

**Czech University of Life Sciences Prague**

**Faculty of Tropical AgriSciences**

**Department of Crop Sciences and Agroforestry**



**IN VITRO ANTIOXIDATIVE EFFECT OF  
MEDICINAL PLANTS FROM GHANA**

**Diploma thesis**

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**PRAGUE**

# CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

Faculty of Tropical AgriSciences

## DIPLOMA THESIS ASSIGNMENT

B.Ed. Benjamin Berkoh Addai

Tropical Crop Management and Ecology

Thesis title

In vitro antioxidative effect of medicinal plants from Ghana

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### Objectives of thesis

1. Selection the most prospective plant species for further phytochemical analysis focused on isolation and identification of the main antioxidant principles of the plants.
2. Screening of selected ethnobotanicals for anti-oxidant activity using the DPPH anti-oxidant assay. The concentration of selected ethnobotanicals to reduce 50% of DPPH radicals (IC<sub>50</sub>) will be determined.
3. Summarization of information on botany, origin and geographical distribution, and ethnopharmacological uses of selected plant species.

### Methodology

Dried plant material of each species will be finely ground and extracted at room temperature in 80% ethanol. Extracts from each specimen will be subsequently filtered and evaporated to dryness in vacuo at 40 °C. Dry residues will be then stored at -20 °C until tested.

DPPH test is prepared by Sharma and Bhat method whereby two-fold serial dilutions of each extract is prepared in methanol in 96-well microtiter plates. In this test 25 µL of freshly prepared 0.4 mM methanol solution of DPPH was mixed with the extracts in each well creating a range of concentrations from 64 to 0.25 µg/mL to obtain 200 µL volume in other to start the radical-antioxidant reaction. The mixture is kept in the dark at 25°C and the absorbance of extracts is measured after 30 min at 517nm using Biotek cytation 3 Microplate Photometer. Also the half maximal inhibitory concentration (IC<sub>50</sub>) values were calculated using Gen 5 version 2.07 software and the results are expressed as an average of three separate experiments, each performed in three replicates.

The proposed extent of the thesis

50

Keywords

Crude plants extracts, Oxidative stress, Antioxidants, In vitro, Inhibition, Dilution, Testing, Concentration, Purity.

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## **DECLARATION**

I, Addai Benjamin Berkoh, declare that this diploma thesis, submitted in partial fulfilment of the requirements for the degree of Ing. in the Faculty of Tropical AgriSciences in the Czech University of Life Sciences Prague, is wholly my own work unless otherwise referenced or acknowledged.

**Prague, June 2016**

*Addai Benjamin Berkoh*

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## ABSTRACT

The *in vitro* antioxidative activity of crude extracts derived from sixteen Ghanaian plant species (*Allophylus spicatus*, *Cnestis ferruginea*, *Ficus thonningii*, *Indigofera hirsuta*, *Indigofera pulchra*, *Launaea taraxacifolia*, *Leea guineense*, *Mikaniopsis tedliei*, *Millettia thonningii*, *Philenoptera cyanescens*, *Salacia senegalensis*, *Senna occidentalis*, *Stachytarpheta indica*, *Tiliacora warneckeii*, *Triumfetta rhomboidea*, *Vernonia colorata*) were tested in frame of this study using 1,1-diphenyl-2-picrylhydrazyl radical scavenging capacity assay. The extract from the bark of *Indigofera hirsuta* has been found as the most active sample ( $IC_{50} = 12.84 \pm 0.82 \mu\text{g/mL}$ ), because its activity is very close to the positive control Trolox and ascorbic acid. We expect that this finding may be used by pharmaceutical industry for development of new herbal-based nutraceuticals and pharmaceutical preparations.

**Keywords:** Medicinal plants, Crude extracts, Antioxidative effect, DPPH test.

## ABSTRAKT

*In vitro* antioxidační aktivita surových extraktů odvozena od šestnácti druhů rostlin Ghany (*Allophylus spicatus*, *Cnestis ferruginea*, *Ficus thonningii*, *Indigofera hirsuta*, *Indigofera pulchra*, *Launaea taraxacifolia*, *Leea guineensis*, *Meconopsis tedliei*, *Millettia thonningii*, *Philenoptera Cyanescens*, *Salacia senegalensis*, *Senna occidentalis*, *Stachytarpheta indica*, *Tiliacora warneckii*, *Triumfetta rhomboidea* a *Vernonia colorata*) byly testovány v rámci této studie za použití 1,1-difenyl-2-picrylhydrazyl testu akceptor radikálů kapacity. Extrakt z kůry *Indigofera hirsuta* bylo zjištěno, jako nejvíce aktivní vzorek ( $IC_{50} = 12.84 \pm 0,82$  ug / ml), protože její činnost je velmi blízko k pozitivní kontrole Troloxu a kyseliny askorbové. Očekáváme, že toto zjištění může být používáno ve farmaceutickém průmyslu, při vývoji nových rostlinných bází nutraceutik a farmaceutických přípravků.

**Klíčová slova:** Léčivé rostliny, surové výtažky, Antioxidační účinek, DPPH test.

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## LIST OF ABBREVIATIONS

ALD	Alcohol-induced liver disease
AD	Alzheimer's disease
CVD	Cardiovascular disease
DNA	Deoxyribonucleic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
GA	Ghana
IC <sub>50</sub>	Inhibitory concentration 50%
LDL	Low-density lipoproteins
MCI	Mild cognitive impairment
PD	Parkinson's disease
RNS	Reactive Nitrogen Species
ROS	Reactive Nitrogen Species

## **FORWARD**

Higher plants, are good provision of medicinal compounds and critical in playing a vital role in the sustainance of physical health over the past years. More than fifty percent of all the recent medical drugs are obtained from natural plant products in existence (Stuffness and Douros, 1982) and natural plant products perform a crucial role in drug manufacturing programs from the pharmaceutical industries (Baker et al., 1995; Cordell, 1995). In developing countries like Ghana, especially in rural areas, people more often consult traditional healers when become ill and plants of ethnobotanical uses are mostly administered for use. In understanding the chemical and biological associations of plants in the past two centuries has made provision for compounds used in the manufacturing of current synthetic drugs and in the emergence of therapeutic agents (Roja and Rao, 2000).

Currently, several scientists and organizations are in the inculcating the need of administering traditional remedies as subsequent source of medicine (Cortella and Pocheltino, 1999, OMS, UICN and WWF, 1993). Thus several plants used in traditional remedies are sold all over urban settlement in order to meet the needs of the public and this has resulted in ; industrialization and huge scale manufacturing of a high number of products of botanical origin mostly consumed. The African continent is one of the continents blessed with enormous biodiversity worldwide, with an array of many food plants used as medicinal herbs, as well as healthy foods and also other remedies for many therapeutic purposes. This is mostly because of the geographical widespread of vast land of approximately 216,634,000 hectares within enclosed forest locations. Over 5,000 several species of plant materials have been identified from these areas, and several others have also been identified to be beneficial in traditional medicine for prophylaxis and in the treatment of diseases (Iwu, 1993). This large biodiversity therefore provides the economic assurance and in the quick emergence of the biotechnological industries.

Thus, we decided to also investigate the antioxidative potential of selected medicinal plants species in order to explore new biologically active extracts, which could possibly be applied to improve human or animal health and nutrition.

## 1.0. INTRODUCTION

### 1.1.0. Oxidative stress

Oxidative stress involves free radical which is any species that contains one or more unpaired electrons as part of its atomic structure and is capable of autonomous existence (Halliwell and Gutteridge, 1999). An unpaired electron is an electron that occupies a nuclear alternatively sub-atomic orbital by itself. Free radicals can be created from the loss or gain of a solitary electron from a non-radical. Moreover, free radicals can also be built by homolytic fission whereby the splitting of a covalent bond in between two atoms occurs (Halliwell and Gutteridge, 1999). Due to this, a single electron from each of the distributed pair remains with each isolated atom:



Within the equation  $A^{\cdot}$  is an A-radical whereas  $B.$  is a B-radical. In the same sense, homolytic fission of a single covalent bond within the water molecule would result in a hydrogen radical ( $H^{\cdot}$ ) and also a hydroxyl radical ( $\cdot OH$ ).

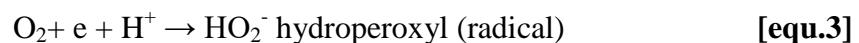
In other words, free radicals can be structured by heterolytic fission in which a single atom accepts two electrons during the breakdown of a covalent bond (Halliwell and Gutteridge, 1999):

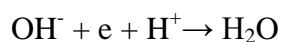
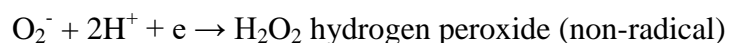


The additional electron makes A an anion and B, which is a free radical a cation.

### 1.1.1. Free Radicals and their Formation

Aerobic existence needs oxygen for the oxidation and burning of hydrogen and carbon rich substrates. This process equips the build-up of chemical energy which is beneficial for survival. It also results in the reduction of the oxygen molecule to various free radicals (Gutteridge, 1993). Systematic reduction of oxygen takes place as follows:





Systematic decreasing of the oxygen atoms results in a number of distinctive radicals as displayed above. Further, the oxygen atoms in the ground state, as it structures naturally which qualifies as a free radical. Furthermore, those oxygen atoms undergoes dismutation processes resulting in the build-up of hydrogen peroxide which plays a crucial part in the structuring of highly sensitive free radical species (Gutteridge, 1993; Halliwell and Gutteridge, 1999). Nitric oxide ( $\text{NO}^-$ ) is an alternative free radical located in different biological frameworks. It is formed by the vascular endothelium and different cells (Moncada and Higgs, 1991). It has been accounted to adhere to the superoxide anion which results in a peroxynitrite intermediate ( $\text{ONOO}^-$ ) which is able to destroy several biological atoms (Beckman et al., 1990; Saran et al., 1990). Transition metals located in the premiere row of the d-block of the Periodic Table holds free electrons. Due to this, they are categorized under the wide definition of free radicals. Furthermore, they show alternate valencies which make them conceivably sensitive. Several other free radicals are found in biological frameworks such as peroxy, alkoxy and thioyl free radicals (Halliwell, 1995).

### ***1.1.2. Diseases related to the oxidative stress***

Oxidative stress play a crucial part in the pathogenesis of several illness. The occurrence of free radicals in vivo occurs haphazardly and for required metabolic purposes. An inequality between free radical generation and physical gaurd mechanisms brings about oxidative harm to most cell structures, examples are DNA, proteins, lipids, etc. Reports show that various pathological states and diseases such as cancer, cardiovascular disease, arthritis, immune system impairment, Parkinson's disease, atherosclerosis, Alzheimer's disease, diabetes and premature body maturing can possibly be associated to the build-up of very sensitive free radicals (Benavente-Garcia et al., 1997, Vaughan, 1997). Reactive oxygen species (ROS) formed in vivo, such as superoxide anion, hydroxyl radical and hydrogen peroxide, are often reactive and potentially destroying

transient chemical species. These are frequently manufactured in the human body, and are essential for energy supply, detoxification, chemical signaling and immune function.

ROS are regulated by endogenous superoxide dismutase, glutathione peroxidase and catalase but due to abundant-production of reactive species, induced by contact with external oxidant substances or a failure in the protective mechanisms, destroy cell structures, DNA, lipids and proteins (Valko, Rhodes, Moncol, Izakovic, & Mazur, 2006) occurring which increases risk of more than 30 different disease processes (Aruoma, 1998). The most dangerous among them being neurodegenerative conditions like Alzheimer's disease (AD) (Smith et al., 1996, Smith, Rottkamp, Nunomura, Raina, & Perry, 2000), mild cognitive impairment (MCI) (Guidi, Galimberti, Lonati, & Novembrino, 2006) and Parkinson's disease (PD) (Bolton, Trush, Penning, Dryhurst, & Monks, 2000). Other diseases include highly disabling vascular pathologies like cardiovascular disease (CVD) and cardiac failure (Jha, Flather, Lonn, Farkouh, & Yusuf, 1995), alcohol-induced liver disease (ALD) (Arteel, 2003) and Ulcerative colitis (Ramakrishna, Varghese, Jayakumar, Mathan, & Balasubramanian, 1997) and cancer caused by a complex of several causes, of which ROS/RNS is a component. Valko et al. (2007) have done an extensive review on the effect of free radicals and antioxidants in normal physiological functions and human disease. Furthermore, it has been proposed that free radicals play an important role in the pathogenesis of ischaemic injury (Cotelle et al., 1996).

### ***1.1.3. Antioxidants***

An antioxidant is any substance that when present at low concentrations, compared to those of the oxidizable substrate, effectively delays, or inhibits, oxidation of that substrate (Halliwell and Gutteridge, 1999). Anti-oxidants can react by a number of mechanisms including removal of oxygen or decreasing local oxygen concentrations; removing catalytic metal ions; removing reactive oxygen species (ROS) such as superoxide anions and hydrogen peroxide; scavenging of the starting free radicals such as hydroxyl free radicals; scavenging of singlet oxygen; and chain-breakage of an oxidation sequence (Gutteridge, 1993). Superoxide dismutase (SOD), catalase and glutathione peroxidase are examples of enzymatic innate biological anti-oxidants inherent to the human body. A number of nonenzymatic anti-oxidants also exist such as



albumin, ascorbic acid,  $\alpha$ -tocopherol,  $\beta$ -carotene, uric acid and bilirubin (Prior and Cao, 1999).

Natural antioxidants are also found in various vegetables (Pratt, 1965), soybean (Pratt and Birac, 1979), citrus peel (Harel and Kanner, 1984), sesame seed (Budowski, 1964), olives (Sheabar and Neeman, 1988), carob pod (Farr et al., 1988), and green tea leaves (Zhao et al., 1989). Natural antioxidants was recently reviewed by Namiki (1990), Pratt and Hudson (1990), Schuler (1990), and Loliger (1991). Plant tissues are the main biological systems that synthesize  $\alpha$ -tocopherol, ascorbic acid, and carotenoids, but in addition they are also rich in a wide variety of phenolic compounds (Dugan, 1980; Horesteen, 1983; Namiki, 1990; Loliger, 1991). Common foods of plant origin contain a variety of hydroxylated flavonoids and other phenolics in amounts ranging from traces to several grams per kilogram of fresh weight (Dugan, 1980; Pratt and Hudson, 1990; Macheix et al., 1990). Flavonoids and other plant phenolics have been reported to have multiple biological effects such as antioxidant activity (Chipault et al., 1956; Chang et al., 1977; Hudson and Lewis, 1983; Bros and Saran, 1987). Grapes, wines, and grape byproducts contain large amounts of phenolic compounds, mostly flavonoids, at high concentrations of 1000-1800mg/ml (Singleton, 1982; Brasseur et al., 1986; Macheix et al., 1990). A large part of the phenolics in grapes, wines, and byproducts may act as antioxidants. Some of these compounds may act selectively at very low concentrations to inhibit ex vivo low-density lipoproteins (LDL) oxidation in vitro (Frankel et al., 1993)(Kanner et al., 1994).

#### ***1.1.4. Medicinal Plants as Antioxidants***

Recently, there is a considerable interest in preventive medicine in the development of “natural antioxidants” from plants material. (Loliger, 1991). Earlier, Chipault et al. (1956) and others (Chang et al., 1977; Dugan, 1980; Loliger, 1991) evaluated the antioxidant properties of many plants like rosemary (*Rosmarinus officinalis*) and sage (*Salvia officinalis*) and discovered that they provided the most effective extracts. Commonly used medicinal plants with antioxidant activity known worldwide are from several families, especially Lamiaceae (oregano, marjoram, basil, thyme, mints, balm), Apiaceae (cumin, fennel, caraway), and Zingiberaceae (turmeric, ginger) and depends on the plant, its variety, environmental conditions, climatic and seasonal variations,

geographical regions of growth, degree of ripeness, growing practices, and many other factors such as postharvest treatment and processing (Skrovankova et al., 2012). In addition, a number of phytochemicals which display anti-oxidant activities have been isolated from medicinal plants. These include polyphenols such as tannins and flavonoids (Yokozawa et al., 1998), tocopherols and catechines (Dapkevicius et al., 1998).

#### ***1.1.5. Antioxidant activities of medicinal plant extracts in West Africa***

Several reports on the *in vivo* antioxidant potential of West African plants collected from Nigeria, Ghana, Mali and Niger Republic have previously appeared in the literature. For example, the antioxidant potential of *Sacoglottis gabonensis* stem bark, a traditional Nigerian alcoholic beverage additive, on 2,4 dinitrophenylhydrazine-induced membrane peroxidation *in vivo* has been reported (Maduka and Okoye, 2002). The authors concluded that the mechanism of antioxidant action of the extract was multifactorial/multi-system involving inhibition of catalase, enhancing the superoxide dismutase (SOD) capability of the liver and red blood cells, and sparing tissue depletion/utilization of vitamin C (ascorbic acid) and vitamin E (tocopherol). Using streptozotocin-induced non-insulin dependent diabetes rat model, Ugochukwu and Babadu (2002) demonstrated that aqueous and ethanolic extracts of *Gongonema latifolium* leaves harvested from eastern Nigeria significantly increased the activity of superoxide dismutase and the level of reduced activities of the plant could be mediated through its antioxidant properties.

Methanol extract of leaves of *Mallotus oppositifolium* harvested from Western Nigeria has been shown to possess antioxidant and anti-inflammatory activities in  $\beta$ -carotene linoleate model system and the carrageen-induced rat paw oedema animal model (Farombi et al., 2001). Thin layer chromatographic analysis of this extract revealed the presence of four phenolic spots, two of which were flavonoids. Germano et al. (2001) have reported the hepatoprotective properties of root decoction of *Trichilia roka*, a plant used in Mali folk medicine, against carbon tetrachloride-induced hepatotoxicity and correlated this effect to the polyphenol antioxidant component of the fraction. In another study involving the screening of 20 Malian medicinal plants, Diallo et al. (2001)

demonstrated that 20% of the plants, including *Cussonia barteri*, *Glinus oppositifolius*, and *Lannea velutina* possessed potent antioxidant activity in DPPH test.

Work combining *in vivo* and *in vitro* assessment of some Ghanaian medicinal plants revealed that *Thonningia sanguinea* possess free-radical scavenging capacity and strong hepatoprotective activity inhibiting hydrogen peroxide-induced lipid peroxidation, galactosamine-induced hepatitis and carbon tetrachloride-induced hepatotoxicity (Gyamfi et al., 1999). Another report on *T. sanguinea* harvested from Adzopé in Ivory Coast confirmed its antioxidant potentials and free-radical scavenging activity (Ohtani et al., 2000). In fact these authors isolated ellagitannins, thonningianin A (1) and thonningianin A (2) as the major antioxidant principles of this plant.

In another work, Cruz-Garcia, 2006 determined the antioxidant capacity of aqueous extracts of 17 Nigerian plants that are used for food and traditional medicines. The antioxidant contents of the aqueous extracts were compared to those of spinach and potato. Of the 17 plants, 11 had greater antioxidant content than spinach and 14 had greater antioxidant content than potato. The leaves of *Tapinanthus globiferus* had the greatest antioxidant content. In general, leaves contained more antioxidants than either fruits or seeds. The leaves of *Entada africana* have also possessed antioxidant action *in vitro* (Cook et al., 1998).

#### ***1.1.6. Characteristics of Ghanaian antioxidant selected plant species***

In this section is described the botanical characterization of Ghanaian plant species, their geographical distribution and traditional uses, in accordance with the aim of this thesis.

##### ***1.1.6.1. Allophylus spicatus***

**Family:** Sapindaceae

**Synonyms:** *Schmidelia spicata* DC.

**Vernacular names:**

- **English names:** red berry tree

- **Tribal names:** GA tetedua TWI tetudua

**Origin and geographic distribution:** *A. spicatus* originated from Southern Africa. It is distributed all over Southern and Western Africa. It is typically found at altitudes of 450 to 1900 m (Nansen et al., 2001). It is the sole West African species of the genus *Allophylus*, which is distributed in tropical Africa through tropical Southeast Asia. *A. spicatus* is found in the coastal savannah zones in rocky areas and by streams, especially in the savannah regions of Ghana (Harrington, 2005).

**Description:** It is many-stemmed shrub; branchlets silver-grey, appressed-pubescent when young, and soon glabrescent. Leaves are 3-foliolate; petiole 0.5–3.5 cm long, has appressed- or crisped-pubescent; with terminal leaflet usually 1.5 times as long as the lateral ones; and petiolules are up to 1 mm long or leaflets subsessile; lamina of terminal leaflet are within 1–4.5 × 0.5–2.5 cm. It has papyraceous, shortly and sparsely appressed- or crisped-pubescent on the midrib and lateral nerves and with occasional tufts of hairs on the under surface of the axils of the latter, otherwise glabrous, lobed usually to about halfway to the midrib (at least in some leaves). Lobes are generally rounded but apex occasionally acute, base cuneate and often narrowed; lateral nerves 3–6 pairs. Inflorescence 1.5–6 cm long, unbranched, and appressed-pubescent. Flowers whitish, in few-flowered are very shortly pedunculate cymules; pedicels are up to 2 mm long. Outer sepals of the plant are 1.3 × 1.3 mm, subcircular, and the inner sepals are 1.3 × 1 mm, elliptic, and all glabrous or nearly glabrous. Petals are 1.2 × 1 mm, and obovate. Stamens are with pilose filaments 2 mm long; Staminodes with filaments 0.7 mm long in female flowers. Style is 1 mm long. Fruit (not known from our area) 3.5 mm in diameter, and globose (Exell, 1966).

**Traditional uses:** *A. spicatus* is a pain killer and also used as treatment to several pulmonary diseases (Burkill, 1985). Also it is very good to put in broth for a sick man or woman, it causing strength. Root bark in hot infusion a remedy for diarrhoea (Soelberg et al., 2015).

#### **1.1.6.2.        *Cnestis ferruginea***

**Family:** Connaraceae

**Synonyms:** None

**Vernacular names:**

- **English names:** Tooth cleaning fruit
- **Tribal names:** ASANTE aposé FANTE akitasé Ga ahuidade

**Origin and geographic distribution:** It is distributed around tropical Africa from Gambia to east Zaire, South Sudan, Southern to Northern Angola. In deciduous forests, forest groups in savannahs, sometimes abundant in pasture reforestation, near beaches, and around villages. *C. ferruginea* is native to tropical Africa (Neuwinger, 1996).

**Description:** It is a shrub, 8–12 ft high, the branches terete, and the young ones densely grey- or ferruginous-velvety. It has fully developed leaves 1 ft long, the petioles 2 in. It has leaflets in 8–10 pairs and a terminal one, the upper ones are oblong or ovate-oblong, 2–2.5 in long, bluntish, the base is cordate, the lateral ones are not quite opposite; the texture is subcoriaceous, upper surface glabrous, and lower coated all over with grey-ferruginous tomentum. It flowers in terminal panicles with long, narrow, racemose, erecto-patent branches; pedicels are about equalling the calyx, which is campanulate, under 0.1 in. deep, the divisions are lanceolate, and densely brown-velvety on the back. Petals are whitish, rotundate-cuneate, considerably shorter than the calyx. Carpels are 1–5, 1–1.5 in long, 0.5 in thick below, considerably curved and narrowed upwards, deeply wrinkled with longitudinal grooves, and clothed with bright red velvety tomentum (Baker, 1868).

**Traditional uses:** *C. ferruginea* is used to treat diseases like fever, and pain (Toyin & Olaide, 2012). Also the teeth being washed with a decoction of this in water, is good for the scurvy in the mouth (Soelberg et al., 2015).

#### **1.1.6.3. *Ficus thonningii***

**Family:** Moraceae

**Synonyms:** *Urostigma thonningii*

**Vernacular names:**

- **English names:** Fig
- **Tribal names:** No GA name recorded

**Origin and geographic distribution:** Widespread in subsaharan Africa from Cape Verde Islands to Ethiopia, and southwards to Namibia and South Africa (Cape Province). Common habitat for *F. thonningii blume* includes Miombo woodland, swamp forest (mushitu), riverine vegetation, Baikiaea thickets (mukusi, mutemwa) on Kalahari Sand, wooded grassland and dambos, often on termitaria. Survives in altitudes of withing 0-2300m above sea level (Arbonnier, 2002).

**Description:** It is a tree up to 15(30) m tall, or a shrub, either terrestrial or hemi-epiphytic. It is made of leafy twigs 1.5–8 mm thick, minutely puberulous to hirtellous or white- to brown-pubescent, or glabrous on the stipule scars, sometimes entirely glabrous, and periderm usually not flaking off. Leaves are spirally arranged, occasionally subopposite; lamina elliptic to oblanceolate or subobovate to subovate, (1.5)3–12(18) x (1)1.5–6(9) cm, subcoriaceous; the leaf apex acuminate to obtuse or rounded; the leaf base cuneate to rounded or subcordate, often slightly inaequilateral; margin entire; superior leaf surface is glabrous or sparsely puberulous to pubescent, the midrib is more dense, the inferior surface glabrous or sparsely to densely white- to brownish-puberulous or pubescent on the whole surface. The lateral veins are (5)7–12(16) in pairs, main vein or midrib often reaching the apex of the lamina (even in leaves with a rounded apex), tertiary venation reticulate or parallel to the lateral veins; leaf petiole (0.5)1–4(6) cm long, 1–2 mm thick. Leaves are often (not depending on the size of the lamina or the position of the leaf on the twig) variable in length on the same twig, glabrous or puberulous, hirtellous or pubescent; stipules between 3–10(20) mm long, with white to brown pubescent, puberulous or only ciliolate, and caducous or subpersistent. Figs in pairs in the leaf axils, sometimes also below the leaves, sessile or on peduncles up to 1 cm long; basal bracts 2–4 mm long, are persistent. Receptacle are globose to ellipsoid, within 5–10(12) mm in diameter when fresh, 4–12(17) mm in diameter when dry, glabrous or sparsely to densely white to brown puberulous or pubescent, reddish, yellowish or brownish at maturity; wall thin, mostly smooth or

slightly wrinkled when dry; and apex plane to strongly protruding when dry (Berg. 1991).

**Traditional uses:** *F. thonningii* is used in treatment of urinary tract infections, diabetes mellitus, respiratory infections and mental illnesses (Dangarembizi et al., 2013). Als it is warmed near fire, and applied to a boil, breaks it (Soelberg et al., 2015).

#### **1.1.6.4.        *Indigofera hirsuta***

**Family:** Leguminosae

**Synonyms:** *Indigofera indica*

**Vernacular names:**

- **English names:** Hairy indigo
- **Tribal names:** No GA name recorded

**Origin and geographic distribution:** Its native Asia and Africa. It was cultivated as a green manure in Bogor in the 19<sup>th</sup> Century and was first tried as such in Malaysia in 1913. *I. hirsuta* was introduced int the United States in 1908 and proved suitable for cultivation in the coastal regions of Florida and Texas. It is now cultivated through out the tropics (Jansen et al., 2007).

**Description:** It is herbaceous, erect, densely and softly hairy. Branches angular. Leaves are short-petioled with the common petiole gland-stipelled. The leaflets are elliptic-oblong, subobtuse, mucronulate, villous on both sides, the terminal petioled; stipules setaceo-subulate, long; racemes subspicate, hirsute peduncles. It is densely many-flowered; bracts minute; calyces hirsute, the segments subulate-acuminate; legumes short, turgid, mucronate, deflexed, densely hirsute, few-seeded. Stem one or two feet high; the whole plant densely clothed with long, soft, pale hairs. Leaves 1.5–2 in long, and closely set. Leaflets are 4–6 lines long, and 2–3 lines wide. The flowers are small, closely set, subsessile, on peduncles 4–6 in long. (Harvey, 1894).

**Traditional uses:** *I. hirsuta* is used for treatment of stomachache and body pain (Suvarnalatha et al., 2014). Also it is boiled and drunk, causes the great sort of pox (syphilis) to skin and dry (Soelberg et al., 2015).

#### **1.1.6.5. *Indigofera pulchra***

**Family:** Leguminosae

**Synonyms:** None

**Vernacular names:**

- **English names:** None
- **Tribal names:** No GA name recorded

**Origin and geographic distribution:** This species is native to tropical Africa. *I. pulchra* is distributed all over tropical and subtropical regions of Nigeria, Niger, Togo, Benin, Ghana and Chad. It survives in altitudes within 100–900 m above sea level (Kuate, 2013).

**Description:** It is erect branching annual up to 1 m tall, pubescent in all its parts, there are hairs on the fruit, the calyx and bracts often yellowish-brown, those elsewhere are white. The leaves are usually dimorphic, with the upper part 1–3 foliolate and the lower part up to 11 foliolate, mostly in all foliolate; stipules; subulate, 1–3 mm long, often reflexed; The rachis are prolonged beyond lateral leaflets, up to 3 cm long including a petiole of up to 6 mm. The leaflets are elliptic-oblongate or cuneate, up to 12 mm long and 6 mm wide. The inflorescences terminal racemes are sometimes condensed and subcapitate, the bracts are leafy and passing gradually into the foliolate leaves below; pedicels are stiffly erect, up to 7 mm long, jointed 1 mm below the calyx, with a slender bracteole at the joint. Calyx is brownish, cleft to the base; lobes lanceolate, fimbriate,  $\pm$  3 mm long in flower, 5 mm in fruit. Standard and keel  $\pm$  2.5 mm long, strigose outside. Stamens are all fertile,  $\pm$  2.5 mm long. Fruit  $\pm$  4 mm long, 1.3 mm wide, 1 mm thick, pubescent, 2 (rarely 1)-seeded; endocarp slightly spotted (Gillett, 1971).



**Traditional uses:** *I. pulchra* is used for treatment of gastrointestinal pain and malaria (Kuate, 2013). Also the leaves are dried, ground to a fine powder and strewn on old leg injuries, which thereafter are bathed with a decoction of the same (Soelberg et al., 2015).

#### **1.1.6.6. *Launaea taraxacifolia***

**Family:** Asteraceae

**Synonyms:** *Sonchus taraxacifolius*

**Vernacular names:**

- **English names:** Wild lettuce
- **Tribal names:** GA agbloge TWI nne-noa

**Origin and geographic distribution:** Native to Senegal and Ethiopian highlands. *L. taraxacifolia* has been domesticated in Nigeria and cultivated in home gardens in Benin, Ghana and Senegal. In North. Kenya hybrids with *L. petitiana* have been found (N. Kilian, (1997)). Its distributed over Senegal, Sierra Leone, Ghana, Benin, Nigeria, Sudan, Ethiopia (Shackleton et al., 2009).

**Description:** It is an annual herb, erect, 1–2 m high; stem solitary, branching in the distal part of the plant, and glabrous. Leaves are cauline, sessile, oblanceolate to broadly elliptic, pinnatilobed with spreading lobes, 6–20 cm long, 2–10 cm wide, with base subauriculate, lobes deltoid, to 4 cm long, margins dentate, apex obtuse and apiculate, glabrous. Capitula many, subsessile in large, lax panicles with long side branches; the stalks of individual capitula is between 1–10 mm, sometimes pilose; involucre within 10–12 mm long, conical-cylindric to spreading. Phyllaries are green, tinged with brown, ovate (outer) to lanceolate (inner), 1.5–12 mm, with the outer extending down the stalk, acute and margins not or hardly hyaline, glabrous or minutely ciliate at the very apex. Corolla is yellow, tube 4–5.5 mm long, distally pilose, ligule 5 mm long. Achenes grey, fusiform, 2.5–3 mm long, with 4 main ribs and 8 thinner ones, all muricate, the apex slightly narrowed; pappus white, 7–8 mm long, of mixed setae and downy hairs (Beentje, 2000).

**Traditional uses:** *L. taraxacifolia* is used for treatment of vomiting, teeth pain and diabetes (Fern, 2014). Also the leaves produces expressed sap used for alleviation of pain in fresh wounds (Soelberg et al., 2015).

**1.1.6.7.        *Leea guineensis***

**Family:** Vitaceae

**Synonyms:** *Leea manillensis*, *Leea coccinea*, *Leea sambucina*

**Vernacular names:**

- **English names:** West Indian holly
- **Tribal names:** ASANTE akataki, okatakyi

**Origin and geographic distribution:** This species is native to tropical South East Asia and Africa. *L. guineensis* distributed from Senegal to Gambia and to other areas. In swamp forest, fringing forest and forest edges (Jean & Barabe, 1998).

**Description:** It is a shrub 1 m tall or a tree up to 10 m. tall (outside our area). Branches are soft-wooded, and glabrous. Leaves are bipinnate or occasionally tripinnate at the base; petiole 12 cm long, glabrous or pubescent; rachis with reddish swellings at the insertion of the pinnae in the fresh state but these swellings changing to constrictions in herbarium specimens; stipules 2–4 × 1–3 cm, very caducous; pinnae 5; Leaflets are 3–5(7), imparipinnate, opposite, petiolule to 2 mm long, leaflet-lamina 4–20 × 2–7 cm, elliptic, acuminate at the apex, margin serrate-dentate, rounded at the base, somewhat discolorous, glabrous or occasionally pubescent on the nerves on the lower surface; nerves are prominent below. Cymes usually 2-branched from the base, up to 20 cm across; branches are reddish, pubescent or glabrous; bracts and bracteoles 0.5 × 0.5 mm, triangular, very caducous, glabrous; pedicels 0.5 mm long, glabrous or pubescent. The flowers globose in bud, 2–5 mm in diameter, red outside, orange or yellowish inside. Calyx is 1.5 mm long, glabrous or pubescent. Corolla 5 mm long, glabrous. Staminal tube with 5 truncate 2-dentate lobes, shorter than the petals. Ovary is ovoid, 4–6-locular; style is 2.5 mm long; stigma subglobose-capitate. The fruits are red turning

black when ripe, up to 8 mm in diameter, and glabrous with a persistent calyx. Seeds is 4 × 3 mm, brownish, and smooth (Wild, 1966).

**Traditional uses:** *L. guineensis* is used for treatment of diseases like muscular pain, arthristis, rheumatism, vertigo, oedema, abscess and furuncle (Herrmann, 2012). Also the bark of the tree is rubbed on chronic swellings (Soelberg et al., 2015).

#### **1.1.6.8. *Mikaniopsis tedliei***

**Family:** Asteraceae

**Synonyms:** *Senecio tedliei*

**Vernacular names:**

- **English names:** None
- **Tribal names:** For Senecio biafrae: FANTEyankonfeh

**Origin and geographic distribution:** *M. tedliei* is native to West Africa and distributed all over Togo, Senegal, and widespread over Ghana, Cameroon, Uganda and Congo (Kinshasa). Favourable to altitudes of 1150m above sea level (Hawthorne & Jongkind, 2006).

**Description:** It is a woody climber reaching several m in length; branches striate, tomentose-pubescent and glandular on young parts, and becoming puberulous with age. Leaves cordate, 6–10.8 cm long, 4.5–10.1 cm wide, base subcordate, margins sparsely dentate, apex acuminate, dark green and puberulous above, grey-green and tomentose beneath; palmately 5–7-veined from the base; petiole 3–6.5 cm long. Capitula is numerous in terminal and axillary corymbs to 22 cm long, disciform; stalk of individual heads 5–7.3 cm long; involucre hemispherical, 7.5–8 mm long; bracts of calyculus 3–4, lanceolate, 2.5–4 mm long; phyllaries 8–14, lanceolate, 7.5–8 mm long, acute, puberulous, and margins scarious. Outer florets female, up to 8 mm long; inner hermaphrodite, corolla 6–8 mm long, lobes 1.3–2 mm long; and style-arms with short hairy subulate appendages. Achenes 0.6–2.1 mm long, 5-ribbed, glabrous; pappus 6.5–8 mm long, and cream or dirty white (Beentje et al., 2005).

**Traditional uses:** *M. tedliei* is used in the treatment of cough, cold, headache, purgative and laxative (Banzouzi et al., 2008). Also the pounded leaves of *M. tedliei* are applied to cuts and contusions (Soelberg et al., 2015).

1.1.6.9. *Millettia thonningii*

**Family:** Leguminosae

**Synonyms:** *Robinia thonningii*

**Vernacular names:**

- **English names:** None
- **Tribal names:** GA taatso, tatso

**Origin and geographic distribution:** *M. thonningii* is native to West tropical Africa and distributed all over Ghana, Togo, Benin, Nigeria, Cameroon, Gabon, Congo, DR Congo, and northern Angola. Found in mixed deciduous forest; savannah, often by streams. It is a savannah and secondary forest species, often found on riverbanks, it occurs as clumps or thickets on plains usually associated with termitaria (Hanelt, 2001).

**Description:** A tree 30-40 ft. high, with glabrous branches. Stipules none or deciduous. Petiole about an inch long, the rachis of the leaf 3–6 in long, both glabrous; leaflets 7–9, the upper oblong acuminate, 3–5 in long, 2–2.5 in broad, the lowest pair considerably shorter, the petiolules about 2 lines long, with small spreading setaceous deciduous stipelle, subcoriaceous, both sides glabrous, and the veins beneath not prominent. The flowers are in sessile drooping axillary racemes 4–8 in long. Bracts linear or lanceolate, shorter than the slender pedicels, which are 2 lines long, and about equal the glabrous or slightly pubescent broadly campanulate calyx. Calyx-teeth deltoid, reaching about a quarter down, the lowest the narrowest, and the two uppermost connate. Corolla 0.5–0.6 in deep, lilac or violet, standard orbicular, 0.4 in broad, and glabrous. The upper stamen is free above the middle. Pod linear-oblong, 6–8 in long, 0.8 in broad, glabrous, firm, subligneous, slightly curved, 5–6-seeded (Baker & Oliver, 1871).

**Traditional uses:** *M. thonningii* is used for treatment of laxative, purgative, blood purification and pain reliever (Fern, 2014). Also the bark of *M. thonningii baker* beaten

soft is used for applying to old leg-injuries for cleansing the wound (Soelberg et al., 2015).

**1.1.6.10. *Philenoptera cyanescens***

**Family:** Leguminosae

**Synonyms:** *Robinia cyanescens*

**Vernacular names:**

- **English names:** Yoruba indigo vine
- **Tribal names:** ASANTE- FANTE-TWI- GA akase

**Origin and geographic distribution:** *P. cyanescens* native to Tropical Africa. Distributed over Togo, Cameroon, Equatorial Guinea and Ghana. It is cultivated particularly in Sierra Leone and Ghana and occasionally else where in the tropics (Jansen & Cardon, 2005).

**Description:** A woody climbing or straggling shrub, in cultivation forming a shrub 7–8 ft high; branchlets silky when young, the young leaves turning bluish-black in drying; flowers reddish, turning blue, in panicles up to a foot long; in coastal districts and in forests. The Yoruba Indigo is cultivated in coastal areas and in forests (Roberty, 1954).

**Traditional uses:** *P. cyanescens* is used in the treatment of bronchitis and cough (Iwu, 2014). Also the leaves of *P. cyanescens* are crushed and applied to old leg- injuries for cleansing the wound (Soelberg et al., 2015).

**1.1.6.11. *Salacia senegalensis***

**Family:** Celastraceae

**Synonyms:** None

**Vernacular names:**

- **English names:** African mammey tree
- **Tribal names:** No GA name recorded

**Origin and geographic distribution:** *S. senegalensis* is native to Tropical Africa. Distributed all Sierra Leone and Ghana (Hall & Swaine, 1981).

**Description:** It is a glabrous shrub; the extremities usually verruculose, with minute simple or lobed warts. Leaves (drying reddish-brown, paler beneath) are rather coriaceous, oblong-elliptical, shortly and obtusely pointed or acuminate, rounded or cuneate at base, obtusely and often obscurely serrulate or subentire, shining above, 2.5–4.5 in. long, 1–1.8 in broad; petiole channelled above, 0.2 – 0.3 in. It flowers in axillary sessile fascicles, often rather crowded; pedicels 3–5 lines, rather slender and not thickened upwards. Calyx-lobes are ovate to rotundate; and the outer smaller. Petals are ovate-elliptical, and imbricate. Filaments from the short thick disk surrounding the base of the sessile ovary, subulate, at length recurved, nearly as long as the style; anthers 2-celled, dehiscing longitudinally; and the lobes slightly divergent below. The fruit is (probably immature) broadly ellipsoidal, 1–1.3 in long. Found in forest and forest regrowth (Oliver, 1868).

**Traditional uses:** *S. senegalensis* is used for treatment of mild aperient (laxative) and treatment of stomach pain (Odugbemi, 2008). Also it is boiled in wine and drunk is good in treating smallpox (Soelberg et al., 2015).

#### **1.1.6.12. *Senna occidentalis***

**Family:** Leguminosae

**Synonyms:** *Cassia occidentalis*

**Vernacular names:**

- **English names:** stinking weed, negro-coffee, mogdad coffee, coffee weed, and septicweed
- **Tribal names:** No GA name recorded

**Origin and geographic distribution:** *S. occidentalis* is native to the Tropics. Distributed throughout tropical and subtropical regions of America, Africa, Asia and Australia. It is widespread all over Tropical Africa (Subramoniam, 2016). It is a common weed found throughout India (Kaur et al., 2014).

**Description:** It is erect, sometimes slightly woody herb, 0.2–2 m high. Leaves are 10–25 cm long, with a large squat ovoid or globose sessile gland near base of petiole; leaflets are in (3–)4–5(–6) pairs, lanceolate to ovate-elliptic, (2.5–)5–12 x (1.5–)2–4 cm, acute or acuminate, glabrous except for ciliolate margins and inconspicuous scattered glands beneath. Racemes from upper axils, very short, almost umbellate; peduncles 3–5(–8) mm long; bracts acute. Sepals obtuse. Petals yellow, obovate, 0.9–1.5 cm long. Stamens: 3 large, 4 medium-sized, and 3 small. Pods linear, usually slightly upcurved, (5–)8–13 x 0.5–1 cm, not or tardily dehiscent, subglabrous, and transversely septate within. Seeds are many, transversely arranged, grey-brown, ovate-circular, compressed, 4.5–5 x 3.75–4.5 mm, minutely pimpled and with an elliptic areole on each face (Thulin, 1993).

**Traditional uses:** *S. occidentalis* is used for treatment of several diseases like lack of appetite, clears vocal cord, hysteria, whooping cough, acidity and gastric problems (Sarker et al., 2015). Also it is dried into powder and snuffed, stops the bleeding (Soelberg et al., 2015).

#### **1.1.6.13. *Stachytarpheta indica***

**Family:** Verbenaceae

**Synonyms:** None

**Vernacular names:**

- **English names:** Aeron's Rod, Brazilian Tea, Bastard Vervain, and Jamaica False
- **Tribal names:** No GA name recorded

**Origin and geographic distribution:** *S. indica* is native to the tropics. Distributed all over Tropica Africa, America and Tropical Asia (Seidemann, 2005).

**Description:** It is a much-branched annual, 2–3 ft high with terete nearly glabrous stems. Leaves are opposite, distinctly petioled, oblong, obtuse, deeply crenate, and cuneate at the base, 2–3 in long. Spikes 0.5–1 ft long; rhachis 0.2 in diameter, conspicuously hollowed out opposite the flowers; bracts lanceolate, and acuminate, 0.3

in long. Calyx rather shorter than the bract; and teeth minute. Corolla-tube slightly longer than the calyx; limb 0.3 in diameter, blue, with a white eye. The capsule is rather shorter than the calyx (Baker & Stapf, 1900).

**Traditional uses:** *S. Indica* is used in treatment of diseases like cough, malaria, stress, and active anthelmintic (Odugbemi, 2008). Also it is pounded and rubbed on the legs, kills the worms that lives there (Soelberg et al., 2015).

#### **1.1.6.14. *Tiliacora warneckeii***

**Family:** Menispermaceae

**Synonyms:** *Tiliacora funifera*

**Vernacular names:**

- **English names:** Elbow leaf and Stem fruit climber
- **Tribal names:** FANTE dsidse

**Origin and geographic distribution:** *T. warneckeii* is native to tropical Africa. Distributed from Ghana eastward to Ethiopia and Somalia, and also southward to Angola, Zimbabwe, Mozambique and South Africa (Brink & Achigan-Dako, 2012).

**Description:** It is woody liane. Leaves petiole are slender, 1.5–5 cm long, puberulous to glabrescent; blade ovate-lanceolate, ovate-oblong or broadly ovate, subcordate to rounded or somewhat obtuse at base, obtuse to acute or acuminate at apex, 5–20 cm long, 3–10 cm wide, papery or coriaceous, glabrous; nerves in 3–5 pairs, and sometimes sparsely puberulous beneath. The male inflorescences either of axillary, solitary, or cymules on peduncles 1–1.2 cm long, or of 3–9-flowered cymules arranged in false racemes which are axillary or springing from the old stems, solitary or clustered, and up to 15 cm long; axes and peduncles puberulous. The male flowers are with 6–9 sepals, the 3–6 outer ones triangular to orbicular, 0.8–1.5 mm long and wide, thickened and ciliolate, the 3 inner obovate-elliptic, 3.5–4 mm long, 1.8–2.3 mm wide; petals 6, clawed, thickened on margins, 1.5–2.5 mm long; and stamens exserted, 3–5 mm long, free or slightly united at base. Female inflorescences is similar to the male. Female flowers with 6–9 sepals, the outer ones lanceolate to ovate, 1–2 mm long, the inner



suborbicular, 2.5 mm long and wide; petals (5–) 6, 1–1.5 mm long; and carpels 8–12, about 1 mm long. Drupes obovoid to nearly round, 5–7 mm. long; stipe 1.5–3 mm. long (Troupin, 1956).

**Traditional uses:** *T. warneckei* is used in treating cough and tachycardia (Quattrocchi, 2012). Also it is made into plaster and applied to a cut, cures it (Soelberg et al., 2015).

#### **1.1.6.15. *Triumfetta rhomboidea***

**Family:** Tiliaceae

**Synonyms:** *Triumfetta annua*

**Vernacular names:**

- **English names:** Diamond burbark or Chinese bur
- **Tribal names:** GA tugbe TWI petekuku

**Origin and geographic distribution:** *T. rhomboidea* is native to tropical Africa and Asia. Distributed from Ghana, east to Ethiopia and Eritrea, and south to south Africa. Also in Madagascar as well as southern and eastern Asia (Grubben & Denton, 2004).

**Description:** It is herbaceous or shrubby, glabrous, villose, stellate-pilose or velvety. The stalks of lower leaves are nearly as long as the blades. Leaves are polymorphous, often differing much on the same specimen, ovate, cordate or rhomboid at the base, acute or somewhat 3-lobed at the apex, palmately 3–5–7-nerved, unequally serrate; the lower serratures sometimes reflexed and glandular; and surfaces exceedingly variable in regard to degree of pubescence. Flowers are numerous, in cymose clusters along the sides and ends of the branches. Pedicels are short. Flower-buds are oblong or somewhat clavate, apiculate. Sepals are hispid or downy, oblong, apiculate. Petals are yellow, oblong, tapering at the base. Stamens are 10–15. Capsules are glabrous, the size of a small pea, albido-tomentose, 3–5-valved; valves covered with smooth, hooked, conical prickles (Masters, 1868).

**Traditional uses:** *T. rhomboidea* is used in treating of cough, blood purifier, pimples, impotency and barrenness (Quattrocchi, 2012). The root of *T. rhomboidea* is used for

the sores from Guinea-worm or Pounded, and so applied, is good for a cut (Soelberg et al., 2015).

**1.1.6.16. *Vernonia colorata***

**Family:** Asteraceae

**Synonyms:** None

**Vernacular names:**

- **English names:** Bitters tree and English bitter leaf
- **Tribal names:** GAagoafu, akpa, tà [tso=tree]

**Origin and geographic distribution:** *V. colorata* is native to tropical Africa. Distributed from South Africa, Swaziland and widespread throughout tropical Africa (Schmidt et al., 2002).

**Description:** It is a shrub or small tree (0.75-)1.5-7.5 m tall, trunk up to 30 cm in diameter, bark grey or brown, smooth or stringy; stems densely pubescent with pale brown asymmetrically and with t-shaped hairs. Leaves petiolate, (broadly) elliptic to ovate, 4.8-36 cm long, 2.5-13.5 cm wide, base cuneate to subtruncate, margins sinuate-serrate or entire, often strongly undulate, apex shortly acuminate, apiculate, thinly pubescent or puberulous and  $\pm$  glabrescent except on veins above, finely crispate-pubescent or tomentose beneath; and petiole 0.5-6 cm long. Capitula is numerous in large compound corymbiform cymes; stalks of individual capitula are 3-25 mm, densely pubescent; involucre broadly ovoid-cylindrical to campanulate-cylindrical, 3-14 mm long; phyllaries 4-8-seriate, narrowly to broadly ovate, 1-10 mm long, obtuse and apiculate, green, greenish white or yellowish green, with dark green apices or appendages, ciliate on margins, and distally glandular. Florets are 10-26 per capitulum, scented; corolla white, sometimes tinged pale mauve, lilac or pink, 6.3-12 mm long, with capitate glands all over, and lobes glabrous, 2.3-3.5 mm long. Achenes 2.5-4.2 mm long, 10-ribbed, glabrous, glandular between the ribs; outer pappus caducous, 1-4 mm long, inner pappus buff, stramineous or tawny, turning reddish, 6.5-8.5 mm long, distally spatulate (Beentje, 2000).

**Traditional uses:** *V. colorata* is used in the treatment of diseases like antipyretic, expectorant, laxative and fever (Bever, 1986). For old leg bone injury a decoction of the leaves of *V. colorata* is used for bathing; the soft beaten and moistened bark of the root is applied to the wound itself (Soelberg et al., 2015).

## **2.0. HYPOTHESIS**

Cellular damage or oxidative injury arising from free radicals or reactive oxygen species (ROS) now appears the fundamental mechanism underlying a number of human neurodegenerative disorders, diabetes, inflammation, viral infections, autoimmune pathologies and digestive system disorders.

Despite the rich tradition of medicinal plants used in folk Ghanaian medicine for treatment of diseases likely to be associated with oxidative stress, their potential to eliminate free radicals has previously been tested in limited manner only. Therefore, it is possible to suppose that the screening of their *in vitro* antioxidative properties can lead to the discovery of species with the significant free radical potential.

### 3.0. OBJECTIVES

The aim of this work is to assess sixteen medicinal Ghanaian plants used in traditional folk medicine for treatment of conditions likely to be associated with undesirable oxidative processes for their *in vitro* antioxidative effects determined by DPPH test. It is believed that these results might help in developing new food and pharmaceutical herbal-based products.

The specific objectives were to;

1. Screen selected ethnobotanicals for anti-oxidant activity using the DPPH antioxidant assay. The concentration of selected ethnobotanicals to reduce 50% of DPPH radicals (IC<sub>50</sub>) will be determined.
2. Select the most prospective plant species for further phytochemical analysis focused on isolation and identification of the main antioxidant principles of the plants.
3. Summarize the information on botany, origin and geographical distribution, and ethnopharmacological uses of selected plant species.

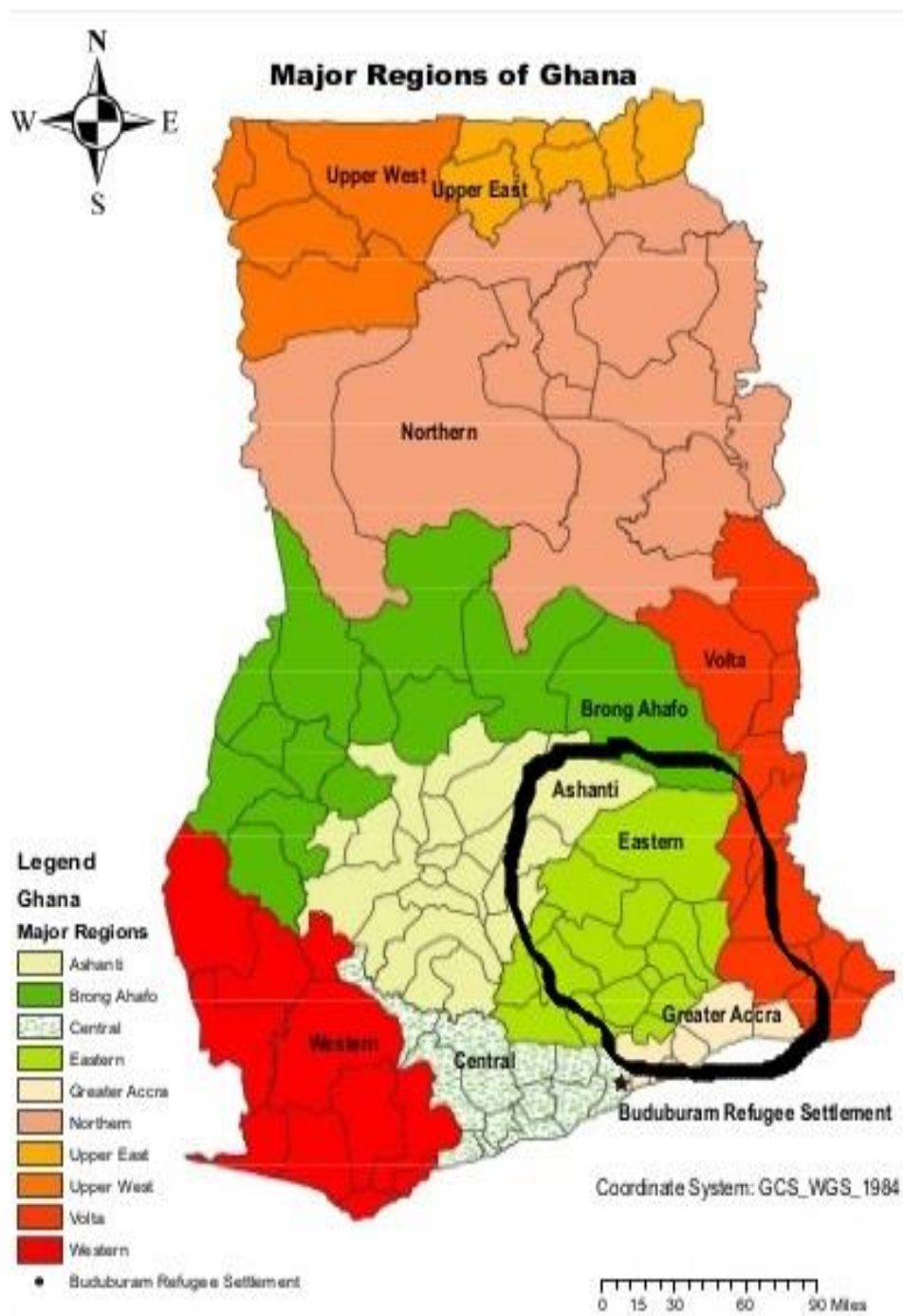
## 4.0. MATERIALS AND METHODS

### 4.1. *Plant material*

The plant samples were mainly collected within the Greater Accra region, Ashanti region and Eastern region of Ghana (Fig. 1). Accra is the capital and largest city of Ghana, with an estimated urban population of 2.27 million as of 2012. The mean monthly temperature ranges from 24.7 °C (76.5 °F) in August (the coolest) to 28 °C (82.4 °F) in March (the hottest), with an annual average of temperature 26.8 °C (80.2 °F). (Anonymous, 2012a). Kumasi (historically spelled Comassie or Coomassie) is a city in Ashanti Region belonging to the largest metropolitan areas of Ghana. It is located approximately 500 kilometres (300 sq mi) north of the Equator and 200 kilometres (100 sq mi) north of the Gulf of Guinea. The climate of the region is tropical wet and dry with relatively constant temperatures throughout the course of the year (Anonymous, 2014b).

The Eastern Region is located on the peninsula of Ashantiland and is one of ten administrative regions of Ghana. Eastern region is bordered to the east by the Lake Volta, to the north by Brong-Ahafo region, to the west by Ashanti region, to the south by Central region and Greater Accra region. It covers an area of 19,323 square kilometres, which is about 8.1% of Ghana's total landform. It has similar temperature and rainfall pattern as that of the Ashanti Region (Anonymous, 2010c). The plants were selected according to the literature data on their use in folk medicine to cure human diseases likely to be associated with undesirable oxidative processes from Ghanaian healers in Ghana. The barks, leaves and roots of plants species were collected in different regions of Ghana, by Mr. Anthony Gyimah in August 2015. The voucher specimens of collected plants authenticated by Mr. Anthony Gyimah are deposited at the University of Ghana-Ghana herbarium.

Figure 1. The area of plant samples collection (adopted from Anonymous, 2015d).



Footnotes: ■ border of the collection area covering Ashanti, Eastern, and Greater Accra

#### 4.2. Extract preparation

The Fresh air-dried plant material of barks, leaves and roots was finely ground on a grinder for two times and 10 g of each sample was weighed. After grinding the powdered form was mixed with 80% ethanol (300 ml) and placed on a shaker for 24 hours. All extracts were filtered after shaking and concentrated through evaporation using vacuum rotary evaporator (Rotavapor R-200, Büchi, Switzerland) at a temperature of 40°C. For determination of antioxidant activity, the dried plant residue was dissolved in dimethyl sulfoxide (DMSO) to obtain 51.2 mg/mL concentrated stock solution and stored under -20°C prior to the use for several tests. The yields of dried residues including the ethnomedicinal uses of the plant samples are shown in Table 1.

**Table 1. Yields and Ethnomedicinal uses of Ghanaian medicinal plants**

Species (voucher specimen no.)	Yield (g/%)	Ethnomedicinal uses
<i>Allophylus spicatus</i> (GA009)	1.47/9.8	Pulmonary diseases, diarrhoea, dysentery
<i>Cnestis ferruginea</i> (GA005)	0.43/2.9	Bronchitis, migraines, sinusitis, tuberculosis, fever, cough, enema, dysmenorrhea
<i>Ficus thonningii</i> (G013)	2.30/15.3	Diarrhoea, urinary tract infections, diabetes mellitus, respiratory infections, mental illnesses
<i>Indigofera hirsuta</i> (GA004)	4.53/30.2	Stomach ache, diarrhoea, body pain
<i>Indigofera pulchra</i> (GA011)	3.40/22.7	Gastrointestinal pain, malaria, dysentery
<i>Launaea taraxacifolia</i> (GA008)	1.35/9.0	Vomiting, teeth pain, diabetes



<i>Leea guineensis</i> (G006)	1.04/6.9	Muscular pain, arthritis, rheumatism, furuncle
<i>Mikaniopsis tedliei</i> (GA016)	2.81/18.7	Bronchopulmonary infections, cough, severe cold, headache, purgative, laxative
<i>Millettia thonningii</i> (GA001)	2.19/14.6	Pain reliever, diarrhoea, dysentery, purgative, laxative
<i>Philenoptera cyanescens</i> (GA012)	4.26/28.4	Bronchitis, cough, diarrhoea, dysentery
<i>Salacia senegalensis</i> (GA014)	2.13/14.2	Mild aperient(laxative), stomach pain reliever
<i>Senna occidentalis</i> (GA002)	3.53/23.5	Lack of appetite, clear vocal cord, hysteria, whooping cough, gastric problems
<i>Stachytarpheta indica</i> (GA007)	2.49/16.6	Cough, malaria, dysentery, stress, active anthelmintic
<i>Tiliacora warneckeii</i> (GA010)	3.63/24.2	Cough, dysentery, amenorrhoea, tachycardia
<i>Triumfetta rhomboidea</i> (GA003)	2.39/15.9	Cough, blood purifier, dysentery, pimples, impotency, barrenness
<i>Vernonia colorata</i> (GA015)	3.67/24.5	Antipyretic, expectorant, laxative, fever, diarrhoea

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### 4.3. Chemicals

Ascorbic acid, Trolox (purity: 98%), 2,2'-azobis (2-methylpropionamide) dihydrochloride (AAPH) (purity 98%), and 2,2-diphenyl-1-picrylhydrazyl (DPPH)

(purity 90%) were bought from Sigma-Aldrich (Prague, CZ). Ethanol (pharmacological grade), methanol (analytical grade) and DMSO (pure) were bought from Lach-Ner (Neratovice, CZ).

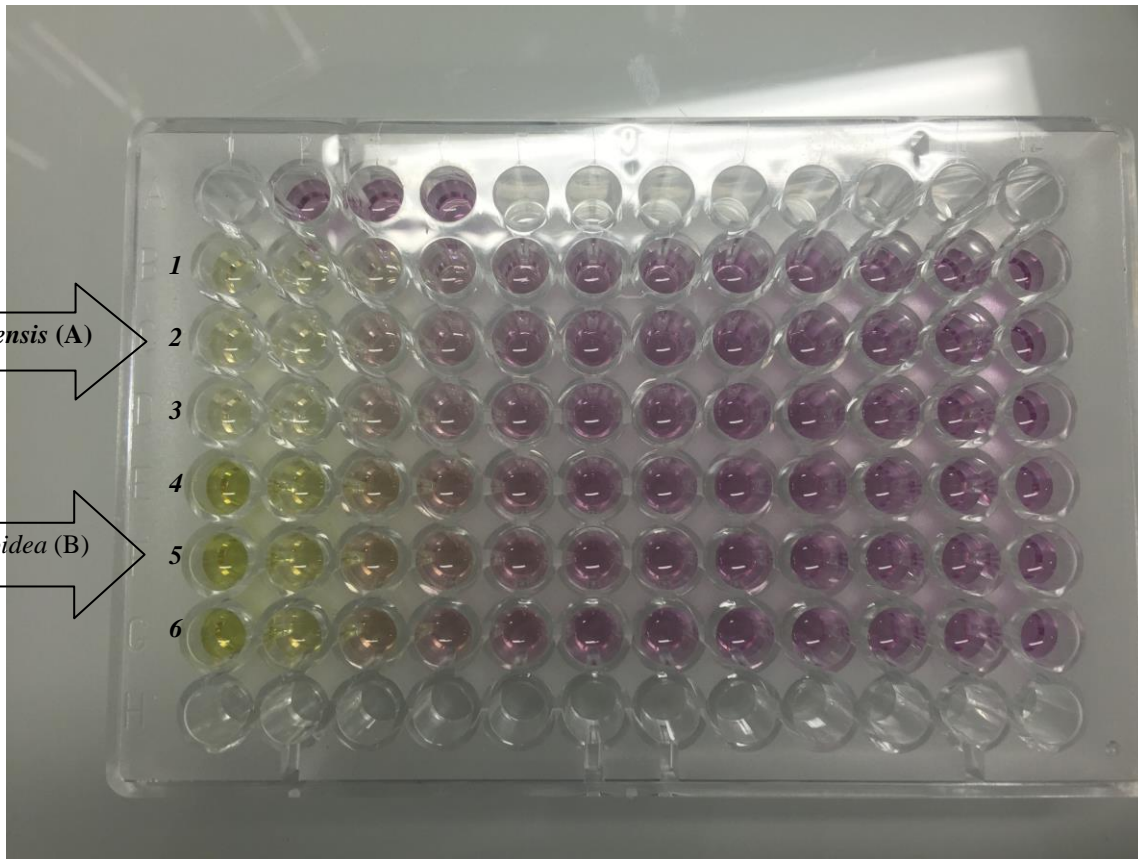
#### **4.4. Antioxidant assay**

##### **4.4.1. 2,2-diphenyl-1-picrylhydrazyl radical scavenging capacity assay**

Total antioxidative activity of extracts was evaluated in vitro using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method described previously by Sharma and Bhat (2009) with slight modifications. In disposable microtiter plates (96 flat-bottomed wells), 10 two-fold serial dilutions of each extract were prepared in concentrations ranging from 0.125 to 64  $\mu\text{g/ml}$  using Tecan Freedom EVO 100 liquid handler (Tecan, Männedorf, Switzerland). Subsequently, 100  $\mu\text{l}$  of freshly prepared 0.4 mM methanol solution of DPPH (Sigma-Aldrich, Prague, CZ) was mixed with the extract in each well (creating a final volume of 200  $\mu\text{l}$ ) to start the radical-antioxidant reaction. The mixture was shaken for 2 minutes and then incubated in the dark at 25 °C (labsystem incubator). The absorbance of samples was read after 30 min at 517 nm using microplate reader (Cytation 3, Biotek, Highland Park, USA). The values of half maximal inhibitory concentrations ( $\text{IC}_{50}$ ) were calculated using Gen 5<sup>TM</sup> version 2.07 software. Trolox and ascorbic acid (Sigma-Aldrich, Prague, CZ) were tested as positive controls. Figure shows two extracts that are *Salacia senegalensis* and *Triumfetta rhomboidea* which were set on the plate during the experiment. In the image 1,2 and 3 represents *S. senegalensis* whiles 4,5 and 6 also represents *T. rhomboidea*. Both extracts showed some level of antioxidant activity.

**Figure 2. Photograph of the microplate design (Addai, 2016)**

*Salacia senegalensis* (A) and *Triumfetta rhomboidea* (B)



## 5.0. RESULTS AND DISCUSSIONS

The results of the antioxidative activity of the Ghanaian medicinal plant extracts are shown in Table 3. All extracts were able to reduce the free radical DPPH to yellow-coloured diphenylpicrylhydrazine in the concentration range 3.14–54.49  $\mu\text{g/mL}$ . The strongest effect was observed for the leaf extract of *I. hirsuta* with  $\text{IC}_{50}$  value of 3.14  $\mu\text{g/mL}$ .

This was followed by the other extracts which are indicated in two groups following a descending sequence according to magnitude: The first group comprises of  $\text{IC}_{50}$  values of extracts up to 10  $\mu\text{g/mL}$  which includes as follows, *S. senegalensis* (3.49  $\mu\text{g/mL}$ ), *A. spicatus* (4.23  $\mu\text{g/mL}$ ), *C. ferruginea* (5.01  $\mu\text{g/mL}$ ), *S. indica* (9.00  $\mu\text{g/mL}$ ). Also the second group comprises of  $\text{IC}_{50}$  values of extracts up till the maximum value which was 54  $\mu\text{g/mL}$  and includes, *L. guineense* (11.42  $\mu\text{g/mL}$ ), *I. pulchra* (12.84 $\mu\text{g/mL}$ ), *P. cyanescens* (14.72  $\mu\text{g/mL}$ ), *V. colorata*, *S. occidentalis* (33.12 $\mu\text{g/mL}$ ), *M. thonningii* (33.74 $\mu\text{g/mL}$ ), *L. taraxacifolia* (35.05 $\mu\text{g/mL}$ ), *M. tedliei* (41.35 $\mu\text{g/mL}$ ), *T. rhomboidea* (51.46  $\mu\text{g/mL}$ ), *F. thonningii* (52.32  $\mu\text{g/mL}$ ), *T. warneckeii* (54.49  $\mu\text{g/mL}$ ).

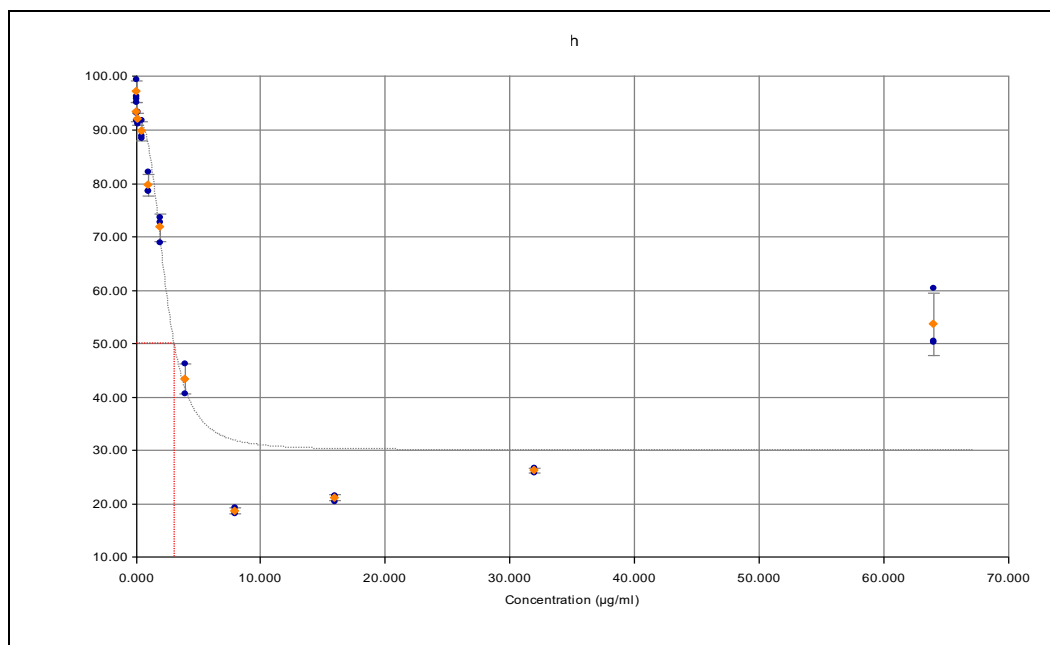
All extracts have shown activity even at lower concentrations between 0.0625  $\mu\text{g/mL}$  and 64  $\mu\text{g/mL}$ , which was the highest used concentration in this experiment. Even though *I. hirsuta* leaf extract has shown the highest antioxidant effect in this part of our experiment, this activity could be approach to the used positive chemical standards Trolox and ascorbic acid, which possess  $\text{IC}_{50}$  value of 0.32  $\mu\text{g/mL}$  and 0.54  $\mu\text{g/mL}$ , respectively. In fig. 3 results are expressed on the third independent experiments where *I. hirsuta* was recorded with a concentration of 3.09  $\mu\text{g/mL}$  in the curve of the DPPH graph shown in the Figure 3. Also ascorbic acid showed promising result in Figure 4 with concentration 0.68  $\mu\text{g/mL}$  in the third independent experiment as shown in the DPPH curve in the graph.

**Table 2. *In vitro* antioxidative activity of Ghanaian medicinal plant extracts**

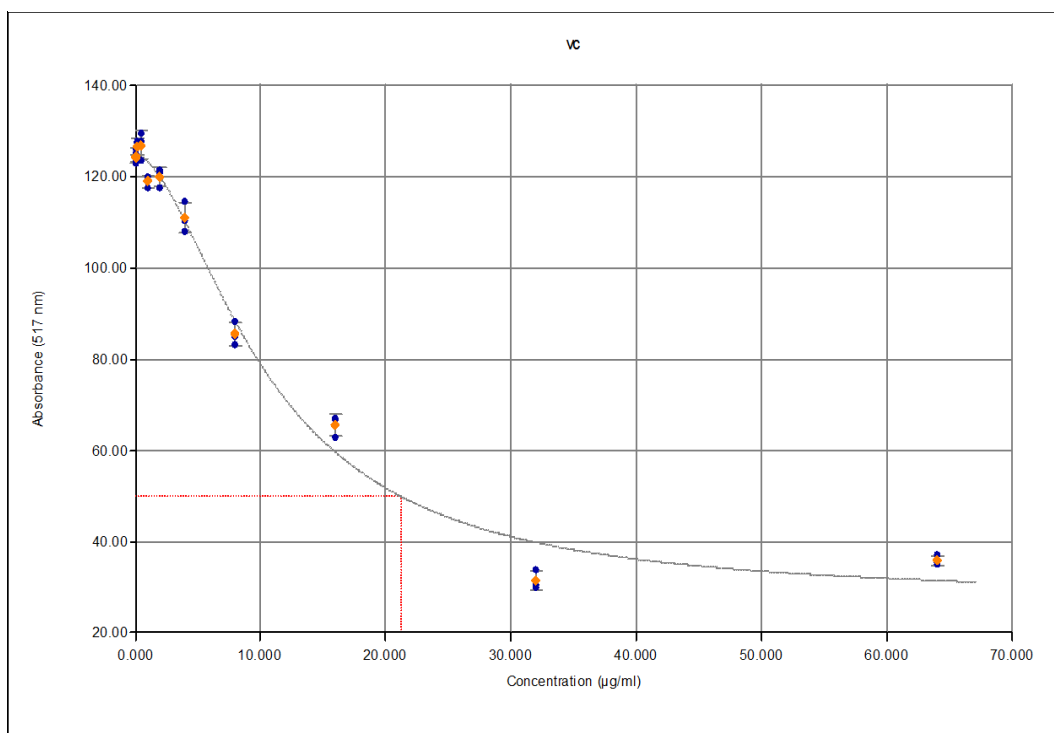
Plant extract	Part tested	DPPH assay			
		Experiment <sup>a</sup>			IC <sub>50</sub> ± SD [µg/mL] <sup>b</sup>
		I	II	III	
<i>Allophylus spicatus</i>	Root bark	5.28	3.19	4.22	4.23±1.04
<i>Cnestis ferruginea</i>	Leaves	6.37	4.12	4.53	5.01±1.20
<i>Ficus thonningii</i>	Leaves	58.96	55.99	42.01	52.32±9.05
<i>Indigofera hirsuta</i>	Leaves	3.50	2.83	3.09	3.14±0.34
<i>Indigofera pulchra</i>	Leaves	12.03	13.67	12.82	12.84±0.82
<i>Launaea taraxacifolia</i>	Leaves	31.50	37.23	36.42	35.05±3.10
<i>Leea guineensis</i>	Bark	6.84	8.25	19.18	11.42±6.75
<i>Mikaniopsis tedliei</i>	Leaves	29.27	47.31	47.49	41.35±10.47
<i>Millettia thonningii</i>	Bark	35.05	30.90	35.26	33.74±2.46
<i>Philenoptera cyanescens</i>	Leaves	12.16	16.48	15.53	14.72±2.27
<i>Salacia senegalensis</i>	Bark	4.02	3.03	3.41	3.49±0.50
<i>Senna occidentalis</i>	Leaves	28.30	40.57	30.48	33.12±6.55
<i>Stachytarpheta indica</i>	Leaves	11.15	7.00	8.85	9.00±2.08
<i>Tiliacora warneckeii</i>	Leaves	57.55	56.24	49.70	54.49±4.21
<i>Triumfetta rhomboidea</i>	Leaves	49.16	49.83	55.38	51.46±3.42
<i>Vernonia colorata</i>	Leaves	27.30	13.33	21.20	20.61±7.00
Vitamin E		0.57	0.72	0.73	0.68±0.09
Vitamin C		0.26	0.56	0.68	0.50±0.22

<sup>a</sup>each experiment performed in triplicate, <sup>b</sup>mean of experiments ± standard deviation

**Figure 3. DPPH antioxidant activity of *Indigofera hirsuta* ( $\mu\text{g/mL}$ )**



**Figure 4. DPPH antioxidant activity of Ascorbic acid ( $\mu\text{g/mL}$ )**



Many of the plants tested in this study are used in treating diseases associated with oxidative stress such as pulmonary diseases, cough, stress, fever, diabetes, and many others (Soelberg et al., 2015). In correspondence with this, most of them showed significant antioxidative activities in the DPPH test.

The results obtained in frame of this thesis on antioxidative action of *I. hirsuta* are corresponding with report previously published by Moura et al. (2011). This study revealed the DPPH scavenging ability of methyl galatte ( $IC_{50} = 5 \pm 0.3 \mu\text{g/mL}$ ), galic acid ( $IC_{50} = 5 \pm 0.2 \mu\text{g/mL}$ ) and rutin ( $IC_{50} = 21.6 \pm 0.6 \mu\text{g/mL}$ ), isolated from methanol extract ( $IC_{50} = 67.7 \pm 0.9 \mu\text{g/mL}$ ) of *I. hirsuta* leaves. Since the chemical composition of plants could be affected by climatic and soil conditions, agronomical techniques and extraction method used (D'Antuono et al., 2002; Colombo et al., 1991; Sanchez et al., 2007, Bekaci-Ali et al., 2007; Al-Saleh et al., 2006; Burt et al., 2005), we suppose that differences between  $IC_{50}$  values recorded in our study and those determined by Moura et al. (2011) can be affected by above mentioned factors.

Despite the previously published paper showing potential of methanol extract from *C. ferruginea* roots to induce production *in vivo* antioxidants in experiments with adult rats (Ishola et al., 2012), this is the first report on antioxidant action of leaves of this plant and its direct antioxidative action. In addition, according to our best knowledge, three other extracts that produced promising activities in our test, namely *A. spicatus*, *S. indica* and *S. senegalensis*, have never been reported for any antioxidant activity.

## 6.0. CONCLUSION

The antioxidative activities of sixteen crude extracts derived from Ghanaian medicinal plant species, namely *A. spicatus*, *C. ferruginea*, *F. thonningii*, *I. hirsuta*, *I. pulchra*, *L. taraxacifolia*, *L. guineense*, *M. tedliei*, *M. thonningii*, *P. cyanescens*, *S. senegalensis*, *S. occidentalis*, *S. indica*, *T. warneckeii*, *T. rhomboidea*, and *V. colorata* have been tested in the frame of this thesis. In summary, all the samples exhibited certain degree of the antioxidative action when tested *in vitro* using DPPH assay. According to our results, *A. spicatus*, *C. ferruginea*, *I. hirsuta*, *S. indica* and *S. senegalensis*, possessed the best antioxidant action. Because of its significant antioxidative action, *Indigofera hirsuta* can be recommended for development of new herbal-based food and pharmaceutical products. *C. ferruginea*, *S. indica*, and *S. senegalensis* are recommended for further research due to the promising activities obtained from these samples.



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## 8.0. APPENDICES

### 8.1. *Photographic illustrations of plant species*

**Figure 5** *Allophylus spicatus*



Photo by Anonymous, 2007e

**Figure 6** *Cnestis ferruginea*



Photo by Anonymous, 2009f

**Figure 7** *Ficus thonningii*



Photo by Anonymous, 2008g

**Figure 8** *Indigofera hirsuta*



Photo by Anonymous, 2006h

**Figure 10** *Indigofera pulchra*



Photo by Anonymous, 2012i

**Figure 9** *Launaea taraxacifolia*



Photo by Anonymous, 2005j

**Figure 12** *Leea guineensis*



Photo by Anonymous, 2011k

**Figure 11** *Mikaniopsis tedliei*



Photo by Anonymous, 2003l

**Figure 14** *Millettia thonningii*



Photo by Anonymous, 2013m

**Figure 13** *Philenoptera cyanescens*



Photo by Anonymous, 2004n

**Figure 15** *Salacia senegalensis*



Photo by Anonymous, 2002o

**Figure 16** *Senna Occidentalis*



Photo by Anonymous, 2004p

**Figure 18** *Stachytarpheta indica*



Photo by Anonymous, 2014q

**Figure 17** *Tiliacora warneckei*



Photo by Anonymous, 2010r

**Figure 20** *Triumfetta rhomboidea*



Photo by Anonymous, 2009s

**Figure 19** *Vernonia colorata*



Photo by Anonymous, 2011t