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ANTIMICROBIAL EFFICACY OF THE EXTRACT, FRACTIONS AND ESSENTIAL OILS FROM THE LEAVES OF *EUGENIA UNIFLORA* LINN (MYRTACEAE)

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ABSTRACT

Eugenia uniflora leaves is employed in Nigerian traditional system of medicine for the treatment of cough, bronchitis, skin and wound infections. In this study, the *in vitro* antimicrobial activities of the methanolic extract, fractions and essential oils from the leaves of *Eugenia uniflora* were investigated on some multidrug resistant pathogens. The essential oil was obtained by hydrodistillation and its chemical profile was determined through GC and GC-MS analysis while the extracts were obtained by maceration with methanol followed by a mild liquid/liquid fractionation process. Antimicrobial activity of the test samples against *Staphylococcus aureus*, *Klebsiella pnemoniae, Pseudomonas aeruginosa*, *Candida albicans* and *Trichophyton rubrum* were determined using the agar well diffusion technique. The results showed that the GC-MS analysis of the essential oil yielded fifty one compounds; with benzofuran (17.23 %) as the major compound. Ethyl acetate extract exhibited largest inhibition on *S. aureus*, *K. pnemoniae*, *P. aeruginosa, C. albicans* and *T. rubrum* with diameter mean zones of inhibition (DMZI) 25.0 ± 1.0 mm, 23.0 ± 0.4 mm, 9.0 ± 0.3 mm, 22.7 ± 0.3 mm, and 21.0 ± 0.8 mm respectively. Hexane, methanol and aqueous extracts of *E. uniflora* gave DMZI range of 8.0-21.7 mm respectively on the tested isolates while essential oil exhibited DMZI range of 7.7-18.3 mm. The minimum inhibitory concentration (MIC) of the leaf test samples from *E. uniflora* ranged between 6.25-100 mg/ml, minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) ranged between 12.5-100 mg/ml. The death rate on the most susceptible bacterial isolate (*Staphylococcus aureus*) occurred at 45 min in 0.5 ml ethyl acetate (EtOAc) fraction. The mode of action of the EtOAc fraction on *S. aureus* cell was demonstrated to be by protein leakage. The results obtained in this study have implications in the use of *E. uniflora* leaves as an antimicrobial agent and hence may provide a drug lead for pharmaceutical preparations.

Keywords: Antimicrobial efficacy, essential oil, extracts, *Eugenia uniflora*, Myrtaceae, multidrug resistant pathogens

INTRODUCTION

The use of medicinal plants as a source of relief from illness is an art that is as old as mankind. In recent times, due to the increasing resistance of bacterial isolates for antibiotics, efforts to find alternative antimicrobial agents have intensified (Ahmed *et al.*, 2012). In the last four decades, quite a number of new antibiotics have been produced but the clinical efficacy of these existing antibiotics is being threatened by the emergence of multi drug-resistant pathogens (Khond *et al.*, 2009; Awouafack *et al.*, 2013; Bagla *et al.*, 2014). According to the World Health Organization (WHO) medicinal plants would be the best source to obtain a variety of drug compounds as over 80% of individuals from developed countries use traditional medicine which has compounds derived from medicinal plants (Dzoyem *et al.*, 2014). Therefore the need to investigate medicinal plants having ethnomedicinal values with a view of understanding their properties, safety and efficacy in pursuance of the search for new and potent antimicrobial compounds and fractions is of necessity.

Eugenia uniflora Linn (Myrtaceae) is a tropical and subtropical shrub widely distributed in America but distributed all over the world as an evergreen plant. Fresh and dried leaves of *E. uniflora* is reported in folk medicine for treating cough, bronchitis, diarrhoea, influenza, rheumatism, fevers, inflammatory and stomach diseases (Consolini and Sarubbio, 2002; Costa *et al.*, 2009; Fiuza *et al.*, 2009; Lago *et al.*, 2010 and Souza-Moreira *et al.*, 2010). In Nigeria, *E. uniflora* leaves have been reported for antimalarial activities (Famuyiwa and Adebajo, 2012). Pharmacological activities of the leaf extract of *E. uniflora* indicated

inhibitory activity of the xanthine-oxidase enzyme through flavonoid action (Schmeda–Hirschmann *et al*., 1987), a reduction in blood pressure measured by direct vasodilation and a slight diuretic effect (Consolini *et al*., 1999). Extracts of *E. uniflora* leaves have been reported for volatile terpenoid oils, flavonoids, condensed and hydrolysable tannins, leucoanthocyanidins, steroids and or triterpenoids (Santos *et al*., 2004). Lee *et al*., 1997 reported eugeniflorin D1 and eugeniflorin D2, as well as two hydrolysable macrocyclic tannins, in the leaf methanolic extract of *Eugenia uniflora.* Previous reports on the essential oils of *Eugenia uniflora* indicated presence of selina-1, 3, 7(11)-trien-8-one, germacrone, furanodiene and curzerene (Fiuza *et al*., 2009; Thambi *et al*., 2013). Here we report the antimicrobial properties of the methanolic extract, three test fractions and essential oil from the fresh leaves of Eugenia *uniflora* on *Klebsiella pnemoniae* and *Trichophyton rubrum* for the first time.

MATERIALS AND METHODS

Collection and authentication of plant material

Fresh leaves of *E. uniflora* were collected from Covenant University environment, Ota, Nigeria in June, 2015. The leaves were authenticated and identified at the Herbarium section of the Department of Botany, Obafemi Awolowo University, Ile-Ife. A voucher sample of the plant was deposited with accession number 17287.

Preparation of *Eugenia uniflora* extract and solvent fractions

Fresh leaves of *E. uniflora* were rinsed with distilled water and air dried at room temperature for ten days. The sample was ground into powder, using milling machine, and stored in an air-tight container for further use. Dried powdered leaves (740 g) was extracted by maceration in a jar using methanol (MeOH, 7.4L, 1:10, w/v) at ambient temperature for seven days with constant shaking. The extract was strained, filtered using Whatmann filter paper and then concentrated to dryness *in vacuo* at 40 ^{0}C. The MeOH crude extract was dissolved in distilled water and partitioned separately using n-hexane (b.p, 65 ^{o}C) and ethyl acetate (b.p, 71 ^{o}C) to yield hexane, ethyl acetate and aqueous fractions. The aqueous fraction was lyophilized, while the hexane and ethyl acetate fractions were evaporated to dryness. The dried extract and fractions were kept in amber glass bottles and stored in refrigerator prior to use.

Extraction of essential oil from *Eugenia uniflora*

Fresh leaves of *Eugenia uniflora* (554 g) was extracted for 4 h by hydrodistillation in a Clavenger-type apparatus. The essential oil obtained was separated from water and dried over anhydrous sodium sulphate. The extract was kept in sealed sample bottle and preserved in the refrigerator prior to analysis.

Gas Chromatography and Mass Spectrometry (GC–MS) Analysis

Essential oil obtained was analyzed using Agilent GC-7890A series Gas Chromatograph equipped with a flame ionization detection (FID) and DB-1 fused silica capillary column (30 x 0.25 mm i.d, film thickness 0.25 μm). The initial oven temperature was held at 80 ^{o}C for 5 min, and increased at the rate of 15 ^{o}C/min to 250 ^{o}C. Helium was used as the carrier gas at a flow rate of 1 ml/min, and the sample size was 0.1 μl, split ratio, 50:1. The percentage composition of the essential oil was determined with a Class-GC computer programme and the relative percentages of the oil constituents were expressed as percentages by peak area normalization. The samples were diluted at a ratio 1:100, v/v in n-hexane and 1.0 μl were injected manually in the split-less mode. Identification of essential oil components was based on the GC retention indexes calculated by using n-hydrocarbons and mass spectra by computerized matching of essential oil compounds with the National Institute of Standards and Technology (NIST), Timberland Regional Library (TRLIB) and Wiley libraries.

Micro-organisms: The test organisms; *Pseudomonas aeruginosa, Klebseilla pneumoniae, Staphylococcus aureus, Candida albicans and Trichophyton rubrum* used in this study were from clinical isolates, obtained from the Laboratory stock culture of the Sacred Heart Hospital, Microbiology unit, Abeokuta, Ogun State, Nigeria. The organisms were sub--cultured on sterile agar slants and incubated at 37 °C for 48 h. and kept as stock cultures in the refrigerator. Biochemical and molecular analysis were carried

out on the test organisms for re-confirmatory purposes.

Antimicrobial screening: The modified agar well diffusion method of Irobi *et al*., (1994) as previously described by Akinpelu and Onakoya (2006) was employed to determine the antimicrobial activities of the test samples leaf extracts at 100 mg/ml**.** The bacterial and fungal isolates were grown in Mueller Hinton broth and potato dextrose broth before use. Standardized 0.5 McFarland isolates (0.1 ml) were spread onto molten Mueller-Hinton agar plates for bacteria and Mueller-Hinton agar supplemented with 2 % glucose and 0.5 % methylene blue (MH-GMB) for yeast, while the same medium without methylene blue was used for mold (Fothergill, 2012). Wells were bored into the agar medium using a sterile 6 mm cork borer after the medium have set. The wells were filled with 0.5 ml solution of the extracts and the plates were allowed to stand on the laboratory bench for 15 min before incubating at 37 °C for 24 h for bacteria and at room temperature for 72 h for fungi. The plates were observed for zones of inhibition. The effects of the extracts were compared with ciprofloxacin (standard antibiotic) and ketoconazole (standard antifungal) at a concentration of 1 mg/ml.

The minimum inhibitory concentrations (MIC) of each test sample was determined by measuring 0.5 ml of Brain Heart Infusion broth into ten rorhen test tubes. Solution of the test sample(s) was constituted in the broth to give two-fold serial dilutions. The final concentrations in each tube were 100, 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78, 0.39 and 0.195 mg/ml. Standardized isolates suspension (0.1 ml) of each test organisms was then pipetted into the tubes and test carried out for each microorganisms and test sample. Controls were set up by dispensing 0.1 ml suspension of the test organisms into a tube containing 0.5 ml of the broth without the standard drugs/test samples. All the tubes were incubated at 37 °C for 24 h for bacterial isolates and 72 h for the fungal isolates. The MIC was taken as the least concentration of the test sample that inhibited the growth of the test organisms. Based on the MIC results, the concentrations of all the test sample that showed no visible growth of the tests isolates (tubes showing no turbidity) were sub-cultured onto blood agar plates for bacteria and yeast while potato dextrose agar plates for mould. Incubation of the plates was carried out at 37°C for 24 h for bacteria and 72 h for fungi. The minimum bactericidal concentration and minimum fungicidal concentration were taken as the least concentration of all the extracts that did not show any growth on the agar plates.

Data was replicated and analyzed using One-way Analysis of variance (ANOVA). Significant differences of triplicate mean values were determined at $P < 0.05$.

Determination of killing rate: The rate of killing of the most susceptible bacterial isolate by the most active test sample, the ethyl acetate fraction was determined according to method described by Odenholt *et al*., (2001). The bacterial suspension (*S. aureus*, 0.5 ml) was added to 4.5 ml of ethyl acetate fraction, suspension was mixed thoroughly after which 0.5 ml of the mixture was transferred into 4.5 ml peptone water broth containing 3 % Tween-80 and serially diluted up to 10^{-5} in 4.5 ml sterile normal saline. The killing rate was determined over a period of 15 min for 2 h. The final dilution (0.5 ml) was transferred into labeled pre-sterilized molten Mueller-Hinton agar plates. The plates were incubated upside down at 37 °C for 72 h. Controls were set up alongside with the experiment using 0.5 ml of the test organism in 4.5 ml of normal saline and diluted out. Exactly 0.5 ml of the final dilution was spread on agar plates and incubated alongside with the experiment. The plates that showed no growth of the organism was taken as the time the antimicrobial agent killed the organism.

Determination of protein leakage assay

The mode of action of bacterial test cell by protein leakage assay was carried out following the method of Bradford (1976), with slight modifications. Bacterial suspension (*S. aureus*, 0.5 ml) was added to 4.5 ml of the ethyl acetate extract. The suspension was thoroughly mixed, and centrifuged at 7000 rpm. 0.1 ml of the supernatant was added to 5 ml protein reagent (0.01 % (v/v) Coomassie brilliant blue, G-250, 4.7 % (v/v) ethanol and 8.5 % (v/v) phosphoric acid) in a tube and the contents were mixed thoroughly.

To 0.1 ml of bacterial suspension was added to 5 ml standard protein (randox protein kit) in another tube. Both tubes were incubated for 5 min at 37 °C. To 0.5 ml of both the standard and the test were transferred into 3 ml cuvette and the absorbance at 595 nm was measured. The amount of protein in the sample was determined using the formula:

$$C\ (mg/ml) = [\{test/standard\} \times 2.0]$$

Again**,** protein assays of the supernatant obtained were carried out using colorimeter at 260 nm and 280 nm. The concentration of protein present was determined using conversion factor

Concentration (mg/ml) = $(1.55 \times A_{280}) - (0.76 \times A_{260})$, A_{280} is absorbance at 280 nm while A_{260} is absorbance at 260 nm.

RESULTS AND DISCUSSION

The physical colour and yields of extracts, fractions and essential oil from *E. uniflora* are represented in table 1. Hydrodistillation of the fresh leaves from *E. uniflora* afforded a yellow viscous oil (0.4 %) with a pungent odor. The constituents analyzed by GC-MS revealed fifty-one compounds. Benzofuran (17.23 %) was the major compound in the essential oil from *E. uniflora* followed by 1-methyl-1-n-hexyloxy-1-silacyclopentane (10.23%), 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-cyclohexane (7.07 %), cryophyllene (6.31 %), gamma-elemene (5.51 %), beta-copaene (3.88 %) and globulol (2.03 %) (Table 2).

Table 3 shows the susceptibility testing of *E. uniflora* leaf extracts on three bacterial strains (*Staphylococcus aureus*, *Klebsiella pnemoniae* and *Pseudomonas aeruginosa*) and two fungal strains (*Candida albicans* and *Trichophyton rubrum*). Though the effect of all the extracts on *Pseudomonas aeruginosa* was not significant (P >0.05), zones of inhibition were wider at 100 mg/ml with ethyl acetate extract, exhibiting more inhibition than

Table 1: Percentage yields of *E. uniflora* leaf extract and fractions.

Extract/Fractions	Physical colour	Yield (%)
Essential oil	Pale yellow	2.44
Crude methanol	black	12.68
n-hexane	brown	32.31
Ethyl acetate	black	13.82
Aqueous	black	23.48

Table 2: Chemical composition of the essential oil of *E. uniflora* leaves

SN	Compound	RT	% Peak Area
1	beta-Phellandrene	9.580	0.28
2	Alpha- Phellandrene	9.978	0.22
3	(+) - 4 – Carene	11.313	0.52
4	Beta- Ocimene	11.687	1.37
5	3,7-dimethyl-1,6-Octadien-3-ol	13.485	0.56
6	1,5,5-Trimethl-6-methylene-cyclohexene	20.590	0.23
7	1,3-Cyclohexadiene	20.910	3.48
8	7-methylene-2,4,4-trimethyl-2-vinyl-bicyclo[4.3.0] nonane	22.234	0.62
9	1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-cyclohexane	22.501	7.07
10	Caryophyllene	23.243	6.31
11	1,5-dimethyl-8-(1-methylethylidene)-1,5-Cyclodecadiene	23.350	0.26
12	1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-Naphthalene	23.439	0.18
13	Gamma-Elemene	23.611	5.51
14	Alloaromadendrene	23.718	0.34
15	Humulene	24.110	0.45
16	Aromandendrene	24.300	0.70
17	Naphthalene	24.691	0.53
18	Beta-Copaene	24.869	3.88
19	Decahydro-4a-methyl-1-methylene-7-(1-methylethenyl) naphthalene	24.988	0.64
20	1,2,4a,5,8,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl) naphthalene	25.142	0.26
21	6-ethenyl-4,5,6,7-tetrahydro-3,6-dimethyl-5-isopropenyl-benzofuran	25.344	17.23
22	1,3-Benzenediamine	25.493	13.91
23	o-(o-methoxyphenoxy)-phenol	25.599	1.23
24	1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-Naphthalene	25.950	0.44
25	1a,2,3,4,4a,5,6,7b-octahydro-1,1,4,7-tetramethyl-1H-Cycloprop[e] azulene	26.300	0.26
26	1-ethenyl-1-methyl-2-(1-methylethenyl)-4-(1-methylethylidene)- Cyclohexane	26.828	2.91
27	Alpha-Bulnesene	27.048	0.39
28	Decahydro-1,1,7-trimethyl-4-methylene-1H-Cycloprop [e] azulen-7-ol	27.309	0.42
29	(-)- Globulol	27.481	1.67
30	Decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-Naphthalene	27.677	1.29
31	Beta-Elemenone	27.944	1.10
32	Guaia-3,9-diene	28.175	0.26
33	1,4-dimethyl-3-(2-methyl-1-propene-1-yl)-4-vinyl-1-Cycloheptene	28.401	0.20
34	Tau-Muurolol	28.852	0.36

Table 2: Chemical composition of the essential oil of *E. uniflora* leaves contd.

SN	Compound	RT	% Peak Area
35	1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-Naphthalene	29.184	1.83
36	Alpha-Campholenal	29.321	0.56
37	1,4-Benzenediamine	30.051	0.44
38	10-(1-methylethenyl)-3,7-Cyclodecadien-1-one	30.158	1.56
39	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-0l	31.713	0.20
40	4-methoxyphenyl dimethyl ester	31.915	0.29
41	Alpha-Bergamotene	32.306	0.66
42	8,9-dehydro-Neoisolongifolene	33.048	0.19
43	1-Phenyl-1-azaspiro [4.5] decane	33.280	0.21
44	1,2,3,5,7-pentamethyl-1H-Indole	33.695	0.20
45	6-Ethoxyquinaldine	34.111	1.05
46	3-(6-Methoxy-3-methyl-2-benzofuranyl) acrylic acid	34.354	11.16
47	Beta-Vatirenene	34.615	0.33
48	6,7-dimethyl-1-Naphthol	35.618	0.46
49	N-(4-methoxyphenyl)-1,4-Benzenediamine	36.224	0.31
50	1-Methyl-1-n-hexyloxy-1-silaxyclopentane	36.520	10.23
51	1,2,3,4,5,6,7,8-octahydro-9,10-dimethyl-Anthracene	36.663	2.20

Table 3: In vitro antimicrobial activity of test samples from *E. uniflora* leaf extracts/fractions

Test samples	Test Organisms/ mean zone of inhibition (Diameter in mm)				
	Staphylococcus aureus	*Klebsiella pnuemoniae*	*Pseudomonas aeruginosa*	*Candida albicans*	*Trichophyton rubrum*
Crude methanol	12.7 ± 1.2	16.0 ± 1.0	8.0 ± 0.7	12.3 ± 0.5	11.6 ± 1.4
Hexane	17.3 ± 0.8	21.0 ± 1.0	8.0 ± 0.5	15.7 ± 0.8	16.3 ± 0.7
Ethyl acetate	25.0 ± 1.0	23.0 ± 0.4	9.0 ± 0.3	22.7± 0.3	21.0 ± 0.8
Aqueous	10.3 ± 0.7	11.7 ± 0.5	–	18.0 ± 0.6	9.0 ± 0.3
Essential oil	10.0 ± 0.6	9.0 ± 0.3	7.7 ± 0.2	8.0 ± 0.6	12.3 ± 0.4
Ciprofloxacin/Ketoconazole	24.0 ± 1.73	23.0 ± 1.73	20.0 ± 1.3	22.0 ± 2.0	20.0 ±1.3
Methanol (solvent)	6.0 ± 4.3	3.3 ± 4.7	–	3.3 ± 4.7	–
Hexane (solvent)	–	–	–	–	–
Ethyl acetate (solvent)	–	–	–	–	–
Distilled water	-	–	–	–	–
Pet. Ether	3.3 ± 4.7	–	–	–	–

Each value represent mean at ± S.D (n=3) at 100 mg/ml - No inhibition detected, P<0.05

Table 4: Minimum inhibitory concentration of *E. uniflora* leaf extracts

Test samples	Organisms/ MIC (mg/ ml)				
	Staphylococcus. aureus	*Klebsiella pnuemoniae*	*Pseudomonas aeruginosa*	*Candida albicans*	*Trichophyton rubrum*
Crude methanol	25.0	25.0	100.0	25.0	50.0
Hexane	12.5	12.5	50.0	25.0	50.0
Ethyl acetate	6.25	12.5	50.0	25.0	50.0
Aqueous	50.0	50.0	100.0	25.0	50.0
Essential oil	25.0	50.0	50.0	50.0	50.0
Ciprofloxacin/Ketoconazole	3.13	6.25	6.25	6.25	12.5

Table 5: Minimum bactericidal and minimum fungicidal concentrations of *E. uniflora* leaf extracts

Extracts	Organisms/Concentration (mg/ ml)				
	Staphylococcus aureus	*Klebsiella pnuemoniae*	*Pseudomonas aeruginosa*	*Candida albicans*	*Trichophyton rubrum*
Crude Methanol	50.0	50.0	100.0	50.0	50.0
Hexane	25.0	25.0	100.0	50.0	50.0
Ethyl acetate	12.5	25.0	100.0	25.0	50.0
Aqueous	100.0	100.0	100.0	50.0	100.0
Fatty oil	50.0	50.0	100.0	50.0	50.0
Essential oil	50.0	50.0	100.0	50.0	100.0
Ciprofloxacin/Ketoconazole	6.25	6.25	6.25	6.25	25.0

The results of the minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of *E.uniflora* leaf extracts are shown in table 5. The MBC of ethyl acetate extract of *E.uniflora* on *Staphylococcus aureus* ranges between 12.5 - 100 mg/ml, the MBC of the essential oils was 50 mg/ml, while MBC of extracts and essential oil of *E.uniflora* against *Pseudomonas aeruginosa* was 100 mg/ml. In fungal isolates, the MFC ranged between 25-100 mg/ml in the extracts and essential oil from *E.uniflora*.

Figure 1 shows the killing rate of ethyl acetate extract on *Staphylococcus aureus*. There were no surviving cell until 45 min.

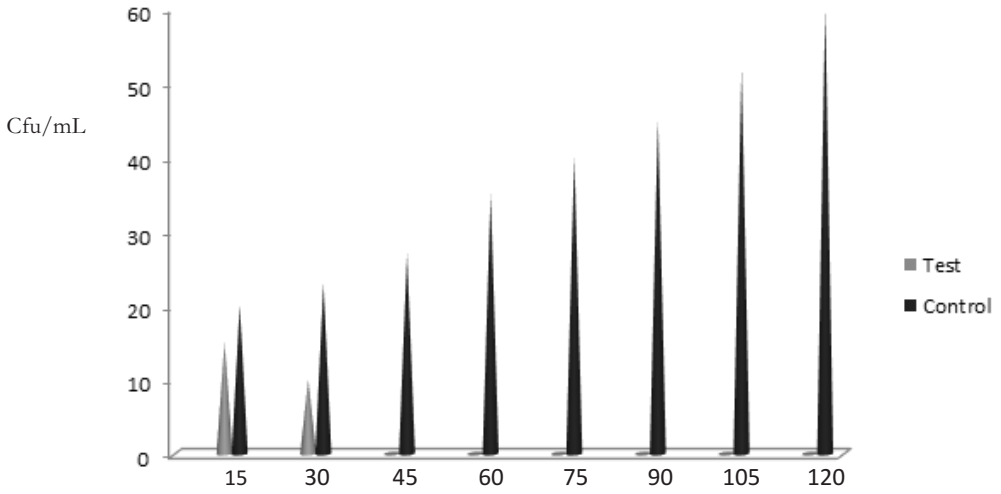

Figure 1: Killing rate of ethyl acetate extract on *S. aureus*.

The results of the mode of action of ethyl acetate extract on *S. aureus* by protein leakage assay was 0.78 ± 0.01 mg/ml (mean protein) while similar assay by colorimetry method yielded 1.5 ± 0.2 mg/ml.

The ethyl acetate fraction of *E. uniflora* exhibited highest significant inhibition on the tested strains, in comparison with other test samples. The presence of bioactive substances have been reported to confer resistance to plants against microorganisms and therefore justifies antimicrobial activity exhibited by the plant test samples used in this study. Generally, *Eugenia* genus has been reported for antimicrobial activities (Lago *et al.*, 2010).

Among the microorganisms tested on the leaf extracts of *E. uniflora, Staphylococcus aureus* (Gram positive bacterium) was more susceptible to the extracts. This corroborates the report of Jigna and Sumitra (2006), that plants extracts are more active against Gram positive bacteria than Gram negative bacteria.

Gas chromatography and mass spectrometry of

the essential oil from *E. uniflora* leaves revealed fifty-one compounds of which benzofuran (17.23 %) was the major compound, including cryophyllene, gamma-elemene and beta-copaene, as previously reported by Melo *et al*., 2007, Lago *et al*., 2011, Thambi *et al*., 2013 in *E. uniflora*. Constituents of essential oil obtained in this study compared with those reported from *E. uniflora* in Brazil, Argentina and India (Melo *et al*., 2007; Lago *et al*., 2011 and Thambi *et al*., 2013.

The bacterial and fungal strains (*Staphylococcus aureus, Klebsiella pnemoniae, Pseudomonas aeruginosa, Candida albicans* and *Trichophyton rubrum*) used in this study were susceptible to extracts obtained from *E. uniflora* leaves. Costa *et al*., 2010 reported that extracts from *E. uniflora* inhibited *Candida albicans*. However, in this report, antimicrobial activities of the leaves from *E. uniflora* on *Klebsiella pnemoniae* and *Trichophyton rubrum* are reported for the first time.

Diseases such as pneumonia, urinary and respiratory tract infections, and opportunistic infections are caused by *Klebsiella* species (Venkatesan *et al*., 2005 and De-Boer *et al*., 2005). The alcoholic leaf extract of *E. uniflora* is reported in folk medicine for treating cough and bronchitis (Costa *et al*., 2009 and Lago *et al*., 2010).

The low minimum inhibitory concentration observed for *Staphylococcus aureus* on the leaves from *E. uniflora* suggests high efficacy against this bacterium. Reports from this study agreed with traditional usage of *E. uniflora* to control infection varying from minor skin infections to severe life threatening infections such as septicemia, surgical wound infection and disseminated abscesses in all organs (Adegoke *et al*., 2010).

The results of minimum inhibitory concentration, minimum bactericidal concentration and minimum fungicidal concentration showed that the leaf extracts from *E. uniflora* have bacteriostatic and bactericidal properties.

The death rate of the most susceptible microorganism (*Staphylococcus aureus*) was low with no surviving cells observed till 45 min/0.5 ml. However, the possible mode of action of ethyl acetate extract on *Staphylococcus aureus* by protein can be speculated to be due to disruption of cell membrane integrity, leading to eventual death of the bacterial cell tested.

CONCLUSION

With the appreciable level of inhibition exhibited by the leaf extracts of *E. uniflora* against the microorganisms used in this study, it suggests that this plant is a potential source of novel antimicrobial drug that can used for the management of cough, bronchitis, skin and wound infections. This is in line with folkloric practice.

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REFERENCES

Adegoke, S. A.; Opata, O. M.; and Olajide, J. E. 2010. Antimicrobial activity of the aqueous, methanol and chloroform leaf extracts of *Cissus multistriata*. *African J. Biotech.* 9 (8):1168-1172.

Ahmed, A. S., Elgorashi, E. E., Moodley, N., McGaw, L.J., Naidoo, V. and Eloff, J. N. 2012. The antimicrobial, antioxidative, anti-inflammatory activity and cytotoxicity of different fractions of four South African Bauhinia Species used traditionally to treat diarrhea. *Journal of Ethnopharmacology* 143 (3): 826-839.

Akinpelu, D. A.; and Onakoya, T. M. (2006). Antimicrobial activities of medicinal plants used in folklore remedies in South-Western Nigeria. *African J. Biotech.* 5(11):1078-1081.

Awouafack, M. D., McGaw, L.J., Gottfried, S., Mbouangouere, R., Tane, P., Spiteller, M. and Eloff, J. N. 2013. Antimicrobial activity and cytotoxicity of the ethanol extract, fractions and eight compounds isolated from Eriosema robustum (Fabaceae). *BMC Complementary and Alternative Medicine* 13: 289-293.

Bagla, V. P., McGaw, L. J., Elgorashi, E. E. and Eloof, J. N. 2014. Antimicrobial activity, toxicity and selectivity index of two biflavonoids and a flavones isolated from Podocarpus henkelii (Podocarpaceae) leaves. *BMC Complementary and Alternative Medicine* 14: 383-390.

Bradford, M. M. 1976. A rapid and sensitive method for quantitation of microgram of protein, utilizing the principle of protein-dye binding. *Anal. Biochem.* 72:248-254.

Consolini, A. E. and Sarubbio, M. G. 2002. Pharmacological effects of *Eugenia uniflora* (Myrtaceae) aqueous crude extract on rat's heart. *J. Ethnopharmacology.* 81(1):57-63.

Consolini, A. E., Baldini, O. A. and Amat, A. G. 1999. Pharmacological basis for the empirical use of *Eugenia uniflora* L. (Myrtaceae) as antihypertensive. *J. Ethnopharmacology.* 66:33-59.

Costa, D. P., Filho, E. G. A., Silva, L. M. A., Santos, S. C.; Passos, X. S.; Silva, M. R. R.; Seraphin, J. C.; and Ferri, P. H. 2010. Influence of fruit biotypes on the chemical composition and antifungal activity of the essential oils of *Eugenia uniflora* leaves. *J. Brazilian Chem. Soc.* 21: 851-858.

Costa, D. P.; Santos, S .C.; Seraphin, J. C.; and Ferri, P. H. 2009. Seasonal variability of essential oils of *Eugenia uniflora* leaves. *Brazilian J. Chem. Soc.* 20:1287-1293.

De-Boer.; H. J.; Kool, A.; and Broberg, A. 2005. Antifungal and antibacterial activity of some herbal remedies from Tanzania. *Journal of Ethnopharmacology* 96:461-469.

Dzoyem, J. P., McGaw, L. J. and Eloff, J. N. 2014. In vitro antibacterial, antioxidant and cytotoxic activity of acetone leaf extracts of nie under-investigated Fabaceae tree species leads to potentially useful extracts in animal health and productivity. *BMC Complementary and Alternative Medicine* 14; 147-152.

Famuyiwa, F. G.; and Adebajo, C. A. 2012. Larvicidal properties of *Eugenia uniflora* leaves. *Agric. and Bio. J. North America.* 3:400-405.

Fiuza**,** T. S.; Silva, P. C.; De- Paula, J. R.; Leonice, M. F. T.; and Saboia-Morais, S. M. T. 2009. Bioactivity of crude ethanol extract and fractions of *Eugenia uniflora* (Myrtaceae) in the hepatopancreas of *Oreochromis niloticus* L. *Bio. Res.* 42:401-414.

Fothergill, A. W. 2012. Antifungal susceptibility testing: clinical laboratory and standards institute (CLSI) methods. Hall, G. S. (Editor). Interactions of yeasts, molds and antifungal agents: how to detect resistance, Springer Science +Business Media, LLC. 21pp.

Irobi, O. N.; Moo-Young, M.; Anderson, W. A.; and Daramola, S. O. 1994. Antimicrobial activity of the bark of *Bridelia ferruginea* (*Euphorbiaceae*). *Int. J. Pharma.* 34:87-90.

Jigna, P.; and Sumitra, C. 2006. *In-vitro* antimicrobial activities of extracts of *Launaea procumbns* Roxb. (Labiateae), *Vitis vinifera* L. (Vitaceae) and *Cyperus rotundus* L. (Cyperaceae). *African J. Biomed. Res.* 9(2):89-93.

Khond, M., Bhosale, J. D., Arif, T., Mandal, T. K., Padhi, M. M. and Dabur, R. 2009. Screening of some selected medicinal plants extracts for *in vitro* antimicrobial activity. *Middle-East Journal of Scientific Research* 4 (4): 271-278.

Lago, J. H. G.; Carvalho, L. A. C.; Da-Silva, F. S.; Toyama, D. D.; Favero, O. A.; and Romoff, P. 2010. Chemical composition and anti-inflammatory evaluation of essential oils from leaves and stem barks from *Drimys brasiliensis* Miers (Winteraceae). *Journal of Brazilian Chemical Society* 21:1760-1765.

Lago, J. H. G.; Souza, E. D.; Mariane, B.; Pascon, R.; and Vallim, M. 2011. Chemical and biological evaluation of essential oils from two species of Myrtaceae – *Eugenia uniflora* L. and *Plinia trunciflora*. *Molecules.* 16: 9827–9837.

Lee, M. H,; Nishimoto, S.; Yang, L. L.; Yen, K. Y.; Hatano, T.; Yoshida, T.; and Okuda, Y. 1997. Two macrocyclic hydrolysable tannin dimers from *Eugenia uniflora*. *Phytochemistry* 44: 1343-1349.

Melo, R. M.; Correa, V. F. S.; Ana- Carolina, A. C.; Ana- Luisa, P. M.; and Rezende, C. M. 2007. Identification of impact aroma compounds in *Eugenia uniflora* L. (Brazilian Pitanga) leaf essential oil. *Journal of Brazilian Chemical Society* 18 (1):179-183.

Odenholt, I.; Lowdin, E.; and Cars, O. 2001. In-vitro pharmacodynamics of telithromycin against respiratory tract pathogens. *Antimicrobial Agents Chemotherapy.* 45:23-29.

Ogunwande, I.; Olawore, N.; Ekundayo, O.; Walker, T.; and Schmidt, J. (2005). Studies on the essential oils composition, antibacterial and cytotoxicity of *Eugenia uniflora* L. *International Journal of Aromatherapy* 15:147–152.

Santos, E. C., Ribeiro, J. P., Guimaraes, D. O., Silva, M. O., Ferri, P. H. Garcia, A. C. F., Pires, J. S., Castro, A. C. M., Silva, M. R. R., Paula, J. R. (2004). Antifungal activity of Eugenia uniflora L. fractions against *Paracoccidiodes brasilienses* (splendore) Almeida. *Revista Brasileira de Plantas Medicinais* 7(1): 30-33.

Schmeda-Hirschmann, G.; Theoduloz, C.; Franco, L.; Ferro, E.; and de- Arias, A.R. 1987. Preliminary pharmacological studies on *Eugenia uniflora* leaves: xanthine oxidase inhibitory activity. *Journal of Ethnopharmacology* 21:183-186.

Souza-Moreira, T. M.; Moreira, R. R. D.; Sacramento, L. V. S.; and Pietro, R. C. L. R. 2010. Histochemical, phytochemical and biological screening of *Plinia cauliflora* (Myrtaceae) Leaves. *Revista Brasielira Farmacognosia* 20:48-53.

Srinivasan, D.; Perumalsamy, L.; Nathan, P.; and Sures, T. 2001. Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. *Journal of Ethnopharmacology* 94:217-222.

Thambi, M.; Tava, A.; Mohanakrishnan, M.; Subburaj, M.; Pradeepkumar, K. M.; and Shafi, P. M. 2013. Composition and antimicrobial activities of the essential oil from *Eugenia uniflora,* L. leaves growing in India. *International Journal of Medicine and Pharmaceutical Sciences* 4:46-49.

Venkatesan, M.; Vishwanathan, M. B.; and Ramesh, N. 2005. Antibacterial potential from Indian *Suregada angustifolia*. *Journal of Ethnopharmacology* 99: 349-352.