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Phytochemical Screening and Antihelminthic Activity of Carallum Dalzielli Extracts Against Cercarial Stage of Schistosoma Mansoni

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Abstract

Phytochemical screening and antihelminthic activity of aqueous, methanolic and hexane extracts of Carallum dalzielli against cercarial stage of Schistosoma mansoni was assessed in vitro. Plants sample were collected and extracted using aqueous, methanol and hexane according to standard procedure. Schistosoma mansoni cercaria were treated with 60, 125, 150, 300 and 600 µg/ml of the extracts for fourty minutes. Quantitative and qualitative phytochemical screening of the extracts revealed the presence of saponins, flavoinoids, tannins, terpenoids, steroids and cardiac glycosides at various concentrations. All the extracts were found to have cercaricidal activity. Aqueous extracts had the highest efficacy, followed by methanol and hexane at 60µg/ml. This study suggests that the extracts possessed metabolites of pharmacological potential with cercaricidal activity. It is recommended that more researches are recommended to taste the plant's efficacy on other life stages of the parasite.

Keywords: Antihelminthic; Cercaria; Extracts; Phytochemical; Schistosomiasis; Screening.

1. Introduction

Schistosomiasis, also known as bilharziasis is an acute and chronic disease caused by fluke of the genus schistosoma [1].

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Five schistosoma species Schistosoma haematobium, Schistosoma mansoni, Schistosoma japonicum, Schistosoma mekongi and Schistosoma intercalatumare naturally known to infect humans [2]. In terms of morbidity and mortality, schistosomiasis is ranked second to malaria as the most common parasitic diseases and is the most neglected tropical disease [3]. At least 236.6 million were infected in 2019 with 90% of the burden occurring in Africa [1]. Nigeria has 29 million cases of schistosomiasis and the highest in sub Saharan Africa with 101.3 million people living in endemic foci [4].

As part of the life cycle, schistosome infects an intermediary host snail species, then undergoes a progression of extensive asexual multiplication within the snail body in order to form the free swimming cercaria that will infect the human host [5]. For schistosome that infect humans, the presence of fresh water snail species of the genera Bulinus, Biophelaria, Oncomelamia are necessary for the transmission [1].

Plants are the richest reserve of drugs of traditional, modern and folk medicines, food supplements, pharmaceutical intermediates and chemical entities for synthetic drugs [6]. Many species are known to have medicinal value and the use of different parts of several plants to cure specific diseases has been a trend since ancient times [7].

Carallumma dalzielii (family: Asclepiadaceae) is a secculent herb with quadrangular branches occurring in West Africa. It is used in traditional medicine as antispasmodic, antemetics, in paralysis, epilepsy, spasm, and analgesic remedy [8]. This plant is perennial, erect and sparsely-branched to 40 cm high, with green stem, quadrangular branches and scattered dark reddish-purple star like flowers. The plant is called Karan Masallaci in Hausa and Gubehi in Fulani languages of northern Nigeria [9]. This study is aimed at determining the antihelminthic activity of the aqueous, methanolic and hexane extracts of Carallum dalzielli extracts against cercarial stage of Schistosoma mansoni.

2. Materials and Methods

Plant collection and identification

Whole plant of Caralluma was collected from Zaria Local Government of Kaduna state, Nigeria where the plants grows abundantly. The plant samples were collected in plastic bags and transported to the herbarium section of the Department of Botany, Ahmadu Bello University Zaria for identification and authentication.

Drying of plant parts

The whole Caralluma was cleaned, washed, cut into small bits with clean knife and air dried at room temperature. The fresh dried plant was pulverized with pestle and mortar into powder and passed through a 0.5mm mesh to standardize the particles. The powder was then stored in glass bottles and kept at room temperature [10].

Extraction of plant materials

Caralluma dalzielii dried plant tissues was extracted in methanol, hexane and aqueous solvent and concentrated using a rotary evaporator at a controlled temperature [11]. Methanol, hexane and distilled water (100ml) each was used to dissolve 100g for each Caralluma dalzielii dried plants tissues and left for 24 hours. The content was filtered using Watman filter paper (No1) and suctioned using suctioning machine. The filtrates was concentrated using rotary evaporator [10].

Test for qualitative and quantitative phytochemical constituents

Tests was done to determine the presence of the active chemicals constituents such as sugar, saponin, flavoinoid, tannins, terpenoid, steroid, alkaloids and cardiac glycosides.

Test for saponins

Frothing test: to a small portion of the plant sample in a test tube, 10ml of distilled water was added, shake vigorously for 30 seconds and allowed to stand for five minutes. The formation of a persistent froth indicates the presence of saponins [12].

Test for flavonoids

Ferric chloride test: Two (2) drops of ferric chloride solution was added to a small portion of the plant sample solution in distilled water. Greenish coloration indicates the presence of flavonoids [12].

Test for tannins

Lead acetate test: to a small portion of the plant sample solution in a distilled water in a test tube, 4 drops of lead acetate solution was added. Formation of cream colored precipitate indicates the presence of tannins [12].

Test for terpenoids/steroids

Salkowski's test: a small portion of the plant was dissolved in 2ml of chloroform, 3 drops of concentrated sulphuric acid were added at the side of the test tube. Formation of a reddish brown coloration at the interface indicates the presence of terpenoids [12].

Test for Alkaloids

Dragendoff's test: a portion of the plant sample was dissolved in 5ml of 5% aqueous hydrochloric acid with continuous stirring in a water bath for five minutes. The solution was cooled and filtered through a watman no 1 filter paper. To filtrate, few drops of dragendoff's reagent were added. Formation of a rose red precipitate indicates the presence of alkaloids [12].

Test for Cardiac glycosides

Keller-kiliani test: a small portion of the plant sample was dissolved in 1ml of glacial acetic acid containing traces of ferric chloride solution. equal volume of sulphuric acid was added, formation of a brown ring at the interface indicates the presence of cardiac glycosides [12].

Quantitative analysis

Quantitative phytochemical determination of Caralluma dalzielii phytochemicals was carried out according to standard procedure [13,14,15,16,17,18,19].

Harvesting of cercaria from snail intermediate host

Snails of the specie Biomphalaria pfeifferi were collected from Samaru, Zaria, Kaduna State Nigeria. These snails were scooped out of water using a sieve attached to a long pole and then transferred into a plastic containing water using a pair of forceps [20] and transported to the laboratory at Department of Parasitology and Entomology, Faculty of veterinary medicine, Ahmadu Bello University Zaria. The snails were exposed to electric light source from a 100 watts bulb and examined for cercaria shedding. Cercaria were collected and maintained in source water at room temperature until required.

Cercaricidal effect of Caralluma dalzielii extracts on cercariae

Extract concentrations of Caralluma dalzielii (60, 125, 150, 300 and 600µg) was prepared and tested for cercaricidal effect. Pipette was used to introduce 50 cercaria into an aliquot which was exposed to each of the five plant concentration and cercaria mortality was monitored using microscope at different time interval. Absence of motility indicates cercarial death. At the end of each experiment, iodine was added for clarity in counting [21].

Statistical analysis

Data obtained was statistically described in form of tables where appropriate. Differences were analyzed using ANOVA. P value less than 0.05 was considered significant. All statistical calculations were done using IBM SPSS statistics 23 software and manitab 16 statistics application.

Table 1: Qualitative Phytochemical Screening of Hexane, Methanol and Aqueous Extracts of Caralluma dalzielii.

	Extract solvent		
Phytochemicals	CDA	CDH	CDM
Saponin	+	_	+
Flavonoids	_		+
Tannins	+	+	_
Terpenoids	+	+	+
Steroid	+	+	+
Alkaloids	_	_	_
Cardiac glycosides	+	+	+

Key

+ = Posiive, - = Negetive, CDH= Caralluma dalzielii hexane extract, CDM= Caralluma dalzielii methanol extract, CDA= Caralluma dalzielii aquaous extract

Table 2: Quantitative Screening of Caralluma dalzielii Phytochemicals in different Solvents.

	Extract solvent		
Phytochemicals	CDA (mg/g)	CDH (mg/g)	CDM (mg/g)
Saponin	30.58	_	13.35
Flavonoids	_		32.35
Tannins	33.33	10.11	_
Terpenoids	39.84	14.37	18.23
Steroid	33.24	49.29	52.91
Alkaloids	_	_	_
Cardiac glycosides	32.78	12.22	19.87

Keys

- = Absence, CDH= Caralluma dalzielii hexane extract, CDM= Caralluma dalzielii methanol extract, CDA= Caralluma dalzielii aquaous extract

Table 3: Cercaricidal activity of C dalzielli aqueous extract at different concentration **.**

Concentration (µg/mL)										
	60		**125**		**150**		**300**		**600**	
TIME	M	N	M	N	M	N	M	N	M	N
0	20	00	20	00	20	00	20	00	20	00
2	5	15	00	20	00	20	00	20	00	20
4	00	20	00	20	00	20	00	20	00	20
6	00	20	00	20	00	20	00	20	00	20
8	00	20	00	20	00	20	00	20	00	20
10	00	20	00	20	00	20	00	20	00	20
12	00	20	00	20	00	20	00	20	00	20
14	00	20	00	20	00	20	00	20	00	20
16	00	20	00	20	00	20	20	20	00	20

Keys M = Motile, N = Non Motile

Table 4: Cercaricidal activity of C dalzielli Hexane extract at different concentration

Concentration (µg/mL)										
	60		**125**		**150**		**300**		**600**	
TIME	M	N	M	N	M	N	M	N	M	N
0	20	00	20	00	20	00	20	00	20	00
2	20	00	20	00	20	20	20	00	00	20
4	20	00	20	00	20	20	10	10	00	20
6	20	00	20	00	00	20	05	15	00	20
8	20	00	20	00	12	08	00	20	00	20
10	15	05	13	07	07	13	00	20	00	20
12	11	09	09	11	04	16	00	20	00	20
14	06	14	04	16	00	20	00	20	00	20
16	03	17	00	20	00	20	00	20	00	20
18	01	19	00	20	00	20	00	20	00	20
20	00	20	00	20	00	20	00	20	00	20

Keys M = Motile, N = Non Motile

Table 5: Cercaricidal activity of C dalzielli methanol extract at different concentration

Concentration (µg/mL)										
	60		**125**		**150**		**300**		**600**	
TIME	**M**	**N**	**M**	**N**	**M**	**N**	**M**	**N**	**M**	**N**
0	20	00	20	00	20	00	20	00	20	00
2	20	00	20	00	20	00	20	00	20	00
6	20	00	20	00	20	00	20	00	18	02
8	20	00	20	00	20	00	20	00	14	06
10	20	00	20	00	20	00	18	02	11	09
12	20	00	00	20	20	00	16	04	07	13
14	00	20	00	20	19	01	15	05	07	13
16	00	20	00	20	19	01	13	07	05	15
18	00	20	19	01	17	03	12	08	04	16
20	00	20	19	01	15	05	10	10	02	18
22	00	20	17	03	12	08	08	12	00	20
24	00	20	16	04	12	08	06	14	00	20
26	00	20	14	06	09	11	04	16	00	20
28	17	03	10	10	05	15	00	20	00	20
30	14	06	06	14	00	20	00	20	00	20
32	11	09	01	19	00	20	00	20	00	20
34	07	13	00	20	00	20	00	20	00	20
36	04	16	00	20	00	20	00	20	00	20
38	02	18	00	20	00	20	00	20	00	20
40	00	20	00	20	00	20	00	20	00	20

Keys M = Motile, N = Non Mo

3. Results and discussion

Qualitative investigation of phytochemical constituents of Caralluma dalzielii using different solvents revealed the presence of saponins, flavonoids, tannins, terpenoids, steroid, alkaloids and cardiac glycosides at varying levels and intensities (Table 1). The screening using hexane as solvent for extraction revealed the presence of

flavonoids, tannins, terpenoids, steroid and cardiac glycosides, using methanol as solvent for extaction, the results revealed the presence of the phytochemicals with the exception of tannins and alkaloid, while aqueous extract indicate all the above phytochemical metabolites with the exception of flavonoids and alkaloid. This is similar to the works of Muhongo and his colleagues 2021; [22] Krishnaveni and his colleagues 2016 [23]on Phytochemical screening and quantitative analysis of hexane, methanol and water extracts of Pechuel-Loeschea leubnitziae and Salicornia virginica which shows that different solvent extracts have varying degree of phytochemicals.

The Quantitative phytochemical screening of the constituents Aqueous, Methanol and Hexane extracts of Caralluma dalzielli revealed the presence of biomolecules at different concentration (Table 2). In the aqueous extracts, saponin concentration (30.58mg/g) varied from methanolic extract (13.52), flavonoid was only present in methanolic extract (32.35), tannins are higher in aqueous extract (33.33) and (10.11) in hexane.

Terpenoids are higher in aqueous extract (39.84) followed by methanolic (18.23) then (14.37) in hexane. Cardiac glycoside are also higher in aqueous extract (32.78) followed by methanolic extract (19.87) then hexane (12.22). in contrast, steroid record highest (52.91) in ethanol extract followed by hexane extract (49.29) while aqueous extract recorded least (33.24). The differences in the phytochemicals recovered may be due the different solvents used for the extraction.

Results shows that that there is variation in the quantity of identified phytochemical compounds obtained using different extract solvent used. This may be due to different solvent used in the extraction. The bioactive constituents are responsible for the efficacy of the plants.

This is similar the work of similar to the works of Muhongo and his colleagues 2021; Akinseye and his colleagues 2017 [24]. These metabolites have also been confirmed to have antischistosomal activity [25] (Albagouri and his colleagues 2014). Hence, it could be concluded that the plant extracts could serve as a source in the synthesis of drugs useful in the treatment of schistosomiasis.

On the cercaricidal activity of Caralluma dalzielli, aqueous, methanolic and Hexane extracts at 60, 125, 150, 300 and 600 µg/ml concentrations (Table 3,4 and 5). Highest efficacy was observed in aqueous extract with 100% cercarial mortality at 60 µg/ml at 4 minutes and 125 µg/ml in 2 minutes.

Hexane extract recorded 100% cercarial mortality at 60 µg/ml at 20 minutes, 125 µg/ml at 16 minute, 150 µg/ml at 14 minute, 300 µg/ml at 8 minute and 600 µg/ml at 2 minutes. Methanolic extract recorded the lowest cecaricidal mortality of 100% at 60 µg/ml in 40 minutes, 125 µg/ml at 34 minutes, 150 µg/ml at 30 minutes, 300 µg/ml at 28 minutes and 600 µg/ml at 22 minutes. Differences in efficacy of cercaricidal activity using different extract solvent might be due the nature and amount of active compound released with different solvents used in the extraction [26,27].

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