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Comparison of the Antiparasitic Activity of *Bauhinia rufescens* Leaves Extracts and Metronidazole Against *Gairdia lamblia*

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Abstract: *Giardia* is a flagellate protozoan with worldwide distribution that causes significant gastrointestinal diseases in a wide variety of vertebrates including cats and human. *Gairdia lamblia* is one of the intestinal protozoa that cause public health problems in most developing countries as well as some developed countries. This study was carried out to evaluate Antigiardial activity of *Bauhinia rufsescens* (Leaves) petroleum ether and methanolic extracts *in vitro* test were perform using four concentrations: (1000, 500, 250 and 125 ppm). The highest activity against *G. lameblia* with respect to time was obtained from petroleum ether extract which exhibited 77.12% mortality within 72 h in 1000 ppm concentration, followed by the same extract which exhibited 76.04% mortality within 72 h with concentration of 500 ppm. On the other hand, the lowest antigiardial activity was recorded by *B. rufescens* petroleum ether extract 82.8% mortality with 125 ppm concentration within 24 hours, with whereas Metronidazol, a pure compound (positive control) showed 83.42% mortality within 72 hours. This result shows that the most potent antigiardial activity was demonstrated in *B. rufescens* petroleum ether extract at all concentrations (i.e. 74% mortality within 72 hours) and that *B. rufescens* species, as claimed also by tradition, is a promising species in treating *G. lamblia.*

Keywords: *Bauhinia rufescens*, *Gairdia lameblia*, Metronidazol

1. Introduction

Medicinal plants are still invaluable source of safe, less toxic, lower price, available and reliable natural resources of drugs all over the world. 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 [1, 2]. 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 [3, 4].

Bauhinia rufescens Lam. is a scan dent shrub or small tree belonging to the giant family Leguminosae, subfamily Leguminosae-caesalpiniodeae; usually 1-3 m high, sometimes reaching 8 m; often scraggy, stunted and multi-stemmed. Bark ash-grey, smooth, very fibrous and scaly when old, slash pink, twigs arranged in 1 plane like a fishbone, with thornlike, lignified, lateral shoots, 10 cm long. The leafs are very small, bilobate almost to base, with semi-circular lobes, glaborous, with long petioles, greyish-green, less than 3 cm long. Flowers are greenish-yellow to white and pale pink, petals 5, spathulate, 15-20 mm long; stamens 10, filaments hairy at the base. Fruits aggregated, long,

narrow pods, twisted, up to 10 cm long, glaborous, obliquely constricted, shining dark red-brown, with 4-10 seeds each [5].

The plant is deciduous in the drier area and evergreen in the wetter area, often found in the dry Savannah region, especially near streams or river banks; occuring throughout West Africa and extends across Africa up to Sudan. It has wide array of medicinal and socio-cultural uses. Several *Bauhinia* species are utilized as folk medicines worldwide, including Africa, Asia, South America and Central America, an extract of the root is used as an astringent or antipyretic in local medicine. Leaves and fruit are applied for the treatment of diarrhea, dysentery and ophthalmic diseases. The bark of the roots and trunk is used to cure chest complaints, syphilis and other venereal diseases, leprosy, diarrhea and dysentery and to reduce fever [6].

Giardia lamblia is one of the most common intestinal pathogenic protozoan parasite [7] It is becoming increasingly important among HIV/AIDS patients. There are reports that some cases of acute and chronic diarrhea in AIDS patients may be associated with giardial infection. However, Metronidazole, the common drug of choice, can cause mutagenicity in bacteria [8] and is carcinogenic in rodents [9] It also possesses undesirable side effects and treatment failures have been reported [10].

The treatment of giardiasis consists of the use of one or more drugs, with metronidazole being the first choice. Other nitroimidazolic derivatives (secnidazole, tinidazole, and ornidazole), benzimidazoles (albendazole, mebendazole), furazolin, quinacrine and paromomycin have also been employed in therapeutic regimens. However, these drugs have adverse effects including gastrointestinal disturbances, nausea, headache, leucopoenia, myopia, neuralgia, and allergic dermatitis and an unpleasant taste in the mouth. Furthermore, they can lead to neurotoxic effects, ataxia, convulsions and vertigo, bringing about the interruption of treatment.

In addition, mutagenic and carcinogenic effects have been described in laboratory animals [11, 12, 13, 14, 15, 16] and thus new antigiardial drugs are probably required. With the purpose of searching for new antigiardial agents, in the present work *Bauhinia rufescens* which are used traditionally for treatment of clinical signs associated with giardiases were selected to evaluate the activity of their petroleum ether and methanol extracts against *G. lamblia* trophozoites *in vitro.*

2. Materials and Methods

2.1. Plant Materials

The plant used in this study was collected from central of Khartoum, Sudan, between January and February 2015. The taxonomic identification of this plant was carried out at Medicinal and Aromatic Plants Traditional Medicine Research Institute, National Center for Research by Dr. Haider Abd al Gader. A voucher specimen was deposited at the herbarium of the institute. The leaves were air-dried and coarsely ground to powder.

2.2. Preparation of Crude Extracts

Extraction was carried out for *Bauhinia rufescens* (leaves) by using overnight maceration techniques according to the method described by Harbone [17]. About 50 g were macerated in 250 ml of methanol 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 in tightly sealed glass vial ready for use. The remaining extracts which is not soluble by successively extracted by petroleum ether using the previous technique. Extracts kept in deep freezer for 48 h, then induced in freeze dryer (Virtis, USA) until 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.

2.3. Parasite Isolate

G. lamblia used in this experiment were isolated from patients of Ibrahim Malik Hospital Khartoum, Sudan. Samples were examined by wet amount preparation. Then the positive sample was transported to the laboratory in Roswell Park Memorial Institute (RPMI 1640) medium. Trophozoites of *G. lamblia* were maintained in RPMI 1640 medium containing 5% bovine serum at 37 ±1°C. The trophozoites were maintained for the assays and were employed in the log phase of growth.

2.4. In Vitro Susceptibility Assays

In vitro susceptibility assays used the sub- culture method [18]. It is described as a highly stringent and sensitive method for assessing the anti-protozoal effects (gold standard) of extracts particularly against *Entamoba histolytica*, *Gairdia intestinalis* and *Trichomonas vaginalis* [19]. Five milligram (5mg) from each extracts were dissolved in 50 µl of dimethyl sulfoxide (DMSO) in eppendorf tube containing 950 µl distilled water 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. Three out of 8 columns of microtitre plate wells were chosen for each extract, 40 µl (micro-liters) of an extract solution (5 mg/ml) were added to the first column wells C-1: On the other hand, 20 µl of complete RPMI medium were added to the other wells in the second and third column (C-2 and C-3). Serial dilutions of the extract were obtained by taking 20 µl of extract to the second column wells and taking 20 µl out of the complete solution in C-2 wells to C-3 wells and discarding 20 µl from the total solution of C-3 to the remaining 20 µl serial solutions in the successive columns. Eighty microlitre (80µl) of culture medium was complemented with parasite and added to all wells. The final volume in the wells was 100 µl. In each test metronidazole (a trichomonocide) pure compound [(1-(2-

hydroxyethl)-2-methyl-5 nitroimidazole], a was used as positive control in concentration 312.5 ppm, whereas untreated cells were used as a negative control (culture medium plus trophozoites).

For counting the samples were mixed with Trypan blue in equal volume. The final number of parasites was determined with haemocytometer three times for counting after 24, 48, and 72 h. The mortality% of parasite for each extracts activity was carried out according to the following formula:

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

2.5. Statistical Analysis

All data were presented as means ± S.D. Statistical analysis for all the assays results were done using Microsoft Excel program 2007.

3. Results and Discussion

The yield% of *B. rufescens* (leaves) methanol and petroleum ether extract was 22.1, 2.3 respectively. For etch test control positive – Metrondozole- pure compound in concentration 312.5 ppm was included in all experiments. Meanwhile, we used media inoculated with *G. lambelia* as negative control. In the present work, petroleum ether extract of *B. rufescens* leaves studies have more mortality effect than the methanol extract against *G. lambelia.* The highest effective concentration of *B. rufescens* petroleum ether extract against *G. lambelia* was 1000 ppm with mortality of 77.11% after 72 hours Figure 2. Meanwhile, the methanol extract give 69.29% mortality in concentration 1000 ppm after 72 hours Figure 1. While 312.5 ppm of Metrondazole was gave 83.42% mortality at the same time. Figure 1-2.

Giardia lamblia is an important cause of acute and chronic gastrointestinal disease throughout the world and has been identified as the etiologic agent in numerous waterborne outbreaks of diarrheal disease. Although *G. lamblia* is among the most prevalent enteric protozoal infections in humans, it is relatively recently that improvements in the *in vitro* cultivation of this organism have allowed reliable, reproducible tests to assess the *in vitro* activity of therapeutic agents against *G. lambelia* [20].

The antigiardial potential of the petroleum ether and methanol extracts of *B. rufescens* leaves at different concentrations (1000, 500, 250 and 125 ppm) and Metronidazole (the reference control) with concentration (312.5 µg/ml) was investigated against *giardia lamblia* trophozoites *in vitro*. Methanol extract of *B. rufescens*, showed 69.29% inhibition at a concentration 1000 µg/ml after 72 h; this was compared with Metronidazole which gave 83.42% inhibition at concentration 312.5 µg/ml at the same time against *G. lamblia* (Figure 1). This result proves the statement that in Sudan had been used as antidiarrhoeal agent. The extracts from the root are used as an astringent or antipyretic in local medicine. Leafs and fruit are applied for the treatment of diarrhea, dysentery and ophthalmic or as tonic. The bark of the root is used to cure chest complaints, syphilis and other venereal diseases, leprosy and reduce fever, the fruit against dysentery [21, 22] reported that methanolic extracts of nineteen plant species of Mexican origin, distributed among thirteen families, and described potent giardicidal activity in six species (*Acalypha phleoides*, *Cnidoscolus tehuacanensis*, *Geranium nievum*, *Hellianthella quinquenervis*, *Heliopsis longipes* and *Teloxys graveolens*), with IC_{50} values less than or equal to 20.64 µg/ml.

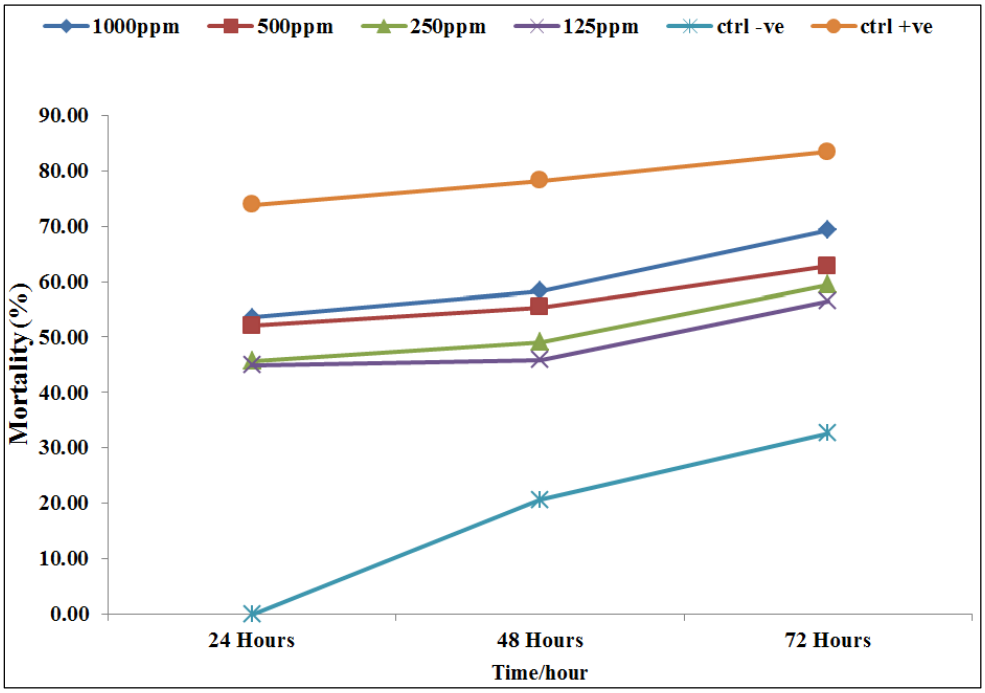

Figure 1. *In vitro activity of B. rufescens (leaves) methanol extract against G. lambelia.*

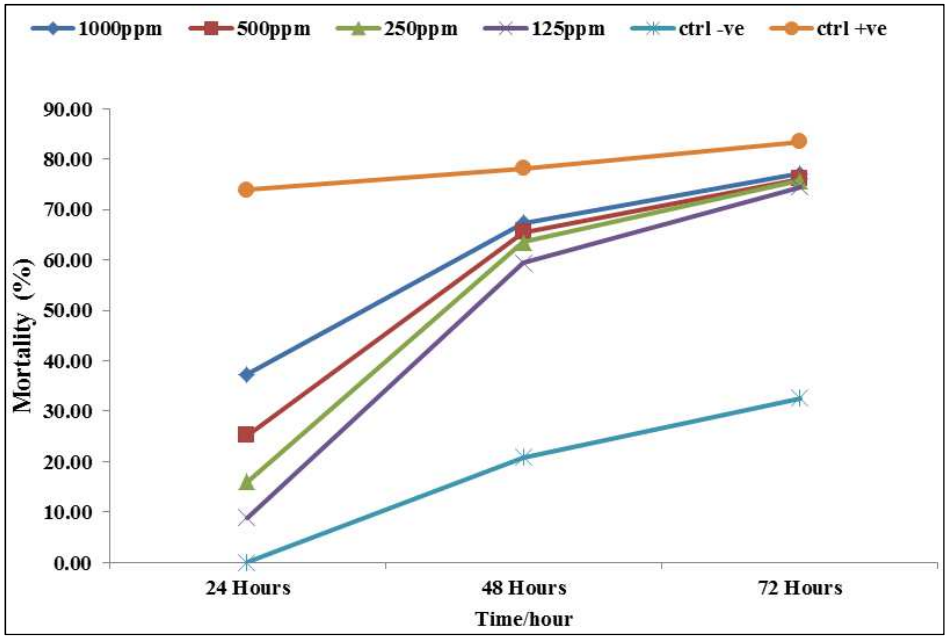

Figure 2. *In vitro activity of B. rufescens (leaves) petroleum ether extract against G. lambelia.*

4. Conclusion

Our results revealed a moderate pharmacological activity against *G. lamblia* we suggested that the extracts have the potential of being used in parasitic infection. The results presented here providing motivation for further exploration of isolation active compounds, particularly as antigiardial agents from *B. rufescens* extracts with important advantages for the development of new anti-parasitic agents.

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