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Phytochemical Analysis and Antihelminthic Effects of Guierasenegalensis on Infected Rabbits

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Abstract

The leaves of the common West African plant *GuireaSenegalensis* were analyzed for the presence of secondary metabolites. The qualitative analysis confirmed the presence of saponins, alkaloids, Flavounoids, tannins and steroids as active constituents of ethanolic extract which are similar to water extract with exception of steroids. Further investigation reveals the antihelminthic effect of *GuireaSenegalensis* by administering 200mg/kg twice of leaves extract over a period of seven days using a rabbit's models; ethanol extracts revealed 50% effective and water extracts 28.57% effective.

Keywords: Phytochemical analysis; *Guirea Senegalensis;* Antihelminthic Rabbits; Helminths

Introduction

Helminths are worm-like parasites with complex reproductive system and life cycles involving intermediate host for the development of larval stages and a definitive host for the adult forms [1]. Research has established that helminthes infections are accompanied by reduced growth rate during childhood and by impaired nutrient utilization [1]. Hookworm infection affects iron status, often to the point where anaemia develops. Many species of helminthes have been reported as causing infections in humans and animal in many part of the world; nematode infection both soil-transmitted helminthiasis and lymphatic filariasis are having public health significance [2,3].

Others include trematodes and cestodes [1]. More than 1.5 billion people, or 24% of the world's population, are infected with soil-transmitted helminth infections worldwide [4,5]. Infections are widely distributed in tropical and subtropical areas, with the greatest numbers occurring in sub-Saharan Africa, the Americas, China and East Asia [6]. Over 270 million preschool-age children and over 600 million school-age children live in areas where these parasites are intensively transmitted, and are in need of treatment and preventive interventions [7,8].

Guiera Senegelensis commonly known as Sabara (in Hausa language) belongs to the Combretaceae family [9]. It is a tropical shrub used in traditional medicine for the treatment of stomach pain, dysenteric diarrhea, syphilis, beriberi, leprosy and impotence [10]. It is also used in veterinary medicine among the Tukolor people in diets designed to increase body weight, reproductive capacity and milk secretion in animals [9]. Powdered dried leaves of *G. senegalensis* associated with *Melantherascandens* are

administered by the nasal route to treat headaches and sinusitis. The leaves are also used as a poultice on tumors and against the guinea worm [11]. Several high quality investigations conducted on *G. senegalensis* have been found to possess pharmacological activities such as antimicrobial, antifungal, antioxidant and anti-inflammatory effects on the central nervous system [11].

Materials and Methods

Collection and Identification of Plant Material

The leaves of *G. Senegalensis* were collected from Alkaleri Local Government Area of Bauchi State, Nigeria. The plant was authenticated at the Postgraduate Biology Laboratory of Abubakar Tafawa Balewa University, Bauchi.

Preparation of Plant Material

Ethanol extraction: 100g of the powdered plant sample was weighed and place in the thimble of the soxhlet extractor. 200ml of ethanol was measured with a measuring cylinder and placed into the flask of the soxhlet extractor. Eight (8) anti-bumping clips were placed in the ethanol in order to prevent overheating and spill over. The soxhlet condenser was then connected and fixed tightly on the soxhlet chamber. It was extracted with ethanol for 6-8hrs at 50-60^{o}C. The crude extract obtained was concentrated in a rotary evaporator to obtain a greenish residue (EE). The weight and the percentage yield were calculated.

Water extraction: The powdered leaves (100g) were extracted with 200ml distilled water using maceration method for 2days with occasional shaking. The extract was filtered using What Man No. 1 filter paper and the filtrate was freed from solvent with the aid of a water bath (30-40^{o}C) to obtain a gummy greenish product subsequently referred to as the water extract (WE). The weight and the percentage yield were calculated.

Phytochemical investigations: The extracts (EE and WE) were subjected to preliminary phytochemical screening for the presence of secondary metabolites such as flavonoids, alkaloids, saponins, tannins etc using standard procedures.

Preparation of animal sample: Three test rabbit were obtained in Bauchi metropolis and kept at school of agric., ATAP laboratory for intensive care. The animals were divided into three groups of one animal each. Group 1 served as the control while groups 2 and 3 served as the test groups. The test rabbits were infected with hookworm via feeding with contaminated feed (larva of hookworm mixed with their food and water).

Mode of administration of extract: The extracts (200mg/kg) were administered to the test animals orally using a syringe while distilled water was administered to the control animal. Group 2 (R1) received ethanol extracts while group 3 (R2) was treated with water extracts.

Determination of Antihelminthic Effect

Stool microscopy: Stool microscopy was carried out weekly during the course of the research work the first was to determine the health status of the animals, then to confirm establishment of *Ascaris* infection and after treatment to assess the effectiveness of the plant extracts as antihelminthic. To carryout stool microscopy, 15 sterile specimen bottles were used in the morning to collect the freshly produced stool by the rabbits. Physiological saline, (0.85g of sodium chloride into 100ml of distilled water) was used for emulsification, and later examined under the ×10 objective of a light microscope. The number of eggs counted was then multiplied by 4 to give the number of eggs per gram of stool.

Counting of Helminth Eggs

Stoll's technique for counting helminth egg was carried out in the study; the counting of the hookworm egg was achieved by weighing 3g of faeces in screw-cap container, 0.1 mol/L solution of sodium hydroxide was added, using a rod, breaking up the faeces and mixing it in NaOH. The container was cupped and shake hardly to complete the mixing, without delay using a wide bore Pasteur pipette about 0.15ml of the suspension was removed and transferred to the slide and was covered with cover slipped.

The entire preparation was examined systematically using x10 objective lens with the condense iris reduced to give good contrast, included in the count any egg's laying outside the edge of the cover glass because these are also contained in the 0.15ml sample. The number of eggs counted was multiplied by 100 to give the number of eggs per gram of faeces.

Results

The percentage yield of extraction for the methanol and aqueous extracts is presented in Tables 1 and 2 respectively.

Phytochemical profile of methanol and water extract of *G. Senegalensis* is shown in Table 3. Diagnosis of the test animals before and after contamination with the hookworm larvae is presented in Tables 4 and 5 respectively.

Antihelminthic effect of methanol and aqueous leaf extracts *G. Senegalensis* on rabbits after seven days of administration is shown in Table 6.

Weight of powered sample	=	100g
Weight of extract and container	=	45.2g
Weight of empty container	=	7.8g
Actual weight of extract = weight of container with extract – weight of container		
= 45.2g – 7.8g	=	37.4g

$$\text{Percentage Yield} = \frac{\text{Final weight of extract} \times 100}{\text{Initial weight of extract}}$$

$$= \frac{37.4 \times 100}{100}$$

$$= 37.4\%$$

Table 1: Percentage yield of ethanol extraction.

Weight of powered sample	=	100g
Weight of extract and container	=	40.8g
Weight of empty container	=	7.8g
Actual weight of extract = weight of container with extract – weight of container		
= 40.8g – 7.8g	=	33.0g

$$\text{Percentage Yield} = \frac{\text{Final weight of extract} \times 100}{\text{Initial weight of extract}}$$

$$= \frac{33.0 \times 100}{100}$$

$$= 33.0\%$$

$$= 33.0\%$$

Table 2: Percentage yield of water extraction.

Constituents	Test	Inferences
		EE WE
Glycosides	Fehling's test	- -
Alkaloids	Mayer's test	+ +
	Dragendorf's test	+ +
Flavonoids	Ferric chloride test	+ +
	NaOH test	+ +
Saponins	Frothing test	+ +
Steroids	Lieberman-Buchard	+ -
	Salkowski test	+ -
Tannins	Lead Sub-acetate	+ +

Key: + = Present - = Absent

Table 3: Phytochemical profile of ethanol and water extract of G. Senegalensis.

Group	Color of specimen	Consistency	No. of eggs	No. of eggs in gram	Grade
RC	Pale	Formed	-	-	-
R1	Pale	Formed	-	-	-
R2	Pale	Formed	-	-	-

Table 4: Screening of rabbit for Helminth at initial stage.
Key R1 = First test animal
R2 = Second test animal
RC = Control

Group	Color of Specimen	Consistency	No. of eggs per preparation	No. of eggs in gram	Grade
RC	Pale	Semi-formed	6	1200	Few
R1	Yellow	Semi-formed	4	800	Few
R2	Pale	Semi-formed	6	1400	Few

Table 5: Result of rabbit's stool after contamination.

Group	Color of Specimen	Consistency	No. of eggs per preparation	No. of eggs in gram	Grade
RC	Yellow	Semi-formed	6	1200	Few
R1	Yellow	Semi-formed	2	400	Scanty
R2	Yellow	Semi-formed	5	1000	Few

Table 6: Result of rabbit's stool after administration of extract.

Discussion

The crude extracts of ethanol and water yields 37.4g% and 33.0% per 100g of the test samples respectively[Table:1&2]. Alkaloids, flavonoids, tannins, saponins and steroids were present in the ethanol extract while the water extract revealed the presence of all the constituents tested in ethanol including alkaloids, flavonoids, tannins and saponins with exception of steroids [Table:3]. These constituents have been reported to be associated with different pharmacological activities of plants ("Phytochemistry, Pharmacology and Ethnomedicinal Uses of Ficus Thonningii [12].

The screening of rabbits for helminth at initial stage [Table: 4] where all the rabbit are found with no infection. However, the investigation of rabbits before and after contaminating their food and water, and with administration of the extracts, results are found with different degree of infections; although they have the same grade of infections [Table: 5,6]. The results obtained shows the average result of investigation of rabbit's stool after administration of extract which shows its effect by reducing the number of eggs of helminth by 50% for ethanol and 28.57% for water.

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