µgµml of metronidazole was gave 100% mortality at the same time. However *A. nilotica* fruit methanol extract in the lowest concentration 250 µg/ml showed the highest activity and 100% after 192 hours, comparing with 100% mortality of metronidazole after 216 hours Fig (2).

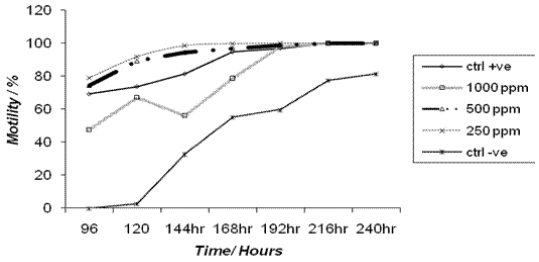

Fig.5. The activity of *Acacia nlotica* bark methanol extract against *Trichomonas vaginalis*

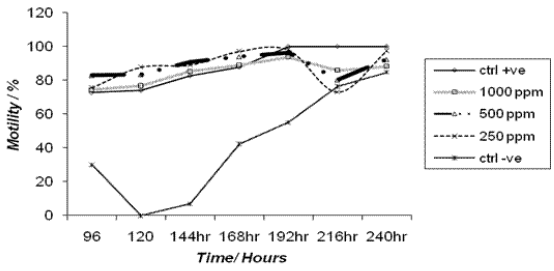

Fig.6. The activity of *Acacia nlotica* bark aqueous extract against *Trichomonas vaginalis*

The activity of *A. nilotica* Fruit aqueous extract was slightly week, the cells viable after 192 hours comparing with 100% mortality of metronidazole in the same time as mentioned previously Fig (3).

Fig (4) showed the activity of *A. nilotica* bark chloroform extract. The highest effective concentration of extract was 250 µg/ml with mortality 100% against *T. vaginalis* within 192 hours. In comparison, all the cells were found dead after 216 hours after incubation. While, the activity of *A. nilotica* bark methanolic extract in low concentration 250 µgµml gave 100% mortality after 192 hours, comparing with 100% mortality of metronidazole at 216 hours Fig (5).

As shown in Fig (6) mortality of *A. nilotica* bark aqueous extract was very low and the cells were viable after 192 hours which gave 97.4% mortality after 240 hours.

DISCUSSION

In the present results we found that both of *A. nilotica* fruit and bark methanol extracts in lower concentrations were equally effective against *T. vaginalis.* While, the chloroform extracts from *A. nilotica* bark was effective only against *T. vaginalis* in the high concentration comparing with metrondizole. The methanolic extracts were more effective against all the test of *T. vaginalis* than chloroform and aqueous extracts. This may be due to the ability of the methanol to elute a wide range of chemical constituents of the plant fruit and bark while the chloroform might have less numbers of the ingredients.

These results agree with traditional uses of garad in Sudan which indicate the plant claimed to be of anti-parasitic properties. The plant extracts were found to exhibit antidiarrhoeal, antibacterial, antimalarial and inhibition of lipid peroxidation (El-Tahir *et al*, 1999; Saleem *et al*, 2001; Rani and Khullar 2004; Agunu *et al*, 2005 and koko *et al,* 2008). El Shanawny, (1996) used *A. nilotica* fruit for treatment of sore throat, cold, bronchitis, pneumonia, ophthalmia, diarrhea, dysentery, leprosy and venereal diseases. Some of these diseases such as, dysentery and venereal diseases (this study did not determine which type of venereal disease) are protozoal diseases and may possibly confirm the antiprotozoal activity of the plant fruit. Indeed, it could explain the antiprotozoal activity of *A. nilotica* on different classes of protozoa such as *Trichomonas vaginalis* and *Entamoeba hystolytica*. Fatima *et al*, (2005) found the crude methanolic extract of *A. nilotica* posses *in vitro* anti leishmanial activity against Leishmania major promastigotes.

Our results revealed a novel pharmacological activity against *Trichomonas vaginalis* and suggested that the methanolic extracts have the potential of being used as topical application in vaginal infection. The results presented here providing motivation for further exploration of isolation active compounds, particularly as anti-trichomnal agents from methanolic extracts with important advantages for

the development of new anti-trichomnal agents from this plant.

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