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Bactericidal Activity of *Psidiumguajava* Leaves Against Some Pathogenic Microbes

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Abstract: *This study was carried out to determine the antimicrobial activity of Psidiumguajava(PG) leavesextracts against some pathogenic microbes. The extracts of P.G leaves was fractionated and purified by preparative TLC technique, then subjected toGas Chromatography-Mass Spectroscopy (GC-MS) to identify the biologically active compounds.Thereafter, thetoxicity of the pure fractions was assessed in vivo using experimental rats. The P.G leaves were extracted with 80% ethanol,then the extracts were fractionated with n-hexane, chloroform, ethyl acetate, n-butanol and water. The antimicrobial activities of the extracts and the fractions of P.G leaves were done on 3 clinical pathogenic strains namely, Staphylococcus (S)aureus, E. coli and Candida (C)albicans. The disc-diffusion and MIC & MBC tests were employed to evaluate the inhibitory effects of the extracts and the fractions. The result of this experiment showed that the ethanolic extract of P.G leaves had a significant (p< 0. 01) inhibitory effect on S. aureushowever the other strains were resistant. The n-hexane fraction of P.G leaves showed a significant (p< 0.01) inhibitory effect on the two tested bacterial strains compared to other fractions. However, C. albicans resisted all the fractions. The zones of inhibition of the sensitive strains significantly (p< 0.05) increased when the concentrations of the ethanolic extracts and fractions were increased. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of ethanolic extracts of PG leaves were nearly equal. The PTLC of n-hexane fraction of P.G leaves showed 4 bands of different colors. These 4 bands were tested for antimicrobial activity and only band 1 gave highest antibacterial activity. Band 1 was further analyzed with GC-MS into variable biological compounds (n-hexadecanoic acid; 9-Octadecenoic acid;Octadecenoic acid; 9, 12Octadecenoic acid (Z,Z)-; Palmitoyl chloride; Hexadecanoic acid; Cyclotridecanone; Eicosanoic acid; Oleoyl chloride; N,N'-bis(trifluoroacetyl)-N,N'-ethylene- bis(stearamide); Glycidol stearate; 1,2-Benzenedicarboxylic acid; 1-Cyclohexyldimethylsilyloxybutane; 14-Beta-H- Pregna; Stigmasta-3,5-dien-7-one). The fractions were found nontoxic to rats. In conclusion, P.G leaves contain nontoxic active antibacterial compounds. Thus P.G leaves can be used as crude or in purified forms to treat patients suffering from intestinal infection caused by S.aureus andE. coli.*

Keywords: *Antimicrobial activity; biologically active; phytochemical components,Psidiumguajava.*

I. Introduction

The*Psidiumguajava*has a long history of folk medicinal uses in Egypt and worldwide as a cough sedative (Karawya, *et al.,* 1999). The problem of microbial resistance is growing and the microbes evolved to overcome these medicines action, therefore the future to overcome this problem is ambiguous. Furthermore, bacteria have the genetic ability to transmit and acquire resistance to medicines(Nascimento, *et al*., 2000). Unfortunately,development of an effective antibacterial agent has been accompanied by the emergence of drug-resistant organisms. The microbes become genetically versatile and resistant to antibiotic and capable to transfer their resistant genes horizontally(Amit and Shailendra, 2006; Aibinu, *et al*., 2007).

Roy, *et al.,* (2006) reported that the aqueous extract of P.Ghas no antibacterial activity against the growth of *E. coli* and *S. aureus*. Differentdegrees of inhibitory effects of P.G on *S. aureus* and*E. coli*has been reported (Neviton, *et al.,* 2005; Goncalves, *et al*., 2008;Joseph, *et al*., 2010; Metwally, *et al.,* 2010;Balakrishnan, *et al.,* 2011; Sushmita, *et al.,*2012).Also,many reports showed that the antifungal activity of P.G leaves on*Candida albicans* (Samy and Ignacimuthu, 2000; Stepanovic, *et al.,* 2003; Metwally, *et al.,* 2010; Adonu Cyril, *et al.,* 2013). On the other hand, the hot water extracts of P.G were reported tohave the least inhibitory effects on fungi (Amit and Shweta, 2011). The extracts of P.Gcan be used as food preservative to improve the shelf life and safety of foods (Rattanachaikunsopon and Phumkhachorn, 2007; Nwanneka, 2013). Additionally, Gutierrez, *et al.,* (2008) reported that the P.Gcontains a number of chemical constituents which possess antimicrobial activities.

Recently, there is an increasing interest in plants as a source of antimicrobial agents. Thus the objectives of this study are to:-

- investigatethe antimicrobial activity of the ethanolic extracts and fractions of P.G against some pathogenic microbes.
- evaluate the safety of the most active compounds.
- isolate and characterize the biologically active ingredients.

II. Materials and Methods

2. 1. Materials

2. 1. 1. Collection and Identification of Plant Materials

The *Psidiumguajava*leaves were collect fromdomestic areas in Sudan. The leaves were collected in large quantity and washed with clear distilled water and dried in shade.

2. 1. 2. Extraction procedure

The dried clean leaves were taken and ground in a mortar with pestle under aseptic condition. Thereafter, 10 grams of the ground P.G leaves were suspended in 200ml of ethanol in Soxhlet apparatus at 78°C for 24hrs, the extract was evaporated aseptically (Nwinyi, *et al.,* 2009; Haniyeh, *et al.,* 2010). The yield percentage was calculated according to the following equation:

$$\text{The yield\%} = \frac{\text{Extracted weight}}{\text{Sample weight}} \times 100$$

Each 100 grams of ground P.Gleavesgavean extract of about 28.3 grams.

2. 1. 3. Fractionation procedure

About 115 grams of the ethanolic extract of P.Gleaves were fractionated according to Sukhdev, *et al.,* (2008).The yield of fractionation was calculated according to the previous equation. The weight of the fractions of n-hexane, chloroform, ethyl acetate, n-butanol and aqueous for the P.G leaves gave 4.574, 11.011, 4.338, 29.3 and 62.3 grams respectively.

2. 1. 4. Antimicrobial strains

The human clinical strains were obtained from the microbiology laboratory of King Khalid Hospital (K.K.H), Najran region, Saudi Arabia. These microorganisms include: *Staphylococcus aureus*, *E. coli* and *Candida albicans*. Furthermore, the Gram negative strainwas further identified by microbact™ to ensure their purity (Mugg and Hill, 1981). The Gram positive and the fungal strains were further identified according to standard microbiological technique (James and Natalie, 2008; Barrow andFeltham, 1993).

2. 2. Methods

2. 2. 1. Antimicrobial Assay

The antimicrobial assay was carried out according to the standard methodsof (Levan, *et al.,* 1979; NCCLS, 2003; Zaidan, *et al.,* 2005; Haniyeh, *et al.,* 2010; Prasannabalaji, *et al.,* 2012;Adonu, *et al.,* 2013).Also, the MIC and MBC tests were determined by the macro broth dilution assay described elsewhere (Greenwood, 1989; NCCLS, 2008;Motamedi, *et al.,* 2010). These experimentswere repeated 4 times.

2. 2. 2. The phytochemical screening

The TLC plates were prepared according to Yrjönen, (2004). The PTLC was performed at the final step of the purification of the pure compound prior to the structure elucidationaccording toKalpesh, *et al.,*(2013).Also, the GC-MS technique was carried out as described elsewhere(John and Kumar, 2012; Srinivasan, *et al.,* 2013; Poongodi, *et al.,* 2015).

2. 2. 3. Toxicity of P.GLeaves in Rats

Thirty male and female rats were distributed and divided randomly into 3 groups each group containing 6 rats. Group 1 as control untreated, Group 2 and Group 3 received pure fraction of n-hexane of P.Gleaves at Dosing 5 and 2.5 ml/rat continued orally for 10 dayrespectively(Sunil, *et al.,* 2001). Each Group of rats was then housed separately in a cage. Furthermore,the clinical signs were recorded. Blood samples were taken at day zero and after 10 days of the treatments. Thereafter, the rats were sacrificed. Also, the specimens of liver, kidney, spleen and heard were collected immediately after slaughtering and were fixed in formalin 70% for histopathology.Additionally, blood samples from rats were collected into clean dry bottles containing the anti-coagulant heparin from the ocular vein. Also, the RBCs, Hb, MCVand MCHC were counted and calculated. However, the blood samples obtained from the ocular vein of rats were used to prepare sera for chemical methods such as enzymes AST, ALT and urea & serum metabolites of total bilirubin and albumin (Reitman and Frankel, 1957; Schmidt and Schmidt, 1963; Schalm, 1965).

2. 2. 4. Statistical Methods

Data were subjected to analysis of variance (ANOVA) followed by Fisher's PLSD. Data are presented as means ± SE. Differences at P<0.05 (Snedecor and Cochram, 1989).

III. Results

3. 1. Effect of EthanolicExtracts of P.GLeaves on theTested Clinical Strains

3. 1. 1. The EthanolicExtract of P.GLeaves

The ethanolic extract of P.G leaveshad a significant (p< 0. 01) inhibitory effect on *S. aureus*only, while the remaining tested strains were resistant to the same extract. When the concentrations of ethanolic extract of P.G leaves were increased, the zone of inhibition on the *S. aureus*significantly (p< 0.05) increased. The means of inhibitory zones of the different concentrations of ethanolic extracts of P.G leaveson *S. aureus* was 8.75 ± 0.48, 10.00 ± 0.41, 10.50 ± 0.29 & 11.75 ± 0.75 for 300, 400, 500 and 600 µg/ml; respectively. Also, the inhibitory effect of P.G leavesextracts on *S. aureus*was superior. Furthermore,The MIC and MBC of P.G leafextracts for *S. aureus*were equal (25 mg/ml).

3. 2. Effect of P.GLeavesFractions on Tested Clinical Strains

3. 2. 1.The n-hexane Fraction of P.GLeaves

The n-hexane fraction of P.G leaveshad a significant (p< 0.01) inhibitory effect on all the tested bacterial strains, while the fungal (*C. albicans*) strain was resistant. When the concentration of n-hexane fraction was increased, the zone of inhibition on sensitive strains significantly (p< 0.01) increased. The means of inhibitory zones of the different concentrations of n-hexane fraction of P.G leaveson *S. aureus*and *E. coli* were 13.00 ± 0.41, 14.00 ± 0.00, 14.00 ± 0.00 & 14.75 ± 0.25 and 12.75 ± 0.25, 13.50 ± 0.65, 14.75 ± 0.25 &14.75 ± 0.25 for 300, 400, 500 and 600 µg/ml; respectively. Also, the inhibitory effect of n-hexane fraction was greater to that of other fractions (Fig. 1 (a &b).

3. 2. 2. The Chloroform Fraction of P.G Leaves

The chloroform fraction of P.G leaveshad a significant (p< 0.01) inhibitory effect on *S. aureus*only. However, this fraction had no effects on the other tested strains. When the concentrations of the same fraction were increased, the zone of inhibition on the *S. aureus*significantly (p< 0.01) increased, The means of inhibitory zones of the different concentrations of chloroform fraction of P.G leaveson *S. aureus*was 8.50 ± 0.29, 8.50 ± 0.29, 9.00 ± 0.00, & 9.00 ± 0.00 for 300, 400, 500 and 600 µg/ml; respectively.

3. 2. 3. The Ethyl acetate Fraction of P.GLeaves

The ethyl acetate fraction of P.G leaveshad a significant (p< 0.01)inhibitory effect on all the tested bacterial strains, whereas the tested fungal (*C.albicans*) strain was resistant to this fraction. When the concentration of the same fraction was augmented, the zone of inhibition on the sensitive strains significantly (p< 0.01) increased. The means of inhibitory zones of the diverse concentrations of ethyl acetate fraction of P.G leaveson *S. aureus*and *E. coli* were 11.50 ± 0.65, 12.25 ± 0.25, 12.75 ± 0.48, & 13.50 ± 0.50 and 9.25 ± 0.48, 9.25 ± 0.48, 10.50 ± 0.29 &10.75 ± 0.48 for 300, 400, 500 & 600 µg/ml; respectively. The inhibitory effect of ethyl acetate fraction was higher than chloroform fraction (Fig. 1 a).

3. 2. 4. The n-butanolFraction of P.GLeaves

The fraction of n-butanol of P.G leaveshad a significant (p< 0.01) inhibitory effect on *E. coli*, whereas *S. aureus*, *P. aeruginosa*and *C. albicans* were resistant to the same fraction. When the concentrations of n-butanol fraction were increased, the zone inhibition on the *E. coli* strain, significantly high (p< 0.05) increased. The means of inhibitory zones of the different concentrations of n-butanol fraction of P.G leaveson *E. coli* was 9.75 ± 0.48, 10.75 ± 0.25, 11.00 ± 0.00 and 11.25 ± 0.25 for 300, 400, 500 and 600 µg/ml; respectively. Also, the inhibitory effect of n-butanol fraction was greater than ethyl acetate fraction (Fig. 1. b). Additionally, All the tested strains were resistant to water fraction of P.G (Fig. 1 (a, &b)).

3. 3. The MICand MBC (mg/ml) of P.GLeavesFractions Against the Sensitive Clinical Strains

The MIC of n-hexane fraction of P.G leavesfor *S. aureus*and*E. coli* were 50 and 100 mg/ml respectively, while the MBC of *S. aureus*was 100 mg/ml and *E. coli* (≥100 mg/ml). Furthermore, the MIC and MBC of chloroform fraction of P.G leavesfor *S. aureus*was equal (25 mg/ml). The MIC of ethyl acetate fraction of P.G leavesfor *S. aureus*was equal to the MBC (12.5). While, MIC of *E. coli* was (25 mg/ml) and MBC was 100 mg/ml (Table 1).

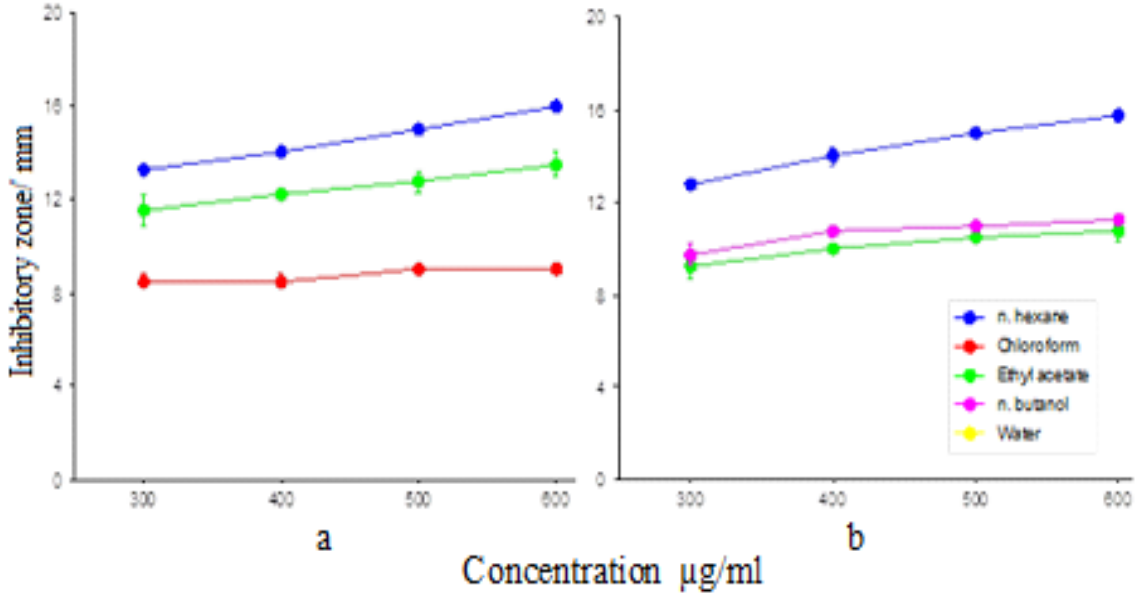

Fig. (1). Inhibitory activity of P.G leavesfractions against the tested clinicalstrains.
a. *S. aureus* b. *E. coli*

Table (1). The mean of MIC and MBC of P.G leaves fractions (Sudan origin) against the tested clinical strains.

Bacterial spp.	P.G leaves fractions									
	n-hexane		Chloroform		Ethyl acetate		n-butanol		Water	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
S. aureus	50.0 ± 0.0	100.0 ± 0.0	25.0 ± 0.0	25.0 ± 0.0	12.5 ± 0.0	12.5.0 ± 0.0	-	-	-	-
E. coli	100.0 ± 0.0	≥ 100. ± 0.0	-	-	25.0 ± 0.0	100.0 ± 0.0	-	-	-	-

3. 4. The Preparative Thin Layer Chromatography (PTLC**)**

The PTLC of n-hexane fraction of P.Gleaves had several pure bands which were determined and examined for antimicrobial for the most active band. Furthermore, the retardation factor values of the PTLCof the n-hexane fraction of P.Gleaves were recorded in the bands (1, 2, 3, and 4) was 0.43, 0.77, 0.87, and 0.97 respectively and their colors were violet, green, red and blue respectively. Moreover, the most active pure band of the antimicrobial activity of the PTLCof P.Gleaves was band number 1 violet color.

3. 5. PhytocomponentsIdentified from the Active Pure Band 1 ofn-hexane Fraction of P.GLeaves

As shown in Fig. (2 & 3) and Table (2) the GC-MS technique showed variable compounds from the pure band 1 of n-hexane fraction of P.Gleavesincludedthe following: n-hexadecanoic acid $C_{16}H_{32}O_2$; 9-Octadecenoic acid $C_{18}H_{34}O_2$, $C_{57}H_{104}O_6$ &$C_{21}H_{40}O_4$;Octadecenoic acid $C_{18}H_{36}O_2$ &$C_{19}H_{36}O_4$; 9, 12-Octadecenoic acid (Z,Z)- $C_{18}H_{32}O_2$;Palmitoyl chloride $C_{16}H_{31}O_{10}$;Hexadecanoic acid $C_{35}H_{68}O_5$ & $C_{19}H_{38}O_4$;Cyclotridecanone $C_{13}H_{24}O$;Eicosanoic acid $C_{20}H_{40}O_2$;Oleoyl chloride $C_{18}H_{33}O_{10}$; N,N'-bis(trifluoroacetyl) - N,N'-ethylene- bis(stearamide) $C_{42}H_{72}F_6N_2O_{10}$;Glycidol stearate $C_{21}H_{40}O_3$, 1, 2-Benzenedicarboxylic acid $C_{16}H_{22}O_4$; 1-Cyclohexyldimethylsilyloxybutane $C_{12}H_{26}OS_i$; 14-Beta-H- Pregna $C_{21}H_{36}$; Stigmasta-3,5-dien-7-one $C_{29}H_{46}O$ and one unknown compound. Also, the retention time, area percentage, molecular weight, compound nature and biological activity were shown in (Table 2).

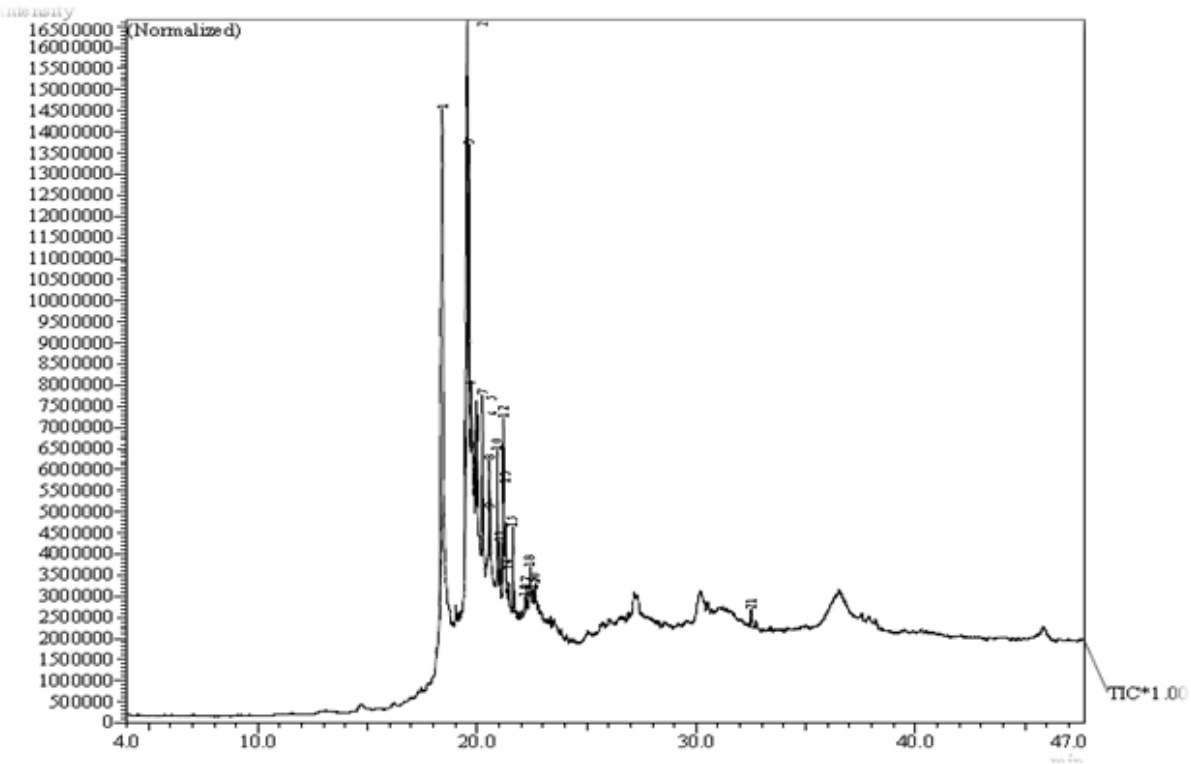

Fig. (2). Chromatogram of GC-MS analysis with pure n-hexane fraction of P.GLeaves.

Table (2). Activity of the phytocomponents identified from pure band 1 of n-hexane fraction of P.G Leaves.

Fig.	Compound Name	Mol Formula	R. time	Area%	Mol Weight	**Compound nature**	**Biological activity**
1	n- hexadecanoic acid	$C_{16}H_{32}O_2$	18.426	35.25	256	Fatty acid	Antimicrobial
2	9-Octadecenoic acid	$C_{18}H_{34}O_2$	19.534	29.48	282	Unsaturated F.A*	Antibacterial
3	Octadecanoic acid	$C_{18}H_{36}O_2$	19.643	6.99	284	Fatty acid	Antimicrobial
4	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	19.748	1.81	280	Unsaturated F.A	Antimicrobial
5	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	19.941	2.60	280	Unsaturated F.A	Antimicrobial
6	Palmitoyl chloride	$C_{16}H_{31}O_{10}$	20.016	2.12	274	Fatty acid	
7	Hexadecanoic acid	$C_{35}H_{68}O_5$	20.257	4.03	569	Unsaturated F.A	Antimicrobial
8	Cyclotridecanone	$C_{13}H_{24}O$	20.568	1.87	196		
9	Eicosanoic acid	$C_{20}H_{40}O_2$	20.609	0.34	312	Fatty acid	Antibacterial
10	Oleoyl chloride	$C_{18}H_{33}O_{10}$	20.936	3.21	300	Fatty acid	Antimicrobial
11	N,N-bis(trifluoroacetyl) - N,N'-ethylene- bis(stearamide)	$C_{42}H_{72}F_6N_2O_{10}$	21.043	0.94	783		
12	9-Octadecenoic acid	$C_{57}H_{104}O_6$	21.208	2.82	884	Fatty acid	Antimicrobial
13	Glycidol stearate	$C_{21}H_{40}O_3$	21.315	0.90	340	Fatty acid	
14	Hexadecanoic acid	$C_{19}H_{38}O_4$	21.442	0.82	330	Fatty acid	Antimicrobial
15	1, 2-Benzenedicarboxylic acid	$C_{16}H_{22}O_4$	21.654	2.10	278		Antimicrobial
16	Octadecanoic acid	$C_{19}H_{36}O_4$	22.176	0.45	312	Fatty acid	Antimicrobial
17	1-Cyclohexyldimethylsilyloxybutane	$C_{12}H_{26}OSi$	22.261	0.60	214		
18	Oleoyl chloride	$C_{18}H_{33}O_{10}$	22.437	1.00	300	Fatty acid	Antimicrobial
19	14-Beta-H- Pregna	$C_{21}H_{36}$	22.568	0.44	288		
20	9-Octadecenoic acid	$C_{21}H_{40}O_4$	22.656	0.80	356	Fatty acid	Antimicrobial
21	Stigmasta-3,5-dien-7-one	$C_{29}H_{46}O$	32.514	0.80	410	Steroids	
22	Unknown			100			

*F.A = Fatty acid, Mol = molecular, R = retention.

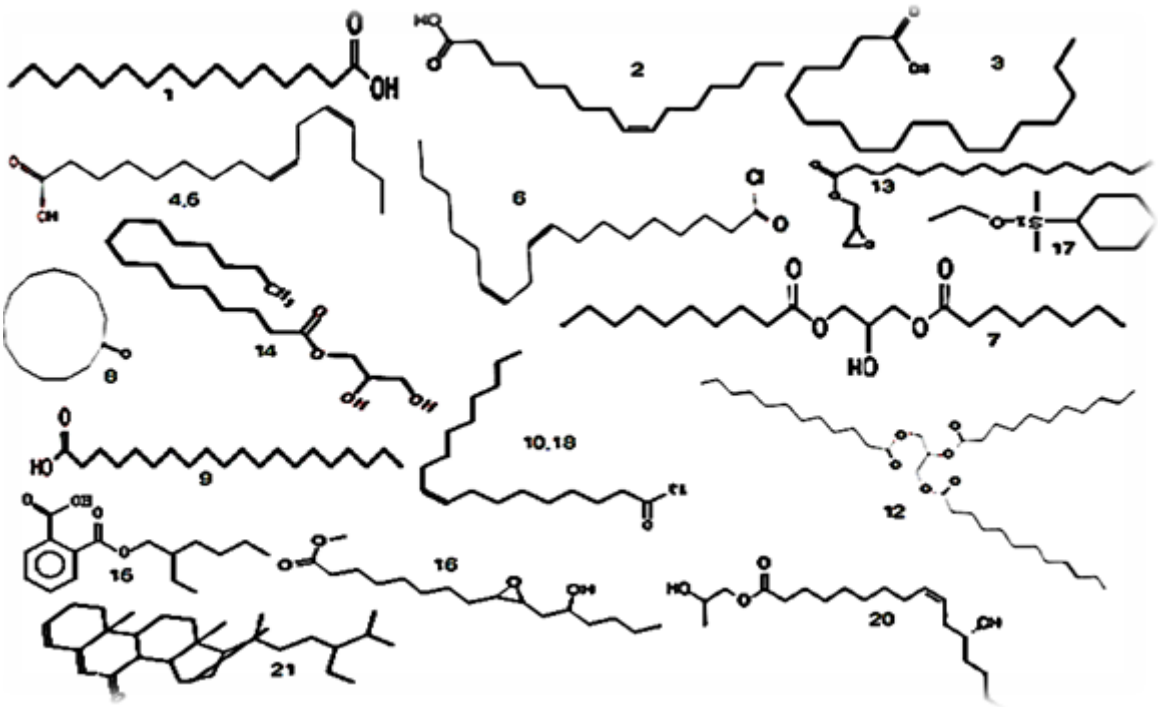

Fig. (3). The structures of pure band 1of n-hexane fraction of P.G leaves.
*The names of the structures are given in Table (2).

3. 6. Acute Toxicity of Pure n-hexane Fractions of P.G leaves in Albino Rats

No toxic reactions or mortalities were recorded at any doses of pure fractions of n-hexane of P.Gleaves. All the rats were apparent alive healthy and active during the observation period. Also, there were no apparent signs of toxicity in all groups treated and no deaths. Also, there were no pathological changes in liver, kidney, heart and spleen in rats in all treatment groups. Moreover, the serum concentration of urea, total bilirubin, albumin and serum enzymes AST, ALT at day 11were not significant ($p > 0.05$) in groups(Table 3).

After 10 days, no significant ($p > 0.05$) differences in RBCs count, WBCs, HGB, HCT, MCV, MCH and MCHC were detected in all groups and the control (Table 4). Additionally, histological sections of liver, kidney, heart and spleen of rats treated with pure fractions of n-hexane of P.Gleaves for 10 successive days showed no histological changes.

Table (3). Means levels of serum constituents in rats treated with pure fractions of P.G leaves.*

Day zero:

Groups	Urea mg/dl	T. bilirubin (mg/dl)	Albumin (g/dl)	AST (i. u/l)	ALT (i. u/l)
G1	44.50 ± 0.34	0.37 ± 0.33	4.00 ± 0.07	79.67 ± 0.49	28.67 ± 0.49
G2	43.33 ± 0.49	0.38 ± 0.05	4.10 ± 0.09	79.83 ± 0.48	28.50 ± 0.56
G3	43.50 ± 0.56	0.35 ± 0.04	4.25 ± 0.14	80.17 ± 0.60	28.33 ± 0.42

After 10 days

Groups	Urea (mg/dl)	T. bilirubin (mg/dl)	Albumin (g/dl)	AST (i. u/l)	ALT (i. u/l)
G1	44.50 ± 0.43	0.40 ± 0.03	4.15 ± 0.07	79.17 ± 0.31	28.00 ± 0.26
G2	44.17 ± 0.48	0.38 ± 0.03	4.35 ± 0.07	80.50 ± 0.76	28.17 ± 0.48
G3	44.17 ± 0.48	0.37 ± 0.03	4.18 ± 0.07	79.33 ± 0.49	29.67 ± 0.84

*Data are presented as mean ± S.E.
G1 = Control, **G2** = Pure P.G. 300 mg/kg, **G3** = Pure P.G. 150 mg/kg, 300 mg/kg = (5 mg/kg/ 6 rats×10 days), 150 mg/kg = (2.5mg/kg/ 6 rats×10 days).

Table (4). Means levels of hematological values in rats treated with pure fractions of P.G leaves.*

Day zero:

Groups	RBC (10^6/µL)	WBC (10^3/µL)	HGB (g/dl)	HCT (%)	MCV (fl)	MCH (Fg)	MCHC (g/dl)
G1	6.71 ± 0.20	7.25 ± 0.08	12.22 ± 0.06	42.57 ± 0.32	60.18 ± 0.29	17.68 ± 0.07	28. 88 ± 0.14
G2	6.77 ± 0.13	7.50 ± 0.11	12.18 ± 0.16	40.90 ± 0.84	60.43 ± 0.67	18.00 ± 0.32	29.22 ± 0.12
G3	6.78 ± 0.15	7.40 ± 0.08	12.75 ± 0.15	43.28 ± 0.83	61.18 ± 0.61	18.05 ± 014	29.48 ± 0.38

After 10 days:

Groups	RBC (10^6/µL)	WBC (10^3/µL)	HGB (g/dl)	HCT (%)	MCV (fl)	MCH (Fg)	MCHC (g/dl)
G1	6.35 ± 0.12	7.33 ± 0.26	12.33 ± 0.18	39.28 ± 1.12	63.78 ± 0.52	18.63 ± 0.42	29.20 ± 0.11
G2	6.56 ± 0.19	7.50 ± 0.29	12.53 ± 0.25	41.89 ± 0.73	63.90 ± 0.39	19.18 ± 0.31	29.25 ± 0.17
G3	6.49 ± 0.18	7.60 ± 0.19	12.25 ± 0.23	41.05 ± 0.40	63.15 ± 0.63	18.93 ± 0.26	28.53 ± 0.67

* Data are presented as mean ± S.E.

G1 = Control, **G2** = Pure P.G. 300 mg/kg, **G3** = Pure P.G. 150 mg/kg, 300 mg/kg = (5 mg/Kg / 6 rats×10 days), 150 mg/kg = (2.5 mg/kg/ 6 rats×10 days).

IV. Discussion

4. 1. EffectofEthanolic Extractsof P.GLeavesonthe Tested Clinical Strains

The present study showed that the ethanolic extract of P.G leaves has an inhibitory effect on the clinical strains of *S. aureus*. This result is in agreement with result of Egharevba, *et al.,* (2010) who reported that the leaf extract of P.Ghas a broad spectrum antibacterial activity against *S. aureus*. Moreover, lower MIC and MBC were documented for ethanolic extract of P.G leaveson the clinical strains of *S. aureus*. This finding indicated that the ethanolic extract of P.G leaveshas a good antibacterial activity. The same extract has no inhibitory effect on the clinical strains of *E. coli*. This finding is in disagreement with result of Balakrishnan**,** *et al.,* (2011) who reported thatthe ethanolic extract of P.G leaves has higher activity on *E. coli*strains. This difference could be due to the environment where the P.G leaves had been grown (ampule irrigation of P.G in Sudan may lead to dilution of the active ingredient) and/or may be due to the lower concentrations (µg/ml) used in this study and/or due to the genetic differences between the different strains.

The same extract has no inhibitory effect on the pathogenic clinical strain of *Candida albicans*. Opposing this finding Adonu Cyril, *et al.,* (2013) and Metwally, *et al.,* (2010) reported an inhibitory effect of ethanolic extract of P.G on *Candida albicans*. The difference may be due to the low concentrations (µg/ml) of P.G ethanolic extract used in this study. This finding is in agreement with result of Nwanneka, *et al.,* (2013) who reported that the presence of sapponins, tannins, glycosides and steroids in leaves of P.G are more bactericidal than fungicidal.When the concentrations of ethanolic extract of P.G leaves were increased, the zones of inhibition on the sensitive clinical strains of bacteria increased. This finding is in agreement with the findings of Mansour and Soudabe, (2012) who reported that higher concentrations induce greater inhibition.

4. 2. Effectof P.G Leaves Fractionon the Tested Clinical Strains

This study indicated that the most active fractions are n-hexane & ethyl acetate and they have prominent inhibitory effect on all the tested clinical strains of bacteria. These findings are in disagreement with the findings of Gnan and Demello, (1999); Geidam, *et al.,* (2007); Baby and Mini, (2010) who reported that n-hexane and ethyl acetate fractions of P.G have limit an inhibitory effect on the *S. aureus*. The ethyl acetate fraction has superior antibacterial activity against all tested bacteria. This is probably due to the properties of ethyl acetate fraction which contains flavonoids and tannins. These fraction are known to possess appreciable antibacterial activities (Narayana, *et al.,* 2001). Futhermore, the n-hexane and ethyl acetate fractions induced higher MIC and MBC on the clinical strains of *E. coli*. This finding agrees with the results of Hassain, (2002) and Ogbonnia, *et al.,* (2008) reported that the quantity of active ingredients of plant origin that is required to cause inhibition of bacterial growth is not a problem, since medicinal plants have been reported to have little or no side effects.

This study also stated that the chloroform fraction has inhibitory effect on the clinical strains of *S. aureus*. This finding is in agreement with finding of Jaiarj, *et al.,* (1999) who reported that the chloroform

fractions of P.Ghas antibacterial activity on *S. aureus* isolated from clinical patients. The n-butanol fraction has a visible inhibitory effect on the *E. coli*clinical strain of bacteria. The effects obtained by these fractions are similar to those obtained by the ethanolic extract of P.G leaves but these fractions have more obvious inhibitory effects. The remaining Gram negative (*E. coli*) bacteria are not susceptible to chloroform fraction. This finding confirmed that the active ingredient that can affect Gram negative bacteria are not found in fraction of chloroform.The water fraction has no inhibitory effect on all the tested clinical strains of bacteria. This finding differs with the result of El-Mahmood, (2009) who reported that the aqueous extracts of P.Gwas more potent in inhibiting the growth of pathogenic *E. coli* and *S. aureus.* This differencemay be due to low concentrations used in this study (µg/ml) and/or due to methods of fractionation used in this study; which known to be accurate than maceration method. When the concentration of all fractions was increased, the zone of inhibition on sensitive clinical strains increased. This result is in agreement with finding of Robbers, *et al.,* (1997) who reported that higher concentrations of active chemical compounds in essential oils of P.Gleaves explain their stronger inhibitory action.

4. 3. The Preparative Thin Layer Chromatography (PTLC)

The present study showed that the analysis of PTLC plate of P.G leaves hexane fraction has four pure bands with difference *Rf* values and colors. The most active pure band was isolated for the antimicrobial activity of the preparative thin layer chromatographyof P.Gleaves is the band number 1 violet color with *Rf*0.43. These finding is in disagreement with result of Hamid and Ali (2004) who reported that only two bands were isolated bypreparative thin layer chromatographyfrom *Peganumharmala* extract and the effective one with *Rf* of 0.33. This difference may be due to the variation in type of plant and/or solvents system used.

4. 4. Phytocomponents IdentifiedfromtheActive Pure Band1 of n-hexane Fraction of P.GLeaves

New seven chemical compounds were discovered by analysis of pure n-hexane fraction of P.Gleaves by GC–MS technique. These compounds are Palmitoyl chloride, Cyclotridecanone, N, N'-bis(trifluoroacetyl)-N,N'-ethylene- bis(stearamide), Glycidol stearate, 1-Cyclohexyldimethylsilyloxybutane, 14-Beta-H- Pregna and Stigmasta-3,5-dien-7-one. All these new compounds were proofed to have antimicrobial activity. Also, the isolated chemical compounds of P.Gleaves which have antimicrobial activity are 9-Octadecenoic acid (most abundant compound of P.G leaves), n- hexadecanoic acid, Eicosanoic acid, Oleoyl chloride, Hexadecanoic acid, Octadecanoic acid, 9, 12-Octadecenoic acid(Z,Z)- and 1, 2-Benzenedicarboxylic acid. This finding is superior to result of (Thenmozhi and Rajan, 2015) who reported antimicrobial activity on the 1, 2-Benzene dicarboxylic acid only.

4. 5. Acute Toxicityof Pure Fractionofn-hexane of P.G Leavesin Albino Rats

The current study showed that there are no deaths and no signs of abnormal behavior observed in the rats treated with pure fractions of n-hexane of P.Gleaves in the acute toxicity assay. Also, there is no appearance of pathological changes in the tested organs of rats in different groups compared to control. There is no significant change in serum constituents and hematological values in all different doses.

4. 6. Conclusions

In conclusion, the present study showed that the extract of P.G leaves has an antibacterial activity on *S. aureus* and *E.coli*. furthermore, increasing the extract concentrations increases its bactericidal effect. Additionally, fractions of P.G leaves produces more purifies chemical compounds that induces better inhibitory effect. These fractions contains new chemical compounds as antibacterial agents discovered (Palmitoyl chloride,Cyclotridecanone,N,N'-bis(trifluoroacetyl)-N,N'-ethylene-bis(stearamide), Glycidolstearate, 1-Cyclohexyldimethylsilyloxybutane, 14-Beta-H- Pregna, Stearic acid chloride, Stigmasta-3,5-dien-7-one.

4. 7. Recommendations

1. The new chemicals compounds of the fractionated from P.G leaves can be manufacturedas new medicines.
2. Further investigation on the effect of P.G on *C. albicans* strainmust be conducted to deny or to confirm its activity on this fungus.

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