Phytochemical And Antimicrobial Screening of The Stem-Bark and Leaf Extracts of Detarium Microcarpum (Leguminosae)

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ABSTRACT

The stem-bark and leaves of Detarium microcarpum which were selected on the basis of their use in traditional medicine to cure dysentery and diarrhoea were extracted with ethanol. The crude extracts were further partitioned with n-hexane, chloroform, ethyl acetate, methanol and water. The fractions were screened for phytochemicals and anti-microbial assay against Salmonella typhi and Staphylococcus aureus. The Salmonella typhi and Staphylococcus aureus were found resistant to all the fractions of the leaf of D. microcarpum at 15, 30 and 60µg/ml. The phytochemical screening revealed the presence of alkaloids, flavonoids, steroids, tannins, saponins and carbohydrates.

Keywords: Stem-bark and leaves of Detarium microcarpum, phytochemicals and anti-microbial, ethanol.

Introduction

Plants offer a large range of natural compounds belonging to different molecular families which have various properties to humans (Herve *et al*., 2008).Plant is man's friend in survival, giving him food, fuel, shelter and medicine from the days beyond the dawn of civilization (Bose and Choudhary, 1991). Although the use of herbs as remedy for treatment of ailments has declined in the west, it continues to exist throughout the developing world (Rahman and Choudhary, 1991).

Many African plants are used in traditional medicine as antimicrobial agents but only few are documented (Bellomaria and Kacon, 1995). Some compounds obtained from medicinal plants are aspirin **(1)** from *Spirea* plant, quinine **(2)** isolated from the bark of *Cinchoma* tree, morphine **(3)** obtained from *opium* tree, vanillic acid **(4)** derived from *Terminalia catappa,* e.t.c. (Chemistry in Britain, 1999; May Dell, 1990).

However, over 80% of the world's population use plant as their primary source of medicine (Cordell, 2000) and in view of the fact that antibiotics are sometimes associated with adverse side effects to the host including hypersensitivity, immunosuppressive and allergic reactions, it is of interest to develop alternative antimicrobial drugs such as medicinal plants for the treatment of infectious diseases (Clark, 1996).

The plant, ***Detarium microcarpum*** is known for many Vernacular names. As "taura" in Hausa, "Sedun" Ogbogbo in Yaruba, "Ofo" in (Igbo), and "gungorrochi" in Nupe. The tree is widely found in northern parts of Nigeria and also found wide spread from Senegal to Sudan in northern tropical Africa, especially in dried savannah areas.

In dry areas this species occurs as a small tree reaching up to 10m high, with a dense rounded crown while in wet areas it may grow up to 25m high, the greenish bark breaks off into rectangular pieces to reveal reddish inner surface. The twigs are covered with a smooth orange bark; their leaves are 8-12cm long and consist 3-6 pairs of alternate, almost opposite leaflets. The leaflets are 5-10cm long and 3-5cm wide and may have a dull green upper surface and grayish-green lower surface (www.sl.ku.dk).

The fruit has a dise shape flattered up to 4cm diameter and 2.5cm thick. The fruit are covered with a brown-brittle skin and contain a sweet green pump, with triangle fibres and not very fleshy, the seed has a circular flattered shape (www.sl.ku.dk).

The endocarp is dull, dark brown and faintly pitted seeds have no endosperm. It contain 7.4% and 3.2% protein seed germinate >60% at 25^0C in river sand. Germination takes about 2weeks when the stone is directly planted.

Two varieties of the species exist, the tall, forest variety produces bitter fruit while the shorter savannah variety produces a sweet, green fruit that is particularly popular in West Africa. The brown pods of sweet-sour fruit have the shape and size of apricots but a shell and pulp akin to its relative the tamarind (Hermann 1891). Usually eaten fresh by children, the fruit is sometimes sun-dried then sold in market. The fruit is boiled with jackal berry and black plum and concentrated to make fruit leathers in northern Nigeria, while in Sierra Leone; it's made into a drink. Deta is richer in vitamin C than guava and has a very good shelf life. It can be returned to its fresh state if it dries out by soaking it in sugar water, and the liquid by-product makes a fruit drink. Boiling the fragrant seed breaks down the seed coat to expose a kernel rich in essential amino acids and fatty acids which is pounded into flour in Nigeria and used to thicken *egusi* soup. Alternatively, cooking oil is extracted from the kernels by crushing them, with the by-products of this process used as an animal feed. When the seeds are not eaten, they are strong together to make fragrant necklaces.

Heated roots produce a sweet scent that is used as a perfume by women in Sudan, and as a mosquito repellent in Chad republic. The plant is resistant to mosquito, weathering, and pests. The dense, hard wood is workable and thus highly desirable for carpentry and joinery when making houses, boats and fence. The wood is also sought for fire wood and charcoal since it lights quickly, even in the presence of moisture.

The bark, leaves and roots of *Detarium microcarpum* help to treat a variety of ailments throughout west and central Africa. Boiled powdered bark is used as a painkiller, fresh bark or leaves are used to dress wounds to prevent infection. In Mali, the bark is used to treat measles and hypertension while the leaves or roots are used to treat meningitis and cramps in people and diarrhoea in cattle. The fruit pulp is used in Burkina Faso to treat skin infections, whereas in Niger Republic and Togo it is used to treat dizziness. In Senegal, the leaves mixed with those of the trees and milk is used to treat snakebites, while in Benin Republic the leaves are boiled to treat fainting and convulsions. The plant is tolerant to heat and drought and capable of thriving on infertile sites. The tree of *D.microcarpum* serves in many places as a good source for reforestation of degraded lands. (Hermann, 1891).

Due to its usefulness, hardiness, nutritive value, and ability the tree is propagated as a good source for domestication. (N.R.C (2008); Kim, 2011). The fruit of this plant is used in the treatment of Dysentery and diarrhoea in both adults and children's since Ancient time (Abu, 2009). The oil obtained from the plant is used in making cream and ointment, and also in the percenteral formulation of injection (keay and Irvine 1964). It was also reported that the leaves are used to treat ulcer and fresh cuts (Irvine, 1961).

The gum extracted from the seeds of *Detarium microcarpum* is used in tablet coating for sustained released effect (Lajide,1995) Phytochemical analysis of *Detarium microcarpum* revealed the presence of butyric acid in the leaves (Irvine, 1961).

Therefore, the purpose of this work is to screen the extracts obtained from the leaves of *D. microcarpum* on *Salmonella typhi* and *Staphylococcus aureus* isolates and analyses the plant materials for phytochemicals.

Materials And Methods

Apparatus

The apparatus used for this experiment are**:** Adhesive tape, Analytical balance, Autoclave, Beakers, Bijour bottles, Filter papers (No 1 whatmann), Funnel, Forceps, Conical flask, Distillation apparatus, Heating mantle, Insuling syringe, Measuring Cylinder, Oven, Petri dish, Punching machine, Retort stand, Rotary evaporator, Spirit lamp, Test tubes, Thermometer, Weighing balance, Wire loop and Hand gloves.

Reagents

The reagent used were all Analar grade:Bismuth nitrate, Chloroform, Conc. HCI, Conc. H_2SO_4, Copper sulphate, Distilled water, Dimethyl sulphoxide (DMSO), Ethanol, Nutrient agar, Nutrient broth, n-hexane, Potassium hydroxide, Petroleum ether-(45/65^{0}C), Sodium hydroxide, Ferric chloride and Ethyl acetate

Collection And Identification Of Plant Material

The fresh samples of the leaves and stem-bark of *(Detarium microcarpum*) was collected from Rigachikun district (IGABI LGA) of Kaduna State. The plant samples were identified and authenticated by Prof. Bala S Aliyu of Biological Science Department, Bayero University Kano. The plant samples were air dried and ground to powder using mortar and pestle in the laboratory.

Extraction

The powdered leaves and stem-bark (250g each) of *D. microcarpum* were perculated in 750ml distilled ethanol at room temperature for two weeks. The filtrate was evaporated using a rotary evaporator at 40^{0}C. The residues obtained were weighed (14.41g) and kept in a deep freezer until used. The crude ethanol extract was labelled as F001.

Fractionation

The crude ethanol extract was macerated with solvents of different polarity.

Maceration with n-hexane

The crude extract was macerated with n-Hexane for several times and the filtrate was concentrated and labeled as F002. (See Scheme 1, Table 1).

Maceration with Chloroform

The residue was further macerated with chloroform and the fraction recovered was dried and labeled as F003.

Maceration with Ethyl Acetate

The residue was further macerated with ethyl acetate and the fraction recovered was also concentrated and labeled as F004.

Maceration with Methanol

The residue was also macerated with methanol; the fraction obtained was dried and labeled as F005.

Maceration with Water

Finally, the residue was further macerated with distilled water; the fraction obtained was allowed to dry and labeled as F006.

Phytochemical Screening

The various fractions (F001, F002, F003, F004, F005 and F006) of the leaves and stem-bark of *D. microcarpum* were subjected to phytochemical screening to determine the presence of alkaloids, carbohydrates, saponins, steroids, tannins, flavonoids, e.t.c.

Preperation Of the Test Reagents

Dragendorffs reagent: Basic bismuth (0.55 g) was dissolved in a mixture of 40 ml distilled water and 10 ml acetic acid. Potassium iodide (8 g) was also dissolved but separately in 20 ml distilled water. The two solutions were mixed and stirred.

Fehling solutions: Fehling solution A and B were mixed together properly and kept before used.

1% (v/v) hydrogen chloride solution: Concentrated HCI (1ml) was poured in 99 ml of distilled water and 1% (v/v) HCI was obtained.

Meyer's reagent: Mercuric chloride (1.35 g) was dissolved in distilled water (50 ml).

Potassium iodide (5 g) was also dissolved in distilled water (50 ml) and the two solutions were mixed together.

5% (w/v) Ferric chloride solution: Ferric chloride powder (5 g) was dissolved in volumetric flask (100 ml) with distilled water. The mixture was stirred until it became homogeneous.

0.5M potassium hydroxide solution: KOH (2.8 g) was dissolved in volumetric flask (100 ml) and filled up to the mark with distilled water.

1M sodium hydroxide solution: NaOH pellets (4 g) were dissolved in volumetric flask (100 ml) with distilled water. The solution was stirred and made up to the mark with the distilled water.

Phytochemical Tests

Each fraction (0.2 g) was placed in different test tubes and dissolved with distilled water and ethanol. The samples were subjected to test for alkaloids. The same preparation was conducted for the test for carbohydrate, saponins, steroids; tannins and flavonoids (Braintona, 1975) below are the procedures for each of the test conducted on the extract.

Test for Alkaloids

1% HCI (2 drops) was added to portion of each fraction. The extract we treated with Meyer and Dragendoffs reagent separately. A creamy white precipitate indicates a positive test for Meyer while an orange brown precipitate indicates a positive test for Dragendoffs.

Test for Carbohydrate (Sugar)

Fehling's test: The mixture (5ml) of equal volumes of Fehling's solutions A and B was added to each test fraction (2ml). The resultant deep blue mixture was boiled for two minutes. A brick red precipitate indicates a positive test.

Test for Saponins (Frothing)

On addition of distilled water to each of the test fraction in a test tube, the test tubes were shaken vigorously. Formation of a froth which remains for a while indicates the presence of saponins.

Test for Steroids

H_2SO_4 (1ml) was added to each fraction (1ml). A red coloration indicates the presence of steroids.

Test for Tannins

5% $Fecl_3$ (2drops) was added to a portion of each fraction. Formation of dirty green precipitate indicates the presence of tannins.

Test for Flavonoids

A little amount of magnesium powder and few drops of HCI were added to about 3ml of test fraction. A red or intense red coloration indicates the presence of flavonoids.

Antimicrobial Activity

Agar-disc diffusion techniques (Bauer and Kirby, 1972) and micro broth dilution techniques (Cheesbrough, 2000 and Scott, 1988) were employed using standard procedures.

Preparation of Sensitivity Discs

Disc of 6mm diameter were prepared from whatmann's No l filter paper using a paper punch. Batches of 50 discs were dispensed in each of the Bijour bottles. They were then sterilized by autoclaving at 121^0C for 15 minutes. The discs were allowed to cool.

Preparation of Stock Solution

The extracts (0.06g) were dissolved in 1ml dimethyl sulphoxide (DMSO). Half (0.5ml) of the stock solution was introduced into 50 sterile discs respectively in bijour bottles to make 60µg/disc concentration. Half (0.5ml) of DMSO was added into the remaining solution making 1ml of the solution, half (0.5ml) of the solution was taken and placed into another bottle containing 50 filter paper discs and labeled 30µg/disc, half (0.5ml) of DMSO was added into the remaining solution making 1ml, another half (0.5ml) of the solution was taking and placed into another 50 filter paper discs and labeled 15µg/disc. Each disc was capable of adsorbing 0.01ml of the solution; the procedure was employed to prepare 15, 30, and 60µg/disc concentrations. The same process of serial doubling dilution as explained above was employed in the preparation of organic solvent extract for disc and mic preparations (Yusha'u and Sadisu, 2011).

- For 60µg/ml disc potency: 0.5ml of the stock was mixed with 0.5ml DMSO and distributed to 50 discs.
- For 30µg/ml disc potency: 0.5ml of the stock was mixed with 0.5ml DMSO the mixture was now distributed to 50 discs.
- For 15µg/ml disc potency: 0.5ml of the stock was mixed with 0.5ml DMSO and the mixture was also distributed to 50 discs.

Preparation of Culture Medium

Nutrient agar (Biotech lab) was used for the investigation of antibacterial activity of *Detarium microcarpum*. The nutrient agar medium was prepared by dissolving 28g of it in 1000ml of distilled water and sterilized by autoclaving for I5minutes at 115^0C. It was cooled to 27^0C and then poured into the sterilized Petri dishes to solidify.

Preparation of Inoculum

A sterile inoculating loop was used to transfer a portion of the colony of each isolate used into a sterilized nutrient broth (biotech lab); this was incubated at room temperature for eighteen hours (18hrs).

Standardization of Inoculum

Barium sulphate (1%w/v) suspension was used as turbidity standard. This was prepared as follows; one percent (1%w/v) solution of sulphuric acid was prepared by adding 1ml of concentrated H_2SO_4 into 99ml of distilled water. One percent (1%w/v) solution barium chloride was also prepared by dissolving 0.5g of dehydrated barium chloride in 50ml distilled water, then 0.6ml of the barium chloride solution was combined with 99.1ml of sulphuric acid solution to yield (1%w/v) barium sulphate suspension. The turbid solution formed was transferred into the test tubes as the standard for comparism (Cheesbrough, 2005)

Bioassay Procedure

The surface of nutrient agar was inoculated with the standard bacterial isolate, using a sterilized inoculating loop, and the prepared discs were placed (one on each position) on the inoculated media using sterile forceps at different locations. And the concentration of each disc was indicated. All these were carried out in the presence of a flaming spirit lamp to eliminate microbes within area. The plates were incubated at 37^0c for 24 hours before observation and measurement of zones of inhibition (Bauer and Kirby, 1972)

Results And Discussion

Screening of Plant Fractions for Anti-microbial Activity

The leaves of *D. microcarpum* were selected in this study for the basis of their use to cure some diseases which are caused by some pathogenic microbes such as *Salmonella typhi* and *Staphylococcus aureus*, e.t.c.

The finding of this research showed that *Detarium microcarpum* yielded more extract when subjected to ethyl acetate extract with brown crystalline appearance as indicated in table 1 and 2; this showed that ethyl acetate has a stronger extraction capacity than n-hexane, chloroform, methanol and water. The phytochemical analysis carried out on the stem-bark and leaf fractions of *D.microcarpum* revealed the presence of alkaloids, reducing sugars, saponins, tannins, steroids and flavonoids Table 3 and 4 even though the flavonoids were only present in the stem-bark and leaf fractions of ethyl acetate. The antimicrobial activity results of various fractions obtained from the leaves of *D. microcarpum* are respectively given in Table 5. All the tested fractions of the leaves of *D. microcarpum* screened for activity against *Salmonella typhi* and *Staphylococcus aureus* were found to be inactive at concentrations of 15µg/ml, 30µg/ml and 60µg/ml.

Table 1: Weight and properties of the leaf extract of *D. microcarpum*

S/N	Solvent	Appearance	Weight (g)
1	Ethanol (F001)	Dark green substance	2.40
2	n-hexane (F002)	Greenish yellow substance	4.30
3	Chloroform (F003)	Brown solid substance	11.20
4	Ethyl acetate (F004)	Brown crystalline substance	20.00
5	Methanol (F005)	Dark green oily substance	7.08
6	Residue (F006)	Dark brown crystalline substance	5.02

Table 2: weight and properties of the stem-bark extract of *D. microcarpum*

S/N	Solvent	Appearance	Weight (g)
1.	Ethanol (F001)	Green solid substance	3.20
2.	n-hexane (F002)	Brown yellow substance	2.00
3	Chloroform (FOO3)	Brown crystalline substance	5.00
4	Ethyl acetate (F004)	Dark green substance	8.50
5	Methanol (F005)	Brown solid substance	3.30
6	Residue (F006)	Brown crystalline substance	2.21

Table 3: Preliminary phytochemical screening of leaf extract of *D. microcarpum*

Fractions	Alkaloids	Flavonoids	Reducing sugars	Steroids	Saponins	Tannins
Ethanol (F001)	-	-	+	-	-	+
n-hexane (F002)	+	-	-	-	-	-
Chloroform (F003)	-	-	-	+	+	-
Ethyl acetate (F004)		+	-	-	-	-
Methanol (F005)	-	-	+	+	-	+
Residue (F006)	+	-	+	-	`+	-

Table 4: Preliminary Phytochemical screening of stem-bark extract of *D. microcarpum*

Fractions	Alkaloids	Flavonoids	Reducing sugars	Steroids	Saponins	Tannins
Ethanol (F001)	+	-	-	-	-	-
n-hexane (F002)	+	-	+	-	-	-
Chloroform (F003)	+	-	+	+	-	-
Ethyl acetate (F004)	-	+	-	-	-	-
Methanol (F005)	-	-	+	-	-	+
Residue (F006)	+	-	-	+	+	+

Key:
+: Present
-: not present

Tables 5: Antimicrobial activity of leaf extract of *D. microcarpum*

S/N	Fractions	Conc. (µg/disc)	Organism		Inhibition zone (mm)
			Salmonella typhi	***Staphylococcus aureus***	
1.	F001	60 30 15	- - -	- - -	0 0 0
2.	F002	60 30 15	- - -	- - -	0 0 0
3.	F003	60 30 15	- - -	- - -	0 0 0
4.	F004	60 30 15	- - -	- - -	0 0 0
5.	F005	60 30 15	- - -	- - -	0 0 0
6.	F006	60 30 15	- - -	- - -	0 0 0

Key: -

F001 Ethanol fraction
F002 n-hexane fraction
F003 Chloroform fraction
F004 Ethyl acetate fraction
F005 Methanol fraction
F006 Water

Conclusion And Comments

The phytochemical analysis of the stem-bark and leaf extracts of *D. microcarpum* showed the presence of almost all the phytochemicals, except flavonoids which is only present in ethyl acetate fraction. Furthermore, the tested fractions of the leaves of *D. microcarpum* exhibited no zones of inhibition on *Salmonella typhi* and *Staphylococcus aureus* at 60µg/disc, 30µg/disc and 15µg/disc. Therefore, further work is recommended on the Plant using higher concentrations.

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