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Investigation of Phenolic Contents and TLC-Direct Bioautography Screening of Four Nigeria Plant Extracts for Antioxidant Activities

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ABSTRACT

In search of lead compounds with no side effect, four medicinal plants; *Euadenia trifoliata*, *Lactuca capensis*, *Alstonia boonei* and *Ficus platyphylla* were screened for antioxidant activity. Dried leaves of the four plants were extracted with EtoAc/MeOH (1:1) and the extracts obtained were screened by TLC-Direct bioautography method (TLC-DB) to identify the most promising extract from the four plants for antioxidant property. The TLC chromatogram revealed that the extract from the leaf of *Euadenia trifoliata* was the most promising and was thus fractionated into dichloromethane, methanol and hexane soluble fractions. The fractions along with the crude extract were investigated for their phenolic contents and antioxidant potentials. Total phenolic content, TPCs, of the dichloromethane, methanol, crude and hexane extracts were 44.37, 28.83, 24.79 and 19.91 mg/g of gallic acid equivalents of extract, respectively. Antioxidant activities of the fractions using phosphomolybdate and free radical scavenging assays were in the order of methanol > dichloromethane > crude > hexane extract. The results of our finding indicated that methanol was the most suitable solvent to extract polyphenols and other antioxidant compounds from *E. trifoliata* leaves. In conclusion high polyphenols content of *E. trifoliata* leaves is an indication that the plant could potentially provide a remedy against disorders caused by oxidative stress.

Keywords: Antioxidant, *Euadenia trifoliata*, Phenolics, Radical, TLC-Bioautography

INTRODUCTION

Reactive oxygen species (ROS) are by-products of normal cell activity. They are produced in many cellular compartments and play a major role in signaling pathways. Overproduction of ROS is associated with the development of various human diseases such as diabetes, atherosclerosis, aging, immunosuppression and neurodegeneration (Ridzuan *et al.*, 2016; Malgorzata Nita and Andrzej Grzybowski 2016). Many endogenous defense mechanisms are available in living organisms to neutralize the effect of ROS (Ames *et al.*, 1993). An imbalance between ROS and the inherent antioxidant capacity of the body leads to the use of dietary and /or medicinal supplements particularly as a result of these diseases. Substantial evidence indicates that there is an inverse relationship between the dietary intake of antioxidant-rich foods and the incidence of human disease (Sies, 1993). However, there is concern that synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) used widely as antioxidants in food processing industry may cause liver damage and carcinogenesis (Grice, 1988; Wichi, 1986). For this reason, interest in the use of natural antioxidants has increased.

Large numbers of medicinal plants have been investigated for their antioxidant properties. Natural antioxidants either in the form of raw extracts or their chemical constituents are very effective in the prevention of the destructive processes caused by oxidative stress (Zengin *et al.*, 2011). It is commonly believed that medicines derived from plant products are safer than their synthetic counterparts. Although the toxicity profile of most of these medicinal plants have not been thoroughly evaluated, (Vongtau *et al.*, 2005; Oluyemi *et al.*, 2007).

E. trifoliolata (Schum. & Thonn.) Oliv. (Capparaceae) is a leafy shrub that grows up to 4 m high and is commonly found in the rain forest of West Africa (Burkill, 1995). The leaves are trifoliate, on 6 inch long or longer petioles. The decoction of the roots, stem-bark and fruits are used in the treatment of tuberculosis, arthritis, otalgia and aphrodisiac (Odugbemi, 2008). Margaret *et al* (2016) reported antinociceptive effect of leaf and stem extracts of *Euadenia trifoliata* with leaf extract showing better activity. They observed suppression of early phase of nociception in formalin test and significant activity in acetic acid-induced writhing. Phytochemical components of the leaves and stem of the plant were also studied by Margaret and her co-workers in 2016. Their results showed that total phenol, flavonoid and proanthocyanidin content of *E.*

trifoliata Leaves were 30.00 ± 0.01 mg GAE/g, 26.53 ± 0.02 mg QE/g and 18.31 ± 0.01 mg CE/g dried extract, respectively. Methanol extract of *E. trifoliata* leaves have been reported to exhibit anti bacteria activities against *Escherichia coli*, *Salmonella enteritidis*, *Pseudomonas aeruginosa and Klebsiella oxytoca* isolated from water, feed, crop, gizzard and faeces of layer chicken (Abiala *et al,* 2016). Lethality study on the ethyl acetate, methanol and petroleum ether fractions of the leaves showed LC_{50} values of 28.77 µg/ml, 55.57 µg/ml and 71.51 µg/ml for ethyl acetate, methanol and petroleum ether respectively (Kayode *et al.*, 2015)

Alstonia boonei is a medicinal plant used widely in Nigeria for the treatment of malaria, insomnia, chronic diarrhea and rheumatic pains (Ojewole, 1984; Iyiola *et al.*, 2011). Previous reports have shown that stem bark extracts possess anti-inflammatory, antipyretic, analgesic, and antimalarial properties. (Iyiola *et al.*, 2011).

Ficus platyphylla Del is one of Burkina Faso medicinal plants use in the treatment of malaria (Nadembega, *et al.*, 2011) and tuberculosis (Kubmarawa *et al.*, 2007). Stem-bark and leaves extracts of this plant have been reported to possess analgesic (Wakeel, *et al.*, 2004), antimalarial (Isma'il, *et al*., 2011), anti-inflammatory, and anticonceptive activities (Amos, *et al.*, 2002). The extracts of *Ficus platyphylla* have also been reported to inhibit gastrointestinal motility (Amos, *et al.*, 2001).

Lactuca capensis is a perennial plant that can grow up to 0.50 metres tall. Its sap contains "lactucarium", which is used in medicine for its anodyne, antispasmodic, digestive, diuretic, hypnotic, narcotic and sedative properties. *L. capensis* extract is also used in the treatment of insomnia, anxiety, neuroses, hyperactivity in children, dry coughs, whooping cough and rheumatic pain (naturalmedicinalherbs.net).

The present work was designed to screen the medicinal plants; *E. trifoliata, L. capensis, A. boonei* and *F. platyphylla*; for their antioxidant activities and to select the most potent of the four for further antioxidant investigation.

MATERIALS AND METHODS

All solvents were redistilled before use and when necessary solvents were dried according to standard procedures.

Plant collection and extraction

Euadenia trifoliata, Lactuca capensis, Alstonia boonei and *Ficus platyphylla* were collected at Akinmanrin area of Oyo in March 2018. The plant samples were identified by the taxonomist at the Department of Botany University of Ibadan, Nigeria.

The plants were air-dried, pulverised and kept at room temperature until extraction. Each pulverized sample, 250 g was extracted with 1:1 MeOH: EtOAc with occasional stirring and shaking for 48 hours. At the end of extraction the extracts were filtered and concentrated on rotary evaporator (RE-52A) under reduced pressure.

TLC-DB

TLC separation-Chemical constituents of the extracts were analyzed by thin layer chromatography using pre-coated commercial aluminium TLC plates (Merck, silica gel 60 F_{254}) Crude extracts of the four plants were spotted manually on 8 x 10 cm TLC plate and was carried out in triplicates. Each of the three plates was developed with one of the three eluent systems, ethyl acetate/methanol/water (40:5.4:5): [EMW] (polar/neutral); chloroform/ethyl acetate/formic acid (5:4:1): [CEF] (intermediate polarity/acidic); benzene/-ethanol/ammonium hydroxide (90:10:1): [BEA] (non-polar/basic), as developed by Kotze and Eloff, (2002). Development of the chromatograms was in a closed tank in which the atmosphere had been saturated with the eluent vapour by lining the tank with filter paper wetted with the eluent. After chromatographic separation, the adsorbent layers were dried at room temperature for 2 hours. For antioxidant activity, each of the chromatograms was sprayed with 0.2%, 2, 2, diphenyl-1-picryl-hydrazyl (DPPH) in methanol (Derby and Margotteaux, 1970). The presence of antioxidant compounds was detected by yellow spots against a purple background on TLC plates sprayed with 0.2% DPPH in methanol.

Fractionation of *E. trifoliata* leaves crude extract

Crude extract of *E. trifoliata* leaves, 18. 02 g, was extracted successively with hexane, dichloromethane and methanol to obtain hexane (6.09 g), dichloromethane (3.45 g), methanol (2.16 g) and residual fraction (1.78 g). All fractions were filtered separately through Whatman No. 41 filter paper to remove particles. The particle free fractions were concentrated on rotary evaporator (RE-52A) under reduced pressure. Air-dried fractions were kept at 4 ^{o}C until further used.

Radical scavenging assay

Free radical scavenging activities of the fractions were determined according to the method described by Manzocco *et al*., (1998). Two milliliter of DPPH solution (0.5 mM) was added to 0.2 mL of various concentrations (0.4-1 g/mL) of each of the fraction. The mixture was shaken vigorously and left for 30 minutes to stand in the dark before the absorbance was taken on UV visible spectrophotometer (GS V11) at 517 nm. The percentage of DPPH radical inhibition was calculated by equation 1.

$$\%\, Inh.of\ Free\ Radical = \frac{(A_{br} - A_{ar})}{A_{br}} \quad (1)$$

where A_{br} is the absorbance before reaction and A_{ar} is the absorbance after reaction has taken place.

Total phenolic content

Phenolic content of the fractions were determined using Folin-Ciocalteau reagent. Each fraction, 1 mL (1 mg/ mL) was added to 1 mL Folin-Ciocalteau reagent and left to stand for 5 min. After standing for 5 min, 10 mL solution of Na_2CO_3 (7%) was added to the mixture followed by 13 mL deionized water and the mixture was shaken thoroughly. The mixture was kept in the dark at room temperature for 90 min. before the absorbance was measured on UV visible spectrophotometer (SG V11) at 750 nm (Kim *et al*., 2003). The experiment was carried out in triplicate using gallic acid as the standard for calibration curved. Total phenolic was express as milligram of gallic acid equivalent (GAE) per gram of dried sample.

Total antioxidant capacity

Total antioxidant capacity of each fraction was determined by phosphomolybdate method (Umamaheswari and Chatterjee, 2008). The extract reduced $Mo^{(vi)}$ to $Mo^{(v)}$ to form a green $Mo^{(v)}$ complex in acidic medium. An aliquot of 0.1 mL (0.4-1 mg/mL) of each fraction was added to1 mL reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) in separate test tube. The test tubes were covered and incubated in water bath at 95 ^oC for 90 min. The absorbance of each sample was measured at 695 nm against blank after the sample had cooled down to room temperature. Blank was 1 mL reagent with 0.1 mL of solvent used to dissolve sample. Ascorbic acid was used as a standard. Total antioxidant capacity (TAC) was determined by equation 2.

$$TAC\ \% = \frac{(A_{control} - A_{sample})}{A_{control}}\ x\ 100 \quad (2)$$

RESULTS AND DISCUSSION

TLC Bioautography Assay

To determine the antioxidant capacity of the four plants; *E. trifoliolata, A. boonei, L. capensis* and *F. platyphylla*, a TLC-DPPH screening method was carried out. After separation on TLC plates, the compounds with radical scavenging activity were determined *in situ* with DPPH reagent. As presented in Figures 1a-1c, the samples producing yellowish spots on the purple background were considered as antioxidants. Usually, the purple background colour was visualized after spraying the plate with DPPH reagent (Ruiz-Terán *et al*., 2010; Rumzhum, *et al*., 2012). *Euadenia trifoliata* leaf, EL, gave most promising bands in the two of the media (BEA and EMW) as indicated by the bright yellow colour noticed in Figure 1a and 1b against the purple background after spraying with DPPH. *A. boonei* leaf, SL, also showed antioxidant activity especially in BEA, figure 1a but not as prominent as that of EL. The extract of *Lactuca capensis*, LC, showed active compounds with less activity only in EMW medium, figure 1b. *Ficus platyphylla* back and *Euadenia trifoliata* stem showed no activity in all media. All the four extracts were poorly separated when developed with CEF solvent system, Figure 1c.

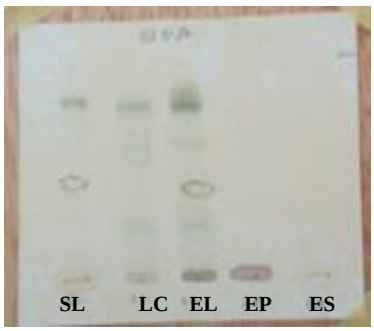


Fig. 1a

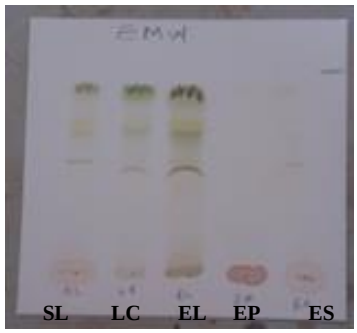


Fig. 1b

Fig. 1c

Fig 1a (BEA): Benzene/ethanol/ammonium hydroxide (90:10:1) non-polar/basic
Fig 1b (EMW): Ethyl acetate/methanol/water (40:5.4:5) polar/neutral
Fig 1c (CEF): Chloroform/ethyl acetate/formic acid (5:4:1) medium polar/acidic

SL = *Alstonia boonei* leaf; LC = *Lactuca capensis* EL = *Euadenia trifoliata* leaf; EP = *Ficus platyphylla* back; ES = *Euadenia trifoliata* stem

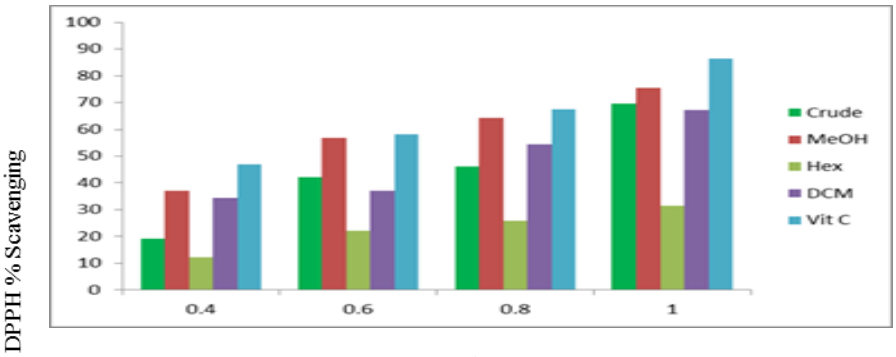
Antioxidant activity

The antioxidant capacities of EL, fractions were measured spectrophotometrically through DPPH free radical scavenging and phosphomolybdenum methods

Free radical scavenging activity of EL was quantified by measuring its scavenging capacity on DDPH free radical. The order of scavenging activity of EL fractions was as presented in figure 2. The methanol fraction showed a higher activity than both crude (1:1 EtoAc/MeOH of *Euadenia trifoliata* leaf) and dichloromethane fraction, which, in turn, were more active than hexane fraction. Both crude and dichloromethane fraction compete favourably with each other, at 0.6 mg/mL crude extract showed higher activity while at 0.8

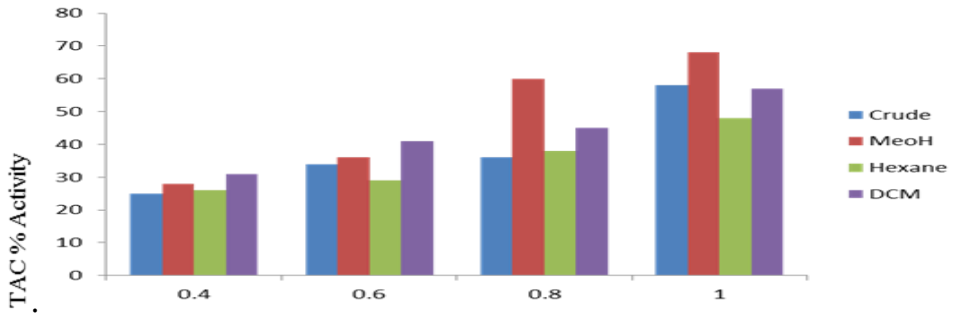
mg/mL dichloromethane fraction was higher in activity. At all concentrations the standard, ascorbic acid, showed higher activity than all the four fractions.

The total antioxidant capacity (figure 3) revealed that methanol fraction showed a higher activity than the other three fractions, while the hexane fraction was the least active. Dichloromethane fraction exhibited a slightly higher activity than crude extract in all concentrations except at 1 mg/mL. This order of activity from polar methanol fraction to nonpolar hexane fraction is quite normal since the polar solvents have a much stronger ability to dissolve and hence extract polar phytochemicals. Antioxidant activities of the fractions were dose dependent.



Concentration mg/mL

Figure 2. DDPH radical scavenging activity of different fractions of *Euadenia trifoliata* leaf

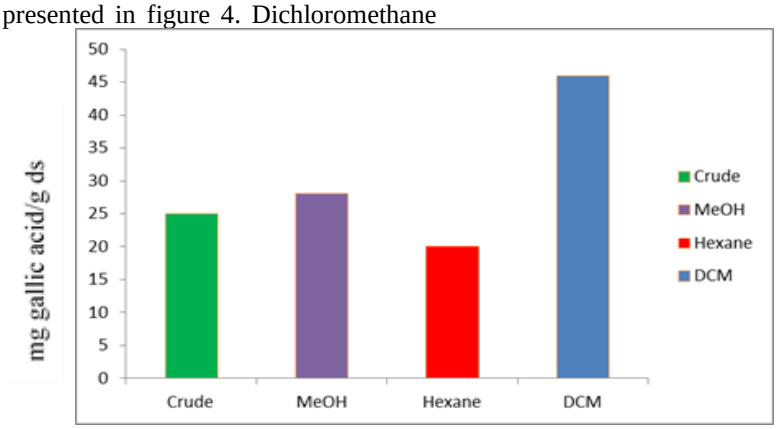


Concentration mg/mL

Figure 3. Total antioxidant capacity of different fractions of *Euadenia trifoliata* leaf

Phenolic content

Total phenolic content of the different fractions of EL fractions was solvent dependent and expressed as mg/g of gallic acid equivalent (GAE) of dried sample as presented in figure 4. Dichloromethane fraction had the highest TPC as shown in Figure 4 followed by the methanol fraction, with the hexane fraction showing the lowest TPC.



Fractions

Figure 4. Total phenolic content of different fractions of *Euadenia trifoliata* leaf

CONCLUSION

In the present study, four Nigeria medicinal plants were screening for antioxidant activity and most promising of the four plants, *E. trifoliata* leaves, fractionated for further antioxidant assay. Free radical activity and total phenolic content of *E. trifoliata* leaves fractions indicated the presence of antioxidant compounds in crude, methanol and dichloromethane fractions. Methanol fraction contained the highest percentage of antioxidant compounds as indicated by its highest activity in most of the assays. On the basis of results of this study it is clear that methanol extract of *E. trifoliata* leaves can be used as a potential source of easily accessible natural antioxidants as well as in pharmaceutical applications. Further studies are required in order to isolate and identify the antioxidant components of the methanol extract.

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