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Trema orientalis L. and *Dialium guineense* Wild. used to manage hypertension in Bénin: phytochemical study and antioxidant activity

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Abstract

Trema orientalis L. (Celtidaceae) and *Dialium guineense* Wild. (Caesalpiniaceae) are used traditionally as remedy against headache, pains, diarrhoea, fever and hypertension. Despite their approved efficacy there are little scientific data available on their biological potential. The present study investigated the phytochemical study and antioxidant activity of *Trema orientalis* and *Dialium guineense*. The phytochemical screening was carried out using standard procedures. The phenolic and flavonoids contents were determined using Folin-Ciocalteu and aluminium chloride reagent. The antioxidant activity was evaluated using 2, 2-diphenyl-1-picryl-hydrazyl, Ferric reducing capacity, superoxide anion and hydrogen peroxide methods. *Tremas orientalis* was the most active in the ferric reducing capacity with $6007.8 \pm 175.57 \mu\text{mol AAE g}^{-1}$. The superoxide anion and hydrogen peroxide scavenging activities ranged from 88.52 ± 0.68 % to 91.33 ± 4.01 %. The DPPH scavenging activity was dose dependent. *Dialium guineense* was the most active with an inhibitory percentage of 96.06 ± 0.34 % comparable to ascorbic acid (99.46 ± 0.37 %).

Keywords: *Dialium guineense*, *Trema orientalis*, antioxidant

Introduction

According to the World Health Organization, more than 80% of the african populations used traditional medicine for primary health care [1]. All over the world, human health is affected by problems such as the ageing and the spread of unhealthy lifestyles. In developed and low income countries, population face the same health problems, of which the most important is non transmissible diseases such as cardiovascular diseases, cancer, diabetes, hypertension and chronic lung diseases [2]. It is well known that a diet rich in vegetables contributes to the decrease of the inflammation and oxidative stress risk, related with chronic diseases such as cardiovascular diseases, arteriosclerosis, cancer, diabetes, and neurological diseases [3-5]. These plant properties are due to phytoconstituents contents among which we find the phenolic compounds. Phenolic compounds are well known for their antioxidant properties which is attributed to the scavenging ability of free radicals, donors of hydrogen atoms, electrons or metal chelate cations [6, 7]. Molecular structures, particularly the number and positions of the hydroxyl groups, and the nature of substitutions on the aromatic rings, confers to phenolic compounds the capacity of inactivating free radicals, which is referred to as structure-activity relationship [8]. Based on the role of phenolic compounds in improving the management of several pathologies, the study of phytoconstituents and their ability to scavenge the reactive oxygen species becomes important. In Bénin, several medicinal plants are used in the treatment of various pathology. Among these plants, *Dialium guineense* Wild. and *Trema orientalis* L. are widely used in folk medicine to manage various ailments [9, 10]. Roots, leaves and bark of *Dialium guineense* are used to cure digestive system disorders, pulmonary troubles, malaria, fever, jaundice, diabetes, coughs, bronchitis, diarrhea, palpitations, dysmenorrhoea, ulcer, anemia, hemorrhoids, febrifuge, anti-dysentery, anti-convulsion, anti-diabetic [11, 9]. The twigs are used as native toothbrushes to protect against tooth decay and dental plaque [12]. *Trema orientalis* is also used in traditional medicine to treat and manage many diseases such as hypertension, malaria, diarrhea, bronchitis, asthma, pneumonia, jaundice, bronchitis, toothache, female sterility. It's also used as inhalant, vapour bath for coughs, anti-helminthic, antidote to general poisoning, febrifuge, anti-dysentery, anti-convulsion, anti-diabetic, analgesic, anti-sickling [10, 13-16]. Therefore, this work was performed

to investigate phytoconstituents, to quantify phenolic compounds and evaluate the antioxidant potential of extracts from *Dialium guineense* Wild. and *Trema orientalis* L.

Material and Method

Chemical

Gallic acid, Foline-Ciocalteu reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid, quercetin, aluminium chloride, sodium acetate, sodium carbonate, vanillin, sulfuric acid, chlorhydric acid, potassium hydroxide, méthanol, sodium hydroxide and acetic anhydride, aluminium chloride were used. Ethanol were purchased from Essential Medicines Purchasing Center of the Ministry of Health in Benin.

Plant materials

Fresh sample of *Dialium guineense* Wild. and *Trema orientalis* L. were collected in Southern Bénin, department of Atlantic in November 2015. The plants were identified and authenticated by taxonomists from the National Herbarium of University of Abomey-Calavi where the voucher specimens (YH 284/HNB; YH 262/HNB) were deposited.

Preparation of extract

The stem bark of *Dialium guineense* and leafy stem of *Trema orientalis* were washed briefly with distilled water and dried in laboratory under air-conditioned ($22^{\circ}\text{C} \pm 2$) and then reduced to powder using an electric grinder (MARLEX Electroline Excella). 300 g of powered plants were extracted with 1 L of ethanol under stirring for 24 hours. The macerate was filtered through a Whatman No.1 paper filter. The extraction process was repeated twice for 2 hours using the first extraction residue. The filtrate was concentrated under reduced pressure with a rotary evaporator (BUCHI Rotavapor RII). The obtained extract was stored at 4°C for assay.

Phytochemical investigation

Thin Layer Chromatography and colorimetric methods were used to determine the presence of phytoconstituents of *Dialium guineense* and *Trema orientalis* extracts. The presence of flavonoids, tannins, alkaloids, triterpenes, coumarins, saponins, essentials oils, lignans, pigments, naphthoquinones, anthracene derivatives and cardiac glycosides was investigated according to standard methods for the detection of plant secondary metabolites using TLC [17] and colorimetric methods [18].

Total phenolic contents

Total phenolics was estimated by Folin-ciocalteu reagent method. This method is based on the reduction in alkaline media of phosphotungstic mixture (WO_4^{2-}) phosphomolybdic (MoO_4^{2-}) of Folin reagent by the oxidizable group of phenolic compounds, leading to the formation of blue reduction products which have a maximum absorption at 765 nm. The blue intensity is proportional to the amount of polyphenols in the sample [19]. Then, 200 μl of diluted sample at 100 $\mu\text{g}/\text{mL}$ were added to 1 ml of diluted Folin–Ciocalteu reagent (1:10). After 4 min, 800 μl of saturated sodium carbonate (75 g/L) were added and the mixture was incubated for 2 h at room temperature. The absorbance of the mixture was then measured at 765 nm. Gallic acid was used as a standard to establish calibration curve ($y = 0,043x - 0,051$; $R^2 = 0,994$). Each assay was done in triplicate and the results expressed as mg of Gallic Acid Equivalents (GAE)/100 mg of extract.

Total flavonoid content

The total flavonoid content was investigated using the aluminium chloride method as described previously [20]. The assay medium was prepared by mixing 1 ml of extract (100 $\mu\text{g mL}^{-1}$), 3 ml of methanol, 0.2 ml of 1 M potassium acetate, 0.2 ml of 10% aluminium chloride with 5.6 ml of distilled water. The mixture was thus incubated at room temperature for 30 minutes. Absorbance of the mixture was read at 415 nm using UV spectrophotometer (VWR UV-1600 PC). Quercetin was used as control to produce the standard curve ($y = 0,325x - 0,363$; $R^2 = 0,995$) and the results were expressed as mg of quercetin equivalent (QE)/100 mg of extract.

Antioxidant evaluation

DPPH radical-scavenging activity

Free radical scavenging activity of *Dialium guineense* and *Trema orientalis* extracts was performed using the method described by Talbi *et al.* [21] with slight modifications. A stock solution of the extracts prepared in methanol at 100 $\mu\text{g}/\text{ml}$ was subjected to two-fold dilutions to make eight concentrations. Initial concentration of extract was 100 $\mu\text{g}/\text{mL}$ and the lowest being 0.78 $\mu\text{g}/\text{ml}$. The test consists of 1.5 ml of freshly prepared 0.2% DPPH methanolic solution and 0.75 ml of extract. Methanolic solutions of DPPH and ascorbic acid were used as blank and reference respectively. Absorbance of mix was read at 517 nm after 15 min incubation in dark using spectrophotometer (VWR UV-1600 PC). All experiments were performed in triplicate. The inhibition of the DPPH radical ability, expressed as a percentage, is calculated according to the formula below:

$$\text{Inhibition (\%)} = \frac{[\text{ABs} - \text{As}]}{\text{ABs}} \times 100$$

As is tested extract absorbance and ABs is the blank absorbance.

Ferric-reducing antioxidant power (FRAP) assay

The total antioxidant potential of extract was determined using the ferric reducing ability assay as described by Meryem *et al.* [22] with minor modifications. The assay was based on the reducing power of an extracts which reduce the ferric ion (Fe^{3+}) to the ferrous ion (Fe^{2+}); the latter forms a blue complex ($\text{Fe}^{2+}/\text{TPTZ}$), which increases the absorption at 700 nm. The test mix consist of 2 ml of extract (100 $\mu\text{g}/\text{ml}$) in ethanol, 2 ml of phosphate buffer (0.2 M, pH 6.6) and 2 ml of potassium ferricyanide $\text{K}_3\text{Fe}(\text{CN})_6$ (10 mg/ml). After 20 min of incubation at 50°C , 2 ml of trichloroacetic acid (100 mg/ml) was added. The obtained solution was centrifuged at 3000 rpm for 10 min. 2 ml of supernatant were mixed with 2 ml of distilled water and 0.4 ml of 0.1% fresh ferric chloride (w/v). The resulting mixture was incubated for 10 min and the absorbance was read at 700 nm. The total antioxidant potential was expressed in μMol Ascorbic Acid Equivalent (AAE)/g of extract.

Superoxide anion scavenging activity

A modified version of the method described previously was used [23]. The superoxide radicals were generated by alkaline Dimethyl Sulfoxide (DMSO). The assay consist of 50 μl of extract (100 $\mu\text{g}/\text{ml}$), 170 μl of alkaline DMSO and 30 μl of nitro blue tetrazolium (NBT) (1 mg/ml in DMSO). The mixture in a 96 wells microplate, was incubated for 5 min at the laboratory temperature ($22^{\circ}\text{C} \pm 2$) and the absorbance at 630 nm was measured against a blank using microplate Reader (Rayto R 6500, China). The blank consist of the test

mixture without extract. Quercetin was used as standard. Superoxide anion scavenging percentage (SSP) was calculated as follow:

$$SSP = [(A_{\text{blank}} - A_{\text{sample}}) / A_{\text{blank}}] \times 100]$$

SSP: Superoxide scavenging percentage; A_{sample} : extract absorbance; A_{blank} : blank absorbance.

Hydrogen peroxide scavenging assay

The ability of ethanolic extracts of *Dialium guineense* and *Trema orientalis* to scavenge hydrogen peroxide was estimated according to the method reported by Mohan *et al.* [24] with little modifications. The assay consist of 0.5 ml of extract (100 $\mu\text{g}/\text{mL}$) diluted in distilled water and 1.5 ml of hydrogen peroxide solution at 40 mM. After 10 min reaction, the absorbance was measured at 295 nm using spectrophotometer (VWR UV-1600 PC). Gallic acid were used as standard compound. Assay was performed three times. The H_2O_2 radical scavenging was calculated in percentage as follow:

$$HSP = [(A_{\text{Blank}} - A_{\text{S}}) / A_{\text{Blank}}] \times 100]$$

HSP is Hydrogen Peroxide Scavenging percentage; A_{S} is extract absorbance; A_{Blank} is the blank absorbance.

Results and Discussion

Phytochemical analysis

The results of phytochemical screening of extracts are showed in Table 1. The secondary metabolites detected in ethanolic extract of *Dialium guineense* were flavonoids, tannins alkaloids, triterpene, coumarin, saponin and anthracene derivatives. A similarity with our results have been reported in previous studies [12, 25, 26]. Flavonoids, lignanes, essential oils, tannin, saponins, Cardiac glycosides were detected in ethanolic extract of leafy stem of *Trema orientalis*. Previous studies on the leaves and bark of the plant showed the presence of phytoconstituents close to our results [27, 16]. The differences observed could be due to the used organs, the extraction solvents or the phenology of the plant [28].

Total phenolic and flavonoids contents

The total phenolic and flavonoid contents of ethanolic extracts of *Dialium guineense* leafy stem and *Trema orientalis* stem bark were respectively expressed as mg of Gallic Acid Equivalent (GAE)/g and Quercetin Equivalent (QE) of dry extract (Table 2). The total phenolic and total flavonoids contents of *D. guineense* were 102.82 mg \pm 2.83 GAE/100 mg and 15.34 \pm 1.03 mg EQ/100 mg. In contrast, the ethanolic extract of *Trema orientalis* showed a low polyphenol and flavonoids contents with concentration of 13.89 \pm 1.01 mg EAG/100 mg and 47.44 \pm 0.41 mg EQ/100 mg respectively. These Results revealed that ethanolic extracts of *Dialium guineense* had higher total phenolics (102.82 mg EGA/g dry matter) content than ethanolic extract of *Trema orientalis* (13.89 \pm 1.01 mg EAG/100 mg). The observed difference could be due to the intrinsic chemical composition of the plant, the solubility or the structure of the polyphenols compound in the extract. Indeed, Solubility of phenolic compounds is governed by the polarity of solvent used, the degree of polymerization of phenols, and interaction between solvent and phenolic compound [29].

Antioxidant activity of extracts

Biological activity of plants depends strongly on the type of active compounds in the extract which is a mixture of secondary metabolites with different functional groups and

polarity. This complexity of extract could lead to scattered results. Accordingly, the approach based on the use of various tests to evaluate the antioxidant potential of the extracts would be the most appropriate procedure. In the present study, the antioxidant activity of extracts was evaluated using DPPH scavenging assay, Ferric Reducing Antioxidant Power assay, superoxide anion and hydrogen peroxide scavenging activity.

DPPH scavenging assay

The antioxidant activity of *Dialium guineense* and *Trema orientalis* were dose-dependent (Figure 1). From 0.78 to 100 $\mu\text{g}/\text{ml}$, DPPH radical scavenging activities of both species were comparable to ascorbic acid. The DPPH radical Scavenging percentage of each concentration of the extracts are showed in Table 3. At 100 $\mu\text{g}/\text{mL}$, the inhibitory percentage of the DPPH radical was evaluated to 83.86 \pm 1.88; 96.06 \pm 1.19 and 99.46 \pm 0.38 respectively for *Trema orientalis*, *Dialium guineense* and Ascorbic acid. Thus, *Dialium guineense* ethanolic extract exhibited the strongest antioxidant activity with an IC_{50} of 4.24 \pm 0.09 $\mu\text{g}/\text{mL}$ in comparison with ascorbic acid (IC_{50} of 4.00 \pm 0.05 $\mu\text{g}/\text{mL}$) used as the standard (Table 3).

Ferric reducing antioxidant power

The ferric reducing capacity of *Dialium guineense* and *Trema orientalis* ethanolic extracts was performed using ferricyanide complex (Fe^{3+}) to ferrous form (Fe^{2+}) reduction assay. In this experiment, the yellow color changes to blue color depending on the concentration of antioxidants compounds in extract. *Trema orientalis* was the most active with reducing power value of 6007.8 \pm 175.57 $\mu\text{mol AAE g}^{-1}$ whereas *Dialium guineense* showed ferric reducing power of 3390 \pm 131.68 $\mu\text{mol AAE g}^{-1}$ (Table 4). It was reported that the scavenging potential and metal chelating ability dependent upon phenolic compounds have redox properties, which allowed them to act as reducing agents, hydrogen donors and singlet oxygen quenchers [30, 31]. The obtained results suggested that both extracts contained compounds which can react with free radicals to convert them into more stable products.

Superoxide anion and hydrogen peroxide scavenging activities

In superoxide anion assay, *Dialium guineense* showed the highest activity with scavenging percentage of 88.52 \pm 0.68 % whereas *Trema orientalis* presented a moderate activity (50.75 \pm 3.23 %). At the same concentration, quercetin showed superoxide anion scavenging percentage of 83.48 \pm 1.21 % weaker than *D. guineense*. Contrary to superoxide anion, both extracts showed moderate activity in hydrogen peroxide scavenging assay with an inhibitory percentage of 35.34 \pm 5.72 % for *D. guineense* and 40.35 \pm 4.95 % for *Trema orientalis* whereas gallic acid activity was 73.89 \pm 1.93 %. All results are summarized in Table 4. The most of the secondary metabolites detected in these extracts are well known for their antioxidant activity [32,33]. Indeed, antioxidants have the ability to neutralize or inhibit the formation of free radical. Previous study reported that polyphenols have the function to scavenge the free radicals in human body and to help maintain healthy body by scavenging or removing the reactive oxygen species (ROS) [36]. In addition, the biological properties of flavonoids and phenolic compounds have been proved by their various mechanisms including inhibition of ROS generation, mitochondrial dysfunction, apoptosis [34, 35]. As far as we know, no study has been

conducted on the determination of antioxidant activity of ethanolic extracts of stem bark of *Trema orientalis*.

Table 1: Preliminary phytochemical screening of extracts of *D. guineense* *T. orientalis*

Phytochemicals	Reagents	<i>T. orientalis</i>	<i>D. guineense</i>
Flavonoid	Sodium hydroxide (10 %), HCl (5 %)	+	+
Tannins	FeCl ₃ (1 %)	+	+
Alkaloids	Hydrochloric acid, Dragendroff's reagent	-	+
Triterpenes	Acetic anhydride ; Sulphuric acid	-	+
Coumarins	Sodium hydroxide (10 %) ; H ₂ O; heating	-	+
Saponins	Distilled water follow vigorous agitation	+	+
Essential oils	Sulphuric vanillin	+	-
Lignanes	Sulphuric vanillin	+	-
Anthocyanines	Sulphuric vanillin	-	-
Naphtoquinones	KOH-MeOH (10 %)	-	-
Anthracenes derivatives	KOH-EtOH (10 %)	-	+
Cardiac glycosides	Sulphuric acid (10 %)	+	-

Table 2: Total flavonoids and polyphenols contents of ethanolic extracts of *Trema orientalis* and *Dialium guineense*

	<i>Trema orientalis</i>	<i>Dialium guineense</i>
Flavonoids (mg EQ/100 mg)	47.44 ± 0.41	15.34 ± 1.03
Polyphenols (mg EAG/100 mg)	13.89 ± 1.01	102.82 ± 2.83

Table 3: The DPPH Scavenged percentage of each concentration of the extracts

Extracts concentration (µg / mL)	DPPH Scavenged percentage (%)		
	<i>Trema orientalis</i>	<i>Dialium guineense</i>	Ascorbic acid
0.78	5.31 ± 0.58	3.28 ± 0.35	4.77 ± 2.02
1.563	8.73 ± 0.52	11.20 ± 0.48	9.13 ± 4.02
3.125	22.33 ± 3.11	20.94 ± 0.71	46.64 ± 2.15
6.25	45.85 ± 0.82	47.65 ± 1.20	46.64 ± 2.15
12.5	56.94 ± 0.80	71.83 ± 2.74	91.44 ± 1.15
25	70.60 ± 3.49	88.78 ± 3.88	96.72 ± 1.03
50	76.42 ± 1.21	92.25 ± 2.87	98.35 ± 0.69
100	83.86 ± 1.88	96.06 ± 1.19	99.46 ± 0.38
	IC ₅₀ (µg/mL)		
	4.80 ± 0.08	4.24 ± 0.09	4.00 ± 0.05

Table 4: FRAP assay, Superoxide anion and Hydrogen peroxide scavenging activity of *Trema orientalis* and *Dialium guineense*

	FRAP assay µmol AAE g ⁻¹	Superoxide anion (%)	Hydrogen peroxide (%)
<i>Dialium guineense</i>	3390.0 ± 131.68	88.52 ± 0.68	35.34 ± 5.72
<i>Trema orientalis</i>	6007.8 ± 175.57	50.75 ± 3.23	40.35 ± 4.95
Quercetin	nd	83.48 ± 1.21	nd
Gallic acid	nd	nd	73.89 ± 1.93

nd : not determinated

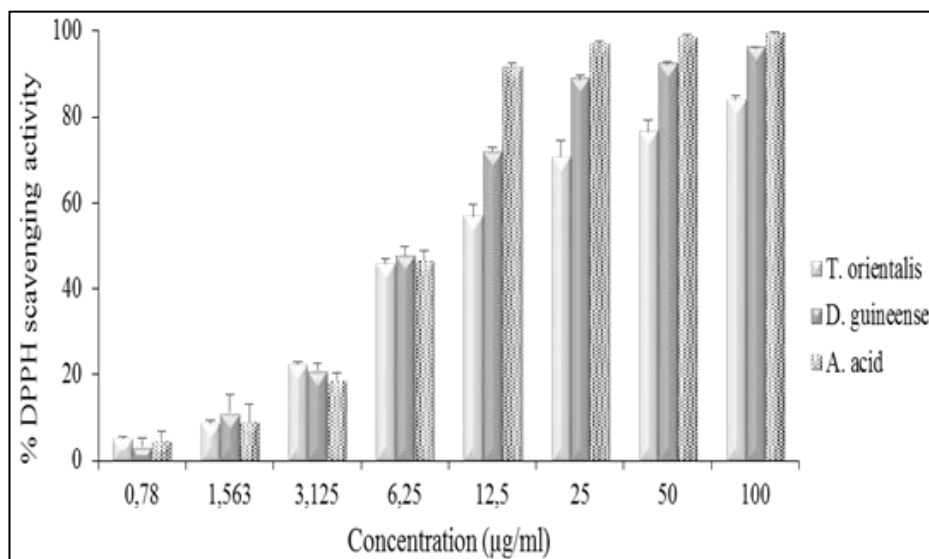


Fig 1: Radical DPPH scavenging activity of *T. orientalis* and *D. guineense* extracts. *T. orientalis*: *Trema orientalis*; *D. guineense*: *Dialium guineense*; A. acid: Ascorbic acid

Conclusion

In conclusion, the results obtained in this study suggested that the ethanolic extract of *Dialium guineense* and *Trema orientalis* exhibited interesting antioxidant activity by neutralizing or inhibiting the formation of free radical. The strong antioxidant, antiradicals potential and phenolic contents of studied plants were described. The presence of phenolic acids and flavonoids contributed to the interesting antioxidant activity of these plants.

References

- World Health Organization: Report of the WHO Inter-regional Workshop on the Use of Traditional Medicine in Primary Health Care, 2009.
- World Health Organization. WHO's Strategy for Traditional Medicine 2014-2023. OMS Ed, 2013, pp1-72.
- Eliassen AH, Hendrickson SJ, Brinton LA, Buring JE, Campos H, Dai Q, *et al.* Circulating carotenoids and risk of breast cancer: Pooled analysis of eight prospective studies. *J Natl Cancer Inst.* 2012; 104:1905-1916.
- Pojer E, Mattivi F, Johnson D, Stockley CS. The case for anthocyanin consumption to promote human health: A review. *Compr Rev Food Sci Food Saf.* 2013; 12:483-508.
- Tanaka T, Shnimizu M, Moriwaki H. Cancer chemoprevention by carotenoids. *Molecules.* 2012; 17:3202-3242.
- Sindhu M, Abraham TE, Zainul AZ. Reactivity of phenolic compounds towards free radicals under in vitro conditions. *J Food Sci Technol.* 2015; 52(9):5790-5798.
- Charalampous P, Konstantina L, Mavromelanidou OK, Panagiotis Z, Sinanoglou VJ. Antioxidant Capacity of Selected Plant Extracts and Their Essential Oils. *Antioxidants.* 2013; 2:11-22.
- Minatel IO, Borges CV, Ferreira MI, Gomez HAG, Chung-Yen OC, Giuseppina PPL. Phenolic Compounds: Chapter 1 : Functional Properties, Impact of Processing and Bioavailability. *In Phenolic Compounds - Biological Activity*, 2017. <http://dx.doi.org/10.5772/66368>
- Osuagwu GGE, Eme CF. The phytochemical composition and antimicrobial activity of *dialium guineense*, *vitex doniana* and *dennettia tripetala* leaves. *Asian Journal of Natural & Applied Sciences.* 2013; 2(3):69-81.
- Ugwoke CEC, Anze SPG, Nweze EA, Chukwube VO. Pharmacognostic evaluation of the leaf of *Trema Orientalis* (Family: Ulmaceae). *International Journal of Biology, Pharmacy and allied sciences.* 2017; 6(1):43-54.
- Moronkola DO, Kunle OF, Olaoluwa O, Ogukwe C. Chemical compositions of *Dialium guineense* Willd. leaf, stem-bark and fruit essential oils. *Journal of Complementary and Alternative Medical Research.* 2017; 3(4):1-8.
- Gnansounou SM, Iskandar S, Robin M, Brunel JM, Dahouenon E, Piccerelle P. *Dialium guineense* Willd. *Parkia biglobosa* (Jacq.) R. Br. Ex Benth. and *Tamarindus indica* L.: Review of known and synergetic bioactive compounds. *J. Med. Plants Stud.* 2018; 6(3):103-111.
- Aboaba SA, Choudhary IM. Chemical Composition and biological activities of the volatile oils of *Palisota hirsuta* (Thunb) K. Schum and *Trema orientalis* (L) Blume. *International Journal of Chemistry.* 2015; 7(2):21-26.
- Oyebola OE, Olajumoke AM, Isaiah OA. In-vivo antimalarial activity of aqueous leaf and bark extracts of *Trema orientalis* against *Plasmodium berghei* in mice. *J Parasit Dis.* 2016. DOI 10.1007/s12639-016-0815-0
- Tuo K, Béourou S, Touré AO, Ouattara K, Meité S, Ako AAB, *et al.* Antioxidant activities and estimation of the phenols and flavonoids content in the extracts of medicinal plants used to treat malaria in Ivory Coast. *Int. J. Curr. Microbiol. App. Sci.* 2015; 4(1):862-874.
- Loumpangou CN, Arnaud WEO, Longin JCBM, Béranger ITK, Peggy UBM, Jean-Maurille O, *et al.* Caractérisation Phytochimique et Toxicité aigüe des feuilles de *Trema Orientalis* (L.) Blume (Cannabaceae). *J. Soc. Ouest-Afr. Chim.* 2016; 42:52-59.
- Wagner H, Bladt S. *Plant Drug Analysis: A Thin Layer Chromatography Atlas.* 2nd éd. Springer-Verlag New York, 2001, 359-364.
- Shah MD, Hossain MA. Total flavonoids content and biochemical screening of the leaves of tropical endemic medicinal plant *Merremia borneensis*. *Arab J Chem.* 2010; 7:1034-1038.
- Amoussa AMO, Sanni A, Lagnika L. Antioxidant activity and total phenolic, flavonoid and flavonol contents of the bark extracts of *Acacia ataxacantha*. *J Pharmacogn Phytochem.* 2015; 4(2):172-178.
- Nadhiya K, Vijayalakshmi K. Evaluation of total phenol, flavonoid contents and *in vitro* antioxidant activity of *benincasa Hispida* fruit extracts. *International journal of pharmaceutical, chemical and biological sciences.* 2014; 4(2):332-338.
- Talbi H, Boumaza A, El-mostafa K, Talbi J, Hilali A. Evaluation of antioxidant activity and physico-chemical composition of methanolic and aqueous extracts of *Nigella sativa* L. *Journal of Material and Environmental Science.* 2015; 6(4):1111-1117.
- Meryem EJ, Rabie K, Marmouzi I, Zerrouki, Yahia C, Katim A. Radical-Scavenging Activity and Ferric Reducing Ability of *Juniperus thurifera* (L.), *J. oxycedrus* (L.), *J. phoenicea* (L.) and *Tetraclinis articulata* (L.). *Advances in Pharmacological Sciences*, 2016, ID 6392656, 6 pages. <http://dx.doi.org/10.1155/2016/6392656>
- Kumar RS, Raj Kapoor B, Perumal P. Antioxidant activities of *Indigofera cassioides* Rottl. Ex. DC. using various in vitro assay models. *Asian Pac J Trop Biomed.* 2012; 2(4):256-261.
- Mohan SC, Balamurugan V, Salini ST, Rekha R. Metal ion chelating activity and hydrogen peroxide scavenging activity of medicinal plant *Kalanchoe pinnata*. *J Chem Pharm Res.* 2012; 4(1):197-202.
- Akinpelu DA, Olaniyi AT, Mayowa AO, Olayinka AA, Anthony OI. Anti-Vibrio and preliminary phytochemical characteristics of crude methanolic extracts of the leaves of *Dialium guineense* (Wild). *J Med. Plants Res.* 2011; 5:2398-2404.
- Orji JO, Alo MN, Anyim C, Okonkwo EC. Antibacterial activities of crude leaf and bark extracts of "Icheku" *Dialium guineense* on bacterial isolates from bronchitis patients. *J Pharm Biol Sci.* 2012; 1:21-25.
- Glory OA, Agumage I, Abdulrahman U. Phytochemical analysis and antibacterial activity of *Trema orientalis* (Ulmaceae) stem bark extracts on respiratory tract bacteria. *Trop J Nat Prod Res.* November. 2018; 2(12):512-516.
- Houghton PJ, Raman A. *Laboratory handbook for the fractionation of natural extracts*, 1st ed. Chappman and Hall, 1998, p199.

29. Khoddami A, Wilkes M, Roberts TH. Techniques for Analysis of Plant Phenolic Compounds. *Molecules*. 2013; 18:2328-2375.
30. Pazos M, Gallardo JMM, Torres JL, Medina I. Activity of grape polyphenols as inhibitors of the oxidation of fish lipids and frozen fish muscle. *Food Chem*. 2005; 92:547-557.
31. Rice-Evans CA, Miller NJ, Paganga G. Antioxidant properties of phenolic compound. *Trends Plant Sci*. 1997; 2(4):152-159.
32. Vijayalakshmi M, Ruckmani K. Ferric reducing antioxidant power assay in plant extract. *Bangladesh J Pharmacol*. 2016; 11:570-572.
33. Kumar S, Kamboj A, Sharma AK. Antioxidant evaluation of ethanolic extract of *Fumaria parviflora* Lam. obtained from root, stem, leaf and fruit and measurement of their total phenols and flavonoids. *The Pharma Innovation Journal*. 2018; 7(4):577-579.
34. Tungmunnithum D, Thongboonyou A, Pholboon A, Yangsabai A. Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: An overview. *Medicines*. 2018; 5(93):1-16.
35. Paredes MD, Romecín P, Atucha NM, O'Valle F, Castillo J, Ortiz MC, *et al*. Beneficial effects of different flavonoids on vascular and renal function in L-NAME hypertensive rats. *Nutrients*. 2018; 10:1-15.