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Bioactivity of Active Extracts of The Root And Stem Bark of *(Detarium microcarpum)*

H.M. Adamu[1], O.A.Ushie[2] B. D.Longbap[2] ,R.J Kuburat and U. Lawal [2]
[1]*Chemistry programme, Abubakar Tafawa Balewa University Bauchi, Nigeria*
[2] *Department of Chemical Sciences, Federal University Wukari, Nigeria*

ABSTRACT

Bioactivity of the acetylated extracts of the root and stem bark of Detarium mirocarpum was done. The procedure employed includes extraction of the bark and root of the plant with different solvents in order of polarity; hexane, acetone, methanol and water, solvent- solvent purification method was carried out for further purification of the extract using different solvents in order of increasing polarity; hexane, methyl acectate, dichloromethane, acetone, and methanol. Acetone and methanol extract were acetylated and were test on some microorganisms to determine the activity of the acetylated extracts.

INTRODUCTION

The human race is depended on its surroundings for survival. The primary benefits of using plant-derived medicines in healing are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatments (Kudi and Myint, 1999). Mathew (1996) observed that in Nigeria, 70% to 80% of the populations rely on plants for their primary health care needs. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body and these chemical substances are called phytochemicals. These are non-nutritive chemicals that have protective or disease preventive property (Subhashini *et al*., 2010).

Detarium microcarpum, commonly known as sweet detar, sweet dattock or tallow tree, is an under utilized tree legume that grows naturally in the drier regions of West and Central Africa. It is known as taura in Hausa. The results of evaluation of proximate composition and functional properties of *D. microcarpum* seed flour showed that the seed flours have good nutritional quality and the functional properties confirmed their suitability for use in various food preparations (Akpata and Miachi 2001). *D. microcarpum* is highly appreciated by local peoples due to its variety of uses; it is said to be one of the most appreciated in the environments where it occurs naturally (Paulette Taïta

2003). The fruit can be eaten raw or cooked, but for the most part, its pulp is transformed into flour (Kouyaté A.M. and N. Lamien 2011). The seed flour is a traditional emulsifying, flavouring and thickening agent used to prepare cakes, bread, couscous, baby food and local beer (Kouyaté, A.M. and P. van Damme 2002). Its seed kernels are added to egusi soup, or are cooked and eaten as vegetables. The leaves are used as a condiment or vegetables, as are its flowers (Kouyaté A.M. and N. Lamien 2011).

Medicinal properties of *Detarium microcarpum* are in the roots, stems, bark, leaves and fruits to treat ailments including tuberculosis, meningitis and diarrhea (Abdalbasit et al 2009). The species showed strong inhibitory effects on HIV-1 or HIV-2 infection in methanol extracts (Kouyaté, A.M. and P. van Damme 2002). It was found to have the highest total phenolic, flavonoid and antioxidant values among fourteen wild edible fruits from Burkina Faso (Abdalbasit et al 2009). A study revealed that the antimicrobial principles in the seed coat of D. microcarpum to be steroidal saponins and flavonoids with the possibility of synergistic action. The study also reveals the potentials of phytoalexins in the seeds coat of *D. microcarpum* and by extension, other seeds in the management of infectious diseases (Ebi and Afieroho 2011). The Species Detarium senegalense are used in the treatment of syphilis, dysentery, bronchititis, leprosy, sore throat pneumonia, diarrhoea, malaria and meningitis (Abreu et al., 1998, 1999). Detarium sengalense showed cytotoxicity at 400µg /100 ml and antiviral activity against canine parvovirus, poliovirus, astrovirus and herpes simplex viruses (Kudi and Myint, 1999). Wahedi and David (2013) concluded that the *Detarium microcarpum* fruit pulp has great effects on haematological parameters, and the body weight gained by the rats. This implies that the prolonged consumption of the fruit pulp of *D. microcarpum* by humans to cure certain ailments could affect the haematological parameters and body weight gain at excess medicinal doses.

The research is aimed at purifying the active crude using solvent of different polarity, acetylating the active isolate and subjecting the acetylated extract to bioactivity test.

MATERIALS AND METHODS

Preparation and Extraction

The Stem barks and the roots of *Detarium mirocarpum* were collected from of Anguar Kanawa, Bauchi state in the fresh forms. The sample was dried under shade at room temperature.
The dried bark of *D. microcarpum* was pulverized in a wooden mortar. 50% of the powered bark of the sample was subjected to differential solvent extractions. The cold maceration method was employed in the extraction. This involved the use of your different solvent in the order, hexane, acetone, methanol and water (order of increasing polarity). 50g of the processed plant sample was weighed and poured into a 500ml bottle that was air tight, and about 250ml of hexane was then added. The mixture was covered, stirred and allowed to percolate for 48 hours at room temperature. The extract was obtained by filtration and the solvent was allowed to evaporate at room temperature in a fumed cupboard. The plant residue was allowed to dry at room temperature and the procedure was repeated using the remaining three solvents in order of increasing polarity.

Solvent Extraction Method

Solvent- solvent extraction was further used to purity the extract by washing each extract in hexane, methanol in the giver, order of increasing polarity. This involved the washing of the each extract in five different solvent which includes: hexane, methylacetate, dichloromethane, acetone and

methanol in that order of increasing polarity. The extracts were recovered by concentration of the filtrate under fumed cupboard.

Acetylation

0.5g of anhydrous zinc chloride and 6.5ml of acetic anhydride was poured into a 100ml round - bottomed flask attached to a leibig reflex condenser. The mixture was heated on a boiling water bath for 5 – l0 mins with occasional shaking until the $ZnCl_2$ has largely dissolved 1.5g of each powdered crude extract was added slowly, the mixture was shaking gently during the addition to control the vigorous reaction which follows. The mixture was then heated under reflux for 1 hour on boiling water bath. After 1 hr the content of the flask was pour into 125 ml of ice - water and stirred vigorously to assist the hydrolysis of un-reacted acetic anhydride after about 30 mins. The oil which first separated gradually solidified. This was filtered washed thoroughly with cold water and was recrystallize several times with ethanol until melting point is constant.

Biological Assay

Biological Assay is the study of antimicrobial activity of the acetylated the active isolate of stem barks and the roots against micro-organism. It was used as a guide to determine the active components of the leaves of *Detariun microcarpum.* The organisms used include: *Escheriichia coll, Staphylococcus aureus. Proteus mirabilis* and *Pseudomonas aeroginqsq*

Sterilization of Apparatus

The petri dishes, steels maculating, steel borer, and the nutrient agar used were sterilized in an autoclave at pressure of 15 atmosphere and a temperature of 121°c for 5 minutes.

Preparation of Media

28 g of the agar was weighed, and poured into a conical flask. To it, 1000m! of distilled water was added. This was shaken thoroughly for proper dissolution. It was further dissolved by placing it on a heating mantel and shaking at intervals until the solution was clear. This solution was hen sterilized in an autoclave at a pressure of 15 aims and a temperature of 1210c for 45mins. After autoclaving the solution was allowed to cool at room temperature. To each of the sterilized plates, 5ml of the nutrient agar was dispensed and allowed to gel. The entire procedure was carried out under skeptical conditions.

Agar Plate Inoculation

The agar diffusion method was employed. The organisms to be used were removed from the fridge to be used, were removed from the fridge and incubated in an oven (35-37°c) for at least 24 hours to re-activate them. To the solidified agar 0.1ml of the inoculums was spread evenly on the media using an inoculating loop. This was carried out in a sterile environment. This process was repeated for each plate using [different organisms. The organisms used include: *Escheriichia coll, Staphylococcus aureus. Proteus mirabilis* and *Pseudomonas aeroginqsa.* Wells were bored using 5ml steel borer and a control well was at the centre. Solutions of the acetylated extracts of 100% concentrations were placed in the different wells with water used as a control. The concentration of the extracts is in table I.

RESULTS AND DISCUSSION

Results

The result of the yields, colour, texture and percentage recovery of the crude extracts of stem bark extracts of *Detarium Mirocarpum* is as shown in below table 1

Table 1: Yields, Colour, Texture and Percentage Recovery of the Crude Extracts of Stem bark Extracts of Detarium Mirocarpum

Solvents	Colour	Texture	Crudes yield of extracts (g)	Percentage Recovery (%)
Hexane	Yellow	Oily liquid	0.01g	0.20
Acetone	Brown	Solid	6.8g	13.60
Methanol	Brown	Crystal	4.9 g	9.80
Water	Brown	Powder	5.5g	11.0

The result of the yields, colour, texture and percentage recovery of the crude extracts of root extracts of *D. Mirocarpum* is as shown in below table 2

Table 2: Yields, Colour, Texture and Percentage Recovery of the Crude Extracts of Root Extracts of Detarium Mirocarpum

Solvents	Colour	Texture	Crude yield of extracts (g)	Percentage recovery
HEXANE	Golden yellow	Oily liquid	0.01g	0.20
Acetone	Dark Brown	Solid	7.1g	14.20
Methanol	Dark Brown	Crystal	5.8 g	11.6
WATER	Black	Powder	5.0g	10.0

Solvent- Solvent Extraction

The result of the recovered crude yield of each extracts of stem bark and root is presented in table 3 and 4.

Table 3: Crude Yields of Solvent-Solvent Extraction of Stem bark Extracts

Solvent	Acetone(g)	Methanol (g)	Water (g)
Hexane	0.05	-	-
Methyl acetate	0.05	0.05	0.10
Dichloromethane	0.10	0.10	0.10
Acetone	3.50	2.10	2.60
Methanol	2.10	2.00	2.40
Residue	0.60.	0.50	0.20

Table 4: Crude Yields of Solvent-Solvent Extraction of Root Extracts

Solvent	Acetone (g)	Methanol (g)	Water (g)
Hexane	0.05	-	-
Methyl acetate	0.05	0.05	-
Dichloromethane	0.10	0.10	0.10
Acetone	3.80	2.00	1.50
Methanol	2.40	2.30	2.50
Residue	0.40.	1.00	0.70

Bioassay

The acetylation reactions carried out on acetone and methanol extracts. The products of the acetylation reactions have the characteristic sweet smell of esters which suggests an hydroxyl group has been converted to ester. The acetylated extracts of acetone and methanol showed remarkable activity on wide range of bacteria. The acetylated extracts indicate some degree of activity on *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeroginosa.* But there was no record on the extracts on *Proteus mirabilis.* To assess the relative activity on the extracts ethanol was used as control for the microbial test. The result of zone of inhibition by the acetylated stem bark extracts *D. Mirocarpum* is shown in table 5 while the result of zone of inhibition by the acetylated root extracts *D. Mirocarpum* is shown in table 6.

Table 5: Zone of Inhibition by the Acetylated Stem Bark Extract of *D. Mirocarpum*

Extract	Conc of extract in (mg/mc)	Organism (bacteria)	Zones of inhibition f (mm) by extracts
	100	*Pseudomonas aeroginosa*	3.0
Acetone Extract wash with methanol solvent	100	*Staphylococous aureus*	9.0
	100	*Proteus mirabiis*	-
	100	*Escherichia coli*	8.0
Methanol Extract wash with Methanol Solvent	100	*Pseudomonas aeroginosa*	2.0
	100	*Staphylocous aureus*	30.0
	100	*Proteus mirabiis*	-
	100	*Escherichia Coli*	8.0

Table 6: Zone of Inhibition by the Acetylated Root Extracts of *D. Mirocarpum*

Extract	Conc of Extract in (Mg/Mc)	Organism (Bacteria)	Zones Inhibition
Acetone	100	*Pseudomonas aeroginosa*	2.0
Extract wash	100	*Staphylococous aureus*	7.0
With Methanol	100	*Proteus mirabils*	-
Solvent	100	*Escherichia coli*	7.0
Methanol	100	*Pseudomona aeroginosa*	7.0
Extract	100	*Staphylocous aureus*	10.0

wash with	100	*Proteus mirabils*	-
Methanol	100	*Escherichia coli*	8.0
Solvent	100	*Escherichia coli*	8.0

Discussion

The acetylated methanol extract showed remarkable activity against the test organism when compared to the acetylated acetone and water extract. The activity was found to be greater on *Escherichia coli* and *staphylococcus aureus* bacteria's that cause urinary tract infection and stomach disorder in human being. The activity of the acetylated extract on *Pseudomonas aeroginosa* was not well marked enough like the first mentioned bacteria while there was no record activity on *Proteus mirabilis.*

The result also suggested that acetylated extract of methanol is more active on these organisms than non - acetylated extract on their organisms. This implies that acetylation of these extracts will also retain the activity of the original crude extracts.

CONCLUSION

This claim by the traditional healers that the extract of *Detarium mirocarpum* is used for treatment of dysentary, diarrheas and bums has been confirmed by the activity on *Staphylococcus aureus, Escherichia coil* and *Pseudomonas aeroginosa* acetylated the extracts increased their activity on there organisms,spectral analyses and acetylation reactions carried out suggested the presence of (-OH-) hydroxyl group and carbonyl group (-C=O-) of esters and the characteristic sweet smell of esters confirmed to the presence of hydroxyl in the extracts.

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