Antimicrobial Activities of *Acalypha Wilkesiana* (Red Acalypha) Extracts in Some Selected Skin Pathogens

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ABSRACT

The study investigated the antimicrobial activities of *Acalypha wilkesiana* (Red acalypha) extracts in some selected skin pathogens. The plant leaves were collected, dried under shade, pulverized and extracted respectively with distilled water, hexane and methanol (in soxhlet extractor apparatus). The respective crude extracts were concentrated using a rotary evaporator and phytochemical screening was performed using standard methods. Isolation of oil from hexane extract was done using vacuum liquid chromatography while characterization was done by gas chromatograhy-mass spectrometry (GC-MS). The result indicated that glycoside, terpenes, and alkaloid were present in the three extract. Major components detected from the oil were 15-hydroxy pentadecanoic acid (Rt: 16.24, 9.84%), 1,2, 3-propanetriyl ester 9-octadecanoic acid (Retention time (Rt) : 17.47, 11.54%), an unsaturated fatty acid and cholesterol (Rt: 17.86, 36.13%). The antimicrobial analysis revealed zones of inhibition at a concentration of 1000mg/ml: 12.5mm (*Escherichia coli*), 17.8mm (*Pseudomonas aeruginosa*), 15.6mm (*Proteus vulgaris*), 18.5mm (*Staphylococcus aureus*) and 16 mm (*Candida albicans*) for aqueous extracts. For hexane and methanol extracts, a dose dependent activity was observed when compared with the standard control antibiotics (Ciprofloxacin). The study revealed that *A.wilkesiana* extracts contains bioactive constituents with high antimicrobial activity against skin microflora germs and could potentially posses rich medicinal values when subjected to further chemical and pharmacological studies. This study has thus corroborated the use of the plant in traditional treatment of skin bacteria by decoction baths.

Key words: *Acalypha wilkesiana,* oil, GC-MS, antimicrobial.

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1. INTRODUCTION

Acalypha wilkesiena(Red acalypha),family-*Euphorbiaceae,* is commonly referred to as red alcalypha due to its reddish colour.*Acalyphawilkesiana is* prominent in the traditional medicinal practice of most tribes in Africa and Asia (Mothana, *et al*. 2008; Duraipandiyan, *et al*. 2006; Sofowora, 1993). *Acalypha wilkesiana* is used in West Africa for the treatment of headache and cold and in Nigeria; the cold extract of the leaves is used to bath babies with skin infection (Adesina, *et al*. 2000).The leaf poultice is deemed good for headaches, swellings and colds in Trinidad (Gills, 1992)

Figure 1. *Acalypha wilkesiana*

Oyelami, *et al*. (2003) carried out a non-comparative study to evaluate the safety and efficiency of *Acalypha wilkesiana* ointment using 32 Nigerians with mycological infections as well as clinical evidence of mycosis. The ointment successfully controlled the mycosis in 73.3% of the affected patients. He concluded that *Acalypha wilkesiana* ointment can be used to treat superficial mycosis. Akinyemi, *et al*. (2006) evaluated crude extracts from six important medicinal plants, namely *Phylantus discoideus*, *Ageratum conyzoides*, *Terminalia avicennioides*, *Bridella ferruginea*, *Acalypha wilkesiana* and *Ocimum gratissimum*, to find activity against methicillin resistant *Staphylococcus aureus* (MRSA). Water and ethanol extracts of these plants were obtained locally and MRSA strains isolated from patients were used. Both ethanol and water extracts showed effects on MRSA with minimum bactericidal concentration (MBC) and minimum inhibitory concentrations (MIC) ranging from 30.4-37.0 µg/ml and 18.2-24.0 µg/ml respectively. A high MBS value was found in two plants and the other four contained traceable amounts of anthraquinones. This study provided scientific support for the use of *Acalypha wilkesiana and other leaves* against MRSA based diseases. According to Ogundaini (2005), the expressed juice or boiled decoction *A. wilkesiana* is used for the treatment of gastrointestinal disorders and fungal skin infections such as *Pityriasis versicolar, Impetigo contagiosa, Candida intetrigo, Tinea versicolor, Tinea corporis and Tinea pedis*. In Southern Nigeria, the leaves of this plant are eaten as vegetables in the management of hypertension (Ikewuchi *et al*. 2008).

The preliminary phytochemical screening of the leaves of *A. wilkesiana* revealed the presence of alkaloids, carotenoids, flavonoids (catechins and flavones), saponins and tannins all of which have potential health promoting effects, at least under some circumstances (Basu, *et al*. 2007).

Oladunmoye (2006) reported the presence of saponins, tannins, anthraquinones and glycosides in the leaves of *Acalypha wilkesiana*, while Adesina *et al* (2000) reported the presence of gallic acid, collagin, geranin, quercentin, 3-0-rutinoside and Kaempferol in the leaves of *A. wilkesiana.*

Recently, there has been much concern on medicinal plants to reduce the adverse effects of various infections due to the resistance caused by some microorganisms to some synthetic drugs and one of such plants is *Acalypha wilkesiana*is. The present study aimed determining the antimicrobial activity of *Acalypha wilkesiana* extracts in some selected skin bacteria.

2. MATERIALS AND METHODS

2.1 Sample Collection and Treatment

The leaves of *A. wilkesiana* were collected fresh from University of Benin environment, Edo state, Nigeria. The plant was authenticated by Prof. J. F. Bamidele, a taxonomist where a voucher specimen was deposited in the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria. The leaves were washed with distilled water, air-dried under shade in the laboratory for four weeks and pulverized to a powdered form. One hundred and fifty grams (150 g) of the powdered plant was macerated in one litre (1 L) of distilled water and left for 24 hours and another fifty grams (50 g) of the powdered leaves was extracted with 500ml of n-hexane for an 8-hour period in a Soxhlet extractor equipped with a reflux condenser. The powdered dry leaves remnants (from the soxhlet extractor) were re-extracted with methanol. The three extracts were concentrated separately using a rotary evaporator (Model, RE 200).

2.2 Phytochemical screening of hexane and methanol extracts

Phytochemical screening was done to identify the presence of chemical constituents such as alkaloids, cardiac glycosides, steroids, flavonoids, saponins, terpenoids, phenolics, and eugenols by using standard procedures by Sofowora (1982) and Trease and Evans (2002).

2.3 Isolation of oil from hexane extract

A 9.8 g hexane extract was partitioned with 100 ml of hexane; Methanol mixture (ratio: 8:2) and shaken vigorously in a separatory funnel. The upper hexane fraction was separated, concentrated and then subjected to vacuum liquid chromatography (VLC), using silica gel (particle size: 200-425 mesh) as the solid phase and hexane: methanol mixture (4:1) as the mobile phase. A yellow oily phase obtained was dried over Na_2SO_4 and concentrated to recover the pure oil.

2.4 Gas Chromatography – Mass Spectrometry (GC-MS) Analysis

The GC-MS of yellow oil (from hexane extract) was obtained on a Schimadzu, GCMS-QP2010.The analysis was carried out on a GC-Mass spectrometer filled with an HP-5 MS (5% phenysiloxane) column at a temperature programme of 70^{o}C (2 minutes) increase at 10^{o}C/min to 280^{o}C and held for 7 minutes. The carrier gas was nitrogen and flow rate, 1.80 mL/min.

2.5 Microorganisms

The microorganisms employed in this study were procured from the University of Benin Teaching Hospital, Benin City which includes clinical isolates of *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris and Candida albican*s

2.6 Media

Nutrient broth and Nutrient agar, all product of Himedia Laboratories Mumbai (India) were used in this study. The composition of the medium was beef extract 3.0g, peptone 5.0g, Sodium chloride 8.0 g, agar 15.0 g.

2.7 Agar Well Diffusion Assay

The antimicrobial activity of the extracts was determined by using the agar well diffusion technique (Monica, 2003). Nutrient agar plates were each seeded with 0.1ml of an overnight culture of each bacterial (10^6 CFU/ml). The 24 hours broth culture of each bacterium were used to seed sterile molten nutrient agar at 45^{0}c, allowed to set and well made by sterile standard cork borer (6.0 mm in diameter) and 200 µg (0.2 ml) of 15 mg/ml solution of extract added into each well. The bacterial plates were incubated at 37^{0}C for 24 hours, after which diameter of zones of inhibition were measured for the three extracts (hexane, methanol and aqueous) (Monica, 2006).

2.8 Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. Minimum inhibitory concentrations are important in diagnostic laboratories to confirm the resistance of microorganism to an antimicrobial agent and also to monitor the activity of a new antimicrobial agent.

The MIC values of each plant extracts were determined using two fold micro-dilution to prepare concentrations of 1000mg/mol, 500 mg/mol, 250 mg/mol, 125 mg/mol of each extract and a drop of the bacterial suspension that had been previously diluted to about 10^6 cfu/ml were aseptically incorporated into molten nutrient agar and allowed to set. The plates were incubated at 37^{0}C for 24 hours. The lowest concentration preventing visible growth for each of the test organisms was recorded as the MIC. The experiment were carried out in triplicate for

each extracts concentration and ciprofloxacin as positive control while distilled water was used as the negative control.

2.9 Determination of Minimum Bactericidal Concentration (MBC)

Minimum bactericidal concentration is the lowest concentration of antibacterial agent required to kill a particular bacterium. It can be determined from broth dilution. The MBC is identified by determining the lowest concentration of antibacterial agents that reduces the viability of the initial bacterial inoculums by ≥ 99.9%. Antimicrobial agents are usually regarded as bactericidal, if the MBC is no more than four times the MIC.

Nutrients agar plates were divided into different sections and labeled with the different concentration on the base of the plates; these were used to plate content of each MIC plate in the respective sections of the plates. The plates were incubated aerobically at 37^0C for 18-24 hrs, after which MBC were recorded. The lowest concentration of MIC tubes with no visible bacterial growth on solid medium was regarded as MBC

3. RESULTS AND DISCUSSION

3.1 Phytochemical screening

3.2 GC-MS ANALYSIS

The GC-MS chromatogram of the isolated yellow oil given in Figure 1 showed 18 peaks indicating from the search list of the chemical abstract service eighteen compounds. The chemical compounds identified in the oil fraction are presented in Table 2.

In Table 1, glycosides, tannins and terpenes were indicated in large amount in methanol extract than aqueous and hexane extract. Saponins, flavonoid and tannins were absent in hexane extract while phenolics and steroid were absent in methanol extract. These phytochemicals are useful bioactive agents that have physiological effect in man (Sofowora, 1982)

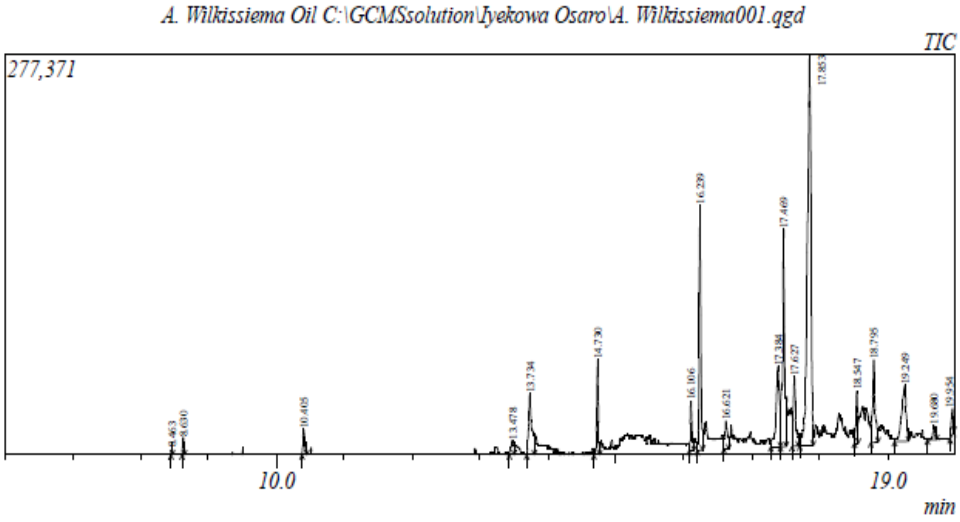
From the GC-MS analysis as shown in Table 2, the major components detected from the isolated yellow oil of *A. wilkesiana we*re 15-hydroxy pentadecanoic acid (T_R: 16.24, 9.84%), a hydroxyl acid; 2-Ethyl – 2-methyl tridecanol (T_R: 17.39, 6.96%), a long chain alcohol; pentadecanal (T_R: 18.80, 5.17%), a saturated aldehyde; n-decanoic acid (T_R: 17.47, 11.54%), a saturated fatty acid and cholesterol (T_R: 17.86, 36.13%) while minor components among others was 2-hexanal (T_R:16.11, 2.22%).

With the presence of unsaturated fatty acid ester and cholesterol (from GC-MS) which are implicated as physiological agents (Doughari 2012) suggest that the plant has rich medicinal properties. This finding is also supported by the phytochemical constituents detected in the three extracts especially with the presence of alkaloids.

The antimicrobial activity of aqueous extract of *A. wilkisiena* (Table 3) displayed activity against *S. aureus* (18.50 mm), *Escherichia coli, P. aeruginosa* (17.80 mm) *and C. albican*s (16.00 mm) as concentration decreases when compared with the standard antibiotic (ciprofloxacin). The acceptable standard zones of inhibition for sensitive organism for the antibiotic ciprofloxacin is greater than 21 mm (National committee for clinical laboratory standard (NCCLS), 1993). In Table 4, the hexane extract was only significant against *E. coli* at all doses but non-significant against *S. aureus, P. aeruginosa, P. vulgaris* and *Candida albican*s at low doses. While the methanol extract displayed the highest significant activity on all the test

organisms with inhibition zones of 10.50-23.00 mm (Table 5). This result supports the work of Alade and Irobi (1993), who stressed that extract of high polarity of *A. wilkisiena* showed antibacterial activity than others. From this study, it's only the methanol extract that compared favourably with the acceptable standard zones of inhibition against *P. aeruginosa, P. vulgaris and C. albican*s which are causative pathogens to skin infections. This findings may support the traditional use of the plant decoction in bathing children with skin diseases like necrotic lesions (caused by *P. aeruginosa*), external ear infection (caused by *P. vulgaris*) and diaper rash in babies (caused by *C. albicans*) (Dagan and Bar-David, 1992).

The minimum bactericidal concentration (MBC) were observed at 125 mg/ml for all the test organisms except *P. vulgaris* (aqueous extract, table 3) and varied with concentration of the extract (table 4) while MBC of 125 mg/ml was observed for methanol extract against all the test organisms.



Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	8.463	8.450	8.475	4434	0.16	5282	0.50	0.84		Butane, 2,2-dimethyl-
2	8.630	8.615	8.650	14456	0.52	12246	1.15	1.18		Cyclopropanecarboxylic acid,-2-methyl,-2(
3	10.405	10.380	10.450	35623	1.27	17732	1.67	2.01		Phenol, 2-amino-4-(1H-1,2,3,4-tetrazol-1-yl
4	13.478	13.435	13.505	29046	1.04	10366	0.98	2.80	V	1,2,3-Trimethyldiaziridine
5	13.734	13.690	13.805	138254	4.93	42768	4.02	3.23		n-Decanoic acid
6	14.730	14.675	14.765	99492	3.55	65931	6.20	1.51	V	Octadecanoic acid, 2-hydroxy-1,3-propaned
7	16.106	16.075	16.150	62300	2.22	34551	3.25	1.80	V	1-Hexyl-2-nitrocyclohexane
8	16.239	16.200	16.280	276101	9.84	169845	15.98	1.63	V	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-
9	16.621	16.590	16.665	49766	1.77	19455	1.83	2.56	V	1-Hexyl-2-nitrocyclohexane
10	17.384	17.280	17.420	195346	6.96	55651	5.23	3.51	V	2-Bromotetradecane
11	17.469	17.420	17.505	323802	11.54	150335	14.14	2.15	V	9-Octadecenoic acid, 1,2,3-propanetriyl este
12	17.627	17.595	17.685	118062	4.21	48113	4.53	2.45	V	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-
13	17.853	17.705	17.910	1013639	36.13	270621	25.46	3.75	V	Cholesterol
14	18.547	18.510	18.570	71342	2.54	36464	3.43	1.96	V	n-Dodecylpyridinium chloride
15	18.795	18.750	18.860	145104	5.17	57115	5.37	2.54	V	Cyclohexane, 1-(1,5-dimethylhexyl)-4-(4-m
16	19.249	19.100	19.310	175962	6.27	38739	3.64	4.54	V	2-Bromotetradecane
17	19.680	19.585	19.720	20842	0.74	10109	0.95	2.06	V	Nitric acid, nonyl ester
18	19.954	19.920	19.975	32026	1.14	17760	1.67	1.80		1-Dodecyn-4-ol
				2805597	100.00	1063083	100.00			

Figure 1. GC-MS Analysis of *A.wikisienna* oil

Table 1. Phytochemical Screening of hexane and methanol extract of *Acalypha Wilkesiana*

S/N	Phytochemical constituents	Name of Test	Aqueous extract	Hexane extract	Methanol extract
1	Glycosides	General Test	++	+	++
2	Saponin	Foam Test	+	_	+
3	Flavonoid	Lead acetate Test	+	_	+
4	Phenolics	Ferric chloride	_	+	_
5	Tannin	Ferric chloride	+	_	++
6	Eugenol	KOH/HCl	+	+	+
7	Steroid	Acetic acid/H_2SO4	_	+	_
8	Terpenes	Salkowski Test	+	+	++
9	Alkaloids	Picric acid Test	+	+	+

- = Absent, + = Present, ++ =Largely Present

Table 2. GC-MS Analysis of isolated yellow oil of *A.wikisienna*

Peak No	Retention Time (Rt)	Name of Compound	Area Percent (%)	Mol. Formula	Mol. Weight
1	8.46	2,2, dimethyl butane	0.16	C_6H_{14}	86
2	8.63	2-methyl cyclopropane carboxylic acid	0.52	$C_{11}H_{18}O_2$	210
3	10.41	2-amino-4-(1h-1,2,3,4-tetrazoy-1-yl)-Phenol	1.27	$C_7H_7N_5O$	177
4	13.48	1,2,3-trimethyl diaziridine	1.04	$C_4H_{10}N_2$	86
5	13.74	n-Decanoic acid	4.93	$C_{10}H_{20}O_2$	172
6	14.73	2,hydroxyl-1, 3-propanediylnester octadecanoic acid	3.55	$C_{39}H_{76}O_5$	624
7	16.11	2-Hexanal	2.22	$C_6H_{10}O$	98
8	16.24	15-hydroxy pentadecanoic acid	9.84	$C_{15}H_{30}O_3$	258
9	16.62	1-Tridecyn-4-ol	1.77	$C_{13}H_{24}O$	196
10	17.39	2-Ethyl-2-methyl tridecanol	6.96	$C_{16}H_{34}O$	242
11	17.47	1,2,3-Propanetryl ester 9-octadecanoic acid	11.54	$C_{57}H_{104}O_6$	884
12	17.63	1-hydroxymethyl, 1,2 ethanediyl ester hexadecanoic acid	4.21	$C_{35}H_{68}O_5$	568
13	17.86	Cholesterol	36.13	$C_{27}H_{46}O$	386
14	18.85	Oxalic acid, allylhexadecyl ester	2.54	$C_{21}H_{38}O_4$	354
15	18.80	Pentadecanal	5.17	$C_{15}H_{30}O$	226
16	19.25	Sulfurous acid, 2-propyl tridecyl ester	6.27	$C_{16}H_{34}O_3S$	306
17	19.68	2-ethyl-3-vinyl oxirane	0.74	$C_6H_{10}O$	98
18	19.96	Bicyclo (3.1.1) heptanes-3-one	1.14	$C_{10}H_{16}O$	152
		Total	**100.00**		

Table 3. Antimicrobial activity of aqueous extract of *A. wilkesiena*

Microorganisms	Minimum inhibitory concentration (MIC) (mg/ml)					
	1000 mg/ml	**500 mg/ml**	**250 mg/ml**	**125 mg/ml**	**Ciplofloxacin**	**MBC (125 mg/ml)**
	Zone of inhibition (mm)					
E.coli	12.50	12.40	10.80	9.40	12.00	9.20
P. aeruginosa	17.80	17.60	11.90	11.20	19.00	10.80
P.vulgaris	15.60	12.70	12.60	12.60	11.00	-
S.aureus	18.50	14.60	12.80	11.40	18.00	12.20
C. albicans	16.00	16.20	16.20	14.10	21.00	12.10

Key: MBC- minimum bacteriacidal concentration

(-) – no activity < 10 mm - non significant activity ; 10-19 mm – significant activity

> 20 mm – high activity (National committee for clinical laboratory standard (NCCLS), 1993)

Table 4. Antimicrobial activity of hexane extract of *A. wilkesiena*

Microorganisms	Minimum inhibitory concentration (MIC) (mg/ml)					
	1000 mg/ml	**500 mg/ml**	**250 mg/ml**	**125 mg/ml**	**Ciplofloxacin**	**MBC**
	Zone of inhibition (mm)					
E.coli	15.10	18.60	14.00	11.00	19.00	9.50 (125 mg/ml)
P. aeruginosa	10.50	10.00	0.00	0.00	16.00	8.70 (500 mg/ml)
P.vulgaris	11.00	1100	0.00	0.00	20.00	8.40 (1000 mg/ml)
S.aureus	11.10	10.10	0.00	0.00	18.00	8.90 (500mg/ml)
C. albicans	12.00	9.00	0.00	0.00	19.00	8.30 (500 mg/ml)

Key: MBC- minimum bacteriacidal concentration

(-) – no activity < 10 mm - non significant activity ; 10-19 mm – significant activity

> 20 mm – high activity (National committee for clinical laboratory standard (NCCLS), 1993)

Table 5. Antimicrobial activity of methanol extract of *A. wilkesiena*

Microorganisms	Minimum inhibitory concentration (MIC) (mg/ml)					
	1000 mg/ml	**500 mg/ml**	**250 mg/ml**	**125 mg/ml**	**Ciplofloxacin**	**MBC** (125 mg/ml)
	Zone of inhibition (mm)					
E.coli	18.00	17.50	12.70	12.30	12.00	11.30
P. aeruginosa	20.50	19.80	12.90	12.50	24.00	10.90
P.vulgaris	21.00	18,60	22.50	14,60	22.00	12.80
S.aureus	15.00	13.00	18.10	12.00	18.00	10.60
C. albicans	23.00	18.90	11.50	10.50	25.00	9.90

Key: MBC- minimum bacteriacidal concentration

(-) – no activity < 10 mm - non significant activity ; 10-19 mm – significant activity

> 20 mm – high activity (National committee for clinical laboratory standard (NCCLS), 1993)

4. CONCLUSION

The research findings have indicated that the oil of the plant may contain bioactive components like terpenes, cholesterol which have physiological effect in humans from GC-MS analysis while the methanol extract indicated the highest antimicrobial activity. Therefore, this work has corroborated the use of the plant in traditional medicine for the treatment of skin infections (from bacteria and fungi) by decoction baths on patients.

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