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ISOLATION, IDENTIFICATION AND CHARACTERIZATION OF 20(29)-LUPEN-3-OL FROM THE ROOT EXTRACT OF *DACRYODES EDULIS* AND ITS ANTIMICROBIAL POTENCIES AGAINST SOME CLINICAL AND PLANT PATHOGENS

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ABSTRACT

Roots of Dacryodes edulis are used in traditional medicine as remedy to stomach ailment, parasitic skin diseases, dysentery and yellow fever. This study was aimed at isolation and characterization of pure compound that enhances the medicinal potency of the plant part by means of spectroscopic methods which include Thin Layer Chromatography, FT-IR, nuclear magnetic resonance spectroscopy (^{1}H-NMR, ^{13}C-NMR and 2D) as well as the antimicrobial activity of the isolated compound against some clinical and plant pathogens. 20(29)-lupen-3-ol was isolated from the plant and its antimicrobial activity was examined on 6 bacteria and 11 fungi and also 4 standard control drugs were used in the study. The antimicrobial screening of the isolated compound showed that the compound has higher potential for medicinal values. The compound exhibited high activity against some of the microbes used. It was sensitive to Aspergillus flavus, Aspergillus fumigates, Coniophora puteana, fibrophoria vaillentii, fasarium proliforatum, Rhizopus sp, Serpula lacryneus with zones of inhibition 32, 30, 27, 28, 29, 30, 28mm respectively. The antibacterial screening shows that the compound was sensitive against Methicillin resist staph aureus, staphylococcus aureus, Escherichia coli and Salmonella typhi with zones of inhibition 30, 27, 28 and 27mm respectively. The results accounts for the trado-medical claims of the plant and thus could be used as a candidate of drug in curing some diseases caused by the microorganisms examined in the study.

KEYWORDS: Aspergillus flavus, Aspergillus fumigates, Coniophora puteana.

INTRODUCTION

Dacryodes edulis is an oliferous fruit tree found in tropic climates. It is believed to have originated from Central Africa and the Gulf of Guinea area (Ayuk *et al*, 1999). It grows in nearly all the western coast of Africa across to Uganda and is an evergreen tree growing to a height of 10-15m in the forest but not exceeding 12m in plantations (Oliver, 2013). The bark is pale gray and rough with droplets of resin, the upper surface of the leaves is glossy and the flowers are yellow and about 5mm across. The main use of the plant is its fruits which can be eaten raw, cooked or roasted. The cooked fruits have a texture similar to butter and thus rich in vitamins. The tree is a source of many herbal medicines for use against stomach upset in oral hygiene and for parasitic skin diseases (Kola *et al;* 2011). The flowers are useful in apiculture. The plant has long been used in traditional medicine of some African countries to treat various ailments such as wounds, skin diseases, dysentery and yellow fever. The plant has been formed to possess a variety of biological and pharmacological properties such as antimicrobial, antioxidant, anti-inflammatory, anti-plasmodic, anti-hypertensive, laxative, diuretic function and anti-sickle cell disease (Ajibesin, 2011). 20(29)-lupen-3-ol is found in vegetables such as white cabbage, pepper, cucumber, tomato, in fruits such as olive, mango, red grapes and the medicinal plants such as American ginseng, shea butter plant (Ogboru *et al.,* 2015). 20(29) Lupen-3-ol otherwise known as Lupeol has been reported to have some pharmacological potentials such as anti-inflammatory, antitumor, antiprotozoal and antimicrobial (Ankita, 2015). In this study, the isolation, characterization and biological activity of the isolated compound against some pathogens was considered and reported.

MATERIALS AND METHODS

The roots of *Dacryodes edulis* were collected from Bunu Tai in Tai Local Government Area of Rivers State and was identified by a plant scientist and assigned to voucher number IAUE/2017/DE/007 and deposited in the herbarium. The roots were air-dried and ground to powder using a mortar and pestle.

EXTRACTION AND ISOLATION

500g of ground root sample of *Dacryodes edulis* was placed in a Soxhet apparatus and extracted for two days using hexane. The extract was evaporated to dryness in a hot evaporator at 40^0C and the extract obtained was dissolved in silica gel and allowed to dry for column chromatography using gradient of ethylacetate and hexane (20:80) (Nande and Igoli, 2017). The elutes were collected in a number of vials and monitored using TLC. The elutes of

similar retardation factor (Rf) were combined and evaporated. One of the fractions was used for further characterization. The isolated fraction was washed with n-heptane to give the compound with fraction code PDE 006. The TLC was done using a pre-coated (MERCK) F254 TLC plates over silica gel using ethylacetate and hexane (3:7). The isolated extract fraction was dspotted on the TLC plate, dip into a TLC tank containing 3:7 ETAC / Hex and allowed for 5 minutes and observed for the distance travelled by the solvent. The plate was removed and subjected to dryness by heating, thereafter, placed in the UV lamp to ascertain its activeness in the lamp. The plate was the sprayed using a mixture of H_2SO_4 and anialdehyde as spraying agent. After spraying, the TLC plate was allowed to dry for the confirmation of the compound colours and distance travelled. The Retardation factor (Rf) was calculated to be 0.51 using the formulated,

Rf= distance of solvent sample ratio / Distance of solvent alone.

THE ANTIMICROBIAL SCREENING

The antimicrobial activities of the isolated compound were determined using some animal and plant pathogens. The animal pathogens were obtained from the department of medical microbiology ABU teaching hospital Zaria. The plant pathogens were obtained from I.A.R A.B.U. Zaria. 0.002mg of the compound was weighed and dissolved in 10mls of DMSO to obtain a concentration of 20µg/ml. Diffusion method was used for screening the compound Mueller Hinton agar sabouraud dextrose agar were the media used as the growth media for the microbes. The media were prepared according to the manufacturer's instructions sterilized at 121°c for 15 minutes, poured into sterile petri dishes and were allowed to cool and solidify. The Mueller Hinton agar was seeded with 0.1ml of the standard inoculum of the test bacteria while the sabouraud dextrose agar was seeded with 0.1ml of the test fungi. The inoculum was spread evenly over the surface of the medium by the use of a sterile swab. By the use of a standard sterile cork borer of 6mm in diameters, well was cut at the centre of each inoculated media. 0.1ml of the solution of the compound of the concentration of 20µg/ml was then introduced into the well on the inoculated media. Incubation was made at 37°c for 24hrs for the bacteria and at 30°c for 1-7days for the fungi after which the plates of the media were observed for the zone of inhibition of growth, the zone was measured with a transparent ruler and the result recorded in millimetres.

Determination of minimum inhibitory concentration (MIC) of the isolated compound

The minimum inhibition concentration of the compound was determined using the broth dilution method. Mueller Hinton broth and sabouraud dextrose broth were prepared, 10mls of the broth was dispensed into test tubes and were sterilized at 121°c for 15 minutes, the broth was allowed to cool. Mc-farland's turbidity standard scale number 0.5 was prepared to give turbid solution Normal saline was prepared, 10mls was dispensed into sterile test tube and the test microbe was inoculated and incubated at 37°c for 6hrs. Dilution of the test microbe was done in normal saline until. The turbidity marched that of Mc-farland's scale by visual comparison at this point the test microbe has a concentration of 1.5 $\times 10^8$cfu/ml. Two-fold seriel dilution of the compound was done in the sterile broth to obtain the concentrations of 20 µg/m/l, 10 µg/ml, 5 µg/ml, 2.5 µg/ml and 1.25 µg/ml. the initial concentration was obtained by dissolving 0.002mg of the compound in 10mls of the sterile broth. Having obtained the different concentrations of the compound in the sterile broth, 0.1ml of the test microbe in the normal saline was then inoculated into the different concentrations incubation was made at 37°c for 24 hours for the bacteria and at 30°c for 1-7days for the fungi, after which the test tubes of the broth were observed for turbidity (growth) the lowest concentration of the compound in the sterile broth which shows no turbidity was recorded as the minimum inhibition concentration.

Determination of minimum bactericidal/ fungicidal concentration (MBC/MFC)

MBC/MFC was carried out to determine whether the test microbes were killed or only their growth inhibition. Mueller agar and sabouraud dextrose agar were prepared, sterilized at 121°c for 15 minutes, poured into sterile petri dishes and were allowed to cool and solidify. The contents of the MIC in the seriel dilutions were then sub-cultured onto the prepared media, the bacteria were sub-cultured onto the Mueller agar while the fungi were sub-cultured onto sabouraud dextrose agar, incubation was made at 37°c for 24 hours for the bacteria and at 30°c for 1-7days for the fungi, after which the plates of the media were observed for colony growth MBC/MFC were the plates with lowest concentration of the compound without colony growth. Results were recorded after 24 hours (Usman *et al*., 2007).

SPECTROSCOPIC CHARACTERIZATION OF PDE006 AS 20(29)-LUPEN-3-OL

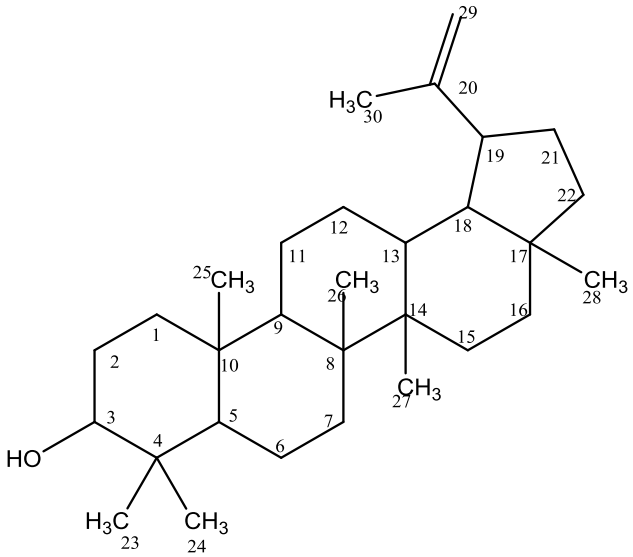
Fraction PDE006 was isolated as a white amorphous solid with melting point 190-192^{0}C. The spot on TLC was inactive in UV lamp but upon exposure to iodine chamber, a brown spot was observed and upon spraying using 5% H_2SO_4 in anialdehyde followed by heating with

hot air gun, a violet coloured spot was observed. The IR spectrum showed absorption for hydroxyl group at 3000cm^{-1} a strong intramolecular hydrogen bonded –OH resulting in the absorption at 3000cm^{-1} (broad), C-H stretching at 2900cm^{-1} and carbon double bond (C=C), 1500cm. the ^{1}H-NMR spectrum showed sevens singlets for methyl groups at δH0.99(H-23), 0.83 (H-24), 0.98 (H-25), 1.07 (H-26), 0.79 (H-27), 0.87 (H-28) and 1.46 (H-30) ppm. The olepinic protons for the methylene group (CH_2) appeared at 4.66 and 4.59 as broad singlets. The ^{13}C-NMR spectrum confirmed the presence of 30 carbon atoms, which is characteristic of triterpenoids. One of the carbons was oxygenated and appeared at δ151.0 and 109.5 were assigned to C-20 and C-29 respectively. The spectrum also showed peaks at δc 18.5, 21.0, 25.2, 27/4, 27.1, 30.0, 34.2, 35.6, 38.7 and 40.0 ppm were for methylene carbons at C-6, C-11, C-12, C-2, C-15, C-21, C-7, C-16, C-1 and C-22 respectively. The methyl carbon atoms were observed at δc15.4, 15.5, 27.1, 16.0, 14.8, 19.32 and 28.0 ppm for C-27, C-24, C-15, C-26, C-28, C-30 and C-23. Based on the spectroscopic data and comparison with literature reports, fraction PDE 006 was identified as 20(29)-lupen-3-ol (Lupeol) previously isolated from many plant species.

Table 1: ^{1}H and ^{13}C NMR chemical shift values of 20(29)-Lupen-3-ol.

C-position	Experimental		Literature Tor-Anyiin & Akpuaka (2011)	
	^{1}H(δ)	^{13}C(δ)	^{1}H(δ)	^{13}C(δ)
1	1.66	38.7	1.66	38.78
2	1.56	27.4	1.37	27.51
3	3.2	79.5	3.19	79.09
4		39.0		38.94
5		55.6		55.36
6		18.5		18.40
7		34.2		34.35
8		41.0		40.90
9	1.28	50.4		50.50
10		37.2		38.11
11	1.16	21.0		21.00
12		25.2		35.20
13		38.1		38.11
14		43.0		42.90
15		27.1		27.50
16		35.6		35.65
17		43.0		43.09
18		48.4		48.36
19		48.7		48.07
20		151.0		151.05

21		30.0	29.90	29.90
22		40.0	40.07	40.07
23	0.99	28.0	0.96	28.07
24	0.85	15.5	0.78	17.47
25	0.98	16.1	0.81	16.21
26	1.07	16.0	1.01	14.63
27	0.79	15.4	0.95	14.63
28	0.87	14.8	0.82	18.08
29	4.66, 4.59	109.5	4.67, 4.56	109.44
30	1.70	19.3	1.67	19.39



Structure of 20(29)-Lupen-3-ol

Table 2: Antifunal activities and zone of inhibition of the isolated compound.

Test organism	Antifungal activity	Zone of inhibition	Fulcin	Kefeconazole
Aspergillus flavus	S	32	S(26)	R(0)
Aspergillus fumigates	S	30	S(28)	R(0)
Aspergillus nigre	R	0	S(30)	S(25)
Coniophora puteana	S	27	R(0)	S(23)
Fibrophoria vaillentii	S	28	S(28)	R(0)
Fomitopsis pinicola	R	0	S(30)	S(27)
Fusarium oxysporm	R	0	S(32)	R(0)
Fusarium proliforatum	S	29	S(26)	R(0)
Rhizopus sp	S	30	S(26)	S(28)
Sclerotium rofsii	R	0	R(0)	S(25)
Serpula lacryneus	S	28	S(30)	S(26)

Table 3: Antibacterial activities and zone of inhibition of the isolated compound.

Test organism	Antifungal activity	Zone of inhibition	Sporfloxacin	Ciprofloxacin
Methicillin resist staph aureus	S	30	S(30)	R(0)
Vancomycin resist enterococci	R	0	S(29)	S(30)
Staphylococcus aureus	S	27	S(32)	S(26)
Escherichia coli	S	28	S(27)	S(27)
Salmonella typhi	S	27	R(0)	S(40)
Pseudomonas aeruginosa	R	0	R(0)	S(25)

Table 4: Minimum inhibitory concentration of the isolated compounds against the test microorganism.

Test organism	20mg/ml	10mg/ml	5mg/ml	215mg/ml	1.25mg/ml
Methicillis resist staph aureus	-	-	-	0*	++
Vancomycin resist enterococci	-	-	-	0*	+
Staphylococcus aureus					
Escherichia coli					
Salmonella typhi	-	-	-	0*	+
Psendomonas aeruginosai	-	-	-	0*	+

Key: - = No turbidity (No growth)

o* = MIC

+ = Turbid (light growth)

++ = moderate turbidity

Table 5: Minimum bactericidal concentration of the compounds against the test organisms.

Test organism	20mg/ml	10mg/ml	5mg/ml	215mg/ml	1.25mg/ml
Methicillis resist staph aureus	-	0*	++	++	++
Vancomycin resist enterococci	-	-	0*	++	+
Staphylococcus aureus					
Escherichia coli					
Salmonella typhi	-	-	0*	++	+
Psendomonas aeruginosai	-	-	0*	++	+

Key: - = No turbidity (No growth)

o* = MIC

+ = Turbid (light growth)

++ = moderate turbidity

Table 6: Minimum inhibitory concentration of the isolated compound against test fungi.

Test organism	20mg/ml	10mg/ml	5mg/ml	2.5mg/ml	1.25mg/ml
Aspergillus flavus	-	-	-	0*	+
Aspergillus fumigates	-	-	-	0*	+
Aspergillus nigre					
Coniophora puteana	-	-	0*	+	++
Fibrophoria vaillentii	-	-	-	0*	+
Fomitopsis pinicola					
Fusarium oxysporm					
Fusarium proliforatum	-	-	-	0*	+
Rhizops sp	-	-	-	0*	+
Sclerotium rofsii					
Serpula lacryneus					

Key: - = No turbidity (No growth)

o* = MIC

+ = Turbid (light growth)

++ = moderate turbidity

Table 7: Minimum fungicidal concentration of the compound against test organisms.

Test organism	20mg/ml	10mg/ml	5mg/ml	2.5mg/ml	1.25mg/ml
Aspergillus flavus	-	0*	+	++	+++
Aspergillus fumigates	-	-	0*	+	++
Aspergillus nigre					
Coniophora puteana	-	0*	+	++	+++
Fibrophoria vaillentii	-	0*	+	++	+++
Fomitopsis pinicola					
Fusarium oxysporm	-	-	0*	+	++
Fusarium proliforatum	-	0*	+	++	+++
Rhizops sp	-	0*	+	++	+++
Sclerotium rofsii					
Serpula lacryneus					

Key: - = No turbidity (No growth)

o* = MIC

+ = Turbid (light growth)

++ = moderate turbidity

Antibacterial Studies

Antimicrobial activity of the isolated compound was screened against few bacterial and fungi by a well diffusion method using agar as nutrient. The following bacteria were used in the study; *methicilin resist staphylococcus aureus (MRSA), staphylococcus aureus, salmonella typhi, vancomycin resist enterocolli, Escherichia coli, salmonella coli* and *pseudomonas aruginosa.* Fungi used in the study were *Aspergillus flavus, Aspergillus fumigates,*

Coniophora puteana, Fibrophoria vaillentii, Fasarium proliforatum, Rhizopus sp, Serpula lacryneus, Sclerotium rofsii, Fusarium oxysporm, Fomitopsis pinicola and *Aspargillus nigre.* The result shows that *Aspergillus flavus* has the highest zone of inhibition (32mm) whereas *Coniophora puteana* recorded the lowest zone of inhibition at 27mm. However, *Fungi such as Aspergillus nigre, Fomitopsi pinicola, Fusarium oxysporn and Sclerotium rofsii* were resistance to the compound but *Aspergillus flavus, Aspergillus fumigates, coniophora puteana, Fibrophoria vaillentii, Fusarium proliforatum, Rhizopus sp* and *Serpula lacryneus* were sensitive to the isolated compound. All the fungi were sensitive to Fulcin except *Coniophora puteana* and *Sclerotium rofsii* that were resistance. *Fusarium oxysporm* recorded the highest zone of inhibition (32mm) with fulcin. *Aspergillus flavus, Aspergillus fumigates, Fibrophoria vaillentii, Fusarium oxysporm* and *Fusarium proliforatum* were resistance to Kefeconazole while others were sensitive with *Rhizopus sp* with the highest zone of inhibition (28 mm). The bacteria such as *Methicillin resist staph aureus, Staphylococcus aereus, Escherichia coli* and *Salmonella typhi* were sensitive to the compound with Methicillin resist staph aureus showing the highest zone of inhibition (30 mm). *Vancomycin resist enterococci* and *Pseudomonas aeruginosa* were resistance to the compound. The clinical isolates were tested with the the standard drugs (Sporfloxacin and Ciprofloxacin). *Methicillin resist staph aureus, staphylococcus aureus, Escherichia coli* and *Vancomycin resist enterococci* were sensitive to Sporfloxacin with *staphylococcus aureus* displaying the highest zone of inhibition (Table 2). All the isolates displayed sensitivity with ciprofloxacin with *Salmonella typhi* showing the highest zone of inhibition (40 mm) (Table 2). Except *Methicillin resist staph aureus* which was resistance to the standard drug. The increased activity of the synthesized compound can be explained electron delocalization and the fractional groups present in the molecule. This increases the lipophilic character of the molecule and favours its permeatics through the lipoid layer of the bacterial membranes. The increased lipophitic character of this compounds seems to be responsible for their enhanced potent antibacterial activity. Thus, it may be suggested that these compounds deactivate various cellular enzymes, which play a vital role in various metabolic pathways of these screened microorganisms (Ugye *et al.,* 2018). Amise *et al* (2016) have reported the potential effect of a specie of the plant against pathogenic microorganism such as staphylococcus aereus, Escherichia coli, salmonella typhi, pseudomonas aeruginosa, candida albicans and candida tropicalis. The antimicrobial activity of the compound could be traceable to the presence of the functional groups present (hydroxyl-OH, and double bond- (c=c) since it has been reported that the OH group are said to be related to their toxicity to microorganisms,

with evidence that increased hydroxylation results in increases toxicity (Vazques *et al.*, 2012). More so, it has been found that the more highly oxidized hydroxyl groups show more inhibitory effects. The mechanisms thought to be responsible for hydroxyl toxicity to microorganisms include enzyme inhibition by the oxidized compounds, possibly through the reaction with sulfhydryl groups or through more nonspecific interactions with the proteins (Anyanwu and Nwosu, 2014).

CONCLUSION

20(29)-Lupen-3-ol is a bioactive compound present in different medicinal plants and a wide range of its bioactivity and bioassays reveals is medicinal potencies against different diseases. Plants are used as remedies to treat different human diseases as such the presence of some secondary metabolites enhances its usefulness for traditional medical practices. Lupeol has been reported for its pharmacological importance in the treatment of diseases under preclinical settings such as oral, intra-peritoneal and intravenous. It has been reported that lupeol has anti-inflammatory, anti-microbial, anti-protozoan, anti-invasive, anti-angiogenic, anti-proliferative and cholesterol lowering agent. This study revealed the anti-fungi and anti-bacterial nature of the compound and thus, it could be used as a precursor for the development of some anti-bacterial and anti-fungi drugs or herbicides used in the treatment of diseases caused by those fungi that shows sensitivity against the compound.

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