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Phytochemical, Cytotoxicity, Antimicrobial and Antioxidant screening of Fractions obtained from *Euadenia Trifoliata* (*Capparaceae*)

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ABSTRACT

The plant, Euadenia trifoliata belongs to the genus Euadenia in the familyCapparaceae. Phytochemical screening of the petroleum ether, ethyl acetate and methanol fractions of the plant leaves indicated the presence of alkaloids, tannins, sterols, triterpenes, cardiac glycosides, flavonoids and saponins. Free and combined anthraquinones were however absent. Brine shrimp lethality test on the fractions revealed that ethyl acetate, petroleum ether and methanol fractions were toxic with a lethality dose (LC_{50}) less than 1000 µ/ml with the ethyl acetate fraction being the most toxic at LC_{50} of 28.77 µg/ml. The antioxidant activity of E. trifoliata fractions as determined using scavenging effect on 2,2-diphenyl-1-picryhydrazyl radical (DPPH) method revealed antioxidant activity slightly lower than that of ascorbic acid and butylated hydroxyl anisole but better than that of α –tocopherol standard antioxidants. The antimicrobial assays of the fractions were carried out by agar diffusion and pour plate method against 13 bacteria and 4 fungi. All the fractions have broad spectrum antimicrobial effect at the various concentrations activities against MRSA (Methicillin-resistant Staphylococcus aureus);Staphylococcus aureus; Streptococcus pyrogenes; Escherichia coli; Salmonella typhi; Shigella dysenterea; Klebsiella pneumoniae, Bacillus subtilis; and the fungi, Candida albicans; Candida krusei; Candida tropicalis. Pseudomonas aeruginosa was resistant to petroleum ether fraction, the bacteria, C. ulcerans; *S. feacalis;P. vulgaris; P. mirabilis; and the fungi, C. stellatoidea were resistant to all the fractions.*

Keywords: *Euadenia trifoliata*, cytooxicity, phytochemical, antimicrobial, antioxidant, 2, 2-diphenyl-1-picrylhydrazyl radical.

INTRODUCTION

Medicinal plants are cheap and renewable source of pharmacologically active substances [1]. They are plants (such as shrub, creeper or tree) in which one or more of its organs contain substances which can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs [2]. Since ancient times, human beings have found remedies within their habitat and have adopted different therapeutic strategies depending upon climatic and their peculiar cultural and socio-cultural strategies. This resulted in what came to be termed as “Traditional Medicine”. In Nigeria, almost all plants are associated with some medicinal value. The application of medicinal plants especially in traditional medicine is currently acknowledged and established as a viable profession endeavor [3].

Natural products have been major source of potential new drugs [4]. The use of, and search forpotential drugs and dietary supplements derived from plants have accelerated in recent years. It is estimated that at least 25 % of all

modern medicines are derived, either directly or indirectly, from medicinal plants, primarily through the application of modern technology to traditional knowledge. In the case of certain classes of pharmaceuticals, such as antitumoral and antimicrobial medicines, this percentage may be as high as 60 %[5]. According to the World Health Organization (WHO), as many as 80 % of the World's population depend on traditional medicine for their primary health care needs and this trend is expected to continue for sometimes to come [6].

Antioxidant chemistry has played major role in the understanding of pathological role of oxidative stress in many chronic diseases such as diabetes, heart disease and cancer. Oxidative stress occurs when the balance between the formation of reactive oxygen species and amount of antioxidants is destroyed and the formation of free radicals increases. Free radicals are the causes of many serious ailments and diseases. Low levels of antioxidants, or inhibition of the antioxidant enzymes, cause oxidative stress and may damage or kill cells. It has been known that phenolic and flavonoid compounds of the plant extracts are responsible for antioxidant and antibacterial effects. These potentials mechanisms of antioxidant action make the diverse group of phenolic compounds an interesting target in the search for natural health-beneficial phytochemicals. There are numerous evidence that free radicals induce oxidative damage to lipids, proteins and nucleic acids, which eventually causes atherosclerosis, ageing, cancer, diabetes mellitue, inflammation, AIDS and several degenerative diseases in humans[7,8,9].

E. Trifoliata plant is a shrub of the dense forest attaining 4 m height and produces a trifoliate leaf with glabrous leaflet membranes of up to 15 x 30 cm. It flowers in May – June and produces fruits of about 30 cm long, each having about 15 seeds. It occurs from Ivory Coast to west Cameroons, in the lowlands and up to 1000 m altitude. In Nigeria, it is commonly found in Edo, Oyo, Ondo, Ogun, Anambra and Enugu States [10].It is called Obo yeho (Vernacular), Olikan and Osoban in Edo, Aka-ato in Ibo and Ologbokiyan in Yoruba [11]. The roots, stem, bark and fruits are used in folk medicine for tuberculosis, arthritis, otalgia, aphrodisiac, rectal prolapsed [12]. In Edo, it is used in the treatment of ear ache and inflammation [13]. The leaf is used for antiemetic and blood disorder. The root decoction is used as antidotes (venomous stings, bites, etc), gargle for gingivitis and sores in the mouth, installation of otitis and purulent ophthalmias, genital stimulants/depressants, pulmonary troubles and pain killer. The pulp root with citron, or the sap, are used in frictions for chest-troubles and kidney pains. In Southern Nigeria, the leaves are used as potherb and they are put into soups or used in cereal dishes [14]. The roots with other ingredients are used as tonic and with lemon are used as a liniment in treating pleurisy, inter-coastal pains and backache [10]. Yoruba people of Nigeria used the root as an anticonvulsant agent and in Enugu state, as cough remedy [15]. However, this plant though used in traditional medicine as herbal remedy and with a lot of pharmacological potentials has not been properly investigated.

Judging the pharmacological potential of the plant and different phytochemicals in different solvents, this research compares the efficiency of the ethyl acetate, methanol and petroleum ether fractions of *E.trifoliata* leaves. According to the literature, little or no work has been carried on the plant leaves to reveal its pharmacological potentials

MATERIALS AND METHODS

Plant collection and identification

The fresh leaves of *Euadenia Trifoliata* were collected from Ibadan, Oyo state, Nigeria, on January 6, 2012, identified and authenticated at the Herbarium in Department of Biological Sciences, Obafemi Awolowo University (OAU), Ile-Ife and a voucher number IFEHERBARIUM 16585 was given at O.A.U.

Test Organisms

The bacteria used for this research were *MRSA (*Methicillin-resistant *Staphylococcus aureus)Staphylococcus aureus; Streptococcus pyrogenes; Escherichia coli; Salmonella typhi; Shigella dysenterea; Klebsiella pneumoniae, Streptococcus feacalis; Bacillus subtilis; Corynebacterium ulcerans; Proteus vulgaris; Proteus mirabilis; Pseudomonas aeruginosa;* and the fungi, *Candida albicans; Candida krusei; Candida tropicalis* and *Candida stellatoidea* were gotten from Department of Medical Microbiology, Ahmadu Bello University, Zaria. All the micro-organisms were checked for purity and maintained in slants of agar.

Sample preparation
The plant material was weighed and air-dried indoors in a well-ventilated room for 3 weeks until the weight was constant and then powdered in a wooden mortar. The pulverized plant material was weighed, packaged, labelled and kept for further analysis.

Extraction
The plant material (2.4 kg) was extracted with redistilled methanol using Soxhlet extractor and the extract was concentrated using a rotatory evaporator. Percentage recovery of the crude extracts was calculated as percentage crude extract of plant material used. The methanol extract was then defatted with petroleum ether (PE) and partitioned into ethyl acetate (EA). The partitioning was done by first dissolving the methanol extract in distilled/deionised water (200 cm^3). The 88 g of the mixture was transferred into separatory funnel and extracted with several 150 cm^3 portions of Petroleum ether and then, ethyl acetate. Both petroleum ether and ethyl acetate fractions collected were concentrated *in-vacuo* and allowed to dry. The water residue was freeze dried and labelled as ML fraction. The three fractions (EA, PE and ML) were used for phytochemical, cytotoxicity, antioxidant and antimicrobial screening.

Phytochemical screening
The three fractions were used to test for the presence of the following plant secondary metabolites; alkaloids, carbohydrates, triterpene/steroids, flavonoids, saponins, tannins, cardiac glycosides, free and combined anthraquinones using standard methods [16,17,18].

Cytotoxicity analysis - Brine shrimp lethality test
The brine shrimp lethality test (BST) was used to predict the toxicity of the fractions [19]. Solutions of the extracts were made in their various solvents of extraction and 2 drops of DMSO was added to each solution, at varying concentrations (1000, 100, and 10 µg/ cm^3). The control was prepared by adding 2 drops of DMSO in sea water. The shrimp's eggs were hatched in sea water for 48 h at room temperature. The harvested shrimps were attracted to one side of the vials with a light source and incubated in triplicate vials with the brine shrimp larvae. Ten brine shrimp larvae were placed in each of the triplicate vials including control. After 24 h the vials were examined against a lighted background and the average number of larvae that survived in each vial was determined. The concentration at 50 % mortality of the larvae (LC_{50}) was determined using the Finney computer programme [20,21].

Antimicrobial screening methods
Preparation of culture media and fractions
Mueller Hinton agar was the medium used as the growth medium for the microbes. The 36 g of the dehydrated bacteriological culture media was weighed and dissolved in 1000 cm^3 distilled water according to the manufacturer's specification. Where necessary, gentle heat was applied to aid dissolution and the resultant suspensions was dispensed into clean conical flask and sterilized at 121^oC for 15 min in an Adelphi bench autoclave and then poured into sterilized petri dishes. The plates were allowed to cool and solidify. Stock solution of each of the fractions (4.0 mg/cm^3) was prepared from 0.04 g of the fraction dissolved in 10 cm^3 of the partition solvent. DMSO (2 drops) was added to ensure complete dissolution of the extract[22].

Antimicrobial Profile
The antibacterial screening was carried out using the agar diffusion method[22]. The prepare medium was seeded with standard inoculums (0.1 cm^3) of the micro-organism. The inoculums were then spread evenly by the use of sterile swab over the surface of the medium, the seeded plants were allowed to dry at 37 ^oC for 30 min inside incubator. A standard Cork borer 6 mm in diameter was used to cut a well at the center of each seeded medium used and the solution of the fractions (4.0 mg/cm^3 of each) was then introduced into each hole on the surface of the medium. The plates were then incubated at 37 ^oC for 24 h (for bacteria) and 27 ^oC for 2 days (for fungi), after which the plates were observed for zones of inhibition. The zones of inhibition were measured with a transparent rule and the results were recorded in mm.

Minimum Inhibitory Concentration
This was done using broth dilution method [23] modified by [24]. Mueller Hinton broth was prepared and 10 cm^3 was dispensed into test tubes and sterilized at 121 ^oC for 10 min, the broth was allowed to cool. Mac-Forland's turbidity standard scale number 0.5 was prepared to give a turbid solution. Normal saline was prepared, 10 cm^3 was dispensed into sterile test tube and the test microorganism was inoculated and incubated at 37 ^oC for 6 h. After

incubation, dilution of the micro-organism in normal saline was done until the turbidity matched that of the Mac-Forland scale by visual comparison, at this point the test microorganism have a concentration of about $1.5x10^8$ cfu/ cm^3.

Two-fold serial dilution of the fraction in the sterile broth was done to obtain the concentration of 4.0 mg/cm^3, 2.0 mg/cm^3, 1.0 mg/cm^3, 0.5 mg/cm^3 and 0.25 mg/cm^3. The initial concentration was obtained by dissolving 0.04 g of the fraction in 10 cm^3 of the sterile broth.

From the suspension of the micro-organism in normal saline, 0.1 cm^3 was inoculated into the different concentrations of the fraction in the Mueller Hinton broth. The broths were then incubated at 37 ^oC for 24 h, after which the test tubes were observed for turbidity (growth). The test tube with lowest concentration of the fraction which showed no turbidity was recorded as the Minimum inhibition Concentration (MIC).

Minimum Bactericidal Concentration

MBC/MFE was carried out to check whether the test microbes were killed or only their growth inhibited. Mueller Hinton agar was prepared according to manufacturer's instruction, sterilized at 121 ^oC for 15 min. It was poured into sterile petri-dishes. The plates were allowed to cool and solidify. The contents of the MIC test tubes in the serial dilution were sub-cultured on to the prepared plates. The plates were then incubated at 37 ^oC for 24 h for the bacteria and 27 ^oC for 2 days for the fungi , after which the plates were observed for colony growth. The MBC/MFC was the plate with lowest concentration of the fraction without colony growth [23,24].

Antioxidant activities - Scavenging Effect on DPPH

The antioxidant activity using the DPPH free – radical scavenging method**.** 0.5 M solution of2.2-diphenyl-l-picryhydrazyl radical (DPPH) solution in methanol was prepared and 3 cm^3 of this solution was mixed with 2 cm^3 of the extract solution at varying concentration (1.0 mg/cm^3, 0.5mg/cm^3 and 0.25 mg/cm^3)[25,26]. The decrease in absorption at 517 nm of DPPH was measured after 10 min of incubation. The actual decrease in absorption was measured against that of control and the percentage inhibition was calculated. The same experiment was carried out using butyrated hydroxyl anisole (BHA), ascorbic acid and α-tocopherol as standard antioxidants. All tests and analysis were run in triplicates and the averages of the obtained results were calculated. The radical scavenging activity (RSA) was calculated as the percentage inhibition of DPPH discoloration using the formula below:

$$\%RSA \text{ or } \% \text{ inhibition} = \{(A_{DPpH} - A_S) / A_{DPPH}\} \times 100$$

Where A_S=Absorbance of the solution A_{DPPH}= Absorbance of the DPPH solution[27].

RESULTS AND DISCUSSION

Phytochemical Screening

Investigations on the phytochemical screening of the leaves extract of *E.trifoliata* revealed the presence of the following secondary metabolites: alkaloid, tannins, flavonoids, saponins, cardiac glycosides and steroid (Table 1). Naturally occurring alkaloids and their synthetic derivatives have analgesic, antispasmodic and bactericidal activities[28,29], correcting renal disorders [30] and potentials for the elimination and reduction of human cancer cell lines [31]. Tannins have been reported to poses antiviral and antibacterial activities[32] and anticancer activities[33]. Flavonoids also have wide range of biological activities like antimicrobial, anti-inflamatory, anti-angionic, analgestic, anti-allergic, cytostatic and antioxidant properties [34,35,36,37].Saponins are known to possess both antimicrobial, antifungal and anti-inflamatory activities [38,39,40] and precipitate and coagulate red blood cells [41]. The leaves of *E. trifoliata*with a wide variety of these secondary metabolites could probably account for its herbal medicinal usein combating many ailments.

The presence of saponins and flavonoids supports the finding in this study that the extract of the plant may be useful in the chemotherapy of fungi infections which the antimicrobial studies revealed. The presence of steroids in the fractions of the leaves of *E.trifoliata* is of great importance and interest due to their relationship with various anabolic hormones including sex hormones [42]. Some steroidal extracts exhibited antibacterial and antiviral properties [43,44]. Therefore, these phytochemicals detected in this plant may be responsible for its antimicrobial potency and might provide a lead bio-prospecting for new pharmaceutical products of herbal origin.

Table 1: Phytochemical screening of the leaves of *Euadenia trifoliata*

	Alkaloid	Tannin	Saponin	Flavonoid	Steroid	Triterpene	C.glycoside	A.quinone
EA	+	+	+	+	-	+	+	-
ML	+	+	+	+	+	+	+	-
PE	-	-	-	-	+	-	+	-

KEY: EA = Ethyl acetate fraction, ML = methanol fraction, PE = petroleum ether fraction, C.glycoside = cardiac glycoside, A.quinone = anthraquinone

Antibacterial activity

All three (EA, ML, PE) fractions of the plant leaves showed varying degree of antibacterial activities against the tested bacterial species (Table2). Antibacterial activities of the EA and ML fractions compared favourably with that of Sparfloxacin (standard drug) and have appeared to be broad spectrum as its activities were independence on gram reaction. All the fractions have activity against *MRSA; S. aureus; S. pyrogenes; E. coli; S. typhi; S. dysenterea; K. pneumonia* and *B. subtilis* while *P. aeruginosa* was sensitive to ML and EA fraction but resistance to PE fraction. The sensitivity of *P. aeruginosa* to ML and EA fractions might be due to the presence of tannin, saponin and flavonoid in EA and MIL fractions which are absence in PE fraction. The bacteria, *C. ulcerans; S. feacalis;P. vulgaris* and *P. mirabilis* were resistant to all the fractions. All the tested bacterial were sensitive to the standard drug (Sparfloxacin) used with the exception of *MRSA.* The zone of inhibition of ML and EA fractions followed the same pattern, ranging from 20 - 32 mm with *S. dysenterea* and *B. subtilis* having the highest zone of inhibition (32 mm) while *S. typhi* give the least (20 mm). EA fraction showed most toxicity with the zone of inhibition ranging from 20 – 32 mm. ML fraction exhibited moderate activity with the zone of inhibition ranging from 20 – 27 mm and PE fraction from 12 -19 mm. The Minimum inhibitory concentration results confirmed EA fraction as the most potent fraction against the tested bacterial with MIC ranging between 0.5 – 1.0 mg/cm^3, MBC between 1.0 – 2.0 mg/cm^3 followed by ML fraction with MIC ranging between 1.0 – 2.0 mg/cm^3, MBC between 2.0 – 4.0 mg/cm^3 and then, PE fraction with MIC at 2.0 mg/cm^3, MBC at 4.0 mg/cm^3. Sparfloxacin did not show any activity against *MRSA* but PE and ML fractions displayed good bactericidal activity against this microbe with MIC at 1.0 cm^3 and MBC at 2.0 cm^3. This suggests their potential as better and alternative drugs than the known drugs that could be used in the treatment of infections caused by this stubborn micro-organism.

It is reported that *MRSA* and *S.aureus* are one of the major causes of infections in human occurring in both community and hospital [45], *P.aeruginosa* is increasingly recognized as an emerging opportunistic pathogen of clinical relevance [46] and particularly difficult to treat due to multiple resistance to available antibiotics. Also, *S. typhi* has reach worrisome scale especially in infrastructure-scale environment due to the prevalence drug resistance strains [47].The significant activities of all the three fractions against these bacterial suggests that new antibacterial drugs can be developed from the leaves of *E. trifoliata*.

Antifungal activity

The antimycotic activities of the leaves of *E. trifoliata* showed that the three fractions have a broad spectrum activity against *C. albicans; C. krusei and C. tropicalis* while *C. stellatoidea* was resistant to all the fractions. The activities of the fractions against the tested fungi was similar to that of the antibacterial activities. All the tested fungi were sensitive to the standard drugs (Fluconazole) used.

The zone of inhibition of ML and EA fractions followed the same pattern, ranging from 20 - 29 mm with *C. krusei* having the highest zone of inhibition (29 mm) while *C. tropicalis* give the least (20 mm). EA fraction showed very high activities against *C. albicans; C. krusei and C. tropicalis* with the zone of inhibition ranging from 22 – 29 mm. ML fraction exhibited moderate activity with the zone of inhibition ranging from 20 – 27 mm and PE fraction from 12 -14 mm. The susceptibility of these fungi to *E. trifoliata* fractions is significant as candidas species used are causative agents responsible for most of the mouth, skin and vaginal infections.

Generally, the EA fraction has the highest activities against both the bacterial and fungal isolates. This was closely followed by ML fraction and the least was observed in PE fraction. The ability of these fractions to inhibit the growth of most of the tested bacterial and fungal species confirmed the antimicrobial potential of the leaves of *E. trifoliata*, which make the plant leaves as an alternative candidate for drug development for the treatment of infections caused by these organisms.

Table 2: Antimicrobial activities of the leaves of *E. trifoliata*

Microbes	Zone of Inhibition (mm)				
	EA	ML	PE	SP	FL
MRSA	27	25	0	0	-
S. aureus 31	22	19	35	-	
E. coli	28	22	15	30	-
S. dysenteria	32	25	16	42	-
S. typhi	25	20	15	30	-
P. aeruginosa	24	24	0	26	-
S. pyogenes	29	25	17	30	-
B. subtilis 32	27	19	32	-	
K. pneumoniae	30	25	16	35	-
C. albicans	25	22	12	-	27
C. krusei	29	27	14	-	29
C. tropicalis	22	2.0	12	-	26

KEY: EA = ethyl acetate fraction; ML = methanol fraction; PE = petroleum ether fraction; SP = sporfloxacin; FL = fluconazole; MRSA =Methicillin-resistant Staphylococcus aureus,

Table 3: MIC and MBC regimes of the fraction of the Leaves of *E. trifoliate*

Microbes	EA (mgcm^{-3})		ML (mgcm^{-3})		PE (mgcm^{-3})	
	MIC	MBC	MIC	MBC	MIC	MBC
MRSA	1.0	2.0	1.0	2.0	2.0	4.0
S. aureus	0.5	1.0	1.0	4.0	2.0	4.0
E. coli	1.0	2.0	1.0	4.0	2.0	4.0
S. dysenteria	0.5	1.0	1.0	2.0	2.0	4.0
S. typhi	1.0	2.0	1.0	4.0	2.0	4.0
P. aeruginosa	1.0	4.0	1.0	4.0	2.0	4.0
S. pyogenes	1.0	2.0	1.0	4.0	2.0	4.0
B. subtilis	0.5	1.0	1.0	2.0	2.0	4.0
K. pneumoniae	0.5	2.0	1.0	2.0	2.0	4.0
C. albicans	1.0	2.0	1.0	4.0	2.0	4.0
C. krusei	1.0	2.0	1.0	4.0	2.0	4.0
C. tropicalis	1.0	2.0	1.0	2.0	2.0	2.0

KEY: EA = ethyl acetate fraction; ML = methanol fraction; PE = petroleum ether fraction; MIC = Minimum inhibitory concentration; MBC = Minimum bactericidal concentration

Brine shrimp lethality test

The ethyl acetate, methanol and petroleum ether fractions of the dried leaves of *E. trifoliata* were subjected to brine shrimp lethality bioassay[19]. The lethality of the fractions to brine shrimps was determined and the LC_{50} values of ethyl acetate, methanol and petroleum ether were found to be 28.77, 55.57 and 71.51 µg/ml respectively as determined by Finney computer programme. These results could probably be correlated with the presence of bioactive phytochemicals especially alkaloids which are among the major powerful poisons known [30]. These toxic chemical compounds are beneficial in the therapy of some ailments involving cell or tumour growth but their usage at high dose should be properly monitored. The ethyl acetate fraction of*E.trifoliata* is the most toxic (LC_{50} = 28.77 µg/ml) followed by methanol fraction (LC_{50} = 55.57 µg/ml) and petroleum ether fraction (LC_{50} = 71.51 µg/ml) is the least. However all the three fractions are moderately toxic and this corroborates the results of antimicrobial screening of the fractions (Table 2). There was no mortality in the negative control groups indicating the test as a valid one and the results obtained are only due to the activity of the test agents.

Antioxidant Activity

The antioxidant activities of the ethyl acetate, methanol and petroleum ether fractions of *E. trifoliate* were evaluated by two methods: scavenging effect on 2, 2-diphenyl-1-picryhydrazyl radical (DPPH) and hydroxyl radical generated by hydrogen peroxide. The results are presented in Tables 3-5.

Scavenging effects on DPPH

The free radical scavenging activity of ethyl acetate, methanol and petroleum ether fractions of the dried leaves of *E.trifoliata* was studied by its ability to reduce the DPPH, a stable free radical which accepts an electron or hydrogen radical to become a stable diamagnetic molecule [48]. Any molecule that can donate an electron or hydrogen to DPPH, can react with it and thereby bleach the DPPH absorption. The reduction in absorbance of DPPH at 517nm

caused by the fractions was measured in triplicate after 10min. At 517 nm, the absorbance of the DPPH solution (2,2-diphenyl-1-picryhydrazyl radical solution) was 0.968 nm. The maximum activities of the tested fractions were observed when compared to the standards used (Table 4). The decrease in absorption at 517 nm indicates that the fractions have hydrogen donating ability or can scavenge free radical. The percentage inhibition was calculated and the fractions gave %inhibition of 72.6 – 89.7% at 0.25 mg/ml with ethyl acetate fraction having the highest inhibition activity. At this concentration, ascorbic acid, butylatedhydroxylanisole (BHA) and α-tocopherol exhibited 91.0%, 96.2% and 29.75% inhibition respectively (Table 4). The activity of the three fractions is however lower than that of ascorbic acid and BHA but better than that of α-tocopherol. The ethylacetate fraction has the highest inhibition activity closely followed by petroleum fraction and then, methanol fraction. The free radical scavenging activities of these fractions might be due to the presence of plant secondary metabolites especially phenolic and flavonoid contents in the leaves of *E.trifoliata*[8].

Table 4: Scavenging effect of DPPH on the fractions from the leaves of E. *trifoliate*

	1.0 mgcm^{-3}		0.5 mgcm^{-3}		0.25 mgcm^{-3}	
	Absorbance	**%inhibition**	**Absorbance**	**%inhibition**	**Absorbance**	**%inhibition**
EA	0.326±0.086	66.3	0.190±0.086	80.4	0.102±0.094	89.7
ML	0.486±0.076	49.8	0.364±0.076	62.3	0.264±0.301	72.6
PE	0.350±0.029	63.8	0.200±0.362	79.3	0.124±0.275	87.2
AA	0.297±0.124	69.2	0.289±0.128	70.1	0.084±0.010	91.0
BHA	0.048±0.002	95.0	0.046±0.008	95.2	0.037±0.006	96.2
α-TL	0.704±0.007	27.2	0.704±0.003	27.3	0.680±0.002	29.8

KEY: EA = ethyl acetate fraction; ML = methanol fraction; PE = petroleum ether fraction; AA = ascorbic acid; BHA = Butylated hydroxyanisole; α-TL = α-Tocopherol

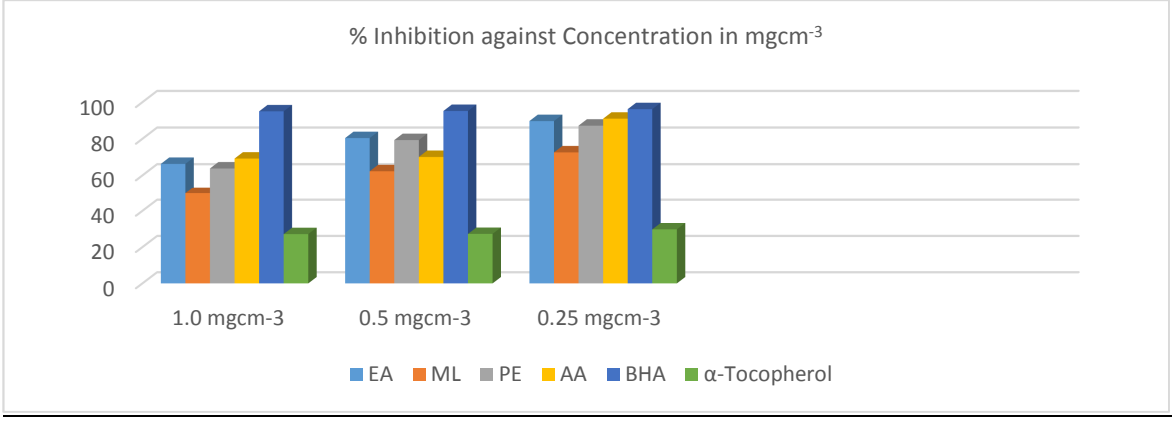

Figure 1: A Plot of % Inhibition against Concentration

CONCLUSION

Euadenia trifoliata is a plant found and used extensively as a herbal remedy in some parts of Nigeria and some countries in West Africa. The phytochemical studies of the plant leaves fractions indicated that the leaves of the plant contained alkaloid, tannins, steroids, cardiac glycosides, flavonoids, carbohydrate and saponnins. The presence of these secondary metabolites in the plant was a great importance in the understanding of the basis of the use of the plant in traditional practice. Tannins and saponnins are known for their cough suppressant effect. metimes to come [17].

Toxicity level as determined by Brine shrimp lethality test showed that the ethyl acetate fraction of the plant *E. trifoliata*is the most toxic (LC_{50} = 28.77 µg/ml) while the methanol fraction is the least toxic (LC_{50} = 71.51 µg/ml), petroleum ether fraction is moderately toxic (LC_{50} = 55.57 µg/ml). The result corroborated the presence in the plant of medicinally active compounds.

The fractions of the plant were evaluated for its biological activities and were found to possess broad spectrum antimicrobial activity against many bacteria and fungi especially *MRSA* which are known to have resistance against many known drugs.

Based upon the results obtained in this study, it was observed that ethyl acetate fraction contains considerably amount of flavonoids and phenolic compounds. Flavonoids and phenolic compounds have good antioxidant potentials and mechanism of action of flavonoids is through scavenging or chelation [49], while phenolic compounds are important because of their hydroxyl groups which confer scavenging ability [50].

These results indicate that the leaves of E. trifoliate is a significant source of natural antioxidant, which could be very useful in the treatment of ailments resulting from oxidative stress such as cancer, inflammation, Parkinson's disease, coronary heart disease, Alzheimer's disease, , cardiovascular disorders, bacterial and viral infections.

This result has adequately authenticated the ethno medicinal claims on the plant, opened way into more areas of its medicinal importance and might provide a lead bio-prospecting for new pharmaceutical products of herbal origin.

Recommendation
This research was limited to the phytochemical studies, cytotoxicity, antioxidant and antimicrobial screening of the fractions of the leaves of *E.trifoliata.* The results revealed the medicinal importance of the plant leaves especially as an antimicrobial and antioxidant agent. However, further work needs to be carried out on the plant in other to determine the active chemical constituents responsible for the observed activities.

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