http://dx.doi.org/10.4314/jpb.v7i2.8
Vol. 7 no. 2, pp. 102-107 (September 2010)
http://ajol.info/index.php/jpb

Journal of
PHARMACY AND BIORESOURCES

Evaluation of larvicidal and antimicrobial potential of *Dacryodes edulis* G. Don; H. J. Lam. (Burseraceae)

Olawale H. Oladimeji[1*], Rene Nia[2,3], E. Nyong[2] and Ndukwe Kalu[4]

[1]Department of Pharmaceutical and Medicinal Chemistry; [2]Department of Pharmacognosy and Natural Medicine, [4]Dept of Pharmaceutics and Pharmaceutical Technology; Faculty of Pharmacy, University of Uyo, Uyo, Akwa Ibom State. Nigeria. [3]Department of Plant and Animal Sciences, University of Buea, Cameroon.

Received 26th May 2010; Accepted 30th July 2010

Abstract
Dacryodes edulis (Burseraceae) is an herbal recipe used in the treatment and or management of earache, fever and headache in ethno-medicine. However, its use in malaria control at the larval stage is yet to be investigated. Hence the larvicidal and anti-microbial studies were undertaken. The larvicidal assay determined in terms of percentage mortality showed that both the crude extracts of the leaves and stem gave remarkably good larvicidal activity (LA%) of 50% and 70% (at 5%w/v) and 70% and 90% ; 80% and 100% (at 10%w/v) at 12 and 24h incubation respectively. However, the larvicidal activity afforded by the crude extract of roots was comparably weaker. The extracts of the leaves and stem inhibited the growth of *B. subtilis* and *S. aureus* while only the leaf extract was active against *S. typhi* and *K .pneumoniae*. Also, the hexane fraction of the leaves demonstrated remarkable activity against *B. subtilis*, *S .aureus*, *S. typhi* and *K .pneumoniae*. The chloroform and butanol fractions were inactive. Neither the extracts nor the fractions showed any activities against the fungal isolates tested. The results obtained in this present study have lent scientific justification to some of the uses of the plant in ethno-medicine.

Keywords: Anti-microbial; larvicidal; extracts; fractions; *Dacryodes edulis*.

Introduction

Dacryodes edulis (G. Don; H. J. Lam) popularly known as African plum and bush butter('sofoutier' in French) belongs to the family, Burseraceae which is made up of 16 genera and about 500 species found growing as shrubs and trees in Africa, tropical America and in the Arabia (Iwu, 1993; Sofowora, 1982; Evans, 1996). *D. edulis* is a native of tropical West Africa. It is used in folkloric medicine for treating earache, fever and headache (Dalziel, 1985). It has huge economic value. It yields timber for furniture making while the fruits and oily seed are used as food, fodder and livestock feed. The plant has also found wide ranging applications in the pharmaceutical and cosmetic industries because of the presence of high quality non-greasy natural oil, lipids (palmitic, oleic and linoleic acids) and proteins in the mesocarp (Akerele and Bonati, 1988).

In spite of the multi-dimensional uses of this plant especially in traditional medicine, the present study was designed with the aim of investigating its potential for larvicidal and antimicrobial activities.

* Corresponding author. *E-mail address*: wale430@yahoo.co.uk, Tel: +234 (0)8023348195
ISSN 0189-8442

Experimental

Collection of materials. The fresh leaves, stem and roots of *D. edulis* were collected in the month of July, 2007 from Urua Ekpa in Itu Local Government Area of Akwa Ibom State. The plant materials were identified and authenticated by R. Nia of the Department of Pharmacognosy and Natural Medicine, University of Uyo, Uyo, Nigeria where voucher specimens NoH54, NoH55 and NoH56 were deposited. *Anopheles gambiae* larvae were bred in plastic buckets and identified.

Chemicals, microorganisms and media. The chemical reagents: butanol, ethanol, chloroform, hexane and methanol (all of AnalaR grade; Aldrich Chemicals Inc; U.S.A.) were purchased in Uyo. Silica gel (254GF), ciprofloxacin, nystatin were obtained from Gemini Pharmaceuticals Limited, Lagos, Nigeria. The micro-organisms (*Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Salmonella typhi, Klebsiella pneumoniae, Candida albicans and Aspergillus niger*) clinically isolated from human specimens; urine, wounds and vaginal swabs were obtained from the Medical Laboratory, University of Uyo Health Center. They were collected in sterile bottles, identified and authenticated by convectional biochemical tests (Gibson and Khoury 1986; Murray *et al*., 1995) and then refrigerated at 0-5^{0}C at the Pharmaceutical Microbiology and Parasitology Unit, Faculty of Pharmacy, University of Uyo, Uyo, Akwa Ibom State prior to use. Also, Mueller Hinton II Agar (Biotec Laboratory Limited, Ipswich England), Sabouraud Dextrose Agar (International Diagnostic Group PLC, Lancaster, England) and Nutrient Broth (Oxoid Limited, Basingstoke, England) were used.

Extraction and processing. The leaves, stem and roots were air-dried and powdered in an electric mill. The resultant ground powders were then extracted with cold 96% aqueous ethanol at room temperature (27± 2^{0}C) for 72h.The filtrates were evaporated to dryness using a rotary evaporator (Buchi CH-920,Laboratorium Technic, Flawk/SG, Switzerland).The dried crude ethanolic extracts were subsequently investigated for plant metabolites(alkaloids, saponins, tannins, cardiac glycosides, terpenes, anthraquinones and flavonoids) according to the laid down phytochemical methods (Sofowora, 1982; Evans, 1996; Harborne, 1984). Also, the extracts were chromatographed on silica gel (254GF) column and gradient elution carried out using hexane: chloroform: butanol (1:1:1) mixture. Eluates which showed similar TLC profiles under UV lamp (λ 366) were pooled and bulked separately to obtain the hexane, chloroform and butanol fractions which were evaporated to dryness and stored in a refrigerator at –4^{0}C prior to the biological tests.

Larvicidal assay

Breeding larvae of Anopheles gambiae. The larvae were bred by keeping outdoor basins of water under growing shrubs near houses for about two weeks. After this period, at least three groups of mosquito larvae were identified accurately in a container using classical methods (Sievers *et al*., 1949). *Anopheles gambiae*, *Aedes aegypti* and *Culex piper-fatigans* responsible for the transmission of malaria, yellow fever and filariasis respectively were so identified. The fourth instar larvae of *Anopheles gambiae* were later selected, separated and the species authenticated at the Department of Entomology, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria before further work.

The method employed for the determination of larvicidal activity was adopted from that described by several authors (Ojewole *et al*., 2000) and WHO directives on such assay with modifications (W.H.O., 1970). Thirty (30) *Anopheles gambiae* larvae in their fourth stage were put

in recovery cups (250ml plastic jars) containing 10ml de-ionized water (pH 7.0) at room temperature (27 ± 2^0C).Three(3ml) volume each of the graded concentrations of the extracts(5 and 10 %w/v) were added to 90ml de-ionized water, mixed thoroughly and then poured into exposure cups(250ml plastic jars containing larvae food). Each aqueous solution of the extract was set up in triplicates. Negative control (containing 90ml de-ionized water and larvae food) and as well as positive control(containing 3ml absolute alcohol,90ml de-ionized water and larvae) were also set up in triplicates. Both the test controls were set up and maintained at room temperature(27 ± 2^0C).The *Anopheles* larvae in each recovery cup were scooped and transferred by means of small nets into test exposure cups containing the sample solutions and or control, larvae food and de-ionized water. The larvae in the test and controls set-up were incubated for a period of 12 and 24h at room temperature(27 ± 2^0C). Therefore, the larvae were gently scooped into small nets, washed with de-ionized water, transferred into recovery cups containing 100ml of de-ionized water, maintained at pH 7.0 and allowed to settle. Prior to mortality determinations, the larvae in recovery cups were gently disturbed and made to go below the water surface by agitating the water with a sterile pipette. The dead and dying larvae which started to float on the surface, were pushed down the recovery cups. The living larvae which were able to swim to the surface were allowed to do so within 5minutes following agitation. The larvae remaining and or staying at the bottom of the recovery cups unable to swim to the surface were regarded as dead.

Antimicrobial sensitivity test

Determination of zone of inhibition. The media were prepared according to Manufacturers' instructions, poured into sterile Petri-dishes (diameter, 13.5cm) and then allowed to set. The bore-hole diffusion method was used for the anti-microbial screening test. The bacteria were cultured in nutrient agar while the fungus was cultured in the sabouraud dextrose agar. The inoculum of each organism was introduced into each Petri-dish. Cylindrical plugs were removed from the agar plates by means of a cork borer to produce wells of approximately 6.0mm.The wells were equidistant from each other and the edge of the plate (Washington, 1995; National Committee for Clinical Laboratory Standards, 2003). Concentrations of 20mg/ml and 40mg/ml of the crude ethanolic extract and the fractions at 5mg/ml dissolved in methanol: de-ionized water (1:1 v/v) were separately introduced into wells. Also, concentrations of 10µg/ml of ciprofloxacin (a standard antibiotic), 1mg/ml of nystatin (a standard anti-fungal drug) and methanol: de-ionized water (1:1 v/v) were introduced into other wells as positive and negative controls respectively. The experiments were carried out in triplicates. The plates were left at room temperature (27 ± 2^0C) for 2h to allow for diffusion. The plates were then incubated at 34 ± 2^0C for 24h.

Results and Discussion

Plant materials. The plant materials used in this present study were identified, authenticated and collected observing basic guidelines of plant collection. The solvents and reagents used were of analytical grade. The phytochemical screening revealed the presence saponins and cardiac glycosides while alkaloids, tannins, anthraquinones, terpenes and flavonoids were absent in all organs investigated (Table 1). This confirms previous studies (Evans, 1996). Secondary metabolites such as saponins and cardiac glycosides and others such as alkaloids, tannins and flavonoids have demonstrated in several studies (Hillar *et al*., 1990; Rios *et al*., 1990; Lamikanra *et al*., 1990; Burapadaja and Bunchoo 1995; Adesina *et al*., 2000) to be responsible for the curative and or

management of many ailments caused by microbes and different kinds of inflammations claimed in the ethno-medicine of plants.

Table 1. Phytochemical Screening of The Crude Extracts of Leaves, Stem and Roots of *D. edulis*.

Plant metabolite	Test	L	S	R
Alkaloids	Dragendoff's	-	-	-
	Mayer's	-	-	-
Saponins	Froth	+ +	+ +	+ +
	Emulsion	+ +	+ +	+ +
Tannins	Ferric chloride	-	-	-
Cardiac glycosides	Salkowski's	+	+	+
	Liebermann-Burchard's	+	+	+
Terpenes	Sulphuric acid	-	-	-
Anthraquinones	Borntrager's	-	-	-
Flavonoids	Shinoda's	-	-	-

Key: L = Leaf ; S = Stem ; R =Roots ; + = Trace(insignificant amounts) ; + + = Moderate; - = Absent

Table 2. Larvicidal Activity (LA%) of The Crude Extracts of Leaves, Stem and roots of *D. edulis* at 5%w/v after 12 and 24h incubation.

Sample	LA%(12h)	LA%(24h)
L	50	70
S	50	70
R	20	30
PC	100	100
NC	0	0

Table 3. Larvicidal Activity(LA%) of The Crude Extracts of leaves ,Stem and Roots of *D. edulis* at 10%w/v after 12 and 24h.

Sample	LA%(12h)	LA%(24h)
L	70	90
S	80	100
R	30	40
PC	100	100
NC	0	0

Key: For Tables 2 and 3, refer to Table 1. PC = Positive Control; NC = Negative Control.

Table 4. Anti-microbial Sensitivity of The Crude Extracts of Leaves, Stem, and Roots and Fractions of *D. edulis* at Different Concentrations in Methanol: De-ionized water(1:1v/v). *Zone of inhibition ± 0.5mm

Test organism	L(mg/ml)		S(mg/ml)		R(mg/ml)20		Lh	Lc	Lb	Sh	Sc	Sb	Rh	Rc	Rb	C	N	MD
	20	40	20	40	40		5mg/ml			5mg/ml			5mg/ml					
B. subtilis	14	15	16	17	6	6	16	6	6	17	6	6	6	6	6	28	NT	6
S. aureus	15	17	13	15	6	6	17	6	6	15	6	6	6	6	6	24	NT	6
E. coli	6	6.5	6	6.5	6	6	6	6	6	6	6	6	6	6	6	26	NT	6
S. typhi	16	18	6	6.5	6	6	16	6	6	6	6	6	6	6	6	25	NT	6
K. pneumoniae	16	17	6	6	6	6	17	6	6	6	6	6	6	6	6	24	NT	6
C. albicans	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	NT	25	6
A. niger	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	NT	27	6

Key: For Table 4, refer to Tables 1, 2 and 3
Lh , Lc, Lb(hexane, chloroform and butanol fractions of the leaves);
Sh, Sc, Sb(hexane, chloroform and butanol fractions of the stem);
Rh, Rc, Rb(hexane, chloroform and butanol fractions of the roots);
C = Ciprofloxacin (standard antibiotic or anti-bacterial drug); N = Nystatin(standard anti-fungal drug);
MD = Methanol: De-ionized water(1:1 v/v); NT = Not Tested.
*Zone of inhibition recorded is diameter of zone and bore-hole cup size[zone diameter(mm)+ 6 mm].

Larvicidal activity. Preliminary larvicidal assay was carried out on the crude extracts of leaves, stem and roots at 5%w/v and 10%w/v and at 12 and 24h incubation. The larvicidal activity (LA%) was calculated in terms of percentage mortality. The lethality furnished after 12 and 24h incubation was concentration and time-dependent (Tables 2 and 3). At 5%w/v (12 and 24h), both the extracts of leaves and stem demonstrated remarkably good larvicidal activity of 50% and 70% while at 10%w/v (12 and 24h),the activities were profound at 70% and 90%; 80% and 100% respectively. However, the extract of the roots showed comparably weaker larvicidal activity of 20% and 30%(at 5%w/v) and 30% and 40% (at 10%w/v) respectively.

The phytochemical screening carried out on the crude extracts revealed the presence of saponins in all the organs .This class of metabolite had been shown in separate studies (Ojewole *et al*., 2000; Bentley *et al*., 1984; Nia *et al*., 2006; Oladimeji *et al*., 2006a; Oladimeji *et al*., 2006b; Oladimeji *et al*., 2007; Oladimeji *et al*., 2008) to be lethal to the fourth instar larvae of *Anopheles gambiae* which prevent the emergence of adult mosquitoes responsible for the transmission of malaria scourging huge populations of people around the world.

Anti-microbial sensitivity test. The extracts and the fractions (obtained from the chromatographic purification of the extracts) were screened for antibacterial and anti-fungal activities using *B .subtilis*, *S. aureus*, *E. coli*, *S. typhi*, *K. pneumoniae*, *C. albicans* and *A. niger* to represent a desirable spectrum of microbes. The extracts were tested at 20mg/ml and 40mg/ml while the fractions were screened at 5mg/ml. The results presented in Table 4 show that the activities elicited were concentration- dependent. The crude extracts of the leaves and stem inhibited the growth of *B. subtilis* and *S. aureus* while only the leaf extract was active against *S. typhi* and. *K. pneumoniae.* However, none of the extracts demonstrated any anti-fungal activities against *C. albicans* and *A .niger*. This could be due to the nature of the fungi because their structures differ from the cell-wall of bacteria and resemble those of higher plants hence limiting the permeation of substance into them (Evans, 1996; Dutta, 1995).

It was observed that among the fractions, only the hexane fraction of the leaves was active against *B. subtilis*, *S. aureus*, *S. typhi* and *K. pneumoniae* while the hexane fraction of the stem demonstrated activity only against *B. subtilis* and *S. aureus*. However, the chloroform and butanol fractions were inactive most probably because of solubility problems encountered with the solvents.

In conclusion, the results of the larvicidal and anti-microbial assays have lent scientific justification to some of the uses of the plant in ethno-medicine.

Acknowledgements

The kind assistance of the Pharmaceutical Microbiology Unit, Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Uyo, Uyo, Akwa Ibom State, Nigeria in the bio-studies is gratefully appreciated.

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