

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/269102118>

In Vitro Anticercarial Activities of some Sudanese Medicinal Plants of the Family Combretaceae

Article · January 2014

CITATIONS

21

READS

511

5 authors, including:



Mahmoud M Dahab

Xishuangbanna Tropical Botanical Garden

41 PUBLICATIONS 200 CITATIONS

[SEE PROFILE](#)



Awatif Elegami

The Research Institute for Tropical Medicine

32 PUBLICATIONS 575 CITATIONS

[SEE PROFILE](#)



Waleed S Koko

Qassim University

111 PUBLICATIONS 1,119 CITATIONS

[SEE PROFILE](#)



Elbadri E. Osman

Elsheikh Abdallah Elbadri University

87 PUBLICATIONS 236 CITATIONS

[SEE PROFILE](#)

In Vitro Anticercarial Activities of some Sudanese Medicinal Plants of the Family *Combretaceae*

Ahmed H. Albagouri, Awatif A. Elegami¹, Waleed S. Koko^{1*},
Elbadri E. Osman², Mahmoud M. Dahab²

¹Medicinal and Aromatic Plants Research Institute, NCR, P. O. Box 2404 Khartoum Sudan

²Faculty of Pure and Applied Sciences, International University of Africa, Khartoum, Sudan

(Received: January 22, 2014; Accepted: February 20, 2014)

Abstract-Eight ethanolic extracts of four Sudanese medicinal plants belonging to the family *Combretaceae* were investigated for their anticercarial activity. Those extracts were of *Combretum aculeatum* (leaves and roots), *C. hartmannianum* (leaves), *C. glutinosum* (leaves, stem and roots) and *Terminalia laxiflora* (leaves and seeds). All extracts showed varying degrees of anticercarial activity against cercariae of *Schistosoma mansoni* within 6 hours period of time. Cercariae exposed to *Terminalia laxiflora* seeds extract showed 100% mortality at concentration 5 ppm after one hour. Lowest calculated LC₉₀ of *C. glutinosum* stem was 3.71 ppm after 3 hours of exposure, LC₉₀ of *C. glutinosum* leaves was 5 ppm after 6 hours, of *C. aculeatum* leaves was 8.78 ppm after 2 hours, of *C. aculeatum* roots was 12.6 ppm after 5 hours, LC₉₀ of *Terminalia laxiflora* leaves was 13.48 ppm after 6 hours, LC₉₀ of *C. glutinosum* roots was 23.64 ppm after 6 hours and LC₉₀ of *C. hartmannianum* leaves was 38.58 after one hour of exposure. Preliminary phytochemical screening conducted on studied plant extracts revealed that they are rich of tannins, unsaturated sterol and/or triterpenes, saponins and flavonoids. MTT assay verified the safety of the examined extracts.

Index Terms: Cerceria, *Schistosoma mansoni*, *Combretaceae*

I. INTRODUCTION

Family *Combretaceae* is widely distributed in the tropical climates of Africa, South America and Asia (but not in Australia) comprises of about 600 species and 20 genera, of which 11 occur in tropical Africa [1]. The largest genus of this family is *Combretum*, comprising of about 250 species and the second largest genus, *Terminalia*, consists of 200 species, both are distributed in the tropics and subtropics. *Combretaceae* in Sudan represents a common constituent of forests on high rainfall savanna on well-drained or alluvial soils along streams, rivers and valleys in south Kassala, Kordofan and south Darfor [2].

About 24 different species of *Combretum* are well known in traditional medicine and used for treatment of a broad range of diseases, such as abdominal pain, back pain, cough, cold, conjunctivitis, diarrhea, dysmenorrhea, earache, fever, headache, fighting worms, infertility in women, fattening babies, leprosy, pneumonia, swelling caused by mumps, scorpion stings and snake bites. As well, many species of *Terminalia* are used for infections, diarrhea, venereal diseases,

and fever [1, 3, 4]. Also *Combretaceae* has its folkloric medicinal uses in Sudan. As some examples, the water extract of the barks and infusion of the leaves of *C. hartmannianum* are used for jaundice, the roots of *Guiera senegalensis* are used for treatment of leprosy, whereas the macerations of the leaves are used as anti-pyretic, antiemetic, anti-blood hypertension and antidiabetic. The decoction of the barks of *Anogeissus leiocarpus* is used against cough, also *Terminalia brownii* barks are used for cough and bronchitis [5, 6]. Also the macerations of the roots of *C. aculeatum* are used to enhance wounds healing and the water extract of its roots is used as a purgative and as a poultice for skin tuberculosis [7]. The cataplasm of bark of *C. glutinosum* is used for swelling while heart wood smoke is used for the rheumatic pains. The barks decoction of *Terminalia laxiflora* is used for malaria [8].

Phytochemical work on *Combretum* and *Terminalia* start since the early 1970s and extends well in the present [3]. Plants of the genus *Terminalia* are known as a rich source of secondary metabolites, such as pentacyclic triterpenes and their glycoside derivatives, flavonoids, tannins and other aromatic compounds, some of which with antibacterial, antifungal, anticancer and hepatoprotective activities [9]. As well, triterpenes, phenanthrene, dihydrophenanthrene, stilbenes and methoxylated flavonoids, were reported from the different *Combretum* species [10].

Several authors have demonstrated that some extracts or purified compounds of the *Combretaceae* species have a broad spectrum of biological activities including antiviral, antibacterial, antiprotozoal, anticancer, analgesic, anti-inflammatory, hepatoprotective and molluscicidal [3, 4, 11].

Schistosomiasis a water-borne parasitic disease infects man and animals in tropical and sub tropical regions, is caused by Schistosoma(s), a genus of digenetic trematode worms. It is the second most prevalent parasitic disease in the world ranked only after malaria regarding the number of people infected and those at risk [12]. More than 90% of the cases are occurring in Africa [13]. According to previous estimates, the disease causes annual loss of 1.7 - 4.5 million disability-adjusted life years (DALYs) [14].

* Corresponding author e mail: wasyko2002@yahoo.com

The major impact of all forms of schistosomiasis worldwide continues to be chronic morbidity [15]. Hepatosplenic involvement is the most important cause of morbidity in *S. mansoni* infection. Hepatic fibrosis and portal hypertension may be life threatening and are irreversible in advanced disease. In endemic communities, hepatomegaly in childhood has been correlated with intensity of infection [15]. *S. mansoni* is found in over 52 countries in Africa, Caribbean, Eastern Mediterranean and Latin America [16, 17].

Recently Sudan has seen a serious increase in endemicity and prevalence of both *S. mansoni* and *S. haematobium* infections since 1990s as a result of expansion in water resource projects, population movements, and unsuccessful control measures. For instance the prevalence in Gezira reaches up to 70% of *Schistosoma mansoni* and reaches 15% of *S. haematobium* [18, 19].

The present work is intended to investigate the cercaricidal activity of 8 ethanolic extracts from 4 plants belong to the family *Combretaceae* against cercaria of *S. mansoni*.

II. MATERIALS AND METHODS

Plants Collection and Extraction

Plants *Combretum aculeatum*, *Combretum glutinosum* and *Terminalia laxiflora* were collected from north Kordofan, while *Combretum hartmannianum* was collected from White Nile area. The plants were compared with authenticated samples in the herbarium of the Medicinal and Aromatic Plants Research Institute.

The preparation of these plants extracts was done according to Harborne [20]. Plants were coarsely powdered using a wooden mortar and pestle. 100 gram of the plant material powder was extracted with ethanol 80% using electric shaker at room temperature. Mixtures were then filtered through a piece of cotton. The filtrate was evaporated to dryness under vacuum at (70°C) using rotatory evaporator. The separated ethanol and residues on the piece of cotton were returned to the mixtures flasks for more extracting. This operation was repeated for twice. The extracts were air-dried, weighed and the percentage yield was obtained for each sample. The extracted powders were then crushed by help of spatula and deposited in small bottles of 50 ml size and then stored at 4°C for further use. Stock solutions of 100,000 ppm concentration, were prepared by dissolving 1g. of the extracted powder in 10ml. of distilled water and also stored at 4°C.

Anticercarial Activity Assay

Naturally infected Snails *Biomphalaria pfeifferi* had been collected from the minor canals of Al Gazira agricultural scheme near Alhasahisa town (about 130 Km south to Khartoum). Snails were transported to the biological assay laboratory in the Medicinal and Aromatic Plants Research Institute where they were put individually in small vials in distilled water and exposed to artificial light and allowed for 2 hours for the shedding of cercariae. Cercariae released during this period were observed by naked eye and under microscope.

24-well microtitre ELISA plates were used for the experiment to test the bioavailability of the plants extracts. Under the microscope, and by help of plastic Pasteur pipette, cercariae were deposited in the wells containing 1 ml. of plants' extracts with concentrations of 500, 250, 125, 100, 50, 20 and 5 ppm. Two wells for each concentration i.e. two replicates, in addition to other 6 wells containing distilled water as control.

The mortality of the cercariae was observed hourly for a period of 6 hours by the morphological and physiological changes of the organism. Dead cercariae were counted and then percentage mortality and percentage average mortality were calculated for each concentration.

Cytotoxicity Screening

Microculture tetrazolium MTT assay was utilized to evaluate the cytotoxicity of the studied plants.

Microculture Tetrazolium (MTT) Assay

Principle

This Colorimetric assay is based on the capacity of Mitochondria succinate dehydrogenase enzymes in living cells to reduce the yellow water soluble substrate 3-(4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into an insoluble, blue colored formazan product which is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells [21].

Preparation of Extracts' Solutions

Using a sensitive balance 5 mg of each extracts were weighed and put in eppendorf tubes. 50 µl of DMSO were added to the extract and the volume was completed to 1 ml with distilled water obtaining a concentration of 5 mg/ml. The mixture was vortexed and stirred by magnetic stirrer to obtain a homogenous solution.

Cell Line and Culturing Medium

Vero (Normal, African green monkey kidney) cells were cultured in a culturing flask containing a complete medium consisting of 10% fetal bovine serum and 90% minimal essential medium (MEM) and then incubated at 37°C. The cells were subcultured twice a week.

Procedure

The monolayer cell culture formed in the culturing flasks was trypsinized and the cells were put in centrifuging tube and centrifuged for 5 minutes separating the cells from the supernatant that flicked out. 1 ml complete medium was added to the cells and all the cell suspension was contained in a basin. In a 96- well microtitre plate, serial dilutions of each extracts were prepared. 3 duplicated concentrations for each extracts i.e. 6 wells for each of 8 extracts. All wells in rows A, B and C were used in addition to first 4 wells from each rows D, E and F. The first 2 wells of row G were used for the negative control and the first 2 wells of row H were used for the positive control Triton X. 20 µl complete medium pipetted in all wells in rows B, C and mentioned wells of rows E and F. Then 20 µl from each extracts were pipetted in rows A and B and first 4 wells of rows E and F. 20 µl taken from row B were pipetted and mixed well

in row C from which 20 µl were taken and flicked out. The same was done from E to F. After that 80 µl complete medium were added to all used wells. Then adjusting the cell account to 3000 cell/well, 100 µl of cell suspension were added completing all wells to the volume 200 µl. Now, we have duplicated three concentrations 500, 250, 125 µg/ml for each extract. Then the plate was covered and incubated at 37°C for 96 hours.

On the fourth day, the supernatant was removed from each well without detaching the cells. MTT suspension stock (5 mg/ml) prepared earlier in 100 ml phosphate buffer solution (PBS) was diluted (1:3.5) in a culture medium. To each well of the 96-well plate, 50 µl of diluted MTT were added. The plate was incubated for further 4 hours at 37°C. MTT was removed carefully without detaching cells, and 100 µl of DMSO were added to each well. The plate was agitated at room temperature for 10 minutes then read at 540 nm using microplate reader.

Phytochemical Screening

Phytochemical screening is of great importance in providing us with information about chemicals found in the plant in term of their nature and range of occurrence. This information would enable us to correlate between the nature and range of occurrence of these chemicals and biological assays held to investigate a certain bioactivity of the mentioned plant. In this study the preliminary phytochemical screening was conducted according to Haborne [20].

Preparation of the Extracts

10 mg of the powdered leaves of each plant were refluxed with 100 ml of ethanol 80% for 4 hours. The cool solution was filtered and enough ethanol 80% was passed through the volume of the filtrate 100 ml. This prepared extract (PE) was used for the various tests.

Test for Unsaturated Sterols and Triterpenes

10 ml of the prepared extract (PE) was evaporated to dryness on a water bath and the cooled residue was stirred several times with petroleum ether to remove most of the coloring materials. The residue was then extracted with 20 ml of chloroform. The chloroform solution was dehydrated over sodium sulphate anhydrous. 5 ml of chloroform solution was mixed with 0.5 ml acetic anhydride followed by two drops of conc. Sulphuric acid. The gradual appearance of green, blue pink to purple color was taken an evidence of the presence of sterol (green to blue) and or triterpenes (pink to purple) in the sample [20].

Test for Alkaloids

7.5 ml of (PE) was evaporated to dryness on a water bath. 5 ml of HCl (2N) was added and stirred while heating on the water bath for 10 minutes, cooled filtered and divided into two test tubes. To one test tube few drops of Mayer's reagent were added. While to the other tube few drops of Valser's reagent were added. A slight turbidity or heavy precipitate in either of the two test tubes was tanked as presumptive evidence for the presence of alkaloids [20].

Test for Flavonoids

95

17.5 ml of the (PE) was evaporated to dryness on a water bath, cooled and the residue was defatted with petroleum ether and the defatted residue was dissolved in 30 ml of ethanol (80%) and filtered.

The filtrate was used for the following tests:

(A) To 3 ml of the filtrate in a test tube 1 ml of 1% aluminum chloride solution in methanol was added. Formation of yellow color indicated the presence of Flavonoids, (Flavones and / or chalcone).

(B) To 3 ml of the filtrate in a test tube 1 ml of 1% potassium hydroxide solution was added. A dark yellow color indicated the presence of the Flavonoids compounds (flavones or flavanones) chalcone and/or flavanol.

(C) To 2 ml of the filtrate 0.5 ml of magnesium turnings were added. Producing of defiant color to pink or red was taken as presumptive evidence that flavanones were present in the plant sample [20].

Test for Tannins

7 ml of the (PE) was evaporated to the dryness on water bath. The residue was extracted several times with n-hexane and filtered. The insoluble residue was stirred with 10 ml of saline solution. The mixture was cooled, filtered and the volume of the filtrate was adjusted to 10 ml with more saline solution. 5 ml of this solution was treated with few drops of gelatin salt reagent. Formation of immediately precipitate was taken as an evidence for the presence of tannin in plant sample. To another portion of this solution, few drops of ferric chloride test reagent were added. The formation of blue, black or green was taken as an evidence for the presence of tannins.

Test for Saponins

1 g of the original dried powdered plant material was placed in a clean test tube. 10 ml of distilled water was added and the tube was stoppered and vigorously shaken for about 30 seconds. The tube was then allowed to stand and observed for the formation of (honeycomb). The appearance of honeycomb, which persisted for least an hour, was taken as an evidence for the presence of Saponins.

Test for Anthraquinone Glycosides

10 g of the powdered plant sample were boiled with 10 ml of 0.5N KOH containing 1 ml of 3% hydrogen peroxide solution. The mixture was extracted by shaking with 10 ml of benzene. 5 ml of the benzene solution was shaken with 3 ml of 10% ammonium hydroxide solution and the two layers were allowed to separate. The presence of Anthraquinones was indicated if the alkaline was found to have assumed pink or red color.

Test for Coumarins

3 g of the original powdered plant sample was boiled with 20 ml of distilled water in a test tube and filter paper was attached to the test tube to be saturated with the vapor after a spot of 0.5N KOH put on it. Then the filter paper was inspected under UV light, the presence of coumarins was indicated if the spot has found to be absorbed the UV light.

Statistical Analysis

Microsoft Excel programme 2007 was utilized to calculate LC₅₀ and LC₉₀ from the obtained results using linear regression equation.

III. RESULTS AND DISCUSSION

Tables 1, 2 and 3 showed the yield percentage of ethanolic water extracts, anticercarial activity and preliminary phytochemical screening of the plants investigated in this study. All plants, extracts showed high *in vitro* potency against cercaria of *schistosoma mansoni*. In table 2 LC₅₀ and LC₉₀ mentioned (low) when LC₁₀₀ was at 5 ppm.

activity against *S. mansoni* cercariae, those were extracts of *T. laxiflora* seeds (100% mortality with concentration 5 ppm in one hour), *C. glutinosum* stem (100% mortality with concentrations 20 and 5 ppm in one hour and four hours respectively), *C. hartmannianum* leaves (100% mortality with concentration 5 ppm in two hours), *C. aculeatum* leaves (100% mortality with concentration 5 ppm in three hours), *C. aculeatum* roots and *C. glutinosum* leaves (Both of 100% mortality with concentration 20 ppm in three hours).

The importance of preliminary phytochemical screening rise on that it could enable us to correlate between the plant chemical constituents and a certain bioactivity test conducted on this plant.

Table 1

Yield Percentage of Ethanolic Water Extracts of Some Sudanese Plants

No.	Scientific Name of Plant	Part Used	Yield Percentage
1	<i>Combretum aculeatum</i>	Leaves	16
2	<i>Combretum aculeatum</i>	Roots	9.2
3	<i>Combretum glutinosum</i>	Leaves	16.6
4	<i>Combretum glutinosum</i>	Stem	9.5
5	<i>Combretum glutinosum</i>	Roots	11.8
6	<i>Combretum hartmannianum</i>	Leaves	31.6
7	<i>Terminalia laxiflora</i>	Leaves	25
8	<i>Terminalia laxiflora</i>	Seeds	10.3

Preliminary phytochemical screening of the plants under study (**Table 3**) showed that the concentration of unsaturated sterol and/or triterpenes in the investigated plants ranged between medium concentration in *C. glutinosum* and high concentration in *C. aculeatum*, *C. hartmannianum* and *T. laxiflora*.

Combretaceae has yielded a number of different pentacyclic triterpenoid structures including oleanoid acid, cycloartane and dammarane. Isolation of oleanene-type pentacyclic terpenoids containing 29-carboxyl-1 α -hydroxyl groups from various species of *Combretum* e.g. *C.molle* and *C. imberbe* confirms chemotaxonomically significant bifurcation in triterpenoids synthesis in *Combretum* species. In related issue of this study mollic acid and imberbic acid were proved to have molluscicidal activity [26].

The aqueous extracts of *C. aculeatum* leaves, *C. glutinosum* leaves and *C. hartmannianum* leaves were investigated before in Sudan against cercaria of *schistosoma mansoni* by Elsheikh [22] reporting that the LC₁₀₀ was 1000 ppm for *C. aculeatum* leaves, *C. glutinosum* leaves and *C. hartmannianum* leaves in 4 hours and 40 minutes period of time, 3 hours and 1 hour and 40 minutes respectively. Those values are very much higher than these we found in present study for the same plants. These differences may due to the use of ethanolic water extracts in our work instead of aqueous extracts used by Elsheikh [22].

Saponins were found at high concentration in both of *C. aculeatum* and *T. laxiflora* while they were at medium concentration in *C. glutinosum* and at low concentration in *C. hartmannianum*.

Medina et al [23] investigated anticercarial activity of diterpene; kaurenoic acid isolated from the Brazilian tree *Croton floribundus* (Euphorbiaceae) against cercariae of *S. mansoni*. The kaurenoic acid showed cercaricidal potency of 100% mortality at concentration of 10 ppm within one hour. This is very comparable with ethanolic water extract of *Terminalia laxiflora* seeds in this study that showed 100% mortality against *Schistosoma mansoni* cercariae at concentration 5 ppm within one hour.

Saponins constitute a vast group of glycosides, which occur in many plants. Triterpene saponins exhibit various pharmaceutical activities [25]. Much known plants of their molluscicidal activity such as *Tetrapleura tetraptera*, *Phytolacca dodecandra* and *Swarzia madagascariensis* are rich of saponins [27].

Investigated plants were found to be of high concentration of tannins. Tannins are polyphenolic compounds of sufficiently high molecular weight which have ability to precipitate proteins. Although they are widespread in plants, their function is still unclear. They may be an effective defense against herbivores [25].

As well the aqueous suspension of *Furcraea selloa marginata*, belonging to family *Agavaceae* was evaluated by Osman et al [24] against cercariae of *S. mansoni*. Cercariae were exposed to concentrations 0, 10, 25, 50 and 90 ppm of the extract for 6 hours. 100% mortality of cercariae fulfilled by the concentration 25 ppm after 6 hours of exposure. For comparison, 6 of extracts in this study showed extreme better

Gallic acid and its derivatives are common occurring constituents in *Combretaceae*. A number of elaborate Tannins have been isolated mainly from *Terminalia* [26]. This is of great harmony with the findings of phytochemical screening conducted within the present study since *T. laxiflora* showed very high concentration of Tannins.

Flavonoids showed very high concentration in *C. glutinosum* and *C. hartmannianum*, high concentration in *C. aculeatum* and traces in *T. laxiflora*.

Flavonoids are conjugated aromatic phenols and they are one of the most diverse and widespread groups of natural constituents. They are of great interest in phytochemistry and

may represent an interesting new therapeutic approach with low toxicity and wide variety of chemical structure [26].

Coumarins were found at low concentration in *C. aculeatum* and *C. glutinosum*, traces of coumarins were found in *C. hartmannianum* while they were absent entirely in *T. laxiflora*. Also the plants were found to be devoid of Anthraquinone glycosides.

The plants were found very poor of alkaloids. Traces of alkaloids were found in both *C. aculeatum* and *C. glutinosum*, while *C. hartmannianum* and *T. laxiflora* were found to be devoid of alkaloids.

Fig.1 demonstrates the results of MTT assay held in this work which showed that the studied plants extracts were of reduced toxicity against *Vero* cell line indicating their safety as cercaricides agents.

Table 2

Anticercarial Activity of Eight Aqueous Ethanolic Extracts of Some Sudanese Medicinal Plants against *Schistosoma mansoni* Cercaria

Scientific Name of Plant	Part Used	Duration of Exposure											
		1 hour		2 hours		3 hours		4 hours		5 hours		6 hours	
		LC ₅₀	LC ₉₀	LC ₅₀	LC ₉₀	LC ₅₀	LC ₉₀	LC ₅₀	LC ₉₀	LC ₅₀	LC ₉₀	LC ₅₀	LC ₉₀
<i>C. aculeatum</i>	Leaves	3.72	58.32	0.0006	8.78	Low	Low	Low	Low	Low	Low	Low	Low
<i>C. aculeatum</i>	Roots	10.72	28.01	4.97	25.55	3.71	14.28	1.98	12.6	1.98	12.6	Low	Low
<i>C. glutinosum</i>	Leaves	27.44	83.64	4.09	24.62	0.20	7.94	0.20	7.94	0.20	7.94	0.02	5
<i>C. glutinosum</i>	Stem	4.49	14.84	1.09	11.17	0.005	3.79	Low	Low	Low	Low	Low	Low
<i>C. glutinosum</i>	Roots	6.99	35.20	5.07	32.07	2.12	27.74	2.12	27.74	2.12	27.74	0.70	23.64
<i>C. hartmannianum</i>	Leaves	8.48	38.58	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low
<i>T. laxiflora</i>	Leaves	11.79	97.64	0.24	37.08	0.01	19.62	0.01	17.23	0.005	13.48	0.005	13.48
<i>T. laxiflora</i>	Seeds	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low

Table 3

Preliminary Phytochemical Screening of Studied Plants

Scientific Name of Plant	Part Used	Unsaturated Sterol And/or Triterpenes	Alkaloids	Flavonoids	Tannins	Saponins	Anthraquinone glycoside	Coumarins
<i>C. aculeatum</i>	Leaves	+++	±	+++	+++	+++	-	+
<i>C. glutinosum</i>	Leaves	++	±	++++	+++	++	-	+
<i>C. hartmannianum</i>	Leaves	+++	-	++++	+++	+	-	±
<i>T. laxiflora</i>	Leaves	+++	-	±	++++	+++	-	-

- = Absent, ± = trace, + = Low concentration, ++ = Medium concentration, +++ = High concentration, ++++ = Very high concentration

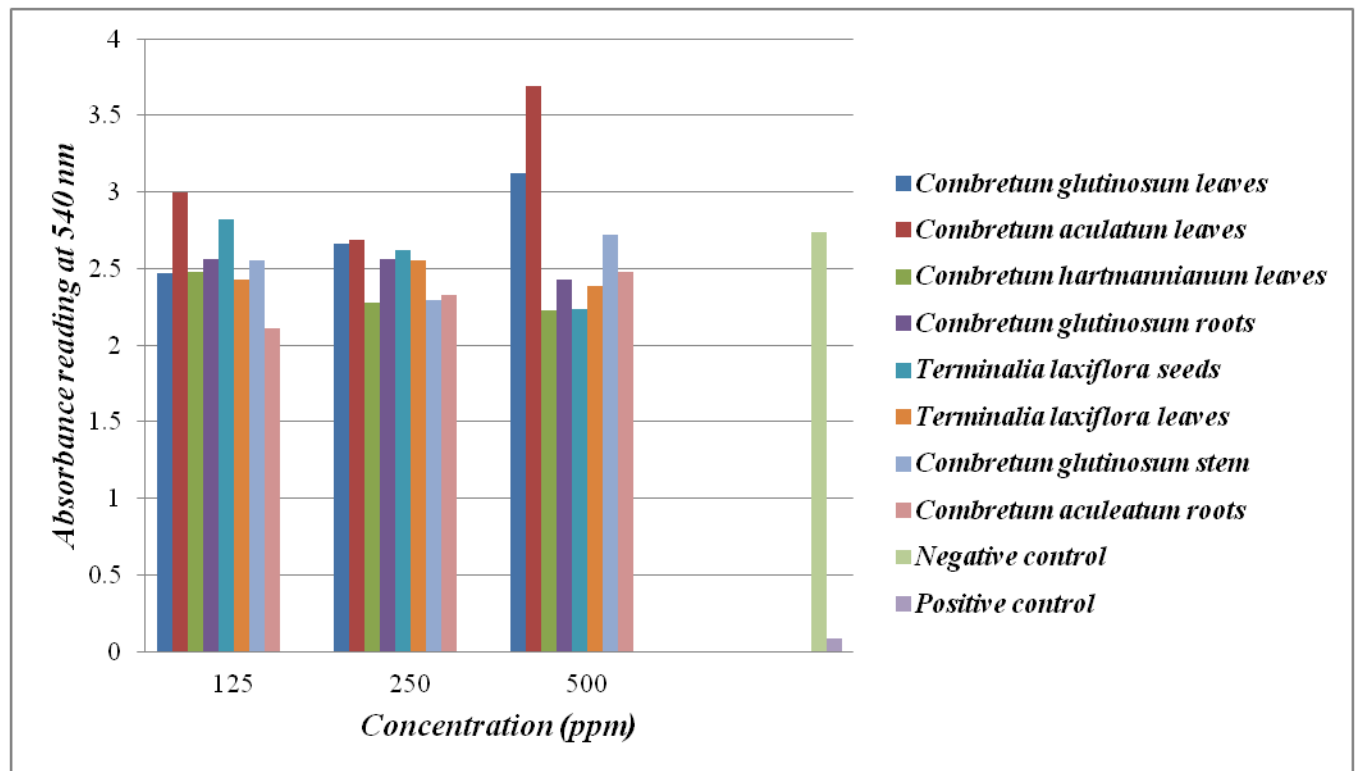


Figure. 1: MTT Assay for Evaluation the Cytotoxicity of Selected Medicinal Plants

IV. CONCLUSION

This study highlights the importance of the Sudanese *Combretaceae* plants as abundant and cheap source of cercaricides recommending at the same time for further chemical investigation for this family in term of isolation and identification of active compounds against cercaria and other stages of the parasite of different forms of *Schistosomiasis*.

REFERENCES

[1]. **Fyhrquist, P. (2007)**. Traditional medicinal uses and biological activities of some plant extracts of African *Combretum* Loefl., *Terminalia* L. and *Pteleopsis* Engl. species (Combretaceae). Academic dissertation.

[2]. **El Amin H. E. (1990)**. Trees and Shrubs of the Sudan. Khartoum University Press.

[3]. **Angeh J.E. (2006)**. Isolation and characterization of antibacterial compound present in members of Combretum section, Hypocrateropsis. A thesis submitted in fulfillment of the requirements for the degree of Doctor of Philosophy (PhD) in the Phytomedicine programme, Department of paraclinical sciences, faculty of veterinary sciences, University of Peritori.

[4]. **Teles, C. B. G., Moreira, L. S., Silva, A. A. E., Facundo, V. Azuliani, J. P., Stabeli, R. G. and Silva-Jardim I. (2011)**. Activity of the Lupane Isolated from *Combretum leprosum* against *Leishmania amazonensis* Promastigotes. *J. Braz. Chem. Soc.*, Vol. 22, No. 5, 936-942.

[5]. **El Ghazali, G. E. B., El Tohami, M. S. and El Egami, A. A. B., Abdalla, W.S. and Mohamed, M. G. (1997)**. Medicinal plants of the Sudan part IV, Medicinal plants of northern Kordofan. National centre for research, Medicinal and aromatic plants research institute, Khartoum, Sudan.

[6]. **El Ghazali, G. E. B., Abdalla, W.S., Khalid, H. E. and Hamad, A. A. (2003)**. Medicinal plants of the Sudan part V, Medicinal plants of Ingassana. National centre for research, Medicinal and aromatic plants research institute, Khartoum, Sudan.

[7]. **El Ghazali, G. E. B., El Tohami, M. S. and El Egami, A. A. B. (1994)**. Medicinal plants of the Sudan part III, Medicinal plants of the White Nile province. National centre for research, Medicinal and aromatic plants research institute, Khartoum, Sudan.

[8]. **Doka, I.G. and Yagi, S. M. (2009)**. Ethnobotanical Survey of Medicinal Plants in West Kordofan (Western Sudan). *Ethnobotanical Leaflets* 13: 1409-1416.

[9]. **Garcez, F. R., Garcez, W. S., Miguel, D. L. S., Serea, A. A. T. and Prado, F. C. (2003)**. Chemical Constituents from *Terminalia glabrescens*. *J. Braz. Chem. Soc.*, Vol. 14, No. 3, 461-465.

[10]. **Mariod, A., Matthäus, B. and Hussein, I. H. (2006)**. Antioxidant activities of extracts from *Combretum hartmannianum* and *Guiera senegalensis* on the oxidative stability of sunflower oil. *Emir. J. Agric. Sci.* 18 (2): 20-28.

[11]. **Ojewole, J. A. O., Nundkumar, N., Adewunmi, C. O. (2004)**. Molluscicidal, cercaricidal, larvicidal and anti plasmodial properties of *Barringtonia racemosa* fruit and seed extracts. *BLACPM* Vol.3 No. 5 September 2005. In LATINDEX from 2004.

[12]. **Okere, P. U. And Odaibo, A.B. (2005)**. Interaction between *Biomphalaria pfeifferi*, the snail intermediate host of *Schistosoma mansoni*, and *indoplanobis exustus*, a possible competitor snail, African Journal of Biotechnology Vol. 4 (7), pp. 676-678. Available online at <http://www.academicjournals.org/AJB>.

[13]. **WHO (2011)**. Weekly epidemiological record. No. 9, 86, 73-80 <http://www.who.int/wer>.

- [14]. **Steinmann, P., Keiser, J., Bos, R., Tanner, M. and Utzinger, J. (2006)**. Schistosomiasis and water resources development: systematic review, meta-analysis, and estimates of people at risk. *Lancet Infect Dis*; 6: 411–25. <http://infection.thelancet.com>.
- [15]. **WHO (2002)**. Prevention and control of schistosomiasis and soil-transmitted helminthiasis. Report of a WHO expert committee.
- [16]. **Boelee, E. and Madsen, H. (2006)**. Irrigation and Schistosomiasis in Africa: Ecological Aspects. International Water Management Institute. 39p. (IWMI Research Report 99).
- [17]. **Hamed, M. A. (2010)**. Strategic control of schistosomes' intermediate host. *Asian journal of epidemiology* 3 (3): 123-140
- [18]. **Kheir, M. M., Eltoum, I. A., Saad, A. M., Ali, M. M., Baraka, O. Z. and Homeida, M. M. A. (1999)**. Mortality due to schistosomiasis mansoni: a field study in Sudan. *Am. J. Trop. Med. Hyg.*, 60(2), pp. 307–310 .
- [19]. **Mudawi, H., Ali, Y., El Tahir, M. (2008)**. Prevalence of gastric varices and portal hypertensive gastropathy in patients with Symmers' periportal fibrosis. *Ann Saudi Med* 28(1): 42-44.
- [20]. **Harborne, J.B. (1984)**. Phytochemical methods. Second edition. Chapman and Hall.
- [21]. **Patel S., Gheewala N., Suthar A., and Shah A. (2009)**. In-Vitro Cytotoxicity Activity of Solanum Nigrum Extract Against *Hela* Cell Line And *Vero* Cell Line. *International Journal of Pharmacy and Pharmaceutical Sciences*, Vol. 1, Suppl 1, Nov.-Dec
- [22]. **Elsheikh, S. H. (1987)**. Toxicity of certain Sudanese plant extracts to the different stages of *Schistosoma mansoni* (Gezira strain-Sudan). Thesis submitted for Master of Science Degree in the department of zoology, Faculty of science, university of Khartoum. <http://www.cfa.uae.ac.ae/research/ejas.htm>.
- [23]. **Medina, J. M., Peixoto, J. L. B., Silva, A. A., Haraguchi, S. K., Falavigna, D. L. M., Zamuner, M. L. M., Sarraggiotto, M. H. and Vidotti G. J. (2009)**. Evaluation of the molluscicidal and *Schistosoma mansoni* cercariae activity of *Croton floribundus* extracts and kaurenoic acid. *Brazilian Journal of Pharmacognosy* 19(1B): 207-211.
- [24]. **Osman, G. Y., Mohamed, A. M., Abdel Kader, A. and Mohamed, A. A. (2011)**. Biological studies on *Biomphalaria alexandrina* snails treated with *Furcraea selloa marginata* plant (family: *Agavaceae*) and *Bacillus thuringiensis kurstaki* (Dipel-2x). *Journal of Applied Pharmaceutical Science* 01 (10); 47-55. www.japsonline.com.
- [25]. **Gurib-Fakim, A. (2006)**. Medicinal plants: Traditions of yesterday and drugs of tomorrow. *Molecular Aspects of Medicine* 27-1-93. www.sciencedirect.com.
- [26]. **Katerere, D. R. P. (2001)**. Phytochemical and Pharmacological Studies of Species of African *Combretaceae*. Thesis submitted for the degree of doctor of philosophy.
- [27]. **Adewunmi C. O. (1999)**. Medicinal Plants, Parasites and Snails in Health. Inaugural lecture. ISSN: 0189-7848 .Obafemi Awolowo University Press Ltd Ile-Ife, Nigeria.