Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Scholars Research Library

Annals of Biological Research, 2016, 7 (1):21-26
(http://scholarsresearchlibrary.com/archive.html)

Annals of Biological Research
Scholars Research Library
ISSN 0976-1233
CODEN (USA): ABRNBW

Screening of crude extracts of *Tamarindus indica* and *Detarium microcarpum* for antibacterial activities

[1]Gera Y, [1]Umeh E. U., [2]Tor-Anyiin T. A. and [1]Iheukwumere C. C.

[1]Department of Biological Sciences, University of Agriculture, Makurdi, Nigeria
[2]Department of Chemistry, University of Agriculture, Makurdi, Nigeria

---

ABSTRACT

Two plants Tamarindus indica and Detarium microcarpum used in Tiv land for treatment of diarrheal infection were screened for their antibacterial potentials. The purpose of the screening is to justify and endorse the use of these plants in ethno-medicine or folklore as traditional treasure to cure diarrheal infection. The phytochemical screening shows secondary metabolites of high therapeutic concern, the alkaloids ,flavonoids, phenols , Tannins, etc,. The antibacterial screening of the stem bark of Detarium microcarpum and root bark of Tamarindus indica shows clear zones of inhibition, by agar well diffusion, demonstrated by the Hexane, ethyl acetate, chloroform and methanol extracts of these plants against the tested bacterial species, which constitutes the etiological agents for the Diarrheal infection. The result of the research provides satisfactory scientific backing for the traditional use of the various extracts of these plants.

Key words: Metabolites, Agar well diffusion, Chloroform, Diarrheal.

INTRODUCTION

The use of plants for food and medicine is as old as the existence of man[1]. They are considered as essential components of the universe and have contributed immensely in mans efforts to establish himself on the face of the earth.[2].

Throughout history, man had depended on plants for his health care needs until the advent of chemotherapeutic agents. With the introduction of the high quality antibiotics to cure both infectious and non infectious diseases, herbalism waned. In recent years the popularity of complementary medicine has increased with the resurgence of interest in investigating plants for medically useful compounds. The search for viral cure plants has reawakened interest in ethanobotany [3] and also with increasing number of multidrug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics coupled with the side effects usually associated with synthetic drugs, WHO 2001,Albinu 2007b, there is the high need and calls for alternative health care strategies , new infection fighting agents to control microbial infections. Plants have provided a good source of anti-infective agents, the secondary metabolites are highly efficient instruments in the fight against microbial infections, Pretorius *et al*,2003, Odugbemi 2006, Akinsulire 2007. Global health research searchlights are now focusing on plants as the main source of health care or at least a compulsory alternative. Many nations are integrating the traditional medicine practice into their primary health care, for example, China, Japan, India, and most other Asian nations.

These two plants(*D. microcarpum and T. indica*) are well known in Northern Nigeria, they are used for the treatment of a wide range of gastro intestinal disorders. Among the Tiv people of Benue state Northern Nigeria the plants are used competitively for the cure of infectious diarrheal.

Tamarindus is of the family fabaceae, a legume. It is of tropical African origin, It produces edible fruits which are used in cuisines. Its medicinal uses are numerous, the pulp are continually imported into USA for drug trade. It is used traditionally for abdominal pain , for diarrheal and dysentery. It has various chemical uses and very rich in phytochemicals. The bark is rich in Alkaloids phenolic compounds , cardiac glycosides, etc,[4].

D. micrcapum, (Gurr.andPerr), is a member of the caesalpinaceae family, a savanna tree with edible fruits . It has a redish brown scarly bark. A tropical plant found widespread in Nigeria, with hard wood resistant to termites. The bark extract is used for dysentery and cuts,[5].

It is the interest of the researchers to screen the two plants for antibacterial activities by subjecting their extracts to antibacterial assays using the human diarrheal, bacterial etiological agents, *E. coli, Staph. aereus, Samonella, Shigella, Vibro cholorae.*

MATERIALS AND METHODS

Plant Collection

Plant materials were collected fresh, Tamarinus from Buruku and the *D. microcarpum* from the new lay out area behind owner occupier houses in makurdi, Benue state of Nigeria. The plants were identified at the College of Forestry University of Agriculture, Makurdi, and also using the guide by[6], and[7].

Preparation of Crude Extracts

The stem bark of *Detarium microcarpum* and root bark *Tamarindus indica* were dried under shade ground into fine powders. 600g of each powdered sample were defatted in chloroform for 72hrs at room temperature, followed by microwave assisted extraction sequentially for hexane ethyl acetate, chloroform ,and methanol solvent. The extracts were filtered with whatman filter paper and then concentrated using rotary evaporator set at 50c.

Test Microorganisms

The bacterial strains used for the screening were selected because they were considered to be directly or indirectly involved in incidences of human diarrheal infection these were *Samonella typhi, Shigella dysenteria Staphylococcus aureus , Escherichia coli, Vibro cholorae*, all isolated from clinical samples at the Federal medical center Makurdi, and at the Bishop murray specialist hospital,Makurdi. These were further subjected to biochemical test In the Lab for further confirmation of identity. Each bacterial strain was suspended in nutrient broth and incubated at 37°c. Nutrient agar (NA) was used for testing the antibacterial activity.

Phytochemical Screening

Phytochemical screening of the extracts were carried out according to the methods of [8] and [9]. The performed tests were:

• **Saponins**: About 2g of each plant extract was shaken with water in a test tube and warmed. Froth persists indicating the presence of saponins

• **Alkaloids**: About O.2g of each extract was stirred with 5m1 of 1 percent aqueous hydrochloric acid on steam bath and filtered; 1mL of the filtrate treated similarly with a few drops Mayer's reagent and portion with Drongendroff's reagent. Turbidity or precipitation with either of the reagents was taken as evidence for the presence of alkaloids in the extract being evaluated.

Flavonoids: Flavonoids form yellow colour at pH 11, and red colour in the presence of Mg and HC1.

Cardiac Glycosides: The extract was dissolved in lmL of chloroform and few drops of concentrated hydrochloric acid were added. A red brown colour at the interface indicates the presence of cardiac glycosides.

Tannins: The extract was dissolved in 1mL of distilled water and filtered. Ferric chloride was added drop-wise to the filtrate. A bluish-green precipitates indicates of tannins.

Phenols: The extract was dissolved in 1mL of distilled water and filtered. Equal volumes of the filtrate and ferric chloride solution were mixed together. A blue precipitate indicates the presence of phenolic compounds.

3.3.4 Determination of antibacterial activity

The antibacterial assays procedures of Perez (1990), were employed with slight modification . Briefly the Mueller Hinton agar was used as the growth medium for the bacterial species. It was prepared according to manufacturers instruction, sterilized at 121^0C for 15 minutes poured into sterile petri dishes and allowed to cool and solidify . The sterilize medium was seeded with 0.1 mL of pure cultures of test organisms. 0.4g of each extract was separately dissolved in 2.0mL of sterile distilled water to make up an initial concentration of 200mg/mL.

A sterile cork borer was used to make holes 6mm in diameter and 2mm deep evenly distributed in the inoculated plates . Each hole was filled with 1mL of extract solution . 200mg/ml of ofloxacin solution was used as positive control while water served as negative control. The inhibition zones diameter was observed after 24hrs of incubation.

RESULTS

Tables 1 and 2 shows the results of the phytochemical screening of *Detarium microcarpum* and *Tamarindus indica* respectively. Table 3-4 indicate the antimicrobial activity of the extracts of *Detarium microcarpum and Tamarindus indica* and the minimum inhibitory concentration (MIC) respectively. All the four extracts showed inhibition on the test organisms. The ethyl acetate showed the highest inhibition followed by the chloroform extracts. The Ofloxacin (positive control) also shows reasonable inhibition against the test organisms.

Table 1: Phytochemical screening of the extracts of the stem

n bark of *Detarium Microcarpum.*

Phyto. bases	Extracts			
	n-hexane	ethylacetate	Chloroform	Methanol
Alkaloids	+	+	-	+
Flavoniods	-	+	-	+
Tannins	-	+	-	+
Saponins	+	-	+	+
Cardiac Glyco.	_	+	+	+
Antraquinone	-	-	+	-
Phenols	-	+	+	+

Table 2: Phytochemical screening of the root bark extracts of v *Tamarindus indica*

.

The bases	Extracts			
	n-hexane	Ethylacetate	Chloroform	Methanol
Alkaloids	+	-	+	+
Flavoniods	-	+	+	+
Tannins	—	+	—	+
Saponins	+ -	+	-	
Cardiac Glycosides	-	+	+	+
Antraquinone	-	+	+	-
Phenols	+	-	+	+

Table 3: Antibacterial activity of the stem bark extracts of I *Detarium microcapum..*

Bacterial spp. and zones of inhibition in (mm).

Extracts	*E.coli*	*Staph.*	*Salmonella*	*Shigella*	*V.cholerae*
n-hexane	15	20	18	16	6
Ethylacetate	28	27	22	25	9
Chloroform	24	25	24	24	8
methanol	23	25	22	21	7
control	30	32	30	27	20

Table 4: Antibacterial activity of extracts of roots of *Tamarindus indica* Bacterial spp. and zones of inhibition in (mm)

Extracts	*E.coli*	*Staph.*	*Salmonella*	*Shigella*	*V.cholerae*
n-hexane	16	18	14	16	10
Ethyl-acetate	24	26	22	24	8
Chloroform	18	12	20	22	11
Methanol	18	22	20	23	7
Control	30	33	30	32	18

Table 5: Minimum inhibitory concentration of stem bark extracts of *Detarium microcapum*

		Microorganisms and zones of inhibition in (mm)				
Extracts/ Conc.(mg|mL).		*E.coli*	*Salmonella*	*Staph.*	*Shigella*	*V.cholerae*
n-hexane	200	15	18	20	16	10
	100	_	_	_	_	_
	50	_	_	_	_	_
	25	_	_	_	_	_
Ethylacetate	200	31	28	22	29	8
	100	2	1.5	2	2	_
	50	_	_	_	_	_
	25	_	_	_	_	_
Chloroform	200	27	27	24	24	11
	100	2.4	2	2.5	1.5	_
	50	_	_	_	_	_
	25	_	_	_	_	_
Methanol	200	23	25	22	21	7
	100	1.5	2	1.8	1.3	_
	50	_	_	_	_	_

The minimum inhibitory concentration of the *D. microcarpum* extract is 100mg/mL. on all the microorganisms, except *vibro cholorae*.

Table 6: Minimum inhibitory concentration of root bark extracts of *Tamarindus indica* on the tested organisms

		Microorganisms and zones of inhibition in (mm)				
Extracts/ Conc.(mg/mL).		*E.coli*	*Salmonella*	*Staph.*	*Shigella*	*V.Cholerae*
n-hexane	200	+	+	+	+	+
	100	_	_	_	_	_
	50	_	_	_	_	_
	25	_	_	_	_	_
Ethyl acetate	200	+	+	+	+	+
	100	_	_	_	_	_
	50	_	_	_	_	_
	25	_	_	_	_	_
Chloroform	200	+	+	+	+	+
	100	_	_	_	_	_
	50	_	_	_	_	_
	25	_	_	_	_	_
Methanol	200	+	+	+	+	+
	100	_	_	_	_	_
	50	_	_	_	_	_

The minimum inhibitory concentration of all the extract is 200mg/mL. on all the microorganisms.

DISCUSSION

Table 1 shows the result of the phytochemical analysis of *D. microcarpum and T. indica.* Alkaloids, saponins flavonoids glycosides, Tannins phenols Antraquinones, are present in both the stem bark and root bark of the plants. These class of compounds may contribute to the activity of the extracts on the pathogens. Hoareau, *et.al*,(2005), reported that these metabolites are plants agents for chemical warfare allowing plants to ward off microorganisms, and that higher plants show a potential source for the new antimicrobial agents. Alkaloids are

molecules of high therapeutic significance with considerable antimicrobial and antiprotozoa potentials. The anti bacterial activity of Alkaloids has long been established Edeoga etal,(2005), Calixto (2000) found the extract of alkaloids effective against a variety of microbes. [14], studied alkaloids ,flavonoids and tannins in many plants and found out that these chemicals were responsible for the antibacterial activities of a wide range of plants. This study is in agreement with these earlier works. Tannins have generally being associated with antibacterial activities ,[12]. Tannins and flavonoids are thought to be responsible for the anti diarrheal activity of these plants, flavanoids exhibits antibacterial action as it causes cell lyses in a variety of pathogenic bacterial [13].

The result obtained for the antibacterial screening of the plants are recorded in table 3and4. It shows that the extracts had inhibited all the tested microorganisms, but the susceptibility of the organisms varies across the extracts with the ethyl acetate extract showing the greatest inhibition followed by chloroform extract of the *Detarium* extracts, while in the *Tamarindus* extract methanol extract followed the ethyl acetate. Several authors have reported on the antibacterial activities of various plants; Similarly the antibacterial activity of the two plants have been reported,[17]. The considerable antibacterial activity displayed by the extracts of these plants suggests the presence in them of growth inhibiting phytochemicals of the order of the flavonoids and tannins, which has already been shown to posses antibacterial potentials,[18].The result obtained is encouraging as almost all the extracts have shown antibacterial activity against the tested organisms. The minimum inhibitory concentration on the susceptible microorganisms is as low as 100mg/mL. Generally the screening and the evaluation of plants extracts against microbes may provide new antimicrobial substances; also plant based antimicrobials have enormous therapeutic potentials as they can serve the purpose with less side effects than are often associated with synthetic antimicrobials,[19].

Since the organisms inhibited are typically those that constitute the etiological agents for the Diarrheal infection, traditional medicine practitioners should be encouraged to continue the exploitation of these plant in the management of diarrheal and other associated illness.

CONCLUSION

The result of this study shows the presence of potent therapeutic compounds which have in earlier works been proved to be bioactive, and therefore could be said to be responsible for the efficacy of these two plants extracts. The plants extracts could be seen as a possible source for useful drugs. The continued use of these plants for the traditional treatment of gastrointestinal and other associated ailments is encouraged, while it is suggested that further work be carried out to isolate and characterize the active constituents responsible for the activity of these plants.

REFERENCES

[1] Malik, H.M.A. (**2001**). Treatment through herbs. *In: medicinal plants of Pakistan* p21.
[2] Cowan M.M., *Plants products as antimicrobial agents* ,**1999**, 12; 564-583.
[3]Moreillion,P.,Y.AQue,andM.P.Glauser,(**2005**).*"Staphylococcu s aureus Including Staphylococca Toxic shock"*. In:GL.Mandell, J.E.Bennett. R.Dolin (eds.).Principles and practice of infectious diseases. Publisheds by churchil livingstone Pennsylvania 6thed.2: 2333-2339.
[4] World Health organization (**2001**). "Traditional Medicine" Fact sheet number 134. Revised May 2003. Available on http/www.int/mediacentre fact sheet/fs/134.
[5] Aibinu,Ibukun, Peters Folake, Amisu Kehinde, Adesida Solayide, Ojo Mathew,and Tulo Odegbemi (**2007b**). *Jn. Am. Sc. 3(3):22-33.*
[6] Pretorius,C.J., Magama, and P.C. Ziestman (**2003**). *South African Journal of Botany* 20 : 188-192.
[7] Odugbemi, T.O., O.R. Akinsulire, I.E. Aibinu and P.O.Fabeku (**2007**). *Afr. Jn. of Trad.,Compl. And Alter.Med.* 4(2)191-198 .
[8] Akinsulire, O.R. E.I. Aibuni, T. Adelowotan,and T. Odugbemi (**2007**). *Afr. Jn. of Tr.,Cpl.and Alt. Medicines 4(3):338-344.*
[9] Balunas M.J.,and Douglas K. (**2005**). Discovery from medicinal plants: *Life science* 78pp.431-441.
[10] Agishi. E.C. (**2004**). Etulo, Idoma. Igede. Tiv and Hausa names of plants. Agitab publishers Ltd Makurdi Nigeria
[11] Burkhil H.M. (**1985**). The useful plants of west tropical Africa. Second edition .Royal botanical gardens, Kewl ppl-6.
[12] Sofowora A . (**1993**) Medicinal plants and traditional medicine in Africa. John wiley and sons limited, 150-153.

[13] Matos , F.J.A. (**1988**).Introduced a fitoquimica experimental. Impresa University. University adiceara for talza, 129pp.
[14] Elmarie, V.W., Johan PC (**2001**). *J Ethpharm*.,76: 87-91.
[15] Enzo A.P. (**2007**). Traditional medicine and herbal remedies used in treatment of diarrheal disease. *In Ahmed I, Agil F. Modern phytomedicine turning medicinal* plants. Wiley,VCH Verlag Gmbh and Co.KgaAWeihem,pp248-260.
[16] Perez, L, Paul M,Bezique,P.. (**1990**). *Aca. Ed. Medicine Experiments* 15:113-154.
[17] Haureau L.and EdgarJ.D.(**1999**). *Elec. Jn. of biotec*. 2(2) pp 56-70.
[18] Edeoga H.O.,Gomina A., (**2000**). *J. Econ. TaxonBot*.24: 7-13.
[19] Calixto J.B. (**2000**). *Brazilian Jn. of Medi. And Bio. Research* 33.pp 179-189.
[20] Mojab F., Javidria K, Yazdani D, Rustaiynan A, essential oil of the aerial parts of teucrium stucksianum boiss .subsp.stocksianum (lamiaceae) from Iran,**2003**, 2;49-53
[21] Jones G.A., Mcallister, T.A.,Muir A.D., Cheng, K.I.J.,(**1984**). *Applied environmental microbiology* 60: 1375-1378.
[22] Shilpa, BML, Sonia Ky, Chetan K (**2009**). *J. Phytol*., 1(6):469-474.
[23] Draghon ,F.A. (**2004**). *Food tecchnol*.. 58(2 20-28.
[24] Iwu M., Angera R.D. and Chin O.O. (**1999**). New antimicrobials of plant origin. *A reprint from Journal of J.P.N.C. and new uses* .ASHS press, Alexander.