Phytochemical Analysis and Antimicrobial Activity of *Punica* granatum L. (fruit bark and leaves)

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ABSTRACT: *Punica granatum* Linn (fruit bark and leaves) were macerated with hexane, ethylacetate, methanol and water successively. The extracts were tested *in vitro* for activity against standard strains microbes and clinical isolates. The zones of inhibition, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) were determined. The *in vitro* antimicrobial screening revealed that the extract exhibited varying activity against different microbes with zones of inhibition ranging from 14-34mm, MIC ranging from 0.625 - 10mg/ml, and MBC/MFC of 1.25-10mg/ml for the sensitive organisms at the tested concentrations. The highest activity was an MIC of 0.625 mg/ml and MBC of 1.25mg/ml. The activities observed could be due to the presence of some of the secondary metabolites like, alkaloids, anthraquinones, sterols, glycosides, saponins, terpenes and flavonoids detected in the plant.

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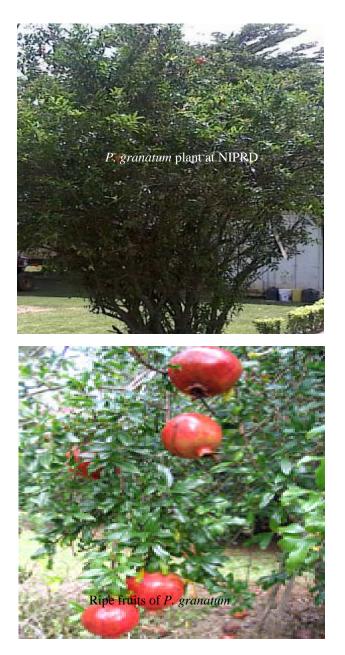
Key words: Punica granatum, phytoconstituents, antimicrobial, MIC, MBC, MFC

1. Introduction

Plants have been known to be a reservoir of secondary metabolites which are being exploited as source of bioactive substance for various pharmacological purposes. The fact that some of these plants have been used traditionally for centuries and modern scientific studies have shown the existence of good correlation between the traditional or folkloric application of some of these plants further strengthens the search for pharmacologically active compounds from plants (Abba et al., 2009; Egharevba and Kunle 2010; Abalaka et al 2009). One of such plants with wide ethnomedicinal use is Punica granatum.

Punica granatum L. commonly known as Pomegranate belongs to the Family Punicaceae. The genus has just two species *P. granatum* Linn. and *P. protopunica* Balf, although the latter is not well known. *Punica granatum* is synonymous to *Punica malus* Linn. *Punica granatum* is a shrub or small tree with several upright, thorny stems, the leaves are elliptic, roughly 2x1 inches, flowers white or red, double-flowered races being also known (figure 1&2). The plant is both selfpollinated and cross-pollinated by insects. The size and fertility of the pollen vary with the cultivars and season. The fruit which is spherical with many seeds embedded

in the pulp is green when unripe and turns pink or yellow when ripe. The plant is propagated widely by stem cutting and sometimes through the seeds. P. granatum is reported to be used for several disease conditions in folklore medicine. Some of its reported uses include gastrointestinal problems, enhancement of semen formation, memory activation, boosting of hemoglobin and as blood purifier. Various parts of the plant are employed in the management of various diseases such as dyspepsia, leprosy, bronchitis and hypertension. The plant has also been used as an antispasmodic and anthelmintic. In locally in Hausa land the flowers are used as vermifuge. The fruit and bark have also being used in tanning in ancient times. The plant is reported to contain over 28% of Gallotannic acid and the alkaloid pelletierine, methypelletierine, isopelletierine, psuedopelletierine, gallic acid, tannic acie, sugar, cacium oxalate, etc. (Irvine 1961). However, the phytochemical constituents of the plant and antimicrobial activity of this plant have not been reported in literature. The present study is aimed at providing information on the type of secondary metabolites contained in the plant and also provide scientific basis for some of its ethnomedicinal use.



2. Materials and Methods

All the solvents and reagent used in the study were of Analar grade and, unless otherwise stated, were sourced from Zayo-Sigma, Abuja, Nigeria.

2.1 Collection and Extraction of Plant Material

The plant was collected on the in April, 2010 from NIPRD medicinal plant garden and authenticated by the Taxonomist in the Department of Medicinal Plant Research and Traditional Medicine of the National Institute for Pharmaceutical Research and Development (NIPRD) Abuja, Nigeria. A voucher specimen number NIPRD/H/6410 was prepared and deposited at the herbarium of the department. The plant with the fruits and leaves was rinsed with clean water and then, the fruits and leaves were removed and separated. The fruits were peeled to separate the bark from the seed. The fruit bark and the leaf were air-dried separately for two weeks, and then crushed with a mortar and pestle. The crushed plant parts were kept separately in an air-tight cellophane bag until used.

The crushed materials were macerated successively for 24hrs in hexane, ethyl acetate, 98% methanol and distilled water. The extracts were concentrated in rotary evaporator and dried over a boiling water bath. The solid extracts were used for phytochemical analysis and antimicrobial screening.

2.2 Phytochemical screening

The presence of some secondary metabolites in the leaf and fruit bark were determined using standard methods (Sofowora 2008; Evans 2002). Proximate analysis was also carried out to determine the moisture content, total ash value, acid insoluble ash value, and alcohol and water extractive values.

2.3 Preparation of Extract Stock Concentration for Antimicrobial screening

A test stock concentration of 10mg/ml for water and methanol extracts were prepared by dissolving 0.1g of each extract in 10mls of distilled water in separate test tubes. For the ethyl acetate and hexane extracts a concentration of 10mg/ml was prepared by dissolving 0.1g in 10mls of dimethyl sofoxide (DMSO). The positive control drugs were sparfloxacin (0.2mg/ml), erythromycin (0.5mg/ml) and flouconazole (0.5mg/ml), all of sigma chemicals UK obtained from Zayo-Sigma Abuja Nigeria.

2.4 Antimicrobial Screening 2.4.1 Organism Source

The organisms used include standard strains, Staphylococcus aureus NCTC 6571, Bacilluc subtilis NCTC 8236, Eschericia coli NCTC 10418, Pseudomonas aeruginosa NCTC 6750, Salmonella typhimurium ATCC 9184, Klebsiella pneumonia ATCC 10031 and Staphylococcuc aureus ATCC 13704, and clinical isolates, Staphylococcus aureus, Methicilin Resistant Staphylococcus aureus, Streptococcus pyogenes, Streptococcus faecalis, Corynebacterium ulcerans Listeria monocytogenes, Bacillus subtilis, Bacillus cereus, Escherichia coli, Klebsiella pneumonia, Klebsialla ozaenae, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas flourescense, Salmonella typhimurium, Shigella dysenteriae, Aspergillus nigre, candida albicans, Microsporum gypseum and Trichophyton rubrum. The typed strains and clinical isolates were obtained from the department of medical Microbiology Ahmadu Bello University

Teaching Hospital (ABUTH) and department of Pharmaceutical Microbiology, Ahmadu Bello University (ABU) Zaria, Nigeria, respectively. All the organisms were checked for purity and maintained at 4°C in slants of nutrient agar and sabouraud dextrose agar (SDA) for bacteria and fungi respectively. Well diffusion method described by Hugo and Russel (1992) was used to determine the antimicrobial activities (zone of inhibition) of the extracts against the organisms.

2.4.2 **Preparation of the Inoculum**

A loopful of the test organism was taken from their respective agar slants and sub-cultured into test-tubes containing nutrient broth for bacteria and sabouraud dextrose liquid for fungi. The test-tubes were incubated for 24hrs at 37° C for bacteria and for 48hrs at 30° C for the fungi. The obtained microorganisms in the broth were standardized using normal saline to obtain a population density of 10^{8} cfu/ml for the bacteria. For the fungi, fungal spores were harvested after 7 days old SDA slant culture was washed with 10ml normal saline in 2% Tween 80 with the aid of glass beads to help in dispersing the spores. The spores suspension were standardized to 10^{5} cfu/ml.

2.4.3 Preparation of Media

The medium was prepared according to manufacturer's instruction (Oxoids Limited Basingstoke, Hampshire, England). 40g of Blood Agar (52g of SDA) were weighed into a conical flask 1000ml of distilled water was added and capped with a cotton wool. The media were boiled to dissolution and then sterilized at 121°C for 15mins. The media were allowed to cool to 45°C and 20ml of the sterilized medium was poured into sterile petri-dishes and allowed to cool and solidify. The plates were labeled with the test microorganism (each plate with a test microbe). The microbes were spread evenly over the surface of the medium with the aid of a glass spreader. The plates were dried at 37°C for 30mins and divided into two sets to be used for the well diffusion method and the disc diffusion method respectively.

2.4.4 Zone of Inhibition - Well Diffusion Method

A standard cork borer of 5mm in diameter was used to cut well at the center of each inoculated plate and the agar removed from the well. 0.1ml of the text solution (extract) was then introduced into the well created at the center for each plate. The bacteria plates were incubated at 37°C for 24hrs while the fungal plates were incubated at 30°C for 1-7days, and observed for the zone of inhibition of growth. The zones were measured with a transparent ruler and the result recorded in millimeters. The screening was done in triplicates. Sterilized distilled water and DMSO were used as negative control.

2.4.5 Minimum Inhibitory Concentration - Broth Dilution Method

MIC of the extracts were also carried out using broth dilution method as described in Ibekwe et al, 2001. The nutrient broth and sabouraud dextrose liquid were prepared according to the manufacturer's instruction (10ml of each broth was dispensed into separate test-tube and was sterilized at 121°C for 15mins and then allowed to cool. Two-fold serial dilutions of the extracts in the broth were made from the stock concentration of the extract to obtain 10, 5, 2.5, 1.25, 0.625 mg/ml for water and methanol, and 5, 2.5, 1. 25, 0.625, 0.3125 mg/ml for ethyl acetate and hexane extracts. 0.1ml of the standardized inoculums of the inoculated the microbes was into different concentrations of the extracts in the broth. The test tubes of the broth were incubated at 37°C for 24hrs and 30°C for 1-7days for bacteria and fungi respectively and observed for turbidity. The lowest concentration which showed no turbidity in the test tube was recorded as the MIC.

2.4.6 Minimum Bactericidal/Fungicidal Concentration - Broth Dilution Method

Blood and sabouraud media were prepared, sterilized at 121°C for 15mins and was poured into sterile petri-dishes and left to cool and solidify. The contents of the MIC in the serial dilution were then subcultured onto the media and incubated at 37°C for 24hrs and 30°C for 1-7days for bacteria and fungi respectively, and observed for colony growth. The MBC/MFC was the plate with the lowest concentration of extract and without colony growth.

2.4.7 Determination of activity index

The activity index of the crude plant extract was calculated as;

Activity index (A.I.) =

Zone of inhibition of the extract

Zone of inhibition obtained for standard antibiotic drug

2.4.8 Determination of proportion index

The proportion index was calculated as;

Proportion index (P.I.) =

<u>Number of positive results obtained for extract</u> Total number of tests carried out for each extract

3. Results

The results of phytochemical screening and proximate analysis are shown in tables 1 and 2, while table 3 shows zones of inhibition and activity index. Table 4 shows the minimum inhibitory concentration (MIC) and minimum bactericidal/fungi concentration MBC/MFC.

		LF	EAF		FRUIT BARK							
Metabolites	Н	Ε	Μ	W	Н	Ε	Μ	W				
Alkaloids	+	+	-	+	+	+	+	+				
Tannins	+	+	+	+	-	+	+	+				
Carbohydrates	-	+	-	+	+	-	+	+				
Flavonoids	-	-	+	+	-	-	+	+				
Phytosterols	-	-	+	+	+	+	-	+				
Phenols	+	+	+	+	-	+	+	+				
Saponins	-	-	-	-	-	-	-	-				
Sterols	-	-	+	+	+	+	+	+				
Terpenes	-	-	+	+	-	+	+	+				
Volatile oils	+	+	+	-	+	-	-	-				
Balsams	+	+	+	+	-	+	+	+				

Table 1: Phytochemical Analysis

 Balsams
 +
 +
 +
 +
 +

 +
 means detected; means not detected; H means N- Hexane; E means ethyl acetate; M means methanol; W means water.

Table 2: Proximate Analysis

Parameter	Val	ues (%)
	LEAF	FRUIT BARK
Moisture content	6.50	11.70
Water-soluble extractive value	22.00	28.00
Alcohol-soluble extractive value	26.00	17.00
Total ash value	4.90	2.50
Acid-insoluble ash value	0.63	0.57

TEST	STRAINS	ZONE OF INHIBITION (mm)							Activity Index								
ORGANISM			Le	eaf			Fruit bark			Leaf				Fruit	t bark		
		W	m	e	h	W	m	e	h	W	m	e	h	w	m	e	h
Staphylococcus	NCTC	32	32	27	28	24	27	30	30	1.45	1.45	1.23	1.27	1.09	1.23	1.36	1.36
aureus	6571																
Bacillus subtilis	NCTC	30	27	22	25	27	28	32	28	1.36	1.23	1.00	1.14	1.23	1.27	1.45	1.27
	8236																
Escherichia coli	NCTC	27	32	0	25	0	0	30	32	1.13	1.33	0.00	1.04	0.00	0.00	1.25	1.33
	10418																
Pseudomonas	NCTC	29	30	22	27	31	0	29	0	1.21	1.25	0.92	1.13	1.29	0.00	1.21	0.00
aeruginosa	6750																
Salmonella	ATCC	32	29	21	0	22	27	30	25	1.19	1.07	0.78	0.00	0.81	1.00	1.11	0.93
typhimurium	9184																
Klebsiella	ATCC	30	30	0	0	27	25	34	26	1.03	1.03	0.00	0.00	0.93	0.86	1.17	0.90
pneumoniae	10031																
Staphylococcus	ATCC	29	27	21	29	27	27	32	27	1.07	1.00	0.78	1.07	1.00	1.00	1.19	1.00
aureus	13704																
Candida albicans	ATCC	24	25	0	0	0	0	31	30	1.09	1.14	0.00	0.00	0.00	0.00	1.41	1.36
	10231																
Staphylococcus	Isolate	24	27	27	0	15	27	32	31	1.14	1.29	1.29	0.00	0.71	1.29	1.52	1.48
aureus																	
Methicilin	Isolate	22	26	22	0	20	32	30	0	0.81	0.96	0.81	0.00	0.74	1.19	1.11	0.00
Resistant Staph.																	
aureua																	
Streptococcus	Isolate	22	26	20	27	19	31	30	30	0.85	1.00	0.77	1.04	0.73	1.19	1.15	1.15
pyogenes																	
Streptococcus	Isolate	20	27	27	27	20	0	30	30	0.69	0.93	0.93	0.93	0.69	0.00	1.03	1.03
faecalis																	
Corynebacterium	Isolate	0	22	24	26	0	27	29	28	0.00	0.73	0.80	0.87	0.00	0.90	0.97	0.93
ulcerans																	
Listeria	Isolate	0	0	0	28	0	29	0	0	0.00	0.00	0.00	1.17	0.00	1.21	0.00	0.00
monocytogenes																	
Bacillus subtilis	Isolate	24	26	17	29	22	26	30	31	0.96	1.04	0.68	1.16	0.88	1.04	1.20	1.24
Bacillus cereus	Isolate	25	30	0	25	27	27	32	29	0.96	1.15	0.00	0.96	1.04	1.04	1.23	1.12
Escherichia coli	Isolate	0	27	0	27	0	29	28	29	0.00	1.35	0.00	1.35	0.00	1.45	1.40	1.45
Klebsiella	Isolate	22	24	22	24	28	27	28	30	1.16	1.26	1.16	1.26	1.47	1.42	1.47	1.58
pneumoniae																	
Klebsiella ozaenae	Isolate	23	27	22	28	22	24	28	0	1.28	1.50	1.22	1.56	1.22	1.33	1.56	0.00
Proteus mirabilis	Isolate	0	0	0	26	0	0	24	29	0.00	0.00	0.00	1.30	0.00	0.00	1.20	1.45
Proteus vulgaris	Isolate	0	27	22	25	0	31	27	0	0.00	1.13	0.92	1.04	0.00	1.29	1.13	0.00
Pseudomonas	Isolate	22	28	24	0	24	27	26	0	1.00	1.27	1.09	0.00	1.09	1.23	1.18	0.00
aeruginosa																	
Pseudomonas	Isolate	24	24	22	0	25	30	0	30	1.00	1.00	0.92	0.00	1.04	1.25	0.00	1.25
flourescenses																	
Salmonella	Isolate	20	28	24	29	27	27	27	29	0.91	1.27	1.09	1.32	1.23	1.23	1.23	1.32
typhimurium																	
Shigella	Isolate	0	22	0	29	30	28	27	31	0.00	1.10	0.00	1.45	1.50	1.40	1.35	1.55
dysenteriae		-		-													
Aspergillus flavus	Isolate	0	0	0	0	0	0	0	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Aspergillus nigre	Isolate	Ő	Ő	Ő	0	0	Ő	28	24	0.00	0.00	0.00	0.00	0.00	0.00	1.22	1.04
Candida albicans	Isolate	0	14	0	25	0	16	28 24	25	0.00	0.58	0.00	1.04	0.00	0.67	1.00	1.04
Microsporum	Isolate	0	0	0	0	0	0	22	0	0.00	0.00	0.00	0.00	0.00	0.00	1.10	0.00
gypseum	1501000	0	0	0	0	0	0		0	0.00	0.00	0.00	0.00	0.00	0.00	1.10	0.00
Trichophyton	Isolate	0	0	0	0	0	0	0	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
rubrum	1301010	0	0	0	U	0	0	U	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 3: Zone of Inhibition and Activity Index

 $^{1}w =$ water extract; m= methanol extract; e= ethylacetate extract; h= hexane extract

²Proportion Index (Leaf): w=0.63; m=0.80; e=0.57; h=0.63

³Propotion Index (Fruit bark): w=0.63; m=0.70; e=0.87; h=0.70

Table 4.	MIC	and MBC/MFC
1 auto 4.	MIC	

TEST	STRAINS	MIC	MIC (mg/ml)									MBC/MFC (mg/ml)									
ORGANISM	Leaf Fruit bark						Lea	f			Frui	t bark									
		W	m	e	h	W	m	e	h	W	m	e	h	W	m	e	h				
Staphylococcus aureus	NCTC 6571	1.25	1.25	2.5	1.25	2.5	2.5	0.625	0.625	5	5	5	2.5	10	5	1.25	1.25				
Bacillus subtilis	NCTC 8236	1.25	2.5	2.5	1.25	2.5	2.5	0.625	1.25	5	5	10	5	10	5	1.25	2.5				
Escherichia coli	NCTC 10418	2.5	1.25	-	1.25	-	-	1.25	0.625	10	2.5	-	5	-	-	2.5	1.25				
Pseudomonas aeruginosa	NCTC 6750	2.5	1.25	2.5	1.25	2.5	-	0.625	-	10	5	10	2.5	5	-	1.25	-				
Salmonella typhimurium	ATCC 9184	1.25	2.5	2.5	-	2.5	2.5	0.625	1.25	10	5	10	-	10	5	2.5	5				
Klebsiella	ATCC 10031	1.25	2.5	-	-	2.5	2.5	0.625	1.25	5	5	-	-	10	5	1.25	2.5				
pneumoniae Staphylococcus	ATCC	2.5	2.5	2.5	1.25	2.5	2.5	0.625	1.25	10	5	10	2.5	10	5	1.25	2.5				
aureus Candida albicans	13704 ATCC 10221	1.25	2.5	-	-	-	-	0.625	0.625	5	5	-	-	-	-	1.25	1.25				
Staphylococcus	10231 Isolate	2.5	2.5	2.5	-	5	2.5	0.625	0.625	10	5	5	-	10	5	1.25	1.25				
aureus Methicilin Resistant Staph. aureua	Isolate	2.5	2.5	2.5	-	2.5	1.25	0.625	-	10	5	10	-	10	2.5	2.5	-				
Streptococcus pyogenes	Isolate	2.5	2.5	2.5	1.25	5	1.25	0.625	0.625	10	5	10	2.5	10	2.5	2.5	1.25				
Streptococcus faecalis	Isolate	2.5	2.5	2.5	1.25	2.5	-	0.625	0.625	10	5	5	2.5	10	-	2.5	1.25				
Corynebacterium ulcerans	Isolate	-	2.5	2.5	1.25	-	2.5	1.25	1.25	-	5	10	2.5	-	5	5	2.5				
Listeria monocytogenes	Isolate	-	-	-	1.25	-	2.5	-	-	-	-	-	2.5	-	5	-	-				
Bacillus subtilis	Isolate	2.5	2.5	5	1.25	2.5	2.5	0.625	0.625	10	5	5	2.5	10	5	2.5	1.25				
Bacillus cereus	Isolate	2.5	1.25	-	1.25	2.5	2.5	0.625	1.25	5	2.5	-	2.5	5	2.5	1.25	2.5				
Escherichia coli	Isolate	-	2.5	-	1.25	-	2.5	1.25	1.25	-	5	-	2.5	-	5	2.5	2.5				
Klebsiella		2.5	2.5	2.5	1.25	2.5	2.5	1.25	0.625	10	5	10	5	5	5	2.5	1.25				
	Isolate	2.3	2.3	2.5	1.23	2.3	2.5	1.23	0.025	10	5	10	5	5	5	2.5	1.23				
pneumoniae Klebsiella	Isolate	2.5	2.5	2.5	1.25	2.5	2.5	1.25	-	10	5	10	2.5	10	5	2.5	-				
ozaenae Drotova mirchilia	Inclata		_		1.25		_	1.25	1.25			_	5	_		5	25				
Proteus mirabilis	Isolate	-		-	1.25	-		1.25	1.25		-				-	5	2.5				
Proteus vulgaris	Isolate	-	2.5	-	1.25	-	1.25	1.25	-	-	5	-	5	-	2.5	2.5	-				
Pseudomonas aeruginosa	Isolate	2.5	2.5	-	-	2.5	2.5	1.25	-	10	5	-	-	10	5	2.5	-				
Pseudomonas flourescenses	Isolate	2.5	2.5	2.5	-	2.5	1.25	-	0.625	10	5	10	-	5	2.5	-	1.25				
Salmonella typhimurium	Isolate	2.5	2.5	2.5	1.25	2.5	2.5	1.25	1.25	10	5	10	5	5	5	2.5	2.5				
Shigella dysenteriae	Isolate	-	2.5	-	1.25	1.25	2.5	1.25	-	-	5	-	2.5	2.5	5	2.5	-				
Aspergillus flavus	Isolate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
Aspergillus nigre	Isolate	-	-	-	-	-	-	1.25	1.25	-	-	-	-	-	-	2.5	5				
Candida albicans	Isolate	-	5	-	1.25	-	5	1.25	1.25	-	10	-	2.5	-	10	5	2.5				
Microsporum	Isolate	-	-	-	-	-	-	1.25	-	-	-	-	-	-	-	5	-				
gypseum Trichophyton	Isolate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
rubrum														not							

 ^{1}w = water extract; m= *methanol* extract; e= *ethyl acetate* extract; h= *hexane* extract; - = not within study concentration range.

4. Discussion

The extracts of exhibited selective activity against most of the food pathogens and human respiratory disease-causing organisms like the *Klebsiella pneumonia, Klebsiella ozaenae, Staphylococcus aureus, Pseudomona flourescences, Salmonella typhimurium,* and enteric organisms like *Streptococcus faecalis* and *Bacillus subtiliss*. The zones of inhibition exhibited against these extracts were comparable to those of the reference drugs. However, the water and methanol extracts of the leaf appeared to be more active than those of the fruit bark as the zones of inhibition were

more although in terms of broad-spectrum activity, there seem to be no significant difference. The reverse was the case for the ethyl acetate and hexane extracts. The observed activity may be due to the presence of some metabolites like alkaloid, saponins, flavonoids and terpenes which have been implicated in various biological activities.

The phytochemical screening of the fruit-bark and leaves indicated the presence of Alkaloid, Tannins, Sterols, Volatile oils, Carbohydrates, Flavonoids, Glycosides, Resins, Balsams, Terpenes and Free-Reducing sugar while Saponins was not detected. The presence of these metabolites suggests great potential for the plant as a source of useful phytomedicines (Kunle *et al.*, 2003). For instance, the presence of flavonoids and resins might be responsible its use as anti-inflammatory properties (Ibrahim *et al.*, 2010;

Structures of some compounds from P. Granatum

Egharevba and Kunle, 2010). Some alkaloids are known to be used as antimalarial agents (Ronan *et al.*, 2009). The presence of tannins could also shows that it is an astringent, help in wound healing and anti-parasitic. The presence of terpenes suggests it possible use as anti-tumor and anti-viral agent as some terpenes are known to be cytotoxic to tumor cells (Okhale *et al.*, 2010).

5. Conclusion

This study supports the folkloric use of *Punica* granatum leaf and fruit bark for the treatment of various infectious diseases in some part of the world. The study also shows that the leaf and fruit bark may be good as an antibacterial recipe but may not be very useful as an antifungus. Work is currently ongoing in our laboratory to isolate the compounds responsible for the activities exhibited.

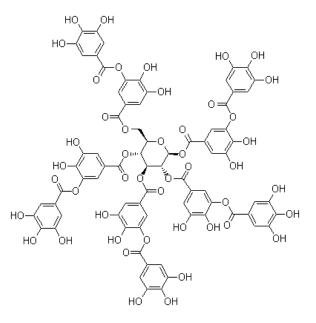


Figure 1:Tannic acid (C76H52O46) Synonyms: gallotannic acid

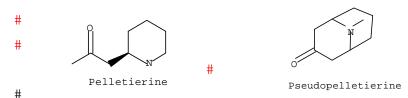


Figure 2: Pelletierine (C₈H₁₅NO) and Pseudopelletierine (C₉H₁₅NO)

Pelletierine synomyms: 2-Acetonylpiperidinepunicineisopelletierine, (.+/-.)-Pelletierine ; Isopelletierine ; 1-(2-Piperidinyl)-2-Propanone ; 2-(2-Oxopropyl)Piperidine; IUPAC Name: 1-Piperidin-2-Ylpropan-2-One (Pelletierine)

Pseudopelletierine Synonyms: 9-methyl-9-azabicyclo[3.3.1]nonan-3-one, 9-Methyl-3-granataninone

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References

- Abba D, Inabo HI, Yakubu SE, Olonitola OS. Phytochemical analysis and Antibacterial Activity of some powdered Herbal Preparations marketed in Kaduna Metropolis. Science World Journal, 2009;4 (1):23-26.
- [2]. Egharevba HO, Kunle OF. Preliminary Phytochemical and Proximate Analysis of the leaves of *Piliostigma thionningii* (Schumach.) Milne-Redhead. Ethnobotanical Leaflets, 2010; 14: 570-77
- [3]. Abalaka ME, Olonitola OS, Onaolapo JA, Inabo HI. Determination of Activity, Time Survival and Pharmacokinetics of Extracts From *Momordica charantia* on Some Bacterial Pathogens. Int. Jor. P. App. Scs., 2009; 3(3):6-13
- [4]. Irvine FR. Woody Plants of Ghana with special reference to their uses. Oxford University press, London. 1961; 65.
- [5]. Sofowora A. Medicinal Plants and Traditional Medicine in Africa. 3rd Edn., Spectrum Books Limited Ibadan, Nigeria, 2008; 199-204.
- [6]. Evans WC. Trease and Evans Pharmacognosy. 15th Edition, Elsevier India, 2002;137-393.
- [7]. Hugo WB, Rusell AD. Pharmaceutical Microbiology 5th ed. Blackwell Scientific Publication, Oxford London, 1992; 258-297.
- [8]. Ronan B, Ademir JSJ, Alaide BO. Plant-derived Antimalarial Agents: New Leads and Efficient Phytomedicine. Part II. Non-Alkaloid Natural

Products – A Review. Molecules, 2009; 14: 3037-3072

- [9]. Ibrahim J, Ajaegbu VC and Egharevba HO. Pharmacognostic and Phytochemical Analysis of *Commelina benghalensis* L. Ethnobotanical Leaflets, 2010; 14: 610-15
- [10]. Kunle O, Okogun J, Egamana E, Emojevwe E, Shok M. Antimicrobial activity of various extracts and carvacrol from *Lippia multiflora* leaf extract. Journal of Phytomedicine, 2003; 10: 59 61
- [11]. Okhale SE, Amanabo MO, Jegede IA, Egharevba HO, Muazzam IW, Kunle OF. Phytochemical and Pharmacognostic Investigation of Antidiabetic, *Scoparia dulcis*.Linn. Scorophulariaceae whole plant grown in Nigeria. Researchers, 2010; 2(6): 7-16.

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