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Research Article

Phytochemical screening and antimicrobial investigation of the ethyl acetate extract fraction of *detarium microcarpum Guill and Perr*. Stem bark to see its therapeutic value on bacterial microbes that will validate its folk-loric uses as remedy for children Ailment Gedigedi/Tando and also its wound healing property

Kalip Datsu Reuben[1*], Olufunke A. Sodipo[2], Yakubu Tarimbuka Ladi[1], Fanna Inna Abdulrahman[3] and Musa DzivamaYohanna[4]

[1]Department of Basic Sciences, Adamawa State College of Agriculture, P.M.B 2088, Ganye, Nigeria
[2]Department of Clinical Pharmacology and Therapeutics, College Of Medical Sciences, University of Maiduguri, Maiduguri, Nigeria
[3]Department of Chemistry, Faculty of Science, University of Maiduguri, P M B. 1069, Maiduguri, Nigeria
[4]Department of Agricultural Engineering Technology, Adamawa state College of Agriculture, P.M.B 2088, Ganye, Nigeria

Corresponding Author E-mail: datsureuben@yahoo.com

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Abstract

The present study was focused to find out the phytoconstituents present in the ethyl acetate extract fraction as well as the antibacterial activities of the extract fraction of Detarium microcarpum stem bark in order to validate its folk-loric use in the treatment of the children ailment Gedigedi/Tando and its wound healing property. The phytochemical test revealed the presence of carbohydrate, tannins, cardiac glycosides, flavonoids and terpenoids. The antibacterial susceptibility test revealed broad spectrum of activity by the extract. Further studies were carried out which revealed the minimum inhibitory concentration and minimum bactericidal concentration respectively. This study thus manifested antibacterial activity that validates the use of Detarium microcarpum for wound healing and for the treatment of the children ailment Gedigedi/Tando traditionally

Keywords: Phytochemical and Antimicrobial Investigation, Ethyl acetate Extract, *Detarium microcarpum*, Stem Bark

Abbreviations:

D. microcarpum: detarium microcarpum, EAE: ethyl acetate, MTE: methanol extract, AQE aqueous extract, MIC: minimum inhibitory concentration, MBC: minimum bactericidal concentration, mm: millimeters, Ec: *Escherichia coli*, St:

almonella typhi, Kp: *Klebsiella pneumonia*, Pa: *Pseudomonas aeroginosa*, Sa: *Staphylococcus aureus*, Sp: *Streptococcus pyogenes*, Bs: *Bacillus subtillis*, Cs: *Corynebacteria species*, Ca: *Candida albicans,* SEM: standard error of mean

INTRODUCTION

Detarium Microcarpum Guill & Perr, Synonym, *Detarium Senegalense*, English Name, Tallo Tree Belong to the family *Caesalpinoideae* and tribe of *Detarieae,* related to *Copaitera* (Arbonnier, 2004; Kouyate and van Damme, 2008). It is also known as *Kwakuragwahiu* and *Taura* in Kilba and Hausa languages (Reuben and Jada, 2013).

D. microcarpum belongs to the tribe *Detarieae* and of the genus *Detarium (Fabiaceae*, sub family *Caesalpiniaceae*) is a native of Africa (Leung et al., 1968; Akah et al., 2012).

Medicinal properties reported of *D. microcarpum* are in the roots, stems bark, leaves and fruits to treat ailments including tuberculosis, meningitis and diarrhea (Wikipedia, the free encyclopedia; Abreu et al., 1998; Kouyate, 2005; Abdalbasit et al., 2009; Okolo et al., 2012). Another, reported medicinal applications of *D. microcarpum* by Kouyate and van Damme, 2008 are: the bark, leaves and roots widely used throughout its distribution areas because of their diuretic and astringent properties. They are prepared as infusions or decoctions to treat rheumatism, venereal diseases, urogenital infections, stomach ache, intestinal worms and diarrhea including dysentery. The fresh bark or leaves are applied to wounds, to prevent and cure infections. The stem bark decoction or infusion is used in the Kilba community of Hong Local Government area, Adamawa State, Nigeria to treat infant ailment known as *Gedigedi* or *Tando* in Hausa.

The numerous reports of the medicinal uses of this plant has arose this study, which is aimed at Scientific investigation into the phytochemical composition and anti – microbial properties of *D*. *microcarpum* stem bark, with the view of authenticating its ethno – medicinal uses.

Objective of the study

The primary objective of the study was to affirm the folk-loric claims about this plant and its therapeutic values that could prove its novel traditional claimed potential with further objective of investigating its bioactive constituents and antibacterial capabilities to evaluate its efficacy as this may lead to discovering novel antibacterial drugs.

MATERIALS AND METHODS

Collection of plant material (parts)

The plant parts: leaves, fruits and stem bark of D. microcarpum Guill and Perr were collected in July 2012 at long. 8^{o} $31^{/}$ $15^{//}$ N and lat. 12^{o} $7^{/}$ $44^{//}$ E, Nyibango, Ganye, Adamawa State Nigeria was authenticated by Peter Mnama and Patrick Boni of the departments of Basic Sciences and Forestry Technology of Adamawa State College of Agriculture Ganye, Nigeria. A voucher specimen with No. 072012L1F2 were deposited in the department of Forestry Technology of the Adamawa State College of Agriculture Ganye, Nigeria.

Justification of Research

The phytochemical screening will help to know the active biochemical constituents present in the plant part, and the antimicrobial studies will reveal the bacterial species that are susceptible to it there by knowing the therapeutic capabilities of the plant extractives

Preparation and Extraction of plant material

The stem bark of *D. microcarpum* was collected and gabled for removal of adulterants and then pulverized (shredded) using wooden pestle and mortar. It was air dried at room temperature for seven days and one thousand grams (1000g)

of the pulverized part was exhaustively and sequentially extracted using petroleum ether, ethyl acetate, methanol and distilled water respectively with soxhelet extractor(gradient extraction). Geidam et a*l*., 2007; WHO, 2000. Figure 1
Detarium microcarpum Guill & Perr Dried pounded stem bark (1000g).
Stem bark (1000g)
Petroleum ether extraction yielded nothing
Ethylacetate extract: light brown powder, 110.00g
Methanol extract: brown shiny powder, 208.00g
Aqueous extract: dark brown shiny crystals, 62.80g

Detarium microcarpum Guill & Perr. Dried Poundered Stem bark(1000g)

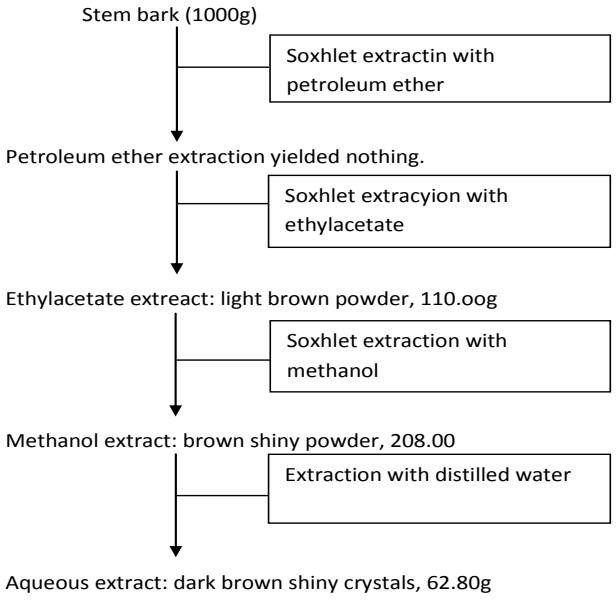

Figure 1. Schematic Diagram of Gradient Extraction and Extract Fractions of *Detarium microcarpum* Stem Bark in Organic and Aqueous Solvents

The extracts were concentrated in vacuum and coded “EAE” for ethyl acetate, “MTE” for methanol and “AQE” for aqueous extracts respectively. All work were carried out in accordance with the general guidelines for methodologies on research and evaluation of traditional medicine (WHO, 2000; Trease and Evans, 2002; Geidam et al., 2007 and Reuben et al., 2012). The petroleum ether did not wash out anything at all. The extracts were then stored aseptically in sterilized sample bottles until use.

Phytochemical screening

The crude ethyl acetate extract was investigated for the presence of bioactive phytochemical constituents, using the standard procedures by Brain and Turner, 1975; Silver et al.,1998 and Trease and Evans, 2002 respectively.

Test organisms

The bacterial and fungal microbes used were*; Escherichia coli*, *Salmonella typhi, Klebsiella pneumonia, Pseudomonas aeroginosa, Staphylococcus aureus, Streptococcus pyogene, Bacillus subtilis, Aspergillus niger, Penicilum reoqueton* and *Trichodama horzranum* respectively. All species were isolates obtained from the human clinical cases at the University of Maiduguri Teaching Hospital (UMTH) Maiduguri, Nigeria.

Susceptibility test

The agar plate-hole assay method as described by (Onoruvwe et al*.,* 1998; Kudi et al*.,* 1999; Ogundipe et al*.,* 2000; Hugo and Russel, 2004; adopted by Reuben et a*l*., 2008ab, 2009 abcd and 2010) was used to determine the growth inhibition of bacteria by the plant extract fraction. The tests were carried out using a stock concentration of 500mg mL^{-1} prepared by dissolving 1g of the extract fraction into 2ml of sterile distilled water. The dilution ratio for grams positive and negative bacteria were 1:1000 and 1:5000 respectively using peptone water while for Candida albicans, sabouraud dextrose roth was used (Usman et al., 2005; Usman et al., 2007 and Reuben et al., 2008a, 2009abc and 2010) which was incubated for 48 hours.

Nutrient agar was prepared and 25ml portion each was poured into sterile Petridis and was allowed to solidify and dry. Using a sterile cock – borer of 9mm diameter, three holes per plate were made in the set agar and were inoculated with 0.5ml suspension of the bacteria (McFaralan standard). Thereafter, the wells were filled with extract solutions at varying concentrations 1000mg, 800mg, 600mg, 400mg, and200mg respectively, then the control. This was done in triplicate and the plates were incubated at 37^{o}C for 24hrs. The antibacterial activities were observed, measured and recorded if the zone of inhibition was ≥ 10mm (Kudi et al., 1999; Vlietinck et al., 1999; Usman et al., 2005; Usman et al., 2007).

Minimum inhibitory concentration (MIC)

MIC is define as the lowest concentration where no visible turbidity is observed in the test tube(Vollekova et al., 2001) with modification by Usman et al., 2007 was employed. In this method, the broth dilution technique was utilized where the plant extract was prepared to the highest concentration of 500mg mL^{-1} in sterile distilled water and serially diluted (two fold) to a working concentration ranging from 6.125mg mL^{-1} to 200mg mL^{-1} using nutrient broth and later incubated with 0.2ml suspension of the test organisms. After 24hrs of incubation at 37^{o}C, the tubes were observed for the presence of turbidity. The lowest concentration where no turbidity was observed was determined and noted as the minimum inhibitory concentration.

Minimum Bactericidal Concentration (MBC)

The MBC is defined as the lowest concentration where no bacterial growth is observed was determined from the broth dilution resulting from the MIC tubes by sub culturing to a microbial free ager as described by Usman et al., 2005; Usman et al., 2007 and Valekova et al., 2001. The lowest concentration of the extract fractions which shows no growth were recorded as the minimum bactericidal concentration.

ANALYSIS OF DATA

The result of the susceptibility test (inhibition zones in “mm”) were subjected to the ANOVA test, using the SAS (9.13) statistical package.

RESULTS AND DISCUSSON

Table 1 The ethyl acetate extract (EAE) was light brown in color, powder in nature and weigh 110.00g, equivalent to 11.00% w/w.

Table 1. The yield of ethyl acetate extract of *D. microcarpum Guill* & *Perr* Stem bark

Parameters	Ethyl acetate extract
Color	Light brown
Form	Powder
Extract weight (mg)	110.00
% yield (w/w)	11.00

NB: Mass of plant sample dried to constant weight used was 1000g; Extraction by Soxhelet apparatus and the solvent used was ethyl acetate.

Table 2 Shows the result of the phytochemical screening for bioactive chemical constituents present in ethyl acetate extract fraction of *D. microcarpum* stem bark.

Table 2. Phytochemical analysis of the ethyl acetate extract fraction of the stem bark plant sample of *Detarium microcarpum* Guill & Perr for bioactive constituents

S/ No.	Bioactive constituents and Test	EAE
1.	Alkaloids	
i.	Dragendroff's test	–
ii.	Mayor' test	–
2.	Carbohydrates test	
i.	General test (molisch's test)	++
ii.	Monosaccharide(barford's test)	–
iii.	Fehling test(free reducing sugar)	++
iv.	Combined reducing sugar	++
v.	Test for ketoses(salivanoff's test)	–
vi.	Test for pentoses	+
3.	Test for soluble starch	+
4.	Test for tannins	
i.	Ferric chloride test($FeCl_3$)	+
ii.	Lead acetate test	++
5.	Test for phlobatannins	–
6.	Test for Anthraquinone glycosides	
i.	Free Anthraquinone	–
ii.	Combined anthraquinone	–
7.	Test for cardiac glycosides	
i.	Salkowski test	+++
ii.	Liebarman-Burchard test	++
8.	Saponins glycoside	
i.	Frothing test	–
9.	Test for flavonoids	
i.	Shinoda's test	+
ii.	Ferric chloride test($FeCl_3$)	+
iii.	Lead acetate test	++
iv.	Sodium hydroxide test	–
10.	Test for Terpenoids	+++

Key

EAE. Ethylacetate extract
+++... High concentration
++... Moderate concentration
+… low concentration
_... Completely absent

This table reveals the presence of terpenes/terpennoids in high concentration as well as cardiac glycosides by salkowski test. Tannins in moderate concentration by the lead acetate test as well as carbohydrates by the molisch's, fehling (free reducing sugar) and combined reducing sugar test respectively. Flavonoids by the lead acetate test also showed moderate concentration. The table also reveals that alkaloids, phlobatanins, anthraquinons and saponins were completely absent. These phytochemicals especially terpenes/terpenoids and flavonoids have been known for their antibacterial and curative properties against several pathogenic bacteria species (Nweze et al., 2004; Hassan et al., 2004; Sartoratto et al., 2004; Nwaogu et al., 2007; Stephen, 2007, Usman et al., 2007).

Table 3 shows the results of the effects of varied concentrations of the ethyl acetate extract (EAE) of *Detarium microcarpum Guill* and *Perr* stem bark on clinical isolates of some bacterial species.

Table 3. The effects of varied concentrations of the ethyl acetate extract (EAE) of *Detarium microcarpum Gill &Perr. stem bark*

Extract	Conc. Ca	Ec	St	Kp	Pa	Sa	Sp	Bs	Cs
EAE	Std. D	21.00_{a}±0.00	23.00_{a}±0.00	22.00_{c}±0.00	20.00_{c}±0.00	24.00_{b}±0.00	21.00_{a}±0.00	22.00_{b}±0.00	23.00_{b}±0.00	19.00_{c}±0.00
	1000	20.33_{a}±0.33	23.33_{a}±0.33	25.33_{a}±0.33	23.33_{a}±0.33	25.33_{a}±0.33	20.33_{a}±0.33	23.00_{a}±0.00	25.33_{a}±0.33	24.33_{a}±0.33
	800	18.00_{b}±0.00	21.66_{b}±0.33	23.33_{b}±0.33	20.33_{b}±0.33	22.66_{c}±0.33	18.33_{b}±0.33	20.33_{c}±0.33	23.33_{b}±0.33	22.33_{b}±0.33
	600	16.33_{c}±0.33	18.33_{c}±0.33	22.33_{c}±0.33	19.33_{c}±0.33	20.33_{d}±0.33	16.00_{c}±0.00	18.33_{d}±0.33	21.33_{c}±0.33	20.00_{c}±0.58
	400	15.00_{d}±0.00	16.00_{d}±0.00	20.00_{d}±0.00	17.33_{d}±0.33	19.33_{e}±0.33	13.00_{d}±0.00	15.33_{e}±0.33	18.00_{d}±0.00	17.33_{d}±0.33
	200	13.33_{e}±0.33	15.33_{d}±0.33	17.33_{e}±0.33	14.33_{e}±0.33	17.33_{f}±0.33	11.33_{e}±0.33	13.33_{f}±0.33	16.33_{e}±0.00	14.33_{e}±0.33
	LSD	0.72	0.83	0.83	0.94	0.94	0.72	0.83	0.83	1.11

All data were average of 3 values plus or minus standard error of mean ($\bar{\times}$ ± SEM).

Ec = *Escherichia coli*, St = *Salmonella typhi*, Kp = *Klebsiella pneumonia*, Pa =*Pseudomonas aeroginosa*, Sa = *Staphylococcus aureus*, Sp = *Streptococcus pyogenes*, Bs = *Bacillus subtillis*,

Cs = *Corynebacteria species*, Ca = *Candida albicans*

The extracts at varying concentrations of 1000, 800, 600, 400 and 200mgs were tested against the various microbes under study. This was replicated three times, and the data obtained was subjected to the ANOVA statistical analysis, using SAS9.13.

The results of the analysis of variance (ANOVA) show that there is a significant difference ($p < 0.05$) in the effects of the varied levels of the concentrations of EAE of stem bark of *D. microcarpum* on the growth inhibition of the bacterial species studied.

From this table, the result shows that all the bacterial species studied were susceptible to the ethyl acetate extract (EAE) of *D. microcarpum Guill* and *Perr* stem bark.

It also reveals that there is a general decrease in the growth inhibition zones of the organisms studied according to the concentrations used, that is 1000mg inhibition zone>800mg>600mg>400mg>200mg respectively.

The table also reveals a broad spectrum of activity of the exact on both gram positive and gram negative microbes including one of the fungal strains studied *Candida albicans*.

It can as well be observed from this table that compared to the standard drug (tetracycline 250mg) used in this research as control, the crude extract showed higher activity on all the microbes studied except for *Escherichia-coli*, and *Streptococcus pyogens* and *Salmonella typhi*. Even of these, that for *Salmonella typhi* is equivalent to the standard drug 23.33a ± 0.33 and 23.00a ±0.00 inhibition zones. This means that this extract has high potential that it can serve as a row material for pharmaceutical companies as a starting material for the synthesis of novel drugs with greater therapeutic value for treatment of ailments caused by these pathogenic bacteria species.

It could also be observed that out of the four fungal microbes studied, only *Candida alibicans* was susceptible while the remaining were resistant.

The broad spectrum antibacterial activity observed of the ethyl acetate extract (EAE) may not be unconnected to the presence of some of the phytochemicals found present in it, typically the terpenes/terpenoids, flavonoide and tanins, as these phytochemicals have been reported to have antibacterial and curative properties (Nweze et al., 2004; Hassan et al., 2004; Sartoratto et al., 2004; Stephen, 2007; Usman et al., 2007).

Table 4 Shows the result of the minimum inhibition concentration (MIC) values for bacterial isolates of the ethyl acetate extract (EAE) of *Detarium microcarpum* stem bark.

Table 4. The minimum inhibitory concentration (MIC) values for bacterial isolates against the ethyl acetate extract of *Detarium microcarpum Guill & Perr* stem bark

Extract	Bacteria	Concentration of extracts (mg)								
		0.780	1.560	3.125	6.25	12.5	25	50	100	200
EAE	Ec	-	-	-	-	-	β	+	+	+
	St	-	-	-	-	β	+	+	+	+
	Kp	-	-	-	-	β	+	+	+	+
	Pa	-	-	-	-	-	β	+	+	+
	Sa	-	-	-	-	β	+	+	+	+
	Sp	-	-	-	-	-	-	β	+	+
	Bs	-	-	-	-	-	β	+	+	+
	Cs	-	-	-	-	β	+	+	+	+
	Ca	-	-	-	-	-	β	+	+	+

Key:
- = Resistance (Growth of bacteria or turbidity)
+ =Extract concentrations that inhibit Inhibition of bacterial growth
β = Least concentration showing no turbidity or MIC (Minimum inhibitory concentration)
Ec = *Escherichia coli*, St = *Salmonella typhi*, Kp = *Klebsiella pneumonia*, Pa =*Pseudomonas aeroginosa*, Sa = *Staphylococcus aureus*, Sp = *Streptococcus pyogenes*, Bs = *Bacillus subtillis*,
Cs = *Corynebacteria species*, Ca = *Candida albicans*

The table reveals that the EAE had MIC of 12.50 mg on four microbes, i.e *Salmonella typhi, Klebsiella pneumoniae, Staphylococcus aureus* and *Corynebacterial* species. While on *Escherichia coli*, *Pseudomonase aeroginosa*, *Barcillus subtillis* and *Candida albican*s, the MIC had been 25.00mg. Out of the four fungal microbes studied, only *Candida albicans* was susceptible, the other three were resistant.

Table 5 shows the result of the minimum bactericidal concentration (MBC) values for bacterial isolates of the ethyl acetate extract (EAE) of *D. microcarpum* stem bark.

Table 5. The minimum bactericidal concentration (MBC) values for bacterial isolates against the ethyl acetate extract of *Detarium microcarpum Guill & Perr* stem bark

Extract	Bacteria	Concentration of extract (mg)								
		0.780	1.560	3.125	6.25	12.5	25	50	100	200
EAE	Ec	-	-	-	-	-	-	β	+	+
	St	-	-	-	-	-	β	+	+	+
	Kp	-	-	-	-	β	+	+	+	+
	Pa	-	-	-	-	-	-	β	+	+
	Sa	-	-	-	-	β	+	+	+	+
	Sp	-	-	-	-	-	-	β	+	+
	Bs	-	-	-	-	-	β	+	+	+
	Cs	-	-	-	-	-	β	+	+	+
	Ca	-	-	-	-	-	-	β	+	+

Key:
- = Resistance (Growth of bacteria or turbidity)
+ = Extract concentrations that kill bacteria
β = Minimum bactericidal concentration (MBC).
Ec = *Escherichia coli*, St = *Salmonella typhi*, Kp = *Klebsiella pneumonia*, Pa =*Pseudomonas aeroginosa*, Sa = *Staphylococcus aureus*, Sp = *Streptococcus pyogenes*, Bs = *Bacillus subtillis*,
Cs = *Corynebacteria species*, Ca = *Candida albicans*

The table reveals that of the nine bacterial microbes susceptible to the EAE, *Klebsiella pneumoniae* and *Staphylococcus aureus* had MBC of 12.50mg, while *Salmonella typhi, Bacillus subtillis* and *Corynebacteria* species had MBC of 25.00mg. *Candida albicans* the only fungal strain susceptible had MBC of 50.00mg together with three other microbes.

CONCLUSION

This study thus provides basis and credence for the wide folk-loric report on this plant for its use as a medication to treat the infant ailment *Gedigedi* or *Tando* as well as its use in wound healing and other various uses. It also justifies the wide folk-loric use and claims about the therapeutic values of this plant as curative agent.

Research Highlights

During this research work, the plant part was first extracted with the Soxhlet apparatus, followed by phytochemical screening and antibacterial susceptibility. Thereafter, the minimum inhibitory concentration and minimum bactericidal concentration studies was done last.

Limitations

Due to lack of facilities we could not separate and purify the various phytochemical constituents present before carrying out the antibacterial studies. If we had done this, we believe we could have recorded better antibacterial result

RECOMMENDATION

Further studies are however encouraged on this novel plant in order to characterize and elucidate the structures of the active phytochemical bioactive agents present in this plant extract with the view of obtaining a more useful chemotherapeutic agent that would have greater efficacy against pathogenic bacteria specie.

Funding and Policy Aspects

The research was not supported by any funding agency. The government or the institution don't support researches, rather, we do it independently.

Author's Contribution and competing interests

Mr. Kalip Datsu Reuben was the principal Investigator, who designed the research, Fanna Inna Abdulrahman supervised the phytochemical screening research and read the manuscript, Olufunke A. Sodipo supervised the antibacterial research and as well read the manuscript, Yakubu Tarimbuka Ladi did the cleaning of the plant part after collection and shredded it, while Musa DzevamaYohanna did the plant sample collection and contributed in the shredding. All Authors declare no conflict of interest. This study was not funded by any funding Agency.

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