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Original Article

In vitro anti-trichomonal activity of three endogenous Sudanese forestry medicinal trees

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ABSTRACT

Trichomoniasis is the major worldwide sexual transmitting protozoal diseases (STPD) especially in the third world, caused by flagellated *Trichomonas vaginalis*. In the present work barks and fruits of three selected medicinal plants (*Acacia seyal, A. senegal* and *Tamarindus indica*) were extracted by methanol, chloroform and water, to be investigated *in vitro* against *T. vaginalis* with different concentrations. After 6 days exposure *A.seyal* bark gave 97, 85 and 78% mortality from methanol, chloroform and water extracts respectively at higher concerntration tested (1000 ppm), followed by *A. Senegal* bark which showed 85, 64 and 66% mortality at the same concentration, while the fruits of *A seyal* revealed 79, 79 and 58% mortality with highest concentration. For A. Senegal fruits 56, 74 and 72% was observed for methanol chloroform and water extracts respectively. However *T. indica* bark and fruit extracts were found the less active among the three investigated plants. Hence *A. Seyal* and *A. Senegal* can be considered of potent antitrichomonal activity rather than *T. indica*.

Keywords: *Trichomonas vaginalis, Acacia seyal, Acacia Senegal, Tamarindus indica*

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Introduction

Trichomonas vaginalis infection is one of the major health problems in the world, and one of the most common transmitted infections in many regions including the developed countries such as United State (Parbara, 2005). *T. vaginalis* infection should not be considered as a simple vaginal infection that serves markers for others sexually transmitted diseases in recent years. 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, despite the bulk work which have been done in this concern by Sudanese researchers; it is undoubtedly that this field remains rich and not wholly exploited (El Ghazali *et al*., 1994 and Koko *et al.,* 2009). Thus the need of alternative drugs to reduce the reliance on synthetic drugs especially after the problem of many cases developed resistance to metronidazole (Pratibha *et al*, 2008).

Thus, with the purpose of searching for new antitrichomonal agents, barks and fruits of three forestry medicinal plants (A. seyal, A. senegal and T. indica) traditionally used in all parts of the Sudan for treatment of clinical signs associated with trichomoniasis such as venereal diseases were selected to be evaluated for the activity of their chloroform, methanol and water crude extracts against *T. vaginalis* trophozoites *in vitro.*

MATERIALS AND METHODS

Plant Material

The fruits and barks of *A. seyal, A. Senegal and T. indica* were collected from central of Sudan between January 2008 and February 2008. . 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 until their uses for extracts preparation.

Preparation of crude extracts

Extraction was carried out for the barks and fruits of selected plants by using overnight maceration techniques according to the method described by Harbone (1984). About 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 under reduced pressure by rotary evaporator at 55°C. Each residue was weighed and the yield percentage was calculated then stored at 4°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 calculated. The extracts were kept in 4°C 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, Khartoum, Sudan. The positive samples were examined by wet mount preparation. Then the positive sample was transported to Medicinal and Aromatic Plants Research Institute (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 and inoculums

The samples were placed into a tube and centrifuged, a drop from deposit urine was put on a slide and covered by cover slip and the deposit examined under high power field 40X of light microscope for parasite viability, this method was described by Ackers and Lumsden (1978).

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 sulfoxide (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 125 µg/ml, whereas untreated cells used as a negative controls (culture medium plus trophozoites). Samples were taken for counting after. For quantification, the samples were mixed with trypan blue in equal volume. The final number of parasites was calculated with haemocytometer in triplicate. The mortality % of parasite for each extracts activity was carried out according to the following formula:

Mortality of parasite (%) =

$$\frac{\text{(No. of viable parasite in control negative – No. of viable parasite in tested sample)}}{\text{No. of viable parasite in control negative}} \times 100\%$$

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. Linear regression was applied for the calculation of IC_{50}.

RESULTS AND DISCUSSION

Experiment was monitored daily and the mortality was calculated after 144-hours. Control positive -metronidazole pure compound was included (125 ppm), also media inoculated with T*. vaginalis* as negative control was used.

Fig. 1 indicates that the mortality of A.seyal fruit chloroformic extract ranging from 54-79% for the all tested concentrations (250-1000 ppm) with IC50 191 ppm, but the barks gave 70-85% mortality for the same concentrations with IC50 49 ppm. On the other hand *A. Senegal* fruits and barks gave mortality ranging from 58-74% and 35 63% with IC50 125 and 484 ppm respectively. However *T. indica* chloroformic extracts for both fruits and bark revealed less than 50% mortality for all tested concentrations.

Fig. 2 indicates that the mortality of *A.seyal* fruit methanolic extract ranging from 62-78% for the all tested concentrations (250-1000 ppm) with IC_{50} 150 ppm, while the barks showed 90-97% mortality for the same concentrations with IC_{50} 0.3 ppm. On the other hand *A. Senegal* fruits and bark gave mortality ranging from 52-57% and 79-86% with IC_{50} 239 and 3 ppm respectively. However *T. indica* methanolic extracts for both fruits and bark revealed less than 50% mortality for all tested concentrations.

Fig. 3 indicates the results obtained from water extracts of the three investigated plants it looks almost similar for the three plants with moderated activity. The mortality ranging from 70-75% and 61-79% with IC50 270 and 108 ppm for both *A. seyal* fruits and barks respectively. While *A. Senegal* fruits and barks extracts gave mortality ranging from 52-72% and 55- 66% with IC50 207 and 132 ppm respectively. In case of T. indica the water extract of fruits and bark revealed mortality ranging from 36 -64% and 54-71% with IC50 461 and 224 ppm respectively.

Despite the previous comprehensive screening of Sudanese medicinal plants for their antiprotozoal activity (Ali *et al.*, 2002; Abdurrahman *et al.*, 2004; Koko, 2005; Dahab *et al.,* 2010; Ahmed *et al.,* 2010), this is the first time for *in vitro* antitrichomonal activity of the above mentioned plants.

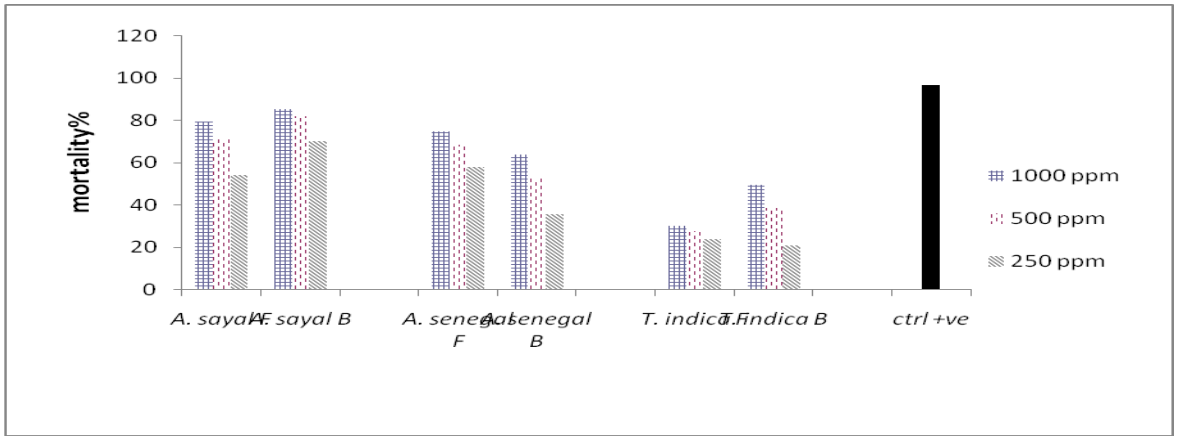

Fig.1. *In vitro* Antitrichomonal activity of Chloroformic extracts of the three selected plants. F; Fruits, B;Barks.

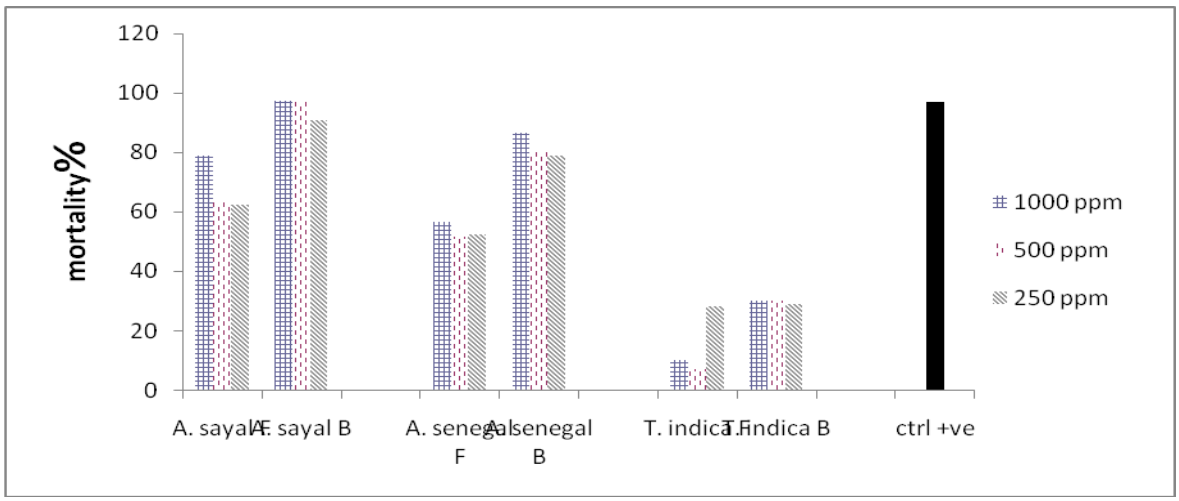

Fig.2. *In vitro* Antitrichomonal activity of methanolic extracts of the three selected plants. F; Fruits, B;Barks

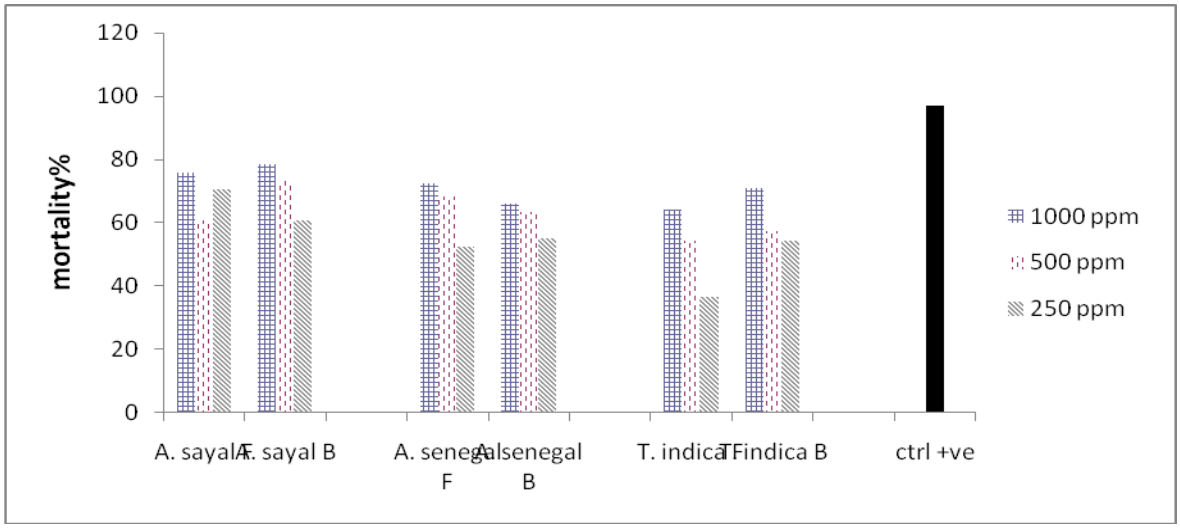

Fig.3. *In vitro* Antitrichomonal activity of water extracts of the three selected plants. F; Fruits

For *in vitro* susceptibility assay, the present study has shown that the antitrichomonal activity of of *A.seyal* bark methanolic extract is the most potent among all examined extracts (IC50 0.3 ppm) followed by the methanolic extracted bark of *A. senegal* (IC50 3.9 ppm). These results agree with previous results obtained from Acacia nilotica fruits and barks methanolic and cholorofomic extracts (Dahab et al. 2010). *A. senegal* was proved to be effective for the treatment of colds, stomach aches, diarrhea, hemorrhages, and syphilis. It is also used as an

aphrodisiac (Kess, 1995). *Tamarindus indica* in general, none of the extracts tested were as active as the other *Acacia* investigated species. In Philippine population Department of Health Circular encourage in herbal and medicinal plants use *Tamarindus indica* in vaginal wash and aromatic bath (Eduardo and Aurora, 2002), but, no data for treatment trichomoniasis in this report. Also, this plant known as laxative wherever it is grown, and can also be used to treat venereal diseases, malaria and infections of the eyes (Kees, 1995) although they did not mentioned which type of sexual disease.

Table.1. Some of Sudanese plants and their related to traditional uses and IC_{50} ppm of all extracts against *T. vagnilis in vitro*.

Name of Plant	**Family**	**Part of sample**	**Solvent use for extract**	**Weight/g**	**Yield (%)**	**IC_{50} ppm**
Acacia sayal	Mimosaceae	Fruit	Chloroform	0.309	0.618	190.8
			Methanol	2.409	4.818	149.6
			Water	2.16.3	4.326	270.4
		Bark	Chloroform	1.098	2.196	46.5
			Methanol	2.884	5.766	0.34
			Water	1.72	3.44	107.8
Acacia Senegal	Mimosaceae	Fruit	Chloroform	1.154	2.438	124.5
			Methanol	2.162	4.919	238.9
			Water	6.4	12.8	206.5
		Bark	Chloroform	0.517	1.054	484.3
			Methanol	2.181	4.362	3.9
			Water	0.92	2.875	131.8
Tamarindus indica	Caesalpiniaceae	Fruit	Chloroform	0.603	1.206	Inactive
			Methanol	21.389	42.778	122.3
			Water	0.31	0.62	460.9
		Bark	Chloroform	0.181	0.362	957.7
			Methanol	3.518	7.036	Inactive
			Water	1.6	3.2	224.2

Hence we can conclude that the genus Acacia is a good source for obtaining antitrichomonal agent/s. These studies clearly demonstrated that plants are rich source of anti-trichomonal substances. Further phytochemical and biological investigations of these plants are highly recommended.

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