Antioxidant Activities and Estimation of Phenol and Flavonoid Contents in The Extracts Of Trema Orientalis Linn Blume

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SUMMARY
Phytochemical analysis is important in the evaluation of bioactive compounds from plants. Oxidative stress has been implicated in the pathology of many diseases such as atherosclerosis, rheumatoid arthritis, cancer, cataract, diabetes, cardiovascular diseases, chronic inflammatory conditions, and stroke. The aim of this study was to evaluate polyphenolic content and antioxidant activity of Trema orientalis. Antioxidant activity was estimated spectrophotometrically using 2,2-diphenyl-1-picrylhydrazyl radical scavenging method. The total polyphenolic and flavonoid contents of the Trema orientalis extracts were determined using standard methods. Independent Sample T–test was used for Data analyses. Phytochemical screening revealed the presence of saponins, tannins, steroids, cardiac glycosides, alkaloids, triterpenes, flavonoids and phenolic compounds. Total phenolic contents were found to be 260.96±2.31 mg GAE/g and 134.08±0.56 mg GAE/g in the ethanol and aqueous extracts respectively. Similarly, total flavonoid contents were between 32.71±0.89 and 4.70±0.23 mg GAE/g. The radical scavenging effect was observed in ethanol extract with IC50 = 9.27 µg/mL. The abundance of polyphenolic compounds and antioxidant activities of the T. orientalis could confirm their good therapeutic potentials in ethnobotany.

Keywords: Trema orientalis, Phenols, Flavonoids, Antioxidant activities

INTRODUCTION
Phenolic compounds are classes of chemicals consisting of a hydroxyl group (–OH) bonded directly to an aromatic hydrocarbon group. They comprise of flavonoids, phenolic acids, tannins, lignans, coumarins, xanthones, among others (Kuete, 2013; Ngameni et al., 2013; Shahat and Marzouk, 2013; Tsopmo et al., 2013). Free
radicals in living systems, drugs and food, are produced primarily through oxidative process (Pourmorad et al., 2006). Antioxidants fight against free radicals. The electron donation ability of natural products can be measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH) purple–colored solution bleaching (Nunes et al., 2008). About 80% of world’s population rely on traditional medicines most of which involve the use of extracts of plants with Trema orientalis Linn. Blume among the most used plants (Sandhya et al., 2006; Panchal et al., 2010; Abiodun et al., 2011; Adinortey et al., 2013).

Trema orientalis is an evergreen flowering plant of the hemp family, Ulmaceae (Adinortey et al., 2013). The leaves vary from 0.012 – 0.072 m wide and 0.020 – 0.20 m long, and taper from the base to apex (FAOSTAT, 2008). Flowers are small, greenish and inconspicuous, carried in short, and dense bunches. The fruits are small (3 – 5 mm long), round, and dark green or purple drupes and change to black when ripe; carried on very short stalks (Wagner et al., 1999; FAOSTAT, 2008). The common names of the plant include charcoal–tree (English), telemukwu (Igbo), afefe (Yoruba), menarong (Malay) and chikan (Hindi) (Malan and Notten, 2005; GRIN, 2007; Orwa et al., 2009). In Africa and some parts of Asia, livestock farmers use various parts of T. orientalis for fodder as feed to cattle, buffaloes and goats because of its high crude protein content and palatability (Motooka et al., 2003; Orwa et al., 2009; Holmström, 2013). The present study aims to quantitatively estimate total phenols, flavonoids and determine the antioxidant potential of T. orientalis.

MATERIALS AND METHODS

Chemicals
Solvents and reagents used in the current study were of analytical grade. Ethanol (95%), tetraoxosulphate (VI) acid (H2SO4), Iron (III) chloride (FeCl3) and dimethylsulfoxide (DMSO) were obtained from R&M Chemicals Co., Essex, UK. Standards acids (garlic acid, GA), Quercetin (98% HPLC) and DPPH were obtained from Sigma-Aldrich Chemicals Co., St Louis, USA. Folin–Ceocalteur’s (FC’s) phenol reagent, NaHCO3, AlCl3 Water (HPLC Gradient grade) was purchased from Loughborough, Leics, UK.

Collection, Identification and Preparation of Leaf Sample
Wild Trema orientalis leaves were collected in November, 2014 in Pasir Akar, Besut, Terengganu, Malaysia. The leaf sample was authenticated by Nashriyah Binti Mat, a Professor of botany at the School of Agricultural Sciences and Biotechnology, Faculty of Bioresources and Food Industry (FBIM), University Sultan Zainal Abidin (UniSZA), Tembila Campus, Besut Terengganu, Malaysia. A voucher specimen was deposited at the Herbarium of School of FBIM, UniSZA, with Voucher No.: 00267. The sample was gently pre–washed using tap water to remove impurities and air dried at ambient temperature until a constant weight was obtained. The dried leaves were then crushed and pulverized to obtain fine homogeneous powder using a Laboratory blender (HGB550, USA). This improved the kinetics of analytic extraction and increased the contact of sample surface with the solvent system. Proper actions were taken to ensure that potential active constituents were not lost, distorted or destroyed during the preparation of the extract.

Determination of Percentage Loss on Drying
Percentage loss on drying (% LOD) was determined gravimetrically according to Geneva, (1998). Five grams of accurately weighed air–dried leaf sample was placed in a previously dried and tared flat weighing bottle. The sample was dried in an oven (Memmert UN 110, Germany) at 105 °C until a constant weight was obtained. The % LOD was calculated using the equation below;

\[
\text{% LOD} = \left( \frac{\text{Loss in Weight}}{\text{Weight of Dried Sample}} \right) \times 100
\]
**Determination of Ethanol Soluble Extractive Value**

The extraction was conducted according to the Quality Control Methods for Medicinal Plants Materials Jain and Argal, (2013). Five grams of oven–dried leaf powder of *T. orientalis* was transferred to a conical flask. One hundred mL of 95 % ethanol was added, and the flask was covered with aluminium foil. It was then placed on an Orbital shaker (2 Tier, 722–2T, Malaysia) during the first 6 hours and allowed to stand for 18 hours separately. After that, it was filtered rapidly taking precaution to minimize the loss of ethanol. Twenty–five mL of the filtrate was collected and transferred to a weighed thin porcelain. It was evaporated to dryness on a water bath and dried completely in an oven at 90 °C until a constant weight was reached. It was kept in a desiccator to cool and the percentage of alcohol soluble extractive yield was calculated with reference to oven–dried leaf according to Sharma and Sharma, 2013; Upreti, et al., 2013 using the following equation;

\[
\text{Percentage Extractive Value} = \left( \frac{\text{Initial Weight} - \text{Final Weight}}{\text{Initial Weight}} \right) \times 100
\]

**Preparation of Crude Ethanol and Aqueous Leaf Extracts**

Approximately 500 g of powdered leaf sample of *T. orientalis* was placed in a 5 L conical flask, completely soaked with 3.5 L of 95 % ethanol, and then covered with aluminium foil. The mixture was allowed to stand at ambient temperature (25°C ± 2) for 72 hours with frequent agitation in order to facilitate dissolution of the soluble matter. The mixture was strained using muslin cloth to remove solid material. The extraction was repeated to ensure maximum yield by soaking the solid material using 1.5 L of the ethanol. Similar procedure was carried out for aqueous extract preparation except for the extraction period which 12 hours. The strained liquids were clarified by filtration by gravitation using Smith filter paper. The filtrate was then concentrated to solid extract under reduced pressure (180 m/bar) at 40°C using Rotary evaporator (BUCHI Rotavapor R–210, Switzerland). The ethanol leaf extract of *T. orientalis* (ELETO) obtained was dark–green in colour.

**Phytochemical Evaluation**

Phytochemical screening was conducted to detect the presence of metabolites according to Godghate et al. (2012) method. Total flavonoid content (TFC), total phenolic content (TPC) and antioxidant activity (AA) were evaluated according to a modified version of Quettier et al. (2000), Kumarasamy et al. (2007) and Stanković, (2011) respectively. Stock solution was prepared at 50 µg/mL.

**Determination of Total Phenolic Content**

The total phenolic content in both aqueous and ELETO was determined using FC’s phenol reagent. The reaction mixture contained 250 µL (1 mg/mL) of diluted crude ELETO, 1.25 mL of freshly prepared diluted 10 % (v/v) FC’s phenol reagent and 1 ml of 7.5 % (w/v) NaHCO₃. The final mixture was diluted to 7 mL with deionized water. The mixture was kept in dark at ambient temperature for 1h to complete the reaction. The sample was prepared in triplicate, and the absorbance was measured at 760 nm using a spectrophotometer (Shimadzu UVmini–1240, Japan). The same procedure was repeated for each sample, and the standard solution of gallic acid, (GA) (Sigma–Aidrich Chemicals, USA) prepared at 150 µg/mL. Control was concomitantly prepared using a mixture containing 1.25 mL 10 % FC’s reagent dissolved in water and 1 mL of 7.5 % NaHCO₃. The calibration line was construed using the GA with a concentration of 4.70, 9.40, 18.80, 37.50, 75.00, and 150.00 µg/mL. Based on the measured absorbance, the concentration of phenols was read (µg/mL) from the calibration line. The TPC in extracts was expressed as gallic acid equivalent (GAE) in mg/g of extract.

**Determination of Total Flavonoid Content**
Total flavonoid content (TFC) in both aqueous and ethanol *T. orientalis* leaf extracts were determined using aluminium chloride (AlCl₃) method. The reaction mixture contained 250 µL of sample at 1mg/mL concentration, 50 µL potassium acetate, and 50 µL of 10% AlCl₃ solution dissolved in 95% ethanol and 2.50 mL of ethanol. The mixture was incubated at ambient temperature for 1 h. The sample was prepared in triplicate, and the absorbance was measured at 415 nm using a spectrophotometer (Shimadzu UVmini–1240, Japan). The same procedure was repeated for each sample and the standard solution of quercetin at 160 µg/mL. The control was prepared to contain an equal amount of all the reagents used except the sample extract. The calibration line was construed at 5, 10, 20, 40, 80, and 160 µg/mL. Based on the measured absorbance, the concentration of flavonoids was read (µg/mL) on the calibration line. The TFC in extracts was expressed as quercetin equivalent (QE) in mg/g of extract.

**Evaluation of Antioxidant Activity**

The DPPH free radicals scavenging activity of the *T. orientalis* was assessed by the standard method with suitable modifications (Kumarasamy *et al.*, 2007) in both aqueous and ethanol the leaf extracts. The test samples and the standard (quercetin) solution were prepared in ethanol at 1 mg/mL each. Dilution was made to obtain a concentration of 500.00, 250.00, 125.00, 62.50, 31.25, and 15.62 µg/mL in each sample. Diluted solution (60 µL each) was mixed with a 200 µL solution of DPPH dissolved in 1% dimethylsulfoxide (DMSO) and kept as 0.1 mM concentration. Control sample contained all the reagents except the extract and quercetin in the case of the standard. After 45 min incubation in darkness at ambient temperature (25 °C ± 1), the absorbance was recorded at 517 nm using Multifunctional Microplate Reader (Tecan Infinite M200PRO, Australia). The percent radical scavenging activity of the samples was determined in comparison with ethanol–treated control groups, whereas half-maximal inhibitory concentration (IC₅₀) values were estimated using linear regression graph. The percentage inhibition (%I) was calculated using the following equation:

\[
%I = 100 - \left( \frac{\text{Absorption of Sample}}{\text{Absorption of Control}} \right) \times 100
\]

**Data analyses**

Total phenolic and total flavonoid contents were analyzed by Independent Sample T–test. Pearson correlation between the TPC, TFC and AA values were evaluated using SPSS version 20. Results are expressed as mean ± standard deviation (SD) of three determinants. *P* values <0.05 were considered significant.

**RESULTS**

**Percentage Loss on Drying**

Mean percentage loss on drying (% LOD) value of *T. orientalis* leaf was found to be 59.80±0.53 with a minimum value of 59.27 and a maximum 60.43 (Table I).

| Fresh weight (g) | Dry weight (g) | Loss in weight (g) | % Loss in weight | Mean % LOD±SD (n = 5) |
|------------------|----------------|--------------------|------------------|---------------------|
| 5.0              | 1.97           | 3.03               | 60.60            | 59.80±0.53          |
| 5.0              | 2.00           | 3.00               | 60.00            |                     |
| 5.0              | 2.02           | 2.98               | 59.60            |                     |
| 5.0              | 2.02           | 2.98               | 59.60            |                     |
| 5.0              | 2.04           | 2.96               | 59.20            |                     |

Percentage loss on drying: % LOD, Standard deviation: SD, Sample size: n

**Ethanol Soluble Extractive Value**

The mean ethanol–soluble extractive value of *T. orientalis* leaves was found to be 14.33±0.32 percentage with a minimum value of 13.90 and a maximum 14.6 (Table II).

| Fresh weight (g) | Dry weight (g) | % Loss in weight | Mean % LOD±SD (n = 5) |
|------------------|----------------|------------------|---------------------|
| 5.0              | 1.97           | 3.03             | 60.60               | 59.80±0.53          |
| 5.0              | 2.00           | 3.00             | 60.00               |                     |
| 5.0              | 2.02           | 2.98             | 59.60               |                     |
| 5.0              | 2.02           | 2.98             | 59.60               |                     |
| 5.0              | 2.04           | 2.96             | 59.20               |                     |

Results of preliminary phytochemical screening on ELETO showed presence of saponins, steroid, cardiac glycoside, alkaloids, triterpenes, flavonoids and phenolic compounds (Table III).
The amount of TPC was found significantly (P<0.05) higher in ELETO (260.96±2.31 mgGAE/g) compared to the aqueous crude extract (134.08±0.56 mgGAE/g) (Table IV, Figure I). Similarly, TFC was also above in ethanol extract (32.71±0.90 mgQE/g) compared to the aqueous extract (4.70±0.24 mgQE/g) (Table IV, Figure II). Ethanol extract exhibited higher values of antioxidants compared to aqueous extract (Figure III). There was a significant positive correlation between the AA and phenolic content of the solvent extracts of the plant (r = 0.798, P < 0.05).

![Figure I: Showing the standard calibration curve for the quantification of total phenolic content in Trema orientalis leaf extract](image)

### TABLE II: Ethanol extractive value of the Trema orientalis leaf

| Weight of dried leaves (g) | Final weight (g) | Weight of extract (g) | % extract value | % mean extractive value ± SD (n=5) |
|----------------------------|------------------|-----------------------|----------------|----------------------------------|
| 5.00                       | 4.27             | 0.73                  | 14.60          | 14.33±0.32                       |
| 5.00                       | 4.27             | 0.73                  | 14.69          |                                  |
| 5.00                       | 4.25             | 0.72                  | 14.30          |                                  |
| 5.00                       | 4.29             | 0.71                  | 14.18          |                                  |
| 5.00                       | 4.31             | 0.70                  | 13.90          |                                  |

Percentage: %, Standard deviation: SD, Sample size: n
TABLE III: Results of phytochemical screening of *Trema orientalis*

| Chemical groups | Saponins | Tannins | Alkaloids | Steroids | Cardiac glycoside | Triterpenes | Flavonoids | Phenolic compounds |
|-----------------|----------|---------|-----------|----------|-------------------|------------|------------|-------------------|
| Ethanol extract  | ++       | ++      | ++        | ++       | ++                | ++         | ++         | +++               |
| Aqueous extract  | +++      | +       | -         | +        | +                 | +          | +          | +                 |

Strong positive: +++, moderately positive: ++, Low positive: +, negative test: –

Figure II: Showing standard calibration curve for the quantification of total flavonoid content in *Trema orientalis* leaf extract

\[ y = 0.0098x + 0.0851 \]
\[ R^2 = 0.9972 \]

TABLE IV: Total phenolic content, total flavonoid content, and antioxidant activity of ethanol and aqueous extracts of *Trema orientalis* leaf (Mean ± SD, n = 5)

| Solvent    | TPC (mg GAE/g) | TFC (mg QE/g) | % Inhibition | IC₅₀ (µg/mL) |
|------------|----------------|---------------|--------------|--------------|
| Ethanol    | 260.96±2.31ᵃ   | 32.71±0.89ᵇ   | 89.11±0.26   | 9.27         |
| Aqueous    | 134.08±0.56ᵇ   | 4.70±0.23ᵃ    | 40.26±0.47   | –            |

Means with different superscripts (a,b) indicate significant differences (P<0.05). Total phenolic content: TPC, Total flavonoid content: TFC, Gallic acid equivalent: GAE, Quercetin equivalent: QE
DISCUSSION
The % LOD and ethanol soluble extractive value of T. orientalis leaf were determined in the present study in order to estimate the amount of fresh and dried leaf that gives a particular amount of solid ELETO; since moisture content is of direct economic importance to the processor and the consumer. The value obtained is slightly higher compared % LOD of Geranium ocellatum leaf with the yield value of 51.7 % (Joseph and George, 2011). The ethanol soluble extractive value obtained is similar to that of Aritochia indica Linn., Zanthoxylum armatum D.C., and Gynura segetum Lour with value 15.53%, 14.66% and 14.13% respectively (Mridula et al., 2011; Devi and Divakar, 2012; Seow et al., 2013; Upreti, et al., 2013). Tuo et al. (2015) reported significant higher extractive value (20%) of ELETO. Other plants with higher ethanol extractive value include Prosopis cineraria Linn. and Catunaregum spinosa Thunb. with value 19.0% and 20.93%, respectively (Shrivastava and Leelavathi, 2010; Ravichandra and Paarakh, 2011; Kumawat et al., 2012; Singh et al., 2013; Komlaga et al., 2014). However, several plants were reported to have lower ethanol extractive yield, including Ficus microcarpa L. (8.0%), Solanum macrocarpon (8.66%) and Balanites aegyptiaca L. (8.79%). Extraction of any plant with a particular solvent yields a solution containing different phyto–constituents. Extractive values are primarily useful for the determination of exhausted or adulterated drugs, evaluation of chemical constituents present and estimation of specific constituents soluble in that particular solvent used for extraction. The preliminary phytochemical screening result obtained in the current study agrees with findings of Tchamo et al. (2000); Gbadamosi et al. (2012) and Adinortey et al. (2013); who reported the presence of saponins, tannins, flavonoids, including cardiacglycoside, alkaloids, triterpenes in T. orientalis leaves extract. However, Ayoade et al. (2014) reported that T. orientalis leaf extract was found to be more abundant in steroids, flavonoids, and alkaloids, while saponins, tannins, glycosides, and terpenoids were present. According to Tuo et al. (2015), T. orientalis leaf extract is devoid of steroids. Several studies of other plants species have shown that saponin and tannins causes inappetance, mass loss, hepatotoxicity, icterus, tachycardia, ruminal stasis, dyspnoea, anaemia, necrosis recumbency and death in animals (Cornick et al., 1988; Shumaik et al., 1988; Miles set al., 1993; Adedapo and Abatan, 2005). According to Graydon et al. (1991), toxic effect of the steroidal saponins is related to their normal metabolism in the rumen. The toxic effects of steroidal saponins are mediated by hydrolysis in the rumen, leading to release of their corresponding sugars and sapogenins (aglycones). The sapogenins are absorbed and
transported to the liver where they form conjugates of epsimilagenin with glucuronic acid and excreted in the bile. Once in the bile, it crystallizes by forming insoluble calcium salts of sapogenin glucuronate precipitate crystals block inside and around the biliary ducts leading to the toxicity signs above mentioned above. Sofowora (1993) reported that saponins precipitate proteins, bind cholesterol, and haemolyse RBC whereas, hydrolysable tannins (astringents) bind plasma and organ proteins causing coagulation and necrosis (Spier et al., 1987).

Higher values of TPC, TFC and AA in ethanol fraction than the aqueous obtained in this study and the strong positive correlation between AA and, TPC and TFC were also reported in several previous studies (Yang et al., 2002; Maksimović et al., 2005; Stratil et al., 2006; Kratchanova et al., 2010; Thoo et al., 2010; Sah et al., 2012). According to Wong et al. (2006), low correlation between TPC and AA occurs because of an error introduced in the assays. However, there are also reports of no such correlation (Bajpa et al., 2005). Assays that were based on the measurement of an end product, one could be measuring the AA of the reaction by-products, rather than the compounds present in the original mixture (Halliwell, 2009). An in vitro technique has been used to determine the AA in order to allow easy screening of drugs since substances that have low AA in vivo, will probably show little activity in vivo (Nunes et al., 2008). A large decrease in the absorbance of the reaction mixture indicates significant free radical scavenging activity of the tested compound under test (Krishnaiah and Sarbatly, 2011). Usually, extracts that contain high amount of polyphenols also show high AA (Wong et al., 2006). High scavenging activity on DPPH radicals could be due to the low molecular weight phenolic compounds in the samples studied. Paixão et al. (2007) reported that DPPH is known to react specifically with low molecular weight phenolic compounds. Factors influencing the recovery of antioxidant of specific sample were antioxidants concentration, extraction medium (time and polarity), pH of medium, temperature, chemical structures and position in the molecule (Prior et al., 2005; Zhang et al., 2007; Thoo et al., 2010). Hence, high yield of individual phenolic compounds may not exhibit a high AA as it is dependent on the synergistic effects of the extracted phenolic compounds. Extraction conditions can significantly influence AA in plants.

Almost every part of T. orientalis is used as traditional medicine in different disease conditions such as pneumonia, pleurisy, tooth ache, hematuria, blood stasis, laxative, hyperglycemia, anticonvulsant, antiplasmodial and helminthiasis (Yanes, 2007; Orwa et al., 2009; N’guessan, 2009; Panchal et al., 2010; Abiodun et al., 2011; Adinortey et al., 2013). These effects may be attributed to its important biologically active compounds such as polyphenols (Adinortey et al., 2013). However, Matuschek and Svanberg (2002) reported that naturally occurring polyphenols bind with non-heme iron in vitro in model systems, possibly reducing its absorption thereby leading to anaemia. The antioxidant activities observed could be ascribed both to mechanisms exerted by phenolic compounds and to synergistic effects of different phyto compounds. The identification of phytochemical constituent defined in this study will facilitate the evaluation of the in vitro models for predicting farm animal toxicity.

**CONCLUSION**

Both ethanol and aqueous extracts of T. orientalis contain saponins, tannins, steroids, cardiac glycosides, triterpenes, flavonoids and phenolic compounds. The TPC and TFC are significantly higher in ethanol extract compared to aqueous. The radical scavenging effect with IC_{50} value of 9.27 µg/mL proved that ELETO has abundance polyphenolic compounds capable of donating hydrogen to free radical to scavenge a potential damage. Hence, *T. orientalis* has good therapeutic potentials in ethnobotany.
ACKNOWLEDGEMENTS
Appreciation goes to the management of Medical Laboratory, Medical Campus UniSZA for their full cooperation during the analysis. Our acknowledgement also goes to the Science officers and Technicians, most especially Miss Rokiah Zainuddin and Noor Muhammed Muzammil for great contributions towards the actualization of this study. We also wish to acknowledge Dr. A.M. Tauheed of Department of Veterinary Pharmacology and Toxicology, A.B.U., Zaria for meticulous prove reading.

REFERENCES
ABIODUN, O., GBOTOSHO, G., AJAIYEBOA, E., HAPPI, T., FALADE, M. and WITTLIN, S. (2011). In vitro antiplasmodial activity and toxicity assessment of some plants from Nigerian ethnomedicine. *Pharmaceutical Biology*, 49: 9–14.

POURMORAD, F., HOSSEINIMEHR, S. J. and SHAHABIMAJD, N. (2006). Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. *African Journal of Biotechnology*, 5: 1142–1145.

ADEDAPO, A.A. and ABATAN M.O. (2005). The effects of pelleted leaves of *Phyllanthus amarus* and *Euphorbia hirta* on the haemograms of rats. *Folia Veterinaria*, 49: 189–192.

ADINORTEY, M.B., GALUYON, I.K. and ASAMOAH N.O. (2013). *Trema orientalis* Linn. Blume: A potential for prospecting for drugs for various uses. *Pharmacognosy*, 7: 67–72.

AYOADE, G.W., OLUSI, T.A., AMOO, I.A. and EKA–ETE, G.E. (2014). Composition of some traditional malaria remedies and their antiplasmodial effects on *Plasmodium berghei*. *International Journal of Scientific and Research Publications*, 4: 2250–3153.

CORNICK, J.L., CARTER, G.K., and BRIDGES, C.H. (1988). Kleingrass–associated hepatotoxicosis in horses. *Journal of the American Veterinary Medical Association*, 193: 932–935.

DEVI, S.L. and DIVAKAR, M.C. (2012). Pharmacognosy and phytochemistry pharmacognostical evaluation on the leaves of *Wrightia tinctoria* (Roxb) R.Br. *Hygeia. Journal for Drugs and Medicines*, 4: 104–111.

FAOSTAT (2008): [http://faostat.fao.org/default.aspx](http://faostat.fao.org/default.aspx).

GBADAMOSII, I.T., MOODY, J.O. and YEKINI A.O. (2012). Nutritional composition of ten ethnobotanicals used for the treatment of anemia in Southwest Nigeria. *European Journal of Medicinal Plants*, 2: 140–150.

GENEVA (1998). Quality Control Methods for Medicinal Plants Materials. World Health Organization, pp. 1–115.

GRIN (2007). *Trema orientalis* information from NPGS/GRIN. Taxonomy for Plants. National Germplasm Resources Laboratory, Beltsville, Maryland: USDA, ARS. National Genetic Resources Program.

HALLIWELL, B. (2009). The wanderings of a free radical. *Free Radical Biology and Medicine*, 46: 531–542.

HOLMSTRÖM, L. (2013). Fodder to ruminants within agroforestry systems in Rwanda – Species and management, pp. 1–4.

SANDHYA, B., THOMAS, S., ISABEL, W. and SHENBAGARATHAI, R., (2006). Ethnomedicinal plants used by the valaiyan community of Piranmalai hills (Reserved forest), Tamil Nadu, India A pilot study. *African Journal of Traditional, Complementary and Alternative Medicines*, 3: 101-114.

JAIN, S. and ARGAL, A. (2013). Preliminary phytochemical screening and micromeretic parameters of *Ocimum*
sanctum  L. Pelagia Research Library. Asian Journal of Plant Science and Research, 3: 126–130.

JOSEPH, L. and GEOGE, M. (2011). Pharmacological profiling of Geranium ocellatum leaves. International Journal of Medicinal and Aromatic Plants, 1: 351–354.

KOMLAGA, G., SAM, G.H., DICKSON, R.A, MENSAH, M.L.K. and FLEISCHER, T.C. (2014). Pharmacognostic studies and antioxidant properties of the leaves of Solanum macrocarpon. Journal of Pharmaceutical Sciences and Research, 6: 1–4.

KRATCHANOVA, M., DEHER, P., CIZ, M., LOZEK, A. and MIHAILRVR, A. (2010). Evaluation of antioxidant activity of medicinal plants containing polyphenol compounds. Comparison of two extraction systems. Acta biochimica Polonica, 57: 229–234.

KRISHNAIAH, D. and SARBATLY, R. (2011). Nithyanandam RR: A review of the antioxidant potential of medicinal plant species. Food Bioprod Process, 89: 217–233.

KUETE, V. (2013). Phenylpropanoids and related compounds from the medicinal plants of Africa. In: Kuete, V. Edr. Medicinal plant research in Africa, 2013. Oxford: Elsevier, pp. 251–60.

KUMARASAMY, Y., BYRES, M., COX, P.J., JASAPARS, M., NAHAR, L. and SARKER, S.D. (2007). Screening seeds of some Scottish plants for free–radical scavenging activity. Phytotherapy Research, 21: 615–621.

KUMAWAT, D.B.K., GUPTA, M. CHAND, T. and SINGH, Y. (2012). Preliminary Phytochemical Investigation on Leaves of Balanites aegyptiaca (L.). Research Journal of Pharmaceutical, Biological and Chemical Sciences, 3: 762–768.

MAKSIMOVIĆA, Z., MALENČIĆB, D. and KOVAČEVIĆA, N. (2005). Polyphenol contents and antioxidant activity of Maydis stigma extracts. Bioresource Technology, 96: 873–877.

MALAN, C. and NOTTEN, A. (2005). Kirstenbosch National Botanical Garden. "Trema orientalis". South African National Biodiversity Institute.

MATUSCHEK, E. and SVANBERG, U. (2002). Oxidation of polyphenols and the effect on in vitro iron accessibility in a model food system. Journal of Food Science, 67: 420–4.

MILES, C.O., WILKINS, A.L., MUNDAW, S.C., FLAØYEN, A., HOLLAND, P.T. and SMITH, B.L. (1993). Identification of insoluble salts of the beta-D-glucuronides of episarsasapogenin and epismilagenin in the bile of lambs with alveld, and examination of Narthecium ossifragum, Tribulus terrestris and Panicum miliaceum for sapogenins. Journal of Agricultural and Food Chemistry, 41: 914–917.

MOTOOKA, PHILIP/CASTRO, LUISA/NELSON, DUANE/NAGAI, GUY/CHING, L. (2003). Weeds of Hawai`i’s Pastures and Natural Areas; An Identification and Management Guide. College of Tropical.

MRIDULA, M., AMITA, T., SUSHMA, BINDU, D. and GHOSH, A.K. (2011). Pharmacognostical and phytochemical investigation of aerial part of Aritochia indica L. International Journal of Research in Ayurveda and Pharmacy, 2:1282–1285.

N’GUESSAN, K., TIÉBRÉ, M.–S., AKÉ–ASSI, E. and ZIRIHI, G.N. (2009). Ethnobotanical study of plants used to treat arterial hypertension, in traditional medicine, by Abbey and Krobou populations of agboville (Côte–d’Ivoire).
Europe Journal of Science Research, 35: 85–88.

NGAMENI, B., FOTSO, G.W., KAMGA, J., AMBASSA, P., ABDOU, T. and FANKAM, A.G., (2013). Flavonoids and related compounds from the medicinal plants of Africa. In: Kuete V, Edr. Medicinal plant research in Africa. Oxford: Elsevier, pp. 301–350.

NUNES, P.X., SILVA, S.F., GUEDES, R.J. and ALMEIDA, S. (2008). Biological oxidations and antioxidant activity of natural products, Phytochemicals as nutraceuticals –Global Approaches to Their Role in Nutrition and Health. 2012. 21Umamaheswari M, Chatterjee TK:In vitro antioxidant activities of the fractions of Coccinia grandis L. leaf extract. The African Journal of Traditional, Complementary and Alternative Medicines, 5: 61–73.

ORWA, C., MUTUA, A., KINDT, R., JAMNADASS, R. and ANTHONY, S. (2009). "Trema orientalis". Agroforestartree Database: a tree reference and selection guide, version 4.0. World Agroforestry Centre.

PAIXÃO, N., PERESTRELO, R., MARQUES, J. C. and CÂMARA, J.S. (2007). Relationship between antioxidant capacity and total phenolic content of red, rosé and white wines. Food Chemistry, 105: 204–214.

PANCHAL, H.S., MASTER, S.M., SHAH, U.D., SALUJA, A.K. and DHOLWANI, K.K. (2010). Anti-convulsion activity of leaf of Trema orientalis. International Journal of Pharmacology Research, 2: 53–55.

PRIOR, R.L., WU, X. and SCHAICH, K. (2005). Standardized methods for the determination of antioxidants capacity and phenolics in foods and dietary supplements. Journal of Agricultural and Food Chemistry, 53: 4290–4302.

QUETTIER, D.C., GRESSIER, B., VASSEUR, J., DINE, T., BRUNET, C., LUYCKX, M.C., CAYIN, J.C., BAILLEUL, F. and TROTIN, F. (2000). Phenolic compounds and antioxidant activities of buckwheat (Fagopyrum Esculentum Moench) hulls and flour. Journal of Ethnopharmacology, 72: 35–42.

RAVICHANDRA, V.D. and PAARAKH, P.M. (2011). Pharmacognostical and Phytochemical Investigation on Leaves of Ficus microcarpa Linn.International Journal of Pharmaceutical Sciences and Drug Research, 3: 137–140.

SAH, S.Y., SIA, C.M, CHANG, S.K., ANG, Y.K. and YIM, H.S. (2012). Antioxidant capacity and total phenolic content of lemongrass (Cymbopogon citrates) leave. Annals Food Science and Technology, 13: 150–155.

SEOW, L.J., BEH, H.K., SADIKUN, A. and ASMAWI, M.Z. (2013). Preliminary Phytochemical and Physicochemical Characterization of Gynura segetum (Lour) Merr (Compositae) Leaf. Tropical Journal of Pharmaceutical Research, 12: 777–782.

SHAHAT, A.A. and MARZOUK, M.S. (2013). Tannins and related compounds from medicinal plants of Africa. In: Kuete V, Edr. Medicinal plant research in Africa. Oxford: Elsevier, pp. 479–555.

SHARMA, R. G. and SHARMA, A. (2013). Study of Physico–Chemical Properties of Drug and Physiological Variation in Leaves of Andrographis paniculata Burm. F. Nees Meenu Sharma. Acta Chimica & Pharmaceutica Indica, 3: 52–64.

SHRIVASTAVA, S. and LEELAVATHI S. (2010). Preliminary phytochemical evaluation of leaf extracts of Catunaregum spinosa Thunb. International Journal of Pharmaceutical Sciences Review and Research, 3: 114.
SHUMAIK, G.M., WU, A.W. and PING, A.C. (1988). Oleander poisoning: Treatment with digoxin–specific Fab antibody fragments. *Annals of Emergency Medicine*, 17: 732–735.

SINGH, S., NARESH, V. and SHARMA, S.KR. (2013). Pharmacognostic Studies on the leaves of *Prosopis cineraria* (L.) Druce. Growing in South Haryana, India. *Journal of Pharmacognosy and Phytochemistry*, 2: 320.

SOFOWORA, A. (1993). Medicinal Plants and Traditional Medicine in Africa. (2nd Ed). Spectrum Books Ltd. Ibadan, pp. 255–256.

SPIER, S.J., SMITH, B.P. and SEAWRIGHT, A.A. (1987). Oak toxicosis in cattle in Northern California: clinical and pathological findings. *Journal of American Veterinary Medical Association*, 191: 959–964.

STANKOVIĆ, M.S. (2011). Total phenolic content, flavonoid concentration and antioxidant activity of *Marrubium peregrinum* L. extracts. *Kragujevac Journal of Science*, 33: 63–72.

STRATIL, P., KLEJDUS, B. and KUBAN, V. (2006). Determination of total content of phenolic compounds and their antioxidant activity in vegetables evaluation of spectrophotometric methods. *Journal of Agricultural and Food Chemistry*, 54: 607–616.

TCHAMO, D.N., DJOUX–FRANCA, M.G., MARIOTTE, A.M., TSAMO, E., DASKIEWICZ, J.B. and BAYET, C (2000). Prenylated xanthones as potential P–glycoprotein modulators. *Bioorganic & Medicinal Chemistry Letters*, 10: 1343–1345.

THOO, Y.Y., HO, S.K., LIANG, J.Y., HO, C.W. and TAN, C.P. (2010). Effects of binary solvent extraction system, extraction time and extraction temperature on phenolic antioxidants and antioxidant capacity from mengkudu (*Morinda citrifolia*). *Food Chemistry*, 120: 290–295.

TSOPMO, A., AWAH, F.M., AND KUETE, V. (2013). Lignans and stilbenes from African medicinal plants. In: Kuete V. Edr. Medicinal plant research in Africa, Oxford: Elsevier, pp. 435–478.

TUO, K., BÉOUROU, S., TOURÉ, A. O., OUATTARA, K., MEITÉ, S., AKO, A.A.B., YAO, S.S., KONFI, D., COULIBAY, B., COULIBALY, A. and DJAMAN, A.J. (2015). Antioxidant activities and estimation of the phenols and flavonoids content in the extracts of medicinal plants used to treat malaria in Ivory Coast. *International Journal of Current Microbiology and Applied Sciences*, 4: 862–874.

UPRETI, K., SEMWAL, A. UPADHYAYA, K., and MASIWAL, M. (2013). Pharmacognostical and phytochemical screening of leaf extract of *Zanthoxylum armatum* DC. *International Journal of Herbal Medicine*, 1: 6–11.

WONG, S.P., LEONG, L.P. and KOH, W.J.H. (2006). Antioxidant activities of aqueous extracts of selected plants. *Food Chemistry*, 99: 775–783.

YANES, C.V. (2007). Germination of a pioneer tree from Equatorial Africa. *Turrialba*, 27: 301–302.

YANG, R.Z., BLAILEANU, G., HANSEN, B.C., SHULDINER, A.R and GONG, D.W. (2002). cDNA cloning, genomic structure, chromosomal mapping, and functional expression of a novel human alanine aminotransferase. *Genomics*, 79: 445–50.

ZHANG, Z.S., LI, D., WANG, L.J., OZKAN, N., CHEN, X.D., MAO, Z.H. and YANG, H.Z. (2007). Optimization of ethanol–water extraction of lignans from flaxseed. *Separation and Purification Technology*, 57: 17–24.