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Full Length Research Paper

In vitro antitrichomonal activity of *Xanthium brasilicum* vell and *Argemone mexicana* L different extracts

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ABSTRACT

Whole plants of *Xanthium brasilicum* and *Argemone mexicana* were extracted by methanol, chloroform and water and then prepared in different concentrations in order to be examined for their trichomonacidal activities *in vitro*. Their activity was compared with metronidazole using strains of *Trichomonas vaginalis* freshly isolated from patients. Methanolic extracts of *X. brasilicum* exhibit 100% inhibition at concentration 500 µg/ml after 192 h, this was compared with metronidazole powder which gave 98.5% inhibition at concentration 312.5 µg/ml at the same time, while the chloroform extracts gave inhibition 100% at 1000 µg/ml after 216 h, mean while, the water extracts gave 100% inhibition at 1000 µg/ml after 192 h. Water extract of *A. mexicana* gave 100% inhibition 1000 µg/ml after 192 h, while the chloroform and methanol extracts 1000 µg/ml gave 100% inhibition after 216 and 192 h respectively. These studies conducted for both *X. brasilicum* and *A. mexicana* they were proved to be potent activities against *T. vaginalis*.

Key word: *Trichomonas vaginalis, Xanthium brasilicum*, Argemone *Mexican,* antitrichomonal activity.

INTRODUCTION

Trichomonas vaginalis is a parasitic protozoan that is the cause of trichomoniasis, a sexually transmitted disease (STD) of worldwide importance. *T. vaginalis* is the most common non viral transmitted infections, with an estimated 170 million cases occurring annually (Sood and Arti, 2008). Moreover, it has been associated with several adverse outcomes including adverse pregnancy, pelvic inflammatory diseases, 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. Sudan is rich in medicinal flora with antiprotozoal, antimicrobial and antifungal activities. Therefore; it is useful to investigate the potential of local plants against these disabling diseases (Koko et al., 2008).

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 have been increased (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); hence new anti-trichomonal drugs are probably required.

With the purpose of searching for new antitrichomonal agents, *Xanthium brasilicum* Vell and *Argemone mexicana* L which were used traditionally for treatment of

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clinical signs associated with trichomoniasis such as venereal diseases, hence the two plants 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 whole plants of *X. brasilicum* and *A. mexicana* were collected between January 2008 and April 2008. All plant parts were collected from River Nile bank, Khartoum State, Sudan. The plants were identified and authenticated by Dr. W. E. Abdalla the taxonomist of Medicinal and Aromatic Plants Research Institute (MAPRI). All plant parts were air-dried with good ventilation and then ground finely in a mill to be used for extract 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 h at room temperature with occasional shaking for 24 h 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 weight) then stored at -20°C for further analysis in tightly sealed glass vial.

The remaining extracts which is 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 h then filtered with Whatman filter paper. Extracts kept in deep freezer for 48 h, 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 patients with pelvic inflammatory diseases or vaginal discharge complaints at Ombadda and Ibrahim Malik Hospitals. The 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 centrifuged, a drop from deposit urine was put on a slide and cover slip applied and the deposit examined under high power field 40X of light microscope for parasite viability, this method was described by Ackers and Lumsden (1978).

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 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 h 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 h. Parasites were counted under the microscope by haemocytometer chamber.

In vitro susceptibility assays

In vitro susceptibility assays used the sub- culture method of Cedillo 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).

5 mg from each extract was dissolved in 50 µl of dimethyl sulfoxzide (DMSO) at eppendorf tube containing 950 µl D.W in order to reach concentration of 5 mg/ml (5000 ppm). The concentrates were stored at -20°C for further analysis. Sterile 96-well microtite plate was used for different plant extracts, positive control and negative control.

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 5 mg/ml) 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 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 the wells was 100 µl.

Each test included metronidazole pure compound [(1-(2-hydroxyethl)-2-methyl-5 nitroimidazole], 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 h.

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:

Mortality of parasite (%) = (Control negative – tested sample with extract) / Control negative x 100%

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 percent of *X. brasilicum* chloroform, methanol

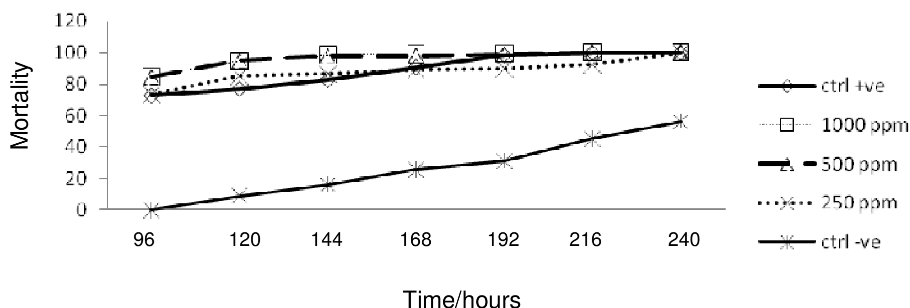

Figure 1. The activity of *X. brazilicum* chloroform extract against *T. vaginalis*.

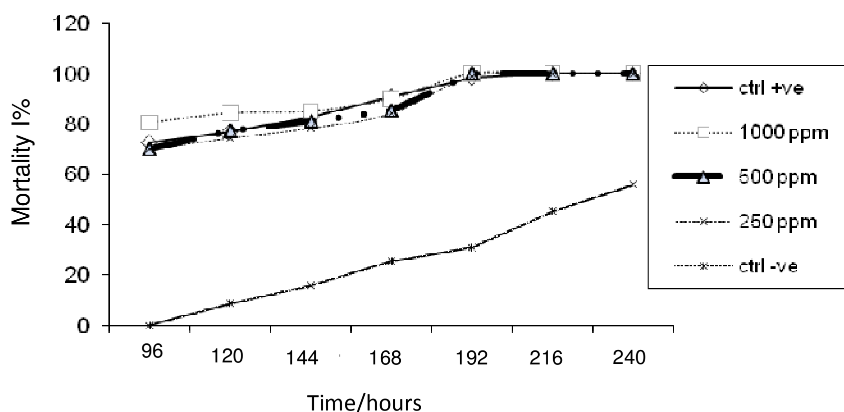

Figure 2. The activity of *X. brazilicum* methanol extract against *T. vaginalis*.

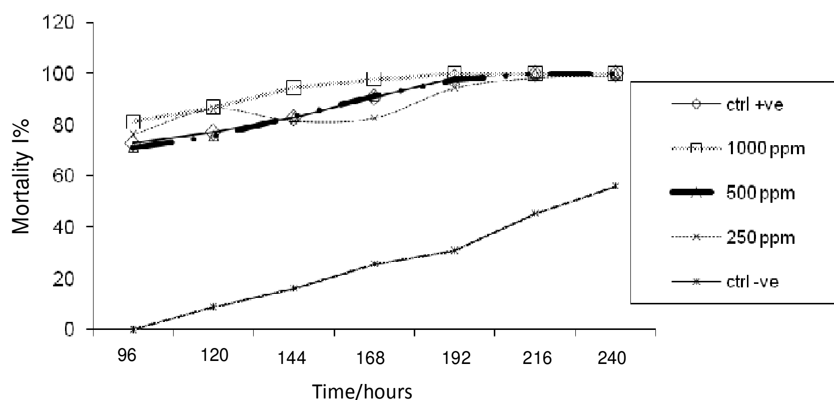

Figure 3. The activity of *X. brazilicum* aqueous extract against *T.*

and water extract was found to be 4.9, 6.6 and 1.6 respectively. While, the yield percent of *A. mexicana* in chloroform, methanol and water extract were 3.1, 10.5 and 3.1 respectively. In our first observations, no growth was observed after 216 h in incubated at 1000 and 500 ppm concentrations of *X. brasilicum* in chloroform extract, it was observed that almost all cells were dead after 216 h of incubation at these concentrations. However, at lower concentrations of 250 ppm, the cells were viable even after 216 h of incubation Figure 1.

In contrast, the activity of methanolic extract of *X. brasilicum* gave inhibition 100% after 192 h in concentration 1000 and 500 ppm after 192 h, while in metronidazole shown 98.5% mortality at the same time Figure 2.

Figure 3, shows the activity of aqueous extract of *X. brasilicum*. The high dose of extract shows 100% inhibition after 192 h. Figure 4 shows the activity of chloroform extract of *A. mexicana*. The high dose of extract gave 100% inhibition after 216 h, this was the

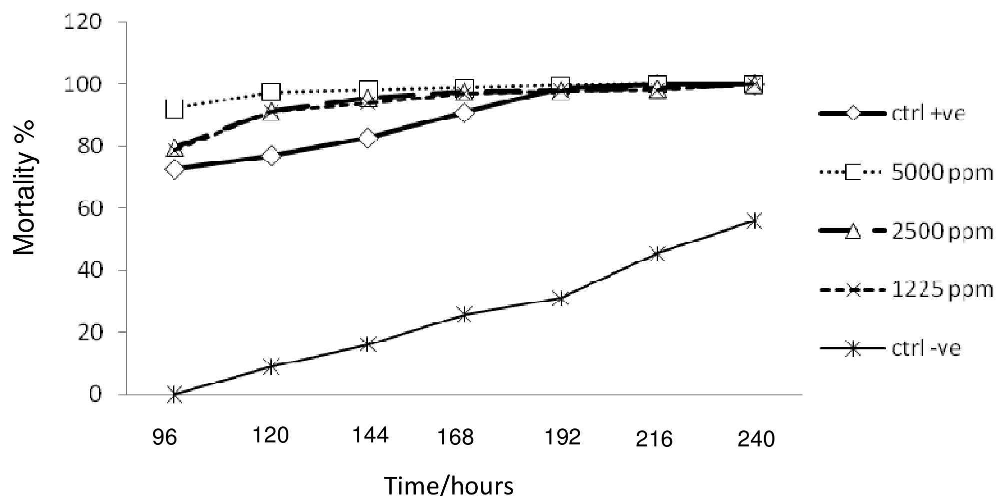

Figure 4. The activity of *A. mexicana* chloroform extract against *T. vaginalis*.

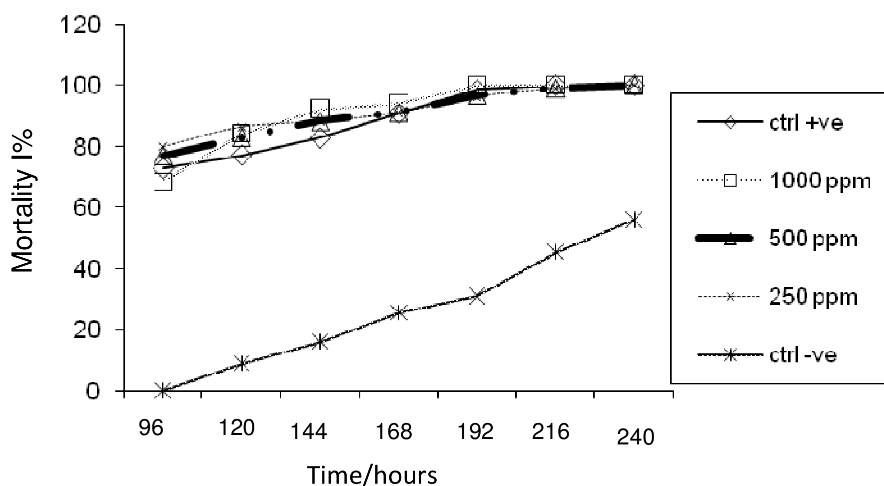

Figure 5. The activity of *A. mexicana* methanol extract against *T.vaginalis*.

same result that appeared with metronidazole.

The activity of methanolic extract of *A. mexicana* was shown in Figure 5. All the cells were dead at concentration 1000 ppm after 192 h, while in control positive gave 98.5% inhibition at the same time.

As show in Figure 6 regarding the activities of aqueous extract of *A. Mexicana*, the high dose of extract showed 100% inhibition at 192 h, comparing with metronidazole gave 98.5% mortality at the same period.

DISCUSSION

The present study, methanolic extracts of *X. brasilicum* exhibit 100% inhibition at concentration 1000 and 500 µg/ml after 192 h, this was compared with metronidazole powder which gave 98.5% inhibition at concentration 312.5 µg/ml at the same time, while the chloroform extracts gave mortality 100% at 1000 and 500 µg/ml after 216 h, mean while, the water extracts gave 100% inhibition at 1000 µg/ml after 192 h. The methanolic extracts were more effective against all test of *T. vaginalis* than chloroform and aqueous extracts. This may be due to the ability of the methanol to extract a wide range of chemical constituents of the plant fruit and park while the chloroform might have extracted less numbers of the ingredients.

Obviously such ways of *X. brasilicum* extracts have more effective against all tests and higher effect than positive control. This means that *X. brasilicum* can be considered to have powerful anti-trichomonal activity. These results agree with the previous study of *X.*

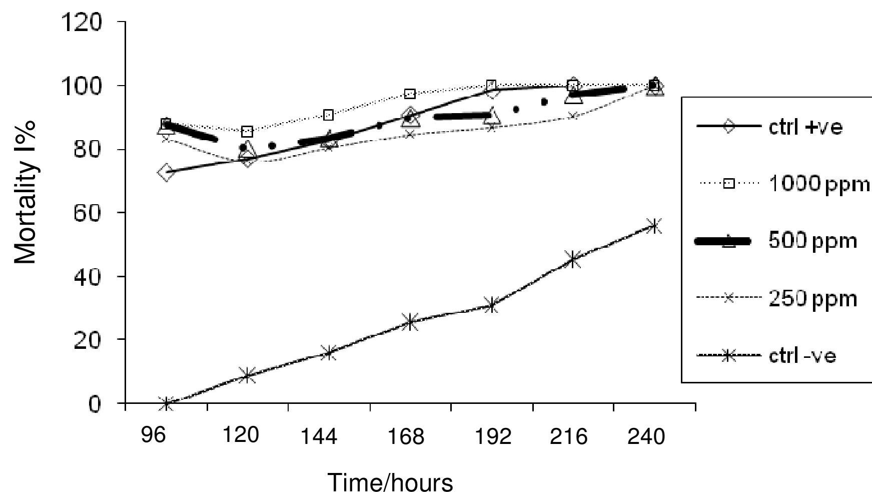

Figure 6. The activity of *A. mexicana* aqueous extract against *T. vaginalis.*

brasilicum as aniprotoazol activities (Koko, 2006). This plant extract also exhibited noticeable activities against *Trypanosoma cruzi* (Chagas disease); *Leishmania donovani* (Kala-Azar) as well as *Plasmodium falciparum* (Amal et al., 2009). Other study in China used *Xanthium spp* traditionally as antimalaria (David, 2004)

In *A. mexicana* we found that both methanol and chloroform extracts were equally effective against *T. vaginalis* in comparing with metronidazole. Meanwhile water extracts showed more activities than metronidazole. Sumeet et al. (2008) found that whole plant was effective for venereal diseases, although they did not mentioned which type of sexual disease, this results approve about the plant of *A. mexicana* have toxicity against *T. vaginalis.*

In conclusion *X. brasilicum* and *A. mexicana* demonstrated promising antitrichomonal and have been selective for further bio-guided fractionation and isolation of active antitrichomonal compound.

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