Chapter one

1- INTRODUCTION

1.1Medicinal Plants

Plants have been utilized as a source of medicine for thousands of years and continue to play an important role globally in primary health care, mostly in developing countries (Balunas and Kinghorn, 2005).

The use of medicinal plants is increasing because people believe that they are safe for human consumption. There is also an increase in infectious diseases worldwide caused by both drug resistance and lack of sufficient affordable medicine for people living in poor communities. The discovery of drugs from medicinal plants may be one of the solutions in the fight against infectious diseases. Thousands of natural products are in clinical trials and some are already prored to be useful in combating some of the diseases.

It is thought that about 80% of the 5.2 billion people of the world live in the less developed countries and the World Health Organization estimates that about 80% of these people rely almost exclusively on traditional medicine for their primary healthcare needs. Medicinal plants are the "backbone" of traditional medicine, which means more than 3.3 billion people in the less developed countries utilize medicinal plants on a regular basis (Maryam *et al*., 2011).

According to the World Health Organization (WHO, 1978) "a medicinal plant" is any plant, which in one or more of its organ contains substances that can be used for the therapeutic purposes or which are precursors for the synthesis of useful drugs. This definition distinguishes those plants whose therapeutic properties and constituents have been established scientifically and plants that are regarded as medicinal but which have not yet been subjected to thorough investigation. The term "herbal drug" determines the parts of a plant (leaves,

flowers, seeds, roots, barks, stems, etc.) used for preparing medicines (Anonymous, 2007). Furthermore, (WHO, 2002) defines medicinal plant as herbal preparations produced by subjecting plant materials to extraction, fractionation, purification, concentration or other physical or biological processes which may be produced for immediate consumption or as a basis for herbal products.

Medicinal plants are plants containing inherent active ingredients used to cure disease or relieve pain (Okigbo *et al*., 2008). The use of traditional medicines and medicinal plants in most developing countries as therapeutic agents for the maintenance of good health has been widely observed (UNESCO, 1996). Modern pharmacopoeia still contains at least 25% drugs derived from plants and many others, which are synthetic analogues, built on prototype compounds isolated from plants. Interest in medicinal plants as a re-emerging health aid has been fuelled by the rising costs of prescription drugs in the maintenance of personal health (Lucy and Edgar, 1999). Furthermore, an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of drugs and chemotherapeutics from these plants as well as from traditionally used herbal remedies (UNESCO, 1998). The medicinal properties of plants could be based on the antioxidant, antimicrobial and antipyretic effects of the phytochemicals in them (Cowman, 1999; Adesokan *et al*., 2008). According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. Therefore, such plants should be investigated to better understand their properties, safety and efficacy (Nascimento *et al*., 2000).

Medicinal plants produce bioactive compounds used mainly for medicinal purposes. These compounds either act on different systems of animals including man, and/or act through interfering in the metabolism of microbes infecting them.

The microbes may be pathogenic or symbiotic. In either way the bioactive compounds from medicinal plants play a determining role in regulating host-microbe interaction in favour of the host. So the identification of bioactive compound in plants, their isolation, purification and characterization of active ingredients in crude extracts by various analytical methods is important (Adesokan *et al*., 2008).

The instant rising demand of plant-based drugs is unfortunately creating heavy pressure on some selected high-value medicinal plant populations in the wild due to over-harvesting. Several of these medicinal plant species have slow growth rates, low population densities, and narrow geographic ranges; therefore they are more prone to extinction.

Conversely, because information on the use of plant species for therapeutic purpose has been passed from one generation to the next through oral tradition, this knowledge of therapeutic plants has started to decline and become obsolete through the lack of recognition by younger generations as a result of a shift in attitude and ongoing socioeconomic changes. Furthermore, the indigenous knowledge on the use of lesser-known medicinal plants is also rapidly declining. Continuous erosion in the traditional knowledge of many valuable plants for medicine in the past and the currently renewal interest prompted the need existed to review the valuable knowledge with the expectation of developing the medicinal plants sector (Kala *et al*., 2006).

1.2 Potential of Herbal Remedies as Sources of New Drugs

Since the beginning of the 19th century, a large number of biologically active secondary metabolites of plant origin have been found to have commercial application as drugs. Recently, there has been an upsurge of interest in the use of plants with folkloric reputations as sources of potentially useful compounds. Analysis of the number and sources of anticancer and anti infective agents,

reported from 1984 to 1995, indicates that over 60% of the approved drugs and pre-NDA (New Drug Application) candidates (for the period 1989-1995), excluding biologics, developed in these disease areas are of natural origin. A recent review reported that at least 119 compounds derived from 90 plant species could be considered as important drugs currently in use in one or more countries, with 77% of these being derived from plants used in traditional medicine. Further evidence of the importance of natural products is provided by the fact that close to half of the best selling pharmaceuticals in 1999 were either natural products or their derivatives (Taylor, 2000).

1.3 Medicinal plants in Sudan

Overview of Medicinal plants in Sudan

In Sudan, it is a common practice to collect medicinal plants from their natural habitats for home consumption and export. Plants collected from different localities or geographic regions may have different concentration of plant compounds. This may be explained by differences in climate, temperature, rainfall, altitude, day length and UV-radiation, all of which play an important role in plant development and affect the biosynthesis of secondary metabolites with biological activity. In general, generation of volatile oils appears to be enhanced at higher temperatures. Continuous rainfall can lead to a loss of water soluble substances from leaves and roots by leaching and also makes collection and drying more difficult. For example, when peppermint is grown in Sudan under long-day conditions, the leaves contain high amounts of menthone and menthol with only trace amounts of menthofuran, whereas peppermint plants grown under short-day conditions contain menthofuran as the major component. The amount of bitter constituents in *Gentiana lutea* increases with altitude, whereas alkaloid contents in *Aconitum napellus* and *Lobelia inflata* and essential oils in thymus

peppermint decrease. *Pyrethrum* delivers the best yield of pyrethrins when cultivated near the equator and at high altitudes (Tuley De Silva, 1996).

Sudanese folk medicine represents a unique blend of indigenous cultures of Islamic, Arabic and African traditions. Consequently, treatments exist for a variety of diseases, both epidemic and endemic. To face these diseases, people have tapped the environmental resources, e.g. plants, minerals and animal products for the management of their health (Khalid *et al.,* 2012).

The development of new and effective drugs against intestinal infections has now made, population-based chemotherapy, available as an important element in control measures. However, chemotherapy alone does not solve the problems and the role of other measures such as sanitation, education, and community participation, traditional and folkloric medicine should be also evaluated. The collection of information from indigenous people about the healing properties of plants is a very useful line of research that national governments and international bodies as World Health Organization are now greatly encouraged (Khalid *et al*. 2012).

1.4 Phytochemistry

1.4.1 Modern Phytochemistry

Approximately 60 percent of all drugs in clinical trials for the multiplicity of cancers were of natural origin. Modern drug discovery approaches applying full automation and robotics, hundreds of molecules can be screened using several assays within a short time, and with very small amounts of compounds. A number of associated techniques are used for identification and analysis of chemical constituents e.g. LC-PDA (liquid chromatography–photo-diode-array detector), LC-MS (liquid chromatography–mass spectrometry detector) and LC-NMR (liquid chromatography– nuclear magnetic resonance spectroscopy). While in the recent past it was extremely difficult, time consuming and labor intensive

to build such a library from purified natural products, with the advent of newer and improved technologies related to separation, isolation and identification of natural products the situation has improved remarkably. Natural products libraries have been established to preserve crude extracts, chromatographic fractions or semi-purified compounds. However, the best result can be obtained from a fully identified pure natural product library as it provides scientists with the opportunity to handle the 'lead' rapidly for further developmental work, e.g. total or partial synthesis, dealing with formulation factors, in vivo assays and clinical trials (Devasagayam *et al*., 2004).

1.4.2 Main chemical constituents in medicinal plants

Is concerned with the enormous variety of organic substances that are elaborated and accumulated by plants and deals with the chemical structures of these substances, their biosynthesis, turnover and metabolism, their natural distribution and their biological function (Harborne, 1998). It is estimated that worldwide there are over 328 640 species of plants (Raven *et al*., 2005). Their sessile nature and lack of a sophisticated immune system has necessitated the development of complex chemical systems. Historically, the compounds produced by plants have been categorized into primary and secondary metabolites (Raven *et al*., 2005). Compounds contributing to fundamental metabolism are termed primary metabolites. In contrast secondary metabolites are limited in their distribution; both throughout the plant and between different species (Raven *et al*., 2005).

The understanding of the important in-planta function of many secondary metabolites is gradually expanding. It is being revealed that many of these secondary metabolites are potent bactericidal, repellent, or even toxic agents to pests and herbivores (Dewick, 1997). Semi chemicals are relied on as a means of

defense against pathogens and predators, as attractants to lure mobile creatures for fertilization and dissemination and also for aerial allelopathy (interplant communication). Volatile organic compounds and pigments are revealed to be attractive to insects that help with fertilization, or warning colors to defend against predators (Dewick, 1997), whilst other plant pigments can provide protection against environmental damage such as free radicals and UV radiation (Raven *et al*., 2005).

Some secondary products perform signaling functions as plant hormones and pheromones. Plants produce an incredible array of secondary metabolites and many of these have been developed into economically important products including; oils, gums, resins, tannins, rubber, waxes, pigments, flavors, fragrances, surfactants, preservatives pesticides and pharmaceuticals (Raven *et al*., 2005).

Plant secondary metabolites represent a tremendous resource for commerce. (Table 1.2). Phytochemical play a fundamental role in the chemical investigation of these plants. Phytochemical studies may be directed towards characterizing the chemical composition of complex essential oils or plant extracts (Harborne, 1998). Some common phytochemical classes reported by Michael Wink (2015) are shown in table (1.1)

Table (1.1): Some Common Phytochemical class

Classes	Chemical structure	Occurrence & properties
Acids	May be saturated or unsaturated.	Widespread (e.g. citric, malic, oxalic, and tartaric acids).
Alcohols	May be free or combined as esters with one or more hydroxyl groups.	Found widely: volatile oils (e.g. geraniol, cinnamyl alcohol and glecrol)
Amino acids	Free molecules; basic units of peptide and proteins.	Nitrogen reserve of plants; important in nutrition.
Bitters	Member of bioflavonoids, glycosides and terpins classes.	Important in garlin &mustard
Esters	Combination of an alcohol and acids	Dispread in volatile oil and balsams.
Fats & lipids	Glycerides of fatty acids.	Various oils.
Fatty acids	Esters of glycerol may be. Saturated or unsaturated.	Found in oil, resins and waxes.
Furanocoumarins	Derivatives of coumarins.	Like psoralens, khellin.
Glucosilonates (Thioclycosides)	Glycosides containing nitrogen and sulfur.	Pungent agents of vegetables & flower.
Iridoids	Monoteroenes.	Bitter agents
Mucilaged & gums	Acidic polysaccharides derived from uronic acids.	Hygroscopic agents used as demulcents & laxative.

Table (1.2): Secondary metabolites of medicinal plants

compounds	Definition	Properties
Alkaloid	Basic amines (Atropine, Hyosine and Morfine)	Includes potent drugs and narcotics. Over 12,000 known; over 13 classes.
Bioflavonoids	Plant pigments; (Kaempferol,, Luteolin)	Most medical effects are questionable. Over 4000 known; over 14 classes.
Essential oils	Used in perfumes and in aromatherapy. Over 9 classes. Also known as volatile oils, ethereal oil, essences	Isoprene derivatives: oxidized terpenes and henylpropanoids (Camphor, Carvone).
Glycosides	Sugar derivatives attached to aglcones (Arabinoside).	Over 10 classes. Over 3000 known.
Resins	Oxidation products of terpenes; resins are insoluble in water (Oleoresins Gum resins, Balsams resins).	Includes oleoresins, gum resins and balsams.
Saponins	Various groups of chemicals. Some are involved in steroid metabolism.	Soap-like glycosides.
Sterols	Steroid and vitamin (Sitosterol, Ergosterol).	Found in soy and other plants; also produced by microorganisms (e.g., sitosterol, stigmasterol).
Tannins	Polyphenolics, mostly based on gallic acid. (Catechin Estter of Galic acid	Astringent compounds, bind to protein (tanning); reduce diarrhea, act as hemostatics.
Terpenes	Derived from 5-carbon iso prene units (10, 15, 20, 30, 40, >40) (Menthol, Menthone).	Over 20,000 known; 6 classes. Most structurally varied phytochemical.

1.5 *Euphorbiaceae* family

Around 7,500 species. Most spurges are herbs, but some, especially in the tropics, are shrubs or trees. Some are succulent and resemble.

1.5.1 Description

The genera in tribe *Euphorbieae*, subtribe *Euphorbiinae* show a highly specialized form of pseudanthium ("false flower" made up of several true

flowers) called a cyathium. This is usually a small, cup-like involucre consisting of fused-together bracts and peripheral nectary glands, surrounding a ring of male flowers, each a single stamen. The fruit is usually a schizocarp, but sometimes a drupe, a capsular fruit with three or more cells, each of which splits open at maturity into separate parts and then breaks away explosively, scattering the small seeds. The family contains a large variety of diterrpene esters, alkaloids, glycosides, and ricin-type toxins (Charles *et al.*, 2007).

1.5.2 Classification of *Euphorbiaceae*

The family *Euphorbiaceae* has been classified into three tribes namely *Euphorbieae, Phyllantheae* and *Crotoneae*, of which Chrozophorinae a sub tribe of crotoneae includes the genus *Chrozophora*. *Chrozophora* is a genus of 11 species, 7 of herbs and under shrubs (Webster, 1994 and Wurdack *et al*., 2005). The common members of the *Euphorbiaceae* family include Acalypha, Phyllanthus, Euphorbia, Aleurites, Glochidion and Breynia (Julius *et al*., 2011).

1.5.3 Distribution of *Euphorbiaceae*

This family occurs mainly in the tropics, with the majority of the species in the Indo-Malayan region and tropical America. A large variety occurs in tropical Africa, but they are not as abundant or varied as in these two other tropical regions. However, Euphorbia also has many species in non-tropical areas such as the Mediterranean Basin, the Middle East, South Africa, and southern USA (Charles *et al*., 2007).

1.5.4 Economic value of *Euphorbiaceae* family

A number of plants of the spurge family are of considerable economic importance. It contains an important starch crops and Prominent plants like cassava (*Manihot esculenta*), castor oil plant (*Ricinus communis*), Barbados nut (*Jatropha curcas*), and the Para rubber tree (*Hevea brasiliensis*). Many are grown as ornamental plants, such as poinsettia (*Euphorbia pulcherrima*). Leafy spurge

(*Euphorbia esula*) and Chinese tallow (*Triadica sebifera*) are invasive weeds in North America. In medicine, some species of *Euphorbiaceae* have proved effective against genital herpes (Heywood, 2007 and Webster, 1994).

1.5.5 *Chrozophora*

1.5.5.1 Species of *Chrozophora*

Is a plant genus of the family *Euphorbiaceae* and the sole genus comprised in the subtribe Chrozophorinae. It comprises of 12 species, which are monoecious herbs or undershrubs (Gbif, 2011)

Species:

C. *obliqua*, *C. burmanni* , *C. parvifolia, C. prostrata, C. tinctoria, C. verbascifolia, C. rottleri*, *C. senegalensis, C. sabulosa, C. oblongifoilia, C. roffleri, C.Gracilis., C.Hierosolyitana.*

1.5.5.2 Distribution of *Chrozophora*

They are found from Africa and the Mediterranean to Southeast Asia.

1.5.5.3 Previous Biological studies on *Chrozophora*

The *in vitro* antimicrobial activities of tested plants extract were assayed using the agar plate diffusion and nutrient broth dilution methods. Several biological studies have been previously carried out on *Chrozophora* genus antimicrobial activity of *Chrozophora Senegalensis* reported by (Usman *et al*., 2007), *Chrozophora rottleri* leaves has Laxative and antihelmintic activity (Priyanka *et al*., 2010), *Chrozophora hierosolymitana* stem had antitumor activity this reported by (Maryam *et al* ., 2012). `

1.5.5.4 Phytochemistry and antioxidant activity of *Chrozophora* species

Chrozophora is a common weed of the family *Euphorbiaceae*. Several antioxidant studies have been previously carried out on *Chrozophora* genus (Narmadaa, 2012) reported that *Chrozophora rottleri* exhibited in vitro

antioxidant and phytochemical analysis, (Khalid, 2001) isolated glycosides from *Chrozophora oblique*, (Delazar *et al*, 2006) had isolated phenolic compounds from *Chrozophora tinctoria*, (Hussein *et al*., 2006) had isolated fatty acids from *Chrozophora brochiana* and finally cytotoxicity of *Chrozophra parvifolia* reported by (Ramesh and Akalanka, 2014).

1.5.5.5 *Chrozophora plicata*

Chrozophora plicata Vahl is belonging to family *Euphorbiaceae*, it as an important medicinal plant of Sudan.

1.5.5.5.1 Taxonomical classification

Kingdom: plantae

Subkingdom: *Viridaeplantae*

Division: Magnoliophyta

Subdivision: *Magnoliophytina*

Class: *Magnolliopsida*

Order: *Euphorbiiales*

Family: *Euphorbiaceae*

Tribe: *Chrozophoreae*

Genus: *Chrozophora*

Species: *Plicata*

(Schmelzer, 2007)

1.5.5.5.2 Vernacular names

Argassi **(Sudan),** giradol **(English),** Khudi-okra **(Bangladish),** Subali and Nilkanthi **India) (**Burkill**,** 1985–2004).

1.5.5.5.3 Synonyms *Chrozophora plicata*

Croton *plicatus***,** *Croton* rottleri (Govaerts *et al.,* 2000).

1.5.5.5.4 Botanical description of *Chrozophora plicata*

C. Plicata is Prostrate or more or less erect, branched annual or perennial herb, up to 50 cm. Most parts densely covered in greyish stellate hairs.Leaves rhombic-ovate, up to 7 × 5 cm with a long petiole, plicate-undulate, especially when young, 3-5-veined from the base with dark purple glands at the base; margin more or less entire or obscurely toothed. Flowers in leaf-opposed or pseudo-axillary inflorescences, covered in stellate hairs, unisexual. Male flowers orange-yellow or pinkish; female flowers, crimson-red, Fruit up to 5 × 9 mm, 3-lobed, densely covered in stellate hairs, reddish or bluish-purple when ripe (Burkill, 1985-2004).

Leaves:

Leaves rhomboid, subhastate or hastate, obtuse, base truncate or cordate, margin irregularly repand, 1–2 in. long, 3/4–1 1/2 in. wide, dark green and thinly hirsute with long hairs above, beneath densely covered with loose woolly tomentum of long hairs, subcoriaceous. Petals pink.

Flowers:

Flowers unisexual, regular, 5-merous; calyx with stellate hairs; male flowers with short pedicel, calyx with lanceolate lobes. 3 mm long, petals elliptical-oblong, 3 mm long, yellowish orange or pinkish; female flowers with long pedicel, extending up to 2 cm in fruit, sepals linear-lanceolate, 1.5–2 mm long, petals minute or absent, ovary superior, densely short-hairy, styles 3, 1.5–2 mm long, fused at base.

Fruits:

Fruit a lobed capsule 4–5 mm × 7–9 mm, densely stellate-hairy, reddish or bluish purple when ripe, Seeds ovoid, 3.5 mm long, smooth or minutely dotted, pale or dark brown to blackish (Germishuizen and Meyer, 2003).

Fig (1.1): *Chrzophora plicata* leaves and fruit

1.5.5.5.5 Distribution of *Chrozophora plicata* in the world

Chrozophora plicata occurs throughout tropical Africa to Northern South Africa, Saudi Arabia, Egypt, Syria, Palestine, and North-Western India to the Mediterranean. It grows in warmer climate and temperate regions. In Sudan it distributed on Blue Nile province and central Sudan, especially on sandy soils and high rainfall savanna (Grin Database, 2006).

1.5.5.5.6 Ecology

Chrozophora plicata occurs on flood plains of rivers, along drainage channels, usually in damp or desiccating black clay soils and alluvial soils, on mudflats and sandbanks, up to 1200 m altitude. It is also weed of arable land.

1.5.5.5.7 Phytochemistery of *C. Plicata*

The leaves of *Chrozophora plicata* plant was reported that it contain triterpenoids and related compounds (sterols, alcohols and hydrocarbons), phenolic compounds (flavonoids, lignans, coumarins, tannins, phenanthrenes, quinones, phenolic acids, etc.) (Kadiri Sunil, 2013). Two new sphingolipids plicatin were isolated from methanol extract of the whole plant. The two compounds showed inhibitory potential against enzyme lipoxygenase with IC_{50}

values 195.1 and 102.3 µm, respectively also the two compounds are possessing antioxidant properties (Naheed *et al*., 2013).

1.5.5.5.8 Medicinal uses of *C. Plicata*

The plant and its parts are widely used by traditional healers and tribal people for curing variety of ailments, the plant is regarded as laxative, and the whole plant is applied to wounds to improve healing and jaundice (Muhammad Farrukh *et al*., 2014), also the plant used as purgative agent, the seeds of the plant is used as blood purifier. Moreover the leaves part is used as antipyretic and chronic persistent fevers, the fruit yield a purplish blue dye, which is used in east Africa as dye agent (Priyanka, 2010).

1.5.5.5.9 Biological activity of *C. Plicata*

1.5.5.5.9.1 Antiulcer activity of *C. Plicata*

Chloroform Leaves were reported to possess antiulcer activity, The gastroprotective activity was assessed by using pylorus ligation induced and indomethacin (40 mg/kg) induced Ulcer in albino rats, The antiulcer activity was determining by comparing gastric volume, acidity, ulcer score and ulcer index in control, test extract and standard (ranitidine 10mg/kg) treated rats. An ulcer index of 1.66 ± 0.42. It is evident from literature that *Chrozophora plicata* leaves possess flavonoids and flavonoids may be responsible for gastroprotective activity (Kadiri and Avanapu, 2013).

1.**5.5.5.9.2 Antianxiolytic activity of *C. Plicata***

(Kadiri and Avanapu, 2013) reported that the crude ethanolic leaves extract of *Chrozophora Plicata* showed significant minor tranquilizing anxiolytic activity, ethanolic extract was found to be less (2.99) when compared to control group rats (12.625). Hence it indicates that the given test ethanolic extract of *Chrozophora plicata* leaf (600 mg/kg) possesses anxiolytic activity.

1.5.5.5.9.3 Antitumor and pro-apoptotic activities of *C. Plicata*

(May *et al*., 2013) reported that the hole plant posses antipro-apoptotic and antitumor activity.

1.5.5.5.9.4 Antioxidant activity of *C. Plicata*

(kumar, 2013) reported that the 70 % ethanol extract of *Chrozophora plicata* plant (Leaves) showed high antioxidant activity using reducing power and scavenging activity of Superoxide anion, Hydroxyl radical and Nitric oxide anion methods, and compared the results with sodium metabisulphite as standard.

1.5.6 *Fabaceae* (family)

1.5.6.1 Description

Fabaceae is the third largest family of flowering plants and consists of 730 genera and over 19,400 species. The members of this family are herbs, less frequently shrubs or trees. Leaves simple flowers papilionate, with often erect, large, adaxial petal , lateral petals (wings) and lower petals usually connate by their lower margins (keel); standard outside and enclosing the other petals in bud. Sepals usually connate into a tube. Stamens 10, either 1 free and 9 fused (diadelphous), all fused (monadelphous) or rarely all free. Fruit usually dehiscent. Seeds often large; endosperm or very scanty (Schrire, 2005); the leaves are usually alternate and compound the Roots host bacteria in their roots within structures called root nodules. These bacteria, known as rhizobia, have the ability to take nitrogen gas (N_2) out of the air and convert it to a form of nitrogen that is usable to the host plant (NO_3 or NH_3), This process is called nitrogen fixation (Watson, 2007).

1.5.6.2 Classification and Distribution of *Fabaceae*

Fabaceae has traditionally been divided into three subfamilies: Caesalpinioideae, Mimosoideae, and Faboideae, each of which has been considered a separate plant family in the past. Classifications based on molecular

analyses now separate Caesalpinioideae into several lineages and recognize the tribe Cercideae as a separate and more basal group in the family (Lewis, Schrire, 2005). The members of *Fabaceae* are found in tropical area, rain and dry forests in America and Africa country. (Lewis, 2005), *Senna (Cassia)* is widely distributed in Central Sudan (Hayati, 2005). It is present in Western and Eastern Sudan, on the Nubian desert, along the River Nile course from Khartoum to Dongla in Northern Sudan and it occurs on all types of soil with best yield on clay soils (Elamin, 1990). Sudan and India are the largest producers and exporters of *Senna* leaves, pods and total sennosides concentrates to the world market.

1.5.6.3 Species of (*Senna*) *Cassia*

Senna is a medicinal plant that has been in use over a thousand years. It is a safe, hazrmless, and some name of *Senna* species: *Senna alata*, *Senna alexandrina*, *Senna artemisioides* , *Senna auriculata* , *Senna bicapsularis, Senna corymbosa, Senna covesii*, *Senna durangensis*, *Senna floribunda, Senna garrettiana*, *Senna hebecarpa*, *Senna hirsuta* , *Senna Singueana*, etc.

1.5.6.4 Previous biological studies on genus *Senna*

(Sumi and Ommen, 2012) reported that the methanol extract of pod *Senna Fistula* showed extremely high antibacterial activity more than the water extract. (Anushia *et al*., 2009). noted that the methanol extract showed high inhibition zone against *Vibrio cholorae* and *Staphylococcus aureus*. (Thirumal *et al*., 2012) reported that *Senna Fistula* anti-inflammatory, antifertility, antipyretic and antitussive. (Odeja *et al*.**,** 2014**)** studied that *Senna Occidentalis* possessive antioxidant activity. (Jinu *et al*., 2012) evaluated antioxidant and anticancer of *Senna Tora.*

1.5.6.5 Phytochemistry of *Senna* species

(Radha *et al*., 2013) assessed that the ethyl acetate leaves extract of *Senna auriculata* extract was found to be effective in different levels of antioxidant activities. *Senna occidentalis* seeds antioxidant carried out by (Sharuti Mehta *et al*., 2010), fatty acids of *Senna Glauca* (Deepak *et al.*, 2013), Metal content of *Senna saligna* and *Acacia polyacantha* reported by (Masvodza, 2013). (Vladimir *et al*., 2014) were isolated 8-O-β-D-glucopyranoside of torachrysone, kaempferol-O-gentiobioside and 1,7-dihydroxy-3- carboxyanthraquinone from *Senna acutifolia.* (Hemen and Lalita, 2012) characterized anthraquinone from *Senna* species, also two alkaloids isolated from *Senna spectabilis* studied by (Christofidis *et al*., 1979), another study for isolation of alkaloids from fruit part of *S. leptophylla* (Bolzani *et al*., 2012). Steroids of Senna species previously reported by (Lalita and Shelley, 2010).

1.5.6.6 *Senna (Cassia) Singueana*

Senna Singueana Vahl is belonging to family *Fabaceae*, it as an important medicinal plant of Sudan and some other countries.

1.5.6.6.1 Taxonomical classification

Kingdom: plantae

Subkingdom: *Viridaeplantae*

Division**:** Tracheophyta

Subdivision: Spermatphtina

Class: Magnoliopsids

Order: Fabales

Family: Fabacea

Genus Senna

Species Singueana (Adzu, *et al*., 2003)

1.5.6.6 .2 Vernacular names

Winter *senna*, sticky pod, scrambled egg (English). Pintcheira do mato (Poland). Mbaraka, mkundekunde (SW). Um saaba (Sudan and Niger) (Burkill, 1995).

1.5.6.6.3 Synonyms *Senna Singueana*

Senna Singueana (1826), *Senna goratensis* Fresen (El-Ghazali *et al*., 1994).

1.5.6.6.4 Botanical Description of *Senna Singueana*

Senna Singueana is a shrub or small tree 1-15 m high; branchlets glabrous to densely pubescent crown open; bark reddish, becoming grey brown and rough with age. Leaves compound, with 4-10 pairs of oval leaflets, 2.5-5 cm long, rachis with a conspicuous gland between each pair of leaflets, rounded at apex, glabrous or nearly so to densely pubescent. Flowers deep yellow, fragrant, in racemes to 15 cm, often aggregated towards branchlet-ends and often produced when the plant is leafless; flower stalks 2-4 cm, with conspicuous glands. Pods linear, straight or somewhat twisted, torulose, slightly compressed, 5- 26 cm long, indehiscent, with stiff and rather hard valves, glabrous to pubescent, rounded to abruptly acute and often apiculate at apex; yellowish when ripe. Seeds dull brown, almost circular, flattened, 5-6 mm in diameter Website (Africa free database).

Fig (1.2): *Senna Singueana*

1.5.6.6.5 Distribution of *Senna Singueana* in the world

Senna Singueana is widespread in Namibia, in Savannah vegetation belt on all soil types. It is widely distributed in Africa in countries such as Niger, northern Nigeria, Mali, Sudan, Eastern and Southern Africa (Galadima, 2008).
The tree is grown wet soil and high rainfull savanna especially on central Sudan it speared on Anngasana city damazin, Rahed and Baw (El-Ghazali *et al*., 1994).

1.5.6.6.6 Ecology

Senna Singueana occurs in thickets, woodland, savanna and dry evergreen forest, often on termite mounds, from sea-level up to 2250 m altitude. It is found in areas with an annual rainfall of 500–1000 mm.

1.5.6.6.7 Economic value

Food: Pods are edible raw or cooked, whereas leaves are eaten as a vegetable.

Fodder: Leaves, pods and seeds are fed to livestock.

Fuel: The wood is commonly used for fuel.

Timber: The conspicuously pitted wood is light brown with a distinct grain. It is used for small furniture, carving and trinket boxes.

Tannin or dyestuff: In Eritrea, the bark is used for tannin production.

Medicine: The root bark is used in Tanzania against convulsions, gonorrhoea, bilharzia, heartburn, stomach-ache, constipation, wounds and snake bites. The ash from the burnt roots mixed with porridge provides a remedy for stomach **pains.**

1.5.6.6.8 Previous phytochemistry of *Senna Singueana*

(Ode and Asuzu, 2014) reported that the methanol 80% extract of *S. Singueana* leaves was fractionaned by column chromatography and obtained 8 fractions the fraction No 8 showed antiulcer activity Structural and molecular elucidation with Nuclear magnetic resonance (NMR) spectroscopy and mass

spectrometry enabled its identification as luteolin. Luteolin is a known compound which is safe and possesses multiplicity of effects against gastric ulceration.

(Olusola, 2011) and (Ode and Onakpa, 2010) were studied the phytochemistry of leaves extracts of *Senna Singueana* the screened provided the leaves contain alkaloids, tannins, triterpenoids and sterols but no flavonoids, saponins, carbohydrates, reducing sugars, starch nor polyuronide.

The GC-MS analysis of ethyl acetate extract of stem bark and the ethanol extract of the root and leaves part indicated that several aromatic compounds, including phenolics, fatty acids, amino acids and terponoids were present in these extracts (Mohammed Auwal *et al*, 2013).

1.5.6.6.9 Biological studies of *Senna Singueana*

1.5.6.6.8.1 Antibacterial activity

The antibacterial activity of ethanol and aqueous crude extracts of *S. Singueana* leaves was studied. The ethanol and aqueous extracts had significant inhibitory effect against Staphylococcus aureus (Olusola *et al*., 2011). Another study reported by (Teklay *et al*., 2014) Root part of the S. Singueana was extracted using methanol, acetone and chloroform; the extracts were screened for their potential antibacterial against seven standard bacteria species, Results showed that the different extracts displayed significant ($p<0.05$) antibacterial activities and the methanol extract was more active**.**

1.5.6.6.8.2 Antiulcer activity

The methanol extract of the leaves of *S. Singueana* was found to have untiulcer activity (Ode and Onakpa, 2010) also (Ode *et al*., 2011) Fractions from crude methanol extract of *S. Singueana* leaves were isolated and showed the most bioactive anti-ulcer agent in the plant leaves.

1.5.6.6.8.3 Hepatoprotective and hypolipidemic activity

The methanol extract of roots of *S. Singueana* was found to have antioxidant compounds that can offer significant protection against hepatic and oxidative injuries (Ottu *et al*., 2013).

1.5.6.6.8.4 Antioxidants activity

In a study the ethyl acetate, ethanol and aqueous extracts of different parts of *S. Singueana.* Leaves, stembark and roots were found to have anti-oxidative activities and can be used as a potential alternative medicine for oxidative stress related non-communicable chronic diseases (Mohammed Auwal *et al*, 2013).

1.5.6.6.8.5 Antimalarial activity

The methanol extract of the root bark of *Senna Singueana* against rodent plasmodia infection, (Plasmodium berghei) in mice; formalin test, yeast-induced pyrexia and egg-albumin-induced inflammation in rats. The results showed that the extract exhibited significant antinociceptive, antipyretic and antiplasmodial activity in all the models used (Adzub, 2003).

1.5.6.6.8.6 Erythrocyte Haemolysis Inhibition

The ethyl acetate solvent fraction from the bark of *Senna Singueana* exhibited concentration dependent erythrocyte haemolysis inhibitory activity indicating that the plant contains constituents that can inhibit erythrocyte haemolysis. This in turn could possibly be due to inhibition of lipid peroxidation. Also, despite the presence of saponins, no observable induction of haemolysis was observed in any of the tested samples (Mebrahtom, 2012).

1.5.7 *Araceae* Family

1.5.7.1 Description

The *Araceae* or Aroids are herbaceous mono cotyledonous plants having heart-shape or cordiform, generally broad and pinnately veined leaves. The

Araceae is a large family comprising about 105 genera and approximately 3000 species of herbaceous mono cotyledons. These are predominantly tropical in distribution with 90% of genera and 95% of species restricted to the tropics. The species are characterized by their inflorescences, which consist of a fleshly spadix surrounded by sessile small flowers and lack floral bracts. The infloresencence covered by a specialized attractive organ called spathe. One of the important character of the family is the inflorescence structure; small flowers born on fleshy axis (spadix) subtended by a modified leaf (spathe), many plants in this family are thermogenic (heat-producing) (Boyce, 1995).

1.5.7.2 Distribution of *Araceae*

The species are normally found in various habitats with special reference to wetlands; ranging from swamps, ponds, lakes, canals, rivers to rice fields. Some species thrive well in forest floors, generally the aroid species are found in tropical areas and also are distributed in Malayan region and tropical America and a large vari ety occurs in tropical Africa (Saswatil, 2013).

1.5.7.3 Classification of *Araceae*

The only classification of the family to date to utilize modern phylogenetic techniques (Mayo *et al*.,1997) recognizes seven subfamilies, of which three are represented in native temperate North American aroid flora: Orontioideae (Orontium, Symplocarpus, Lysichiton); Calloideae (Calla); and Aroideae (Peltandra, Arisaema, and Pistia). Acorus, a genus historically included in Araceae, istreated as a separate family in theat flora based on extensive morphologic and chemical evidence that supports its removal from Arales (Grayum , 1990; Mayo *et al*., 1997; Govaerts *et al*., 2002).

1.5.7.4 Medicinal uses of *Araceae*

Rhizomes of *Acorus calamus* are used in medicines in fever, cough and for improve memory (Sharma, 2012). The young leaves and rhizomes of *Homalomena Aromatica* is used for treating stomach problem, jaundice, diarrhea and rhizomes are used as good source of nutrition also the leaves and fruit of some trees on the family used for treat fever and malaria (Das *et al*., 2013).

1.5.7.5 Economic value of *Araceae*

Aroids or *Araceae* contribute an important part to the carbohydrate content of the diet in many regions in developing countries. They produce edible starchy storage corms or cormels; (Boyce, 1995) reported that the major plants of the family contain oxalate (crystal).

1.5.7.6 Species of genus *Stylochiton*

Is a plant genus of the family *Araceae* and the whole genus comprised in the subtribe *Chrozophorinae*. It comprises of 12 species, which are monoecious herbs or undershrubs (Gbif, 2011).

Species:

S. angolensis, S. Bangneri, S.borumensis, *S.Chevalieri, S. Crassispathus, S. Cuclliferus S.dalzieli, S. euryphyllus, S. kerensis, S. Barteri and S. similis*.(Malaiss and Bamps, 1994).

1.5.7.7 Phytochemistry of *Stylochiton* species

General Phytochemical screening of the rhizome of *Stylochiton lancifolius* (*Araceae*) revealed the presence of steroids triterpenes, saponins, fatty acids and tannins. Extensive Phytochemical investigation of the petroleum compounds ether extract of the rhizome afforded white crystalline mixture of β-sitosterol, stigmasterol and 2-hydroxy hexadecanoic acid methyl ester (Pateh, 2008).

1.5.7.8 Biological activity of *Stylochiton* species

The methanolic extract of the rhizome of *Stylochiton lancifolius* was evaluated for analgesic and anti-inflammatory activities using acetic acid-induced writhing, formalin-induced pain and formalin-induced-inflammation.The methanol extract exhibited significant ($P<0.001$) inhibition of acetic acid-induced writhing in mice and a significant ($P<0.001$) reduction in paw licking time of the second phase of formalin-induced pain in rats. The methanol extract also produced a significant ($P<0.001$) anti-inflammatory effect in formalin-induced inflammation which is comparable to that of the reference drug Piroxicam (10 mg/kg), which is a standard analgesic and anti-inflammatory drug. The intraperitoneal (i.p) median lethal dose (LD_{50}) of the methanol extract of *S. lancifolius* was found to be greater than 5000 mg/kg in mice. The result obtained from this study showed that the methanol extract of *S. lancifolius* possesses analgesic and anti-inflammatory activities and supports the use of the plant in the management of pain and inflammatory conditions (Pateh, 2011).

1.5.7.9 *Stylochiton borumensis* N.E.Br

Stylochiton borumensis is belonging to family *araceae*, it as an important medicinal plant of Sudan and some other countries.

1.5.7.9.1 Taxonomical classification

Kingdom: plantae
Subdivision: Alismatales

Class: Liliopsida

Order: lismatales

Family: *Araceae*

Genus *Stylochiton*

Species *borumensis* (Adzu *et al*., 2003)

1.5.7.9 .2 Vernacular names

Stylochiton borumensis N.E. Br. (*S. angustifolius*) (Timberlake, 1987).

1.5.7.9 .3 Synonyms *Stylochiton borumensis*

Stylochiton lobatus, S. rogersii, S.angustifolius, S. heterophyllus, S. obliquinervis, S. lubulosus (El-Ghazali *et al*., 1994).

1.5.7.9.4 Botanical Description of *Stylochiton borumensis*

Rhizome vertical or horizontal, 0.5-1 cm thick. Leaves several; petiole 4-40 cm long blade very variable in shape, linear to ovate-elliptic, the base often hastate to sagittate; posterior lobes variable, very short to as long as the anterior lobe. Inflorescence appearing before the leaves, borne at or near the soil surface. Peduncle 1.5-6.5 cm long. Spathe glaucous green to pale yellow-green, often with purplish markings, cylindric; apex erect, acuminate, usually less than ¼ the total length of the spathe. Spadix hidden within the spathe tube (Haigh and Boyce, 2012).

Fig (1.3): *Stylochiton borumensis* leaves and root

1.5.7.9.5 Distribution in the world

Stylochiton borumensis is widespread and common in most of its range. In Namibia and Savannah vegetation belt on all soil types. It is widely distributed in Africa in countries such as Niger, northern Nigeria, Sudan, Tanzania and Mozambique.

The tree is grown wet soil and high rainfull savanna. It found on central Sudan it speared in Anngasana city damazin, Rahed, Baw and Nuba hill (El-Ghazali *et al*., 1994).

1.5.7.9.6 Ecology of *Stylochiton borumensis*

S. Borumensis is plant growing in swamp in lowland savanna of tropical Africa. It was found in areas with raise field.

1.5.7.9.7 Medicinal uses of *Stylochiton borumensis*

The root bark is used in Tanzania against convulsions, gonorrhoea, bilharzia, heartburn, stomach-ache, constipation, wounds and snake bites. The ash from the burnt roots mixed with porridge provides a remedy for stomach pains (Galadima, 2008).

General objective

The main aim of this work is to carry out phytochemical investigation of *Chrozophora Plicata, Stylochiton Borumensis* and *Senna Singueana and* to isolate and identify compounds using spectroscopic technique.

Specific objectives

To determine the antifungal and antibacterial activity of *Chrozophora Plicata, Stylochiton Borumensis* and *Senna Singueana* leaves and seeds extracts.

To determine the *in vitro* cytotoxicity of the extracts of tested plants using Brine shrimps lethality and *vero cell* lines.

To determine the total phenolics content of extracts of tested plants using spectrophotometer method.

To determine the antioxidant properties of extracts of studied plants.

To measure the amino acids of tested plants material using amino acids analyzer.

To measure the concentration of heavy metals and trace element using ICP technique.

To evaluate the anti-inflammatory and other possible biological activities of extracts and pure compounds of plants under study.

To isolate and purify compounds present in the crude extracts using thin-layer chromatography (TLC) and column chromatography (CC).

To characterize and elucidate the structural of isolated compounds using FT-IR, NMR and MS spectroscopic methods.

Chapter two

2- MATERIALS AND METHODS

2. 1. MATERIALS

2.1.1 Plant materials

The plant materials were collected from Angassena, Blue Nile province and central Sudan during the period 2011/2012. The collected materials were authenticated specimens, deposited in the herbarium of the Medicinal and Aromatic Plant Research institute. Each part of the plant (seeds, leaves) was shade-dried and ground into powder using hammer mill.

2. 1. 2 Chemicals and Reagents

- **General reagents**

  Acetone, cholorform, ethyl acetate, hexane and methanol all were GPR, were obtained from Romil, United Nation. 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH), Folin ciocalteau reagent, gallic acid, tannic acid and querestin were purchased from England, Sigma Chemical Company. Ferric chloride Potassium hydroxide and sodium hydroxide were obtained from Loba Chemie. India. Formic acid, ceric acid, sodium chloride and vanillin were obtained from Scharlu, Spain. Sodium nitrite, potassioum ferrous cyanide, sodium bicarbonate and aluminum chloride were brought from Techno pharmachem. India.

  Medium for silica gel:

- Silica gel for TLC GF 254 LR for TLC s.d. fine – Chem Limited, Mumbai. India.
- Precotated silica gel 60 / UV 254 for TLC, obtained from SDFCL Industrial Estate, Mumbai.India.
- Silica gel for column chromatography GF 260 - Lobachem / India.

- **Culture medium:**

- Mueller Hinton agar and Sabouraud dextrose agar were obtained from Maharashtra, India.
- RPMI 1640 with L-Glutamine obtained from Gibco-Brl, Life Technology.

- **Chemotherapeutic agents:**

The following reference drugs were used in this study:

- Antibiotics (Ampicillin, Ciprofloxac and Gintamicin) were purchased from Sigma, USA. Metronidazole (metronidazole (flagl®) powder. No. 070501. Manufacture in May, 2012 Expire in May, 2016. Company: Wujiang Li town pharmacetutical. (MTT) 3-(4, 5 Dimethylthiazole-2-yl) 2, 5. diphenyltetrazolium bromide, Sigma, USA.
- Brine shrimps: artemia cystsfrom JBL Gmbh & Co.KG (Neuhofen Germany Cell line used: Vero cells (Normal green monkey kidney).

- **Cell line used:**

- Vero cells (Normal, African green monkey kindney, South Africa).

- **Standard Bacterial micro- organisms:**

The following standard fungi were used in this study:

Bacillus subtilis	NCTC 8236	(Gram positive cocci)
Escherichia coli	ATCC 25922	(Gram negative rods)
Pseudomonas aeruginosa	ATCC 27853	(Gram negative rods)
Staphylococcus aureus	ATCC 25923	(Gram positive cocci)

- **Standard Fungal micro-organisms:**

Aspergillus niger	ATCC 9763 (Filamentous fungi)
Candida albicans	ATCC 7596 (Yeast fungi)

** National Collection of type culture (NCTC), Colindale, England.

*American Type Culture Collection (ATCC), Rockville, Maryland, USA.

The test bacterial was obtained from the department of microbiology, Central lab, Ministry of Science and Communication.

The test fungi were obtained from Central International lab, Khartoum.

Product identification and description (*A. Salina)*

The *Artemia* cysts had been harvested from Great Salt lake, Utah, USA and were identified as *A. salina*, based on zo-ogeography.

2.1.3 Equipments and Instruments

1- **Equipments**

- Analytical balance - Kern / Germany.
- Atomizer for spraying the chromatogram - Desaga / Germany.
- Autoclave - Griffin & George Ltd / England.
- Incubator - Heraeus electronic / Germany
- Oven - Baird and Tat lock / London.
- Rotator evaporator - BÜCHI – 011/ BÜCHI 146.
- Soxhlet -Tsek / Turkey.
- Water Bath - Earth / India.
- Glass columns for chromatography.

2- **Instruments**

Chromato - VUE Cabinet

TLC cabinet for UV detection with two wave length (short (254) long (366) nm from U.S.A.

Amino acids analyser –

High pressure liquid chromatography with UV-VIS detector, Sykum /Japan.

- **FT.IR**

Fourier 8400S, CE to determine the fuctional group – Shimadzu / Japan.

- **Gas chromatography Mass spectrometer**.
- GC-MS. QP. 2010 with capillary column (length 30m, 0.25µm) and mass spectrometer detecter, Shimadzu. Japan.
- **Inductively Coupled Plasma**

  Atomic Emission Spectroscopy elemental analysis (ICP-OES) 725, Varian, U.S.A.
- **Mass spectrometer**

  Mass spectrometer with electron impact ionization mode high resolution tequnine, quderpole, 2010, Shimdazu / Japan.
- **FT-NMR Spectrometer**

  Advance III 400MHz Bruker High performance Digital.
- **UV-Visible spectrophotometer**

  UV-VIS with rang (190-800) nm for qualilitative and qunititative analysis - Jasco / Japan.
- **Ultra spectrophotometer**

  With micro plate (96 plate) and UV-VIS rang (190 -1000) nm. Thermo, U.S.A.

2.1.4 Reagent preparation for qualitatative determination of phytochemical compounds

1- Ferric chloride:

Three gram of ferric chloride dissolved in 100 ml methanol.

2- Gelatin salt reagent:

10 g of gelatin powder dissolved in 100 ml hot distilled water.

3- Lead acetate:

25 ml of lead acetate was dissolved in 100 ml of distilled water.

4- 1 % Potassium hydroxide:

1 g of potassium hydroxide dissolved in 100 ml methanol.

5- Dragendroff's reagent:

About 14 g of sodium iodide with 5.2 g basic bismuth carbonate in 50 ml glacial acetic acid were boiled for a few mintues and allowed to stand overnight and filtered off the precipitate of sodium acetate crystals. To 40 ml of the red-brown, 160 ml of ethyl acetate and 1 ml water filtrate were added. The stock solution was preserved in a brown bottle. When needed, 20 ml of acetic acid were added to 10 ml of this stock solution and made up to 100 ml with water before use.

6- Mayer' reagent:

a- 1.36 g of mercuric chloride dissolved in 60 ml of distilled water.

b- 5 g of potassium iodide dissolved in 10 ml of distilled water.

Solution (a) and (b) mixed and diluted to 100 ml with distilled water.

7- **Wagner's reagent:**

Iodine (1.27 g) and potassium iodide (2 g) were dissolved in 5 ml ofwater and made up to 100 mL in distilled water.

8- Folin ciocalteau reagent

Dissolved 100 g sodium tungstate (VI) dihydrate and 25 g sodium molybdate (VI) dihydrate with 700 ml distilled water, 100 ml concentrated hydrochloric acid, and 50 ml of 85% phosphoric acid to which is added 150 g of lithium sulphate hydrate.

2.1.5 Spray Reagents for thin layer chromatography

1- Anisaldehyde-sulphuric acid

Reagent was freshly prepared by dissolved 0.5 ml of anisaldehyde in cold solution contains 50 ml glacial acetic acid and 1 ml concentrated sulphuric acid.

2- Ceric acid

1g of ceric powder dissolved in cold solution contenaied conc. sulphuric acid and 500 ml distill water.

3- Ferric chloride

5g ferric chloride was dissolved in 100 ml methanol and few drops of hydrochloric acid were added.

4- Vanillin-sulphuric acid

Three grams of vanillin were dissolved in solution contenaied 250 ml cold ethanol and 2.5 ml con sulphuric acid and kept in dark bottle.

2.2 Methods

2.2.1 Sample preparation

The plant materials (leaves and seeds) were harvested and immediately washed with distilled water. The plant was air dried at room temperature (about 30°C) for approximately 5 days. When dried, the material was ground to a powder using a pestle and mortar and stored in a clean container ready for analysis.

2.2.2 Extraction

Plant samples were extracted (hot extraction) using petroleum ether (40-60)°C, chloroform, ethyl acetate and methanol, respectively. About 500 ml of petroleum ether (40-60)°C were added to each of the tested material and extracted using soxhlet apparatus. Extraction was carried out for about four hours for petroleum ether, twelve hours for chloroform, and eighteen hours for ethyl acetate and seventy two hours for methanol. The extracts were filtered to remove any precipitate, evaporated to dryness under reduced pressure using rotary

evaporator, after drying yield percentage of each extract was calculated as followed:

$$\text{Yield \%} = \frac{\text{Weight of extract}}{\text{Weight of the plant}} \times 100$$

2.2.3 Phytochemicals screening of plant extracts

The preliminary phytochemical screening for various extracts, petroleum ether (40-60))°C, chloroform, ethyl acetate and methanol extracts was performed for the Presence of various phytoconstitiuents like steroids, triterpenoids, flavonoids, tannins, alkaloids, coumarin, saponin and lignin. Preliminary screening for major classes of secondary metabolites was carried out using methods described by (Martinez and Valencia, 1999; Sofowora, 1993 and Harborne, 1984).

1. Test for Alkaloids

The various extracts were basified with ammonia and extracted with chloroform. The chloroform solution was acidified with dilute hydrochloric acid. The acid layer was used for testing the alkaloids.

a. Wagner's test (Iodine in Potassium iodide): The acid layer was treated with few drops of Wagner's reagent. Formation of reddish brown precipitate indicates the presence of alkaloids.

b. **Mayer's test** (Potassium Mercuric Iodine solution): The acid layer was treated with few drops of Mayer's reagent. Formation of creamy white precipitate indicates the presence of alkaloids.

c. **Dragendorff's reagent** (Potassium Bismuth Iodide): The acid layer was treated with few drops of Dragendorff's reagent. Formation of reddish brown precipitate indicates the presence of alkaloids.

2. Test for Flavonoids

a. **Shinoda test:** To the alcoholic solution of extract a few fragments of magnesium ribbon and concentrated hydrochloric acid were added. Appearance of red to pink colour after few minutes indicates the presence of flavonoids.

b. **Ferric chloride test:** Few drops of neutral ferric chloride solution were added to little quantity of alcoholic extract. Formation of blackish green colour indicates the presence of phenolic nucleus.

c. **Lead acetate test:** To the extract, few drops of aqueous basic lead acetate solution were added. Formation of yellow precipitate indicates presence of flavonoids.

3. Test for Sterols

Different extracts were dissolved in chloroform, filtered and the filtrate was tested for sterols and triterpenes.

a. **Salkowski test:** Few drops of concentrated sulphuric acid were added to the chloroform solution, shaken and allowed to stand, appearance of red colour in lower layer indicates the presence of sterols.

b. **Liebermann-Burchard test for Sterols:** To the chloroform solution, few drops of acetic anhydride were added and mixed well. One ml of concentrated sulphuric acid was added from the sides of the test tube, appearance of reddish brown ring indicates the presence of sterols.

4. Test for Tri-terpenes

a. **Salkowski test:** Few drops of concentrated sulphuric acid was added to the chloroform solution, shaken and allowed to stand, appearance of golden yellow colour indicates the presence of triterpenes.

b. Liebermann-Burchard test for triterpenes: To the chloroform solution, few drops of acetic anhydride was added and mixed well. one ml of concentrated

sulphuric acid was added from the sides of the test tube, appearance of deep red colour indicates the presence of triterpenes.

5. Test for tannins

a. **Ferric chloride test:** To extracts a few drops of 1% neutral ferric chloride solution were added, formation of blackish blue colour indicates the presence of tannins.

b. **Gelatin test:** To the extracts 1% solution of gelatin were added to solution containing 10% sodium chloride. Formation of white precipitate indicates the presence of tannins.

c. **HNO_3 test:** drops of HNO_3were added to extract formation of blackish blue colour indicates the presence of tannins.

d. Lead acetate test: To the extract, a few drops of aqueous basic lead acetate solution were added. Reddish brown bulky precipitate indicates presence of tannins.

6. Test for Lignin

Labat test: drops of gallic acid were added to the extract, it develops olive green colour indicates the positive reaction for lignins.

7. Test for Saponins

Foam test: Small amount of extract was shaken with little quantity of water, if foam produced persists for 10 minutes; it indicates the presence of saponins.

8. Test for Coumarins

1g of powdered extract kept with water in a test tube, covered with paper soaked in NaOH was diluted and boiled. Yellow fluorescence indicates the presence of coumarins after examination under ultra-voilet lamp.

2.2.4 Quantitative determination of total phenols, flavonoids and tannins contents in plant extracts:

The total phenols, flavonoids and tannins were determined using double beam spectrophotometer.

1. Total Phenolics Content

The total phenolic content was determined by adopting the method as described by Wolfe *et al*., (2003) with some modifications. 1 mg/ml of the extract were taken in a 10 ml glass tube and made up to a volume of 3 ml with distilled water. 0.5 ml Folin ciocalteau reagent (1:1 with water) and 4 ml Na_2CO_3 (7.5%) were added sequentially in each tube. A blue colour was developed in each tube and the intensity of the colour is directly proportional to the phenolic content. The blue coloration in the tube is due to the formation of molybdenum blue as a result of complex redox reaction between phenols and phosphomolybdic acid in Folin ciocalteau reagent in alkaline medium. The test solution kept in dark for 30 minutes, cooled and absorbance was measured at 765 nm.

The total phenolic contents were expressed as gallic acid equivalents (mg/l) using the following equation based on the calibration curve: $y = 0.0008x+0.0397$ where x = concentration of gallic acid (mg/l) corresponding to optical density. A calibration curve was prepared using gallic acid (100-900 mg/l) as standard and used for calculation of total phenolic compounds (Fig 2.1).

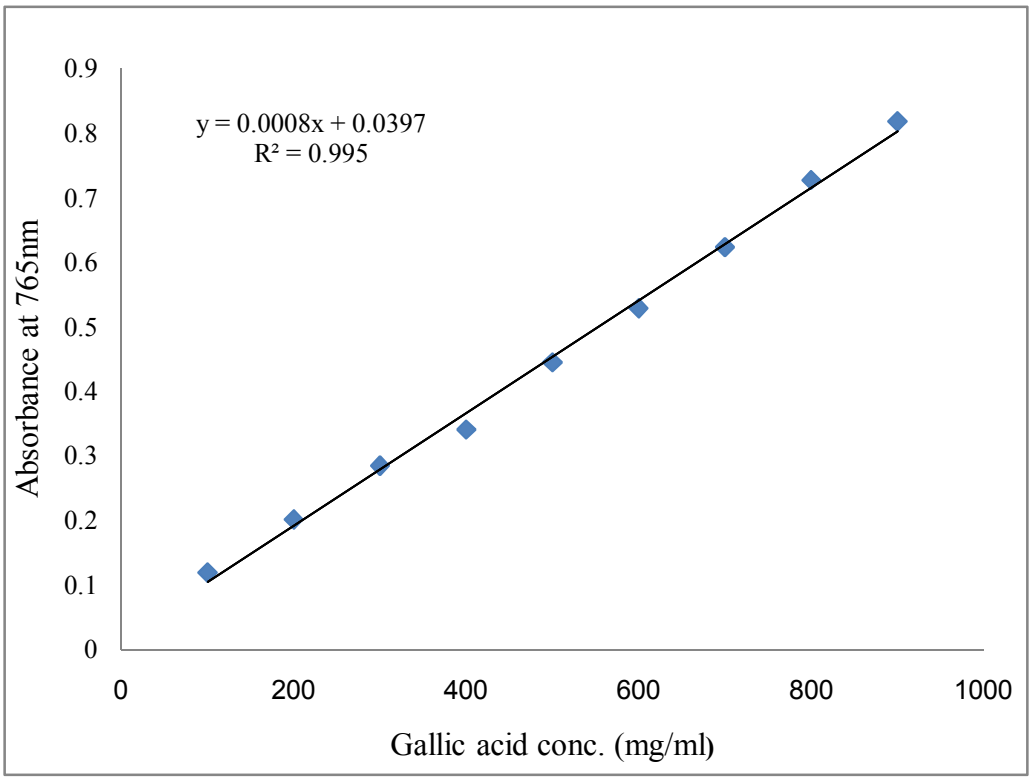


Figure (2.1): standard curve of Gallic acid (mg/l)

2. Total flavonoids content

The total flavonoids content was determined by adopting the method described by Shanukha *et al*., (2012). Aliquots of each extracts were pipette out in series of test tubes and volume was made up to 2 ml with distilled water, 0.3 ml of sodium nitrite (5%) was added to each tube and incubated for 5 min. at room temperature, 0.3 ml of aluminium chloride solution (10%) was added and incubated for 5 min, 2 ml of sodium hydroxide (1M) were added. Absorbance was measured at 415 nm against a reagent blank.Total flavonoids content was expressed as quercetin (mg/l) using the following equation based on the calibration curve: Y =0.0007x+0.0537, where y was the absorbance. A calibration curve was constructed, using quercetin (50-800 mg/l) as standard and

total flavonoids content of the extracts (mg/l) expressed as quercetin equivalents (Fig 2.2).

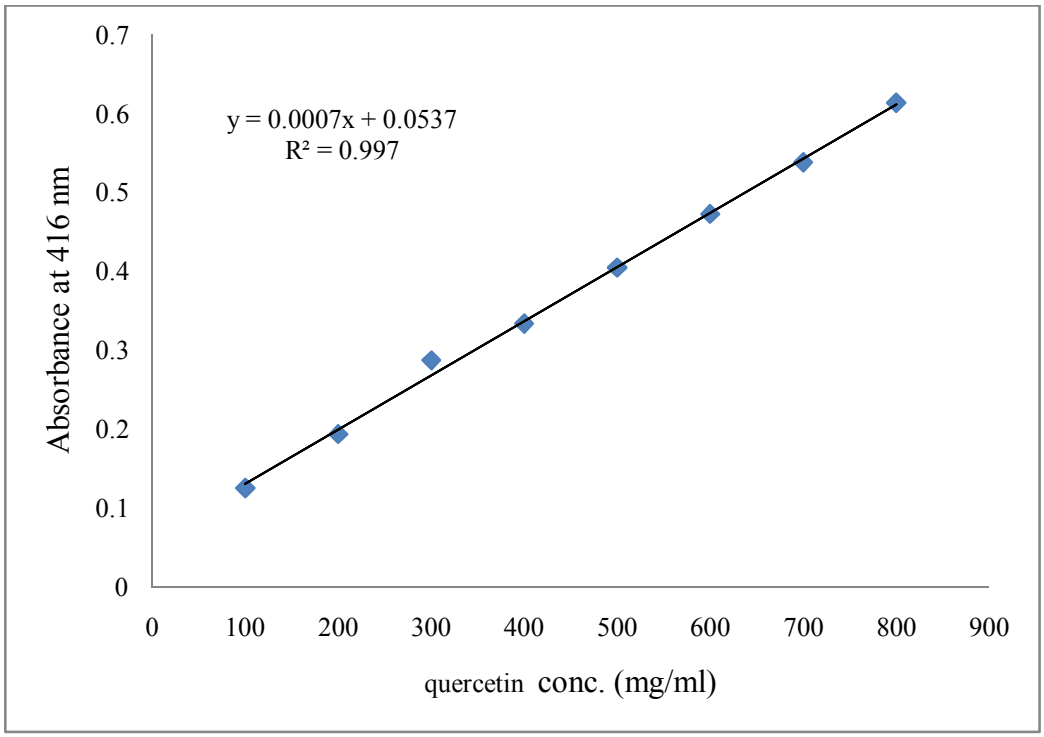


Fig (2.2): standard curve of quercetin (mg/l).

3. Total tannins content

The tannins content was determined by using $FeCl_3$ and gelatin test method as described by Shivakumar *et al.*, (2012) with some modification. About 1 ml of extract (1mg/ml) was transferred to vials, 1ml of 1% $K_3Fe(CN)_6$ and 1 ml of 1% $FeCl_3$ were added, and the volume was made up to 10 ml with distilled water. After 5 min absorbance was measured at 510 nm against a reagent blank. The total tannins content was calculated using the following equation y=0.002x+0.082 where x=concentration of tannic acid (mg/l) corresponding to optical density. a calibration curve was constructed, using tannic acid (100-900 mg/l) as standard

and total tannins content of the extracts (mg/l) expressed as tannic acid equivalents (Fig 2.3) .

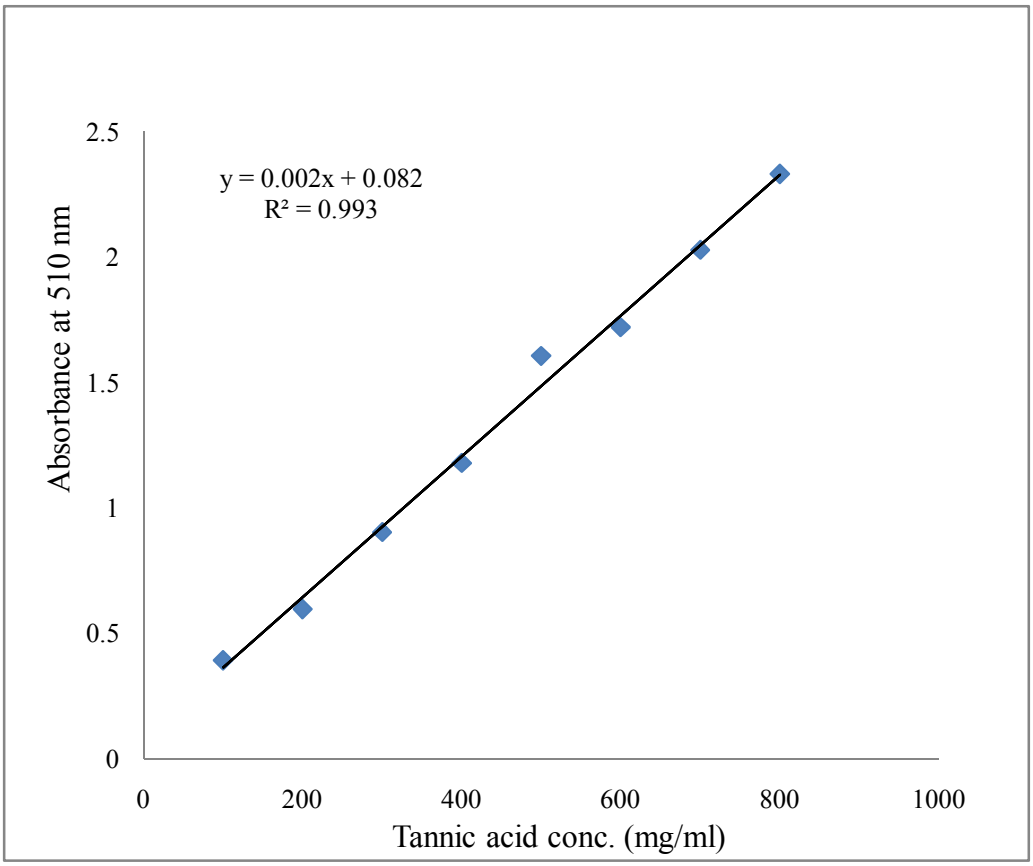


Figure (2.3): standard curve of Tannic acid (mg/l).

Statistical analysis

The experimental results were expressed as mean value. In order to estimate statistically any significant differences among mean values, where it was applicable, the data were subjected to T- test analysis using (SPSS v.16).

2.2.4 Proximate Analysis of Plant Samples

The proximate analysis of the powdered plant samples for protein, fat, fiber, ash and dry matter was determined using the methods described in AOAC (2000).

1- Determination of dry matter of plant samples

The plant sample was throughly mixed with water in a bottle. The water content was determined by weighing out 2 g of the sample into a pettry dish which has been previously ignited and weighed, it was dried in the oven for 24 hour at 100°C, and it was then allowed to cool for 10 minutes in desiccators before weighing.

$$\% \text{ moisture (residual)} = \frac{\text{Wt. of sample taken} - \text{Wt. of sample after drying}}{\text{Wt of sample}} \times 100$$

Wt. of sample Dry matter = 100 - % of moisture.

2- Determination of ash of plant samples

The residue from the moisture was charred over a flame and the furnace was ignited at 5000°C until the ash was grey, the sample was allowed to cool and then weighed.

3- Determination of ether extract (oil) of plant samples

A Soxhlet extractor was fixed with a reflux condenser and a small flask which has been previously dried in the oven and weighed; 1 g of sample was weighed and transferred to a fat free extraction thimble which was plugged lightly with cotton wool. The thimble was placed in the extractor and petroleum ether 40-60 °C was added, once it siphoned more ether was added until the barrel of the extractor is half full. The ether boils gently and it was left to siphon ten times, then the flask was detached and the content was poured into the ether stock bottle. The condenser and the flask were replaced and the ether was distilled until the flask was dry. The flask which now contained all the oil was detached; the oil was cooled and then weighed.

$$\% \text{ ether extracts} = \frac{\text{Wt. of oil}}{\text{Wt. of sample}} \times 100$$

4- Determination of crude protein of plant samples

2 g of the sample was weighed into a Kjeldahl flask, 5 g of anhydrous sodium sulphate and 25 ml of concentrated H_2SO_4 were added to it. Thereafter it was placed in the fume cupboard and heated gently for 5 - 10 minutes. After frothing nearly ceased and the solution gave a green coloration, it was allowed to cool and was diluted with water. The % N_2 in the sample was determined using the micro Kjeldahl apparatus.

5- Determination of crude fiber of plant samples

25 ml of 10 % sulphuric acid was measured with a pipette into a beaker and 175 ml of water was added while the residues from the ether extract was added and allowed to boil. When the liquid had boiled for exactly 30 minutes, it was poured into the funnel and filtered by suction. The residue was washed with hot water until it was free from acid, then the residue was turned into a digesting flask and 200 ml of 1.25% sodium hydroxide solution which was previously boiled was added. It was then filtered through filter paper and washed with boiling water until it was free from acid. The residue was washed twice with 95 % alcohol and three times with petroleum ether using small quantities; the residue was allowed to drain and was transferred into a silica dish. It was thereafter dried in the oven to remove all organic matter and weighed after cooling.

2.2.5 Gas chromatography mass spectroscopy of methyl ester

1- Preparation of methyl ester:

Total lipids were extracted from the seeds of the plants with petroleum ether (b.p- 40- 60˚C) using Soxhlet apparatus. The solvent was evaporated on rotary evaporator under reduced pressure and the produced oil was dried in an oven at 105˚C to constant weight (AOAC, 2000).

The oil fatty acid methyl esters were prepared as described by Christie (1990). One ml of the produced oil was added to seven ml methanolic NaOH (0.5M) followed by seven ml of methanolic H_2SO_4 before shaking. The solution was left overnight. Two ml of n-hexane or heptane were added to the reaction mixture followed by saturated NaCl. This mixture was shaken well and allowed to separate in to two layers. One ml from the upper layer was transferred to a new tube and dried with Na_2SO_4 anhydrous.

2- GC- MS parameter of methyl ester:

Gas Chromatography Mass Spectrometer was performed using Shimadzu instrument (GC- MS- QP- 2010) fitted with electron impact (EI 1.70 eV) mode. The analytical column was RTX 5 (5% phenyl- 95% dimethyl polysiloxane with length of 30 meter x 0.25 µm). Helium gas was used as a carrier gas at a flow rate of 1ml/min. the temperature was programmed at 50˚C for 3 min then increased to 280˚C at rate of 5˚C/min. The temperature of injector was 250˚C. Then a result was taken after comparison with NIST27 library for GC-MS.

2.2.6 Analysis of total amino acids

200 mg of the plant sample was taken in hydrolysis tube, 5 ml of 6 N HCL was added and the tube closed. The tube incubated at 110˚C for 24 hours. Solution was then filtered. 2 ml of the filtrate was evaporated to dryness at 140 ˚C four about one hour. 1 ml of diluting buffer was added to the residue and then measured using amino acid analyzer.

Instrument parameter:

Injetion volume 100µl at run time 75 min with lithum cation column and used buffer solution as mobile phase with PH (2.9, 4.2 and 8) with UV-VIS detector.

2.2.7 Trace and heavy metal analysis by Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES)

1- Grinding and digestion of plant samples

The rinsed plants were dried in shade at 25˚C. The dried samples were grinded into fine powder. The dry digestion procedure was adopted. Two gram of the powdered material of each plant were first kept in the desiccators to eliminate the moisture content. The samples were then kept in the furnace at 100˚C for two hours and then at 600˚C till they were ashed. After ashing each sample was mixed with 10 ml of diluted HCl. All the mixtures were filtered through whatman filter paper and the filtrates were further diluted up to 25 with distilled water. (AOAC, 2000).

2- ICP - Atomic Emission Spectroscopy (ICP-AES Analysis) parameters

The plasma flow: 15 L/min, pump rate: 15 rpm, run time: 1 hour, and the model used was varian- Vista- MPX-CCD, Simultaneous software.

2.2.8 Thin layer chromatography:

Thin layer chromatography was carried out according to the method described by Stahl (1969).

Preparation of plates

30 g of silica gel were shaken with 60 ml distilled water for two minutes using 250 ml stoppered conical flask. The slurry was spread using spreader making 0.25 mm thickness on five glass 20×20 cm plates. The coated plates were then allowed to dry at room temperature and activated at 105°C for one hour. The hot plates were stored and allowed to cool down and stored till used it.

2.2.9 Chromatography and isolation of pure compounds

2.2.9.1 Column fractionation of methanol extract of *Chrozophora Plicata* leaves

An appropriate column sized 5 cm diameter and 80 cm length was used. It was washed with water and rinsed with acetone and dried completely. Little of pure cotton was placed at the bottom of column. Wet method for packing chromatographic columns was used; petroleum ether (40-60) °C was mixed with silica gel (369 gm) in a beaker and poured into the column. The column was allowed 24 h to stabilize after the set-up. Methanol extract of *Chrozophora Plicata* leaves (35 gm) was mixed with 80 gm of graded silica gel until it became free flowing powder. When it reached a defined state it was slowly poured into the column containing petroleum ether (40-60) °C solvent with slight movement of stirring by glass rod to avoid clogging. Little cotton was placed on top of silica gel- extract mixture pack to get neat column pack. The knob at the bottom was slowly opened to release the solvent. The elution was done using petroleum ether, ethyl acetate and methanol in different ratios like petroleum ether (40-60) °C : ethyl acetate 100:0, 99:1, 98:2, 96:4, 94:6, 92:8,90:10, 80:20, 70:30, 60:40, 50:50, 40,60, 30:70, 20:80 and 10:90, 100% ethyl acetate (1 liter) also the same ratio above used between ethyl acetate and methanol finally 100% methanol. A measured volume (500 ml) of each solvent combination was used in the elution process fraction collected in volume 10 ml. As soon as column chromatography was completed, the collected fractions were first concentrated by rotary evaporator and developed on TLC plates using the solvent systems ethyl acetate: hexane (1:1, 2:8, 4:6), ethyl acetate : chloroform (4:6, 3:7), methanol : chloroform (1:9, 2:8, 1:9: drop formic acid) From TLC results, the fractions were combined according to their separation profile into (19) fractions. Fraction No 1 (1g), 4 (1g), 5 (1g), 8 (1g), 10 (1g) of the same column were separately

chromatographic on silica gel preparative TLC (20 X 20 cm) and fraction No (18) 2.3g was subjected to another sub-column to purify compounds.

2.2.9.2 Preparative Thin layer chromatography of sub fractions from column No (1):

The sub-factions from column No (1); Fractions 4 (1g), 5 (1g), 8 (1g), 10 (1g), 15 (1g) was purified using preparative thin layer chromatography.

Preparation of plates

60 g of Silica gel GF254 was added to 120 ml distilled water and shaked for two minutes using 250 ml conical flask. The slurry was spread using spreader making 0.5 mm thickness on five glass 20×20 cm plates. The coated plates were then allowed to dry at room temperature and activated at 105°C for one hour. The hot plates were stored and allowed to cool down and stored till it was use.The following solvent systems were used for each sub-fractions:

Fraction 1 (20-38) (n-hexane: ethyl acetate), (1:1)

Fraction 4 (187-210) (Methanol: Hexane: Chloroform), (1:2:7)

Fraction 5 (211-219) (Hexane: Ethyl acetate), (4:6)

Fraction 8 (304-315) (Hexane: Ethyl acetate), (3:7)

Fraction 10 (323-329) (Hexane: Ethyl acetate), (3:7)

After the solvent mixture reached the height of 15 to 20 cm, the plate was taken out of the tank and exposed to air till solvent was completely evaporated. The plate was inspected in day light, and then examined under UV lamp. Line containing the same spots was scratched and the scratches of all plates were collected together. Collected scratches were then dissolved in methanol and

filtered using filter paper. Concentrated solution was then tested by thin layer chromatography using the following solvent systems:

Chloroform: Methanol (8:2), Hexane: Ethyl acetate (3:7) and Toluene: Ethyl acetate: Formic acid (4:3:1).

Plates were inspected in day light, under UV lamp and sprayed with ceric acid, Vanillin-sulphuric acid and Para-Anisaldehyde

2.2.9.3 Sephadex column Chromatography for fraction No 18

The Fraction No 18 (2.3 gm) from column No (1) of the crude methanol extract was subjected to sub-column Chromatography (Sephadex LH-20) in glass column with length 50 cm and the dimater 3cm, a small piece of cotton was situated at the end of the column with a glass rod. Column filled with 50 g of Sephadex and eluted by mixture of Hexane: Chloroform 50:50 and left to flow down with continues addition of the mixture of mobile phase until the Sephadex was macerated. Air bubbles were replanted by knocking the outer surface of column with a rubber rod. Little cotton was placed on top of sephadex extract mixture pack to get neat column pack. The knob at the bottom was slowly opened to release the solvent. The elution was done using chloroform, ethyl acetate and methanol in different ratios like chloroform : ethyl acetate 90:10, 70:30, 50:50, 100% ethyl acetate; ethyl acetate:methanol:90:10, 50:50, 20:80, 100% methanol; A measured volume (400 ml) of each solvent combination was used in the elution process. The fractions were collected in aliquots of 10 ml in test tubes. Collections were allowed two days to concentrate at room temperature (37^{o}C). As soon as column chromatography was completed, the collected fractions 59 were collected in each vials , then developed on TLC plates using the solvent systems ethyl acetate: hexane (1:1, 2:8, 4:6), ethyl acetate : chloroform (4:6, 3:7),

methanol : chloroform (1:9, 2:8, 1:9: drop formic acid) From TLC results, the fractions were combined according to their separation profile to provide (35) fractions. Fractions from (23-33) contained same compounds was collected together (F) and also subjected to column chromatography to purify compounds.

2.2.9.4 Sub column for fraction F (23-33)

Glass column of 50 cm length and 2 cm internal diameter and 35 g of Sephadex powder was filled in to the column and mixture of Hexane: Chloroform 50:50 were used to macerate the sephadex. About 1.2 gm of the fraction extract was added to the top of the column. Column was then eluted with chloroform, ethyl acetate and methanol by increasing the polarity of the solvent as Chloroform: Ethyl acetate 50:50, 30:70, pure ethyl acetate; ethyl acetate: methanol 80:20, 60:40, 40:60, 20:80, 100 % methanol; ameasured volume was 200 ml for each ratio. Fractions of 10 ml were collected in separated vials and fractions were concentrated ; Collected factions were tested with thin layer chromatography and used the following solvent system: Ethyl acetate: hexane (1:1, 2:8, 4:6, and 0.5:9.5), ethyl acetate: chloroform (4:6, 3:7), methanol: chloroform (1:9, 2:8, and 1:9: drop formic acid) and spread the plates with vanillin sulphuric acid –ceric acid and anise aldehyde as indicators the number of the collected fractions of was found to be 10 fractions (A-J) fraction B was found to be pure compounds and identified by TLC and spectroscopy instruments but fraction D and H was purified using preparative thin layer chromatography using the following solvent system: Methanol: Chloroform (2:8) for C and G fraction.

2.2.9.5 Spectroscopic analysis of isolated compounds

The pure fractions were characterized by a variety of spectroscopic approaches including MS, IR and 2D NMR. The known compounds were

identified by comparison of their spectroscopic data with the values in the literature reported previously, Structures were elucidated by interpretation of their 1D NMR (such as 1H-NMR, 13C-NMR, DEPT-135), 2D NMR data (such as 1H-1H COSY, and HMBC) and MS for the determination of the exact mass and functional groups via fragmentation pathways.

1- Nuclear magnetic resonance spectroscopy (NMR)

The ^{1}H NMR and ^{13}C NMR data were recorded on Avance Bruker High performance Digital FT-NMR Spectrometer advance III 400MHz spectrometers using $CDCl_3$ as a solvent and tetramethylsilane (TMS) as an internal standard. Chemical shifts (δ) were expressed in ppm with reference to the TMS signals.

2- Mass spectrometry (MS)

The spectrometer was operated in either positive or negative ion mode. Mass spectra were acquired at collision energy of 20 eV using Helium as the collision gas at a pressure of 2.0 x 10-5 mbar. The spectra data were processed with Masslynx software version.

2.2.10 Extraction of steroids from chloroform extracts from tested plant

500 mg of dried were defatted and extracted with 100-150 ml petroleum ether (60-80 **°C** B.P.) for 2-4 hours in soxhlet apparatus. Petroleum ether extract was hydrolysed with 5% hydrochloric acid (v/v) in 70% ethanol (v/v) (5 ml HCl in 95 ml 70% ethanol) for four hours on water bath (70-80 **°C**). The hydrolysates (floated residual mass) were extracted with 50 ml ethyl acetate. This step was done for 2-3 times till the residual mass gave colourless solution. All ethyl acetate fractions were collected together and dried in vacuo. 2-4 ml Ethyl acetate or chloroform was added to the residue and used for further analysis of steroids. (Tomita *et al*., 1970)

Sample extract: Residue was redissolved in ethyl acetate or chloroform.

GC-MS condition for steroids analysis

Gas Chromatography Mass Spectrometer was performed using Shimadzu instrument (GC- MS- QP- 2010) fitted with electron impact (EI 1.70 eV) mode. The analytical column was RTX 5 (5% phenyl- 95% dimethyl polysiloxane with length of 30 meter x 0.25 µm). Helium gas was used as a carrier gas at a flow rate of 1ml/min. the temperature was programmed at 200˚C then increased to 270˚C with rate 10 ml/min, finally increased to 320˚C with 7 ml/min. The temperature of injector was 310˚C, with column flow1.5ml/min. Then a result was taken after comparison with NIST27 library for GC-MS.

2.2.11 Microbiological testes

Preparation of culture medium

1- Nutrient broth

This medium contained peptone, yeast extract and sodium chloride. It was prepared according to Barrow and Feltham (1993) by dissolving 13 grams of the medium in one liter of distilled water. The pH of the medium was adjusted to 7.4 and the medium was then distributed into screw capped bottles, 5 ml each and sterilized by autoclaving at 121°C for 15 minutes.

2- Nutrient agar

The medium contained lab- lemco powder (1.0 g), yeast extract (2.0 g), peptone (5.0 g) and agar No.3 (15.0 g). Twenty eight grams of dehydrated medium were dissolved in one liter of distilled water and the pH was adjusted to 7.4. The dissolved medium was sterilized by autoclaving at 121°C for 15 minutes.

3- Preparation of Mueller Hinton agar

Thirty eight grams of the powder of Mueller Hinton agar were weighed, disolved in 1 liter of distilled water and allowed to soak for 10 minutes. The medium was placed in water bath to dissolve, swirled to mix and sterilized by autoclaving for 15 minutes at 121°C, cooled to 47°C mixed well then poured into sterile Petri dishes.

4- Preparation of Sabouraud dextrose agar:

Sixty two grams of the powdered Sabouraud dextrose agar, was weighed, dispread in 1 liter water and allowed to soak for 10 minutes, swirled to mix then sterilized by autoclaving for 15 minutes at 121 °C, cooled to 47 °C, mixed well then poured in to sterile petri dishes.

5- Preparation of reference strains of bacteria

One ml aliquots of 24 h broth culture of tested organisms were aseptically added to nutrient agar slopes and incubated at 37°C for 24 h. The bacterial growth was harvested and washed off by addition of sterile normal saline . The harvested bacteria was suspended in a suitable volume of normal saline to produce a suspension containing about 10^8-10^9 colony forming units per ml (CFU\ml). The suspension was stored in the refrigerator at 4 °C till used. The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique (Miles and Misra, 1938).

2.2.11.1 Testing for antibacterial activity

Disc diffusion method:

The antimicrobial activity test was performed using the disc diffusion assay (Shankar *et al*., 2010). Sterile filter paper discs (Whatman No.1, 0.5 mm in diameter) were impregnated with one ml of each extract (20, 10, 5 mg/ ml) and left to dry so as to remove residual solvent, which might interference with the determination. A bout 0.1 ml of the standardized bacterial stock suspension 10^8 - 10^9 C.F.U/ml was prepared and added to the sterilized medium using sterile cotton swab. Sterilized filter paper discs (6 mm diameter) were soaked in the prepared extracts, and then were placed on surface of the test bacteria plates, each extract was tested in triplicate.The plates were incubated for 24 hours at room temperature and the diameters of the inhibition zones were measured. Reference drugs and 10% DMSO were used as the positive and negative controls, respectively. After incubation period, the diameters of the resultant growth inhibition zone were measured. Mean and standard error values were tabulated.

2.2.11.2 Testing for antifungal activity

The same method described for the bacteria will be adopted to test antifungal activity, *Sabouraud dextrose* agar was used. The inoculated medium will be incubated at 25 °C for two days for two days for the *Candida albicans* and three days for *Aspergillus niger*.

2.2.12 Determination of antioxidant activity

Colorimetric spectrophotometric assay

DPPH (2.2Di (4-tert-octylphenyl)-1-picryl-hydrazyl) radical scavening assay

The DPPH radical scavenging was determined according to the method of Shimada *et al* (1992).with some modification. In 96-wells plate, the test samples were allowed to react with 2.2Di (4-tert-octylphenyl)-1-picryl-hydrazyl stable free radical (DPPH) for half an hour at 37°C. The concentration of DPPH was kept as (300 µl). The test samples were dissolved in DMSO while DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at 517nm. Percentage radical scavenging activity by samples was determined in comparison with a DMSO treated control group. All tests and analysis were run in triplicate and compared with propyl gallate.

IC_{50} calculation:

The IC_{50} (the concentration of test material which possess 50% inhibition of free radicals) of all the extracts and their fractions was determined by monitoring the effect of different concentrations ranging from 500 - 62.25 µg/ml. the IC_{50} of the extracts and their fractions were calculated using EZ-FIT Enzyme kinetic program (Perrella scientific the U.S.A).

Equation

$$\% \text{ decoloration} = \left[\frac{\text{Av controls} - (\text{Av sample DPPH} - \text{Av samplemethanol})}{\text{Av controls}}\right] \times 100$$

Where:

Av controls = average absorbance of all DPPH control wells – average absorbance of all Methanol control wells.

Av sample DPPH = average absorbance of sample wells with DPPH.

Av sample methanol = average absorbance of sample wells with methanol.

2.2.13 Cytotoxicity of tested plants extracts

2.2.13.1 Brine Shrimp lethality test

Culture and harvesting of *A.salina*

A.salina cysts were stored at -20°C before use. *A.salina* cysts were incubated for hatching in a shallow rectangular dish (14 cm x 9 cm x 5cm) filled with 225 ml of a 3.3% solution of artificial sea water. A plastic divider with several 2 mm holes was clamped in the dish to make two unequal compartments. The cysts (1.11 grams) and yeast (0.0827 grams) were sprinkled into the larger compartment which was darkened. The smaller compartment was illuminated by a tungsten filament light and gently sparged with air. After 24 hours, hatched *A.salina* cysts were transferred to fresh artificial sea water and incubated for a further 24 hours under artificial light with air sparging. The phototropic nauplii were collected by pipette from the lighter side having been separated by the divider from the shells (Mclaughlin, 1998).

Preparation of test extract

The extract dissolved in dimethyl sulphoxide (DMSO). Test extracts at appropriate concentration (5 ml, 50 ml, and 500 ml for 10 ppm, 100 ppm and 1000 ppm, respectively) were transferred into vials (9 vials for each dose and 1 for control). Three replicates were prepared for each dose level. The following extracts were used for the bioassay: petroleum ether, chloroform, ethyl acetate and methanol of all extracts of the tested plants.

Bioassay:

Ten shrimps were transferred to each sample vial using disposable pipette, and artificial sea water was added to make 5 ml. Vials were left for 24 hours and numbers of survived larvae were counted. Data were analyzed by Finney Probit Analysis computer program to determine LD_{50} values with 95% confidence intervals.

2.2.13.2 Cytotoxicity of cell line

Microculture tetrazolium (MTT) assay was utilized to evaluate the cytotoxicity of the studied plants according to method described by Berridge *et al*. (2005).

Microculture tetrazoolium (MTT) assay

Preparation of plant solution:

About 5 mg of each extracts were weighed using a sensitive balance and put in Eppendrof tubes. 50µ of DMSO were added to the extract and the volume was completed to 1 ml with distilled water obtaining concentration of 5 mg/ml. the mixture was vortexes and stirred by magnetic stirrer to obtain a homogenous solution.

Cell counting

Cell counts were done using the improved Neubauer chamber. Slip and chamber were cleaned with detergent, rinsed thorough with distilled water and swapped with 70% ethanol and dried. An aliquot of cell suspension was mixed with equal volume of 0.4% in trypan a small blue tube. The chamber was charged with cell suspension. After cells had settled, the chamber was placed under light microscope. Using 40* objective, cells in the 4 large corner squares (each

containing 16 small squares) were counted. The following formula was used for calculating cells:

$$(\text{cells/ml}) = \frac{\text{Number of cells counted x Dilution factor x Volume } (10^4\text{ml})}{4}$$

Procedure:

The monolayer cell culture was mechanically detached, and then the cell count was adjusted to 1x 10^4 - 10^5 cell/ml using medium RPMI- 1640 containing 10% fetal bovine serum (FBS). To each well microtitre plate, 100 µl of diluted cell suspension were added. After 24 hours, when the monolayer formed the supernatant was flicked off and 100 µl of fresh complete medium were added to all wells after that serial dilutions of the crude extracts in growth medium were prepared to give three concentrations and incubated at 37°C in 5% CO_2 incubator for 72 hours and cells were periodically checked for granularity, shrinkage, swelling. The first 2 wells of row G were used for the negative control and the last 2 wells of row H were used for the positive control triton X. after 72 hours, 50 µl of MTT dye were added to each well which prepared with concentration 5mg/ml in PBS. The plates were gently shaken and wrapped in aluminum foil and incubated for 4 hours at 37°C in 5% CO_2 incubator. The supernatant was removed, 100 µl of DMSO were added, and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using ELISA reader at a wavelength of 570nm. The percentage growth inhibition was calculated using formula below:

$$\textbf{Cell inhibition\%} = \frac{\textbf{100- (Ac-At) x 100}}{\textbf{Ac}}$$

Where At: absorbance value of tested compound; **Ac**: absorbance value of control**.**

2.2.14 Antigiardial activity

G. Lamblia used in all experiments was taken from patient of Ibrahim Malik Hospital (Khartoum). All positive samples were examined by wet mount preparation. Then the positive sample was transported to the laboratory in nutrient broth medium containg 5% bovine serum at 37±1°C. the trophozoites were maintained for the assays and were employed in the log phase of growth.

Chemotherapeutic agents:

In all experiments metronidazole (flagyl®) powder manufacture in May, 2012. Expire in May, 2016. Company; Wujiang Li town pharmaceutical was used.

In vitro susceptibility assay:

In the susceptiblity assays used the sub- culture method of (Cedilla *et al*., 2002), which is being described as a highly stringent and sensitive method for assessing the anti- protozoal effects (gold standard) particularly in *Entamoeba histolytica***,** Gairidia intestinalis and Trichomonnnnas Vaginalis (Arguello *et al*., 2004). Five mg from each extract were dissolved in 50 µl of DMSO at Eppendrof tube containg 950 µl distill water in order to reach concentration of 5 mg/ml. the concentrates were stored at – 20°C for further analysis. Sterile 96- well microliter plate was used for different plant extracts positive control and negative control**.** Three out of 8 column of microtitre platte wells (8 columns x12 rows) were chosen for each extract, 40 µl (micro- liters) of an extract solution (5 mg/ml) were added to the first column wells C-1: On the other hand, 20 µl of complete RPMI medium were added to the other wells the second column and third column (C-2 and C-3). Serial dilutions of the extract were obtained by taking twenty µl of extract to the second column wells and taking 20 µl out of the complete solution in C-2 wells to C-3 a solutions in the successive columns.

Eighty µl of culture medium were complemented with parasite and dded to all wells. The final volume in the wells was 100 µl.

In each test metronidazole (atrichomonocide) pure compound [(1-(2-hydroxyethl)-2-methyl-5 nitroimidazole] was used as positive control in concentration 312.5 µl/ml, where as untreated cella were used as a negative controls (culture medium plus trophozoite). For counting, the samples were mixed with trypan blue in equal volume. The final number of parasites was determined with haemocytometer three times for counting after 24, 48 and 72 hours. The mortality % of parasite for each extracts activity was calculated according to the following formula:

$$\text{Mortality of parasite (\%)} = \frac{\text{(control negative – tested sample)}}{\text{Control negative}}$$

Only 100% inhibition of the parasite considered, when there was no motile parasite observed (plate).

Statistical analysis

All data were presented as means ± S.D. statistical analysis for all the assays results was done using Microsoft Excel program. This test was used to determine significant difference between control and plant extracts at level of $P < 0.05$.

Chapter three

3- RESULTS AND DISCUSSION

3.1 Quantities of extracts

3.1.1Quantity of extracts for *Senna Singueana*

Successive extraction of leaves of *Senna Singueana* gave the highest yield with methanol followed by ethyl acetate, petroleum ether and finally chloroform: 20.55; 15.67; 3.063 and 2.58 %, respectively. Regarded to seeds, the highest yield was observed with methanol followed by ethyl acetate, chloroform and finally petroleum ether 17.4; 13.74; 3.44; 1.74% respectively as presented in figure (3-1)

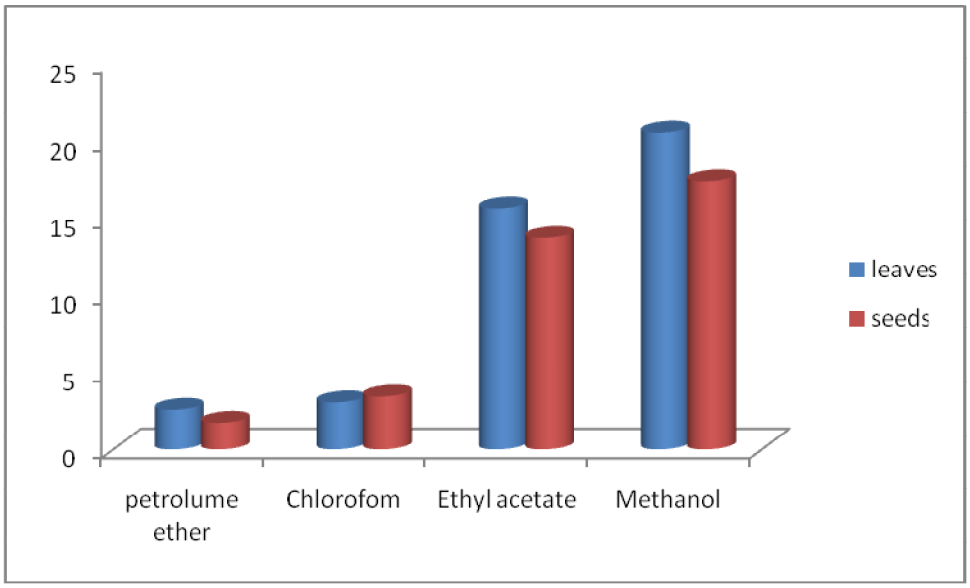


Fig (3.1): Yield (%) of different extracts from *S. Singueana* leaves and seeds

3.1.2 Quantity of extracts of *Chrozophora Plicata*

Successive extraction of leaves of *Chrozophora Plicata* gave the highest yield with methanol followed by Chloroform, petroleum ether and finally ethyl acetate:

16.52; 1.7; 1.4 and 0.89 % respectively. Regard to seeds, the highest yield was observed with methanol followed by ethyl acetate, chloroform and finally petroleum ether 4.42; 4.9; 5.2, 5.7% respectively as showed in figure (3.2).

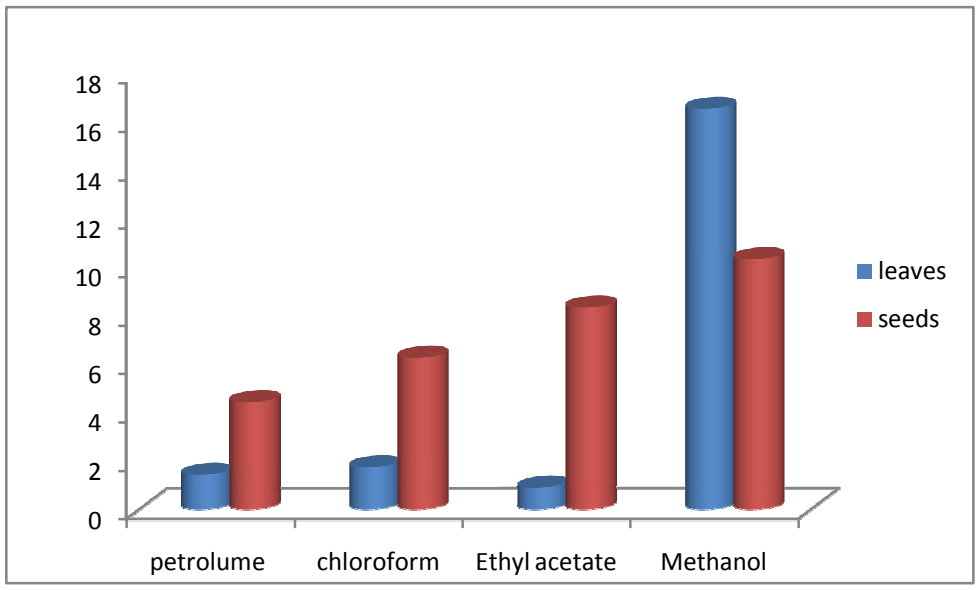


Fig (3.2): Yield (%) of different extracts from *C. Plicata* seeds and leaves

3.1.3 Quantity of extracts for *Stylochiton Borumensis*

Successive extraction of leaves of *Stylochiton Borumensis* was found to have maximum extractive yield gave with methanol followed by petroleum ether, ethyl acetate and finally Chloroform: 10.353; 1.485; 0.43 and 0.069 % respectively. As shown in figure (3-3).

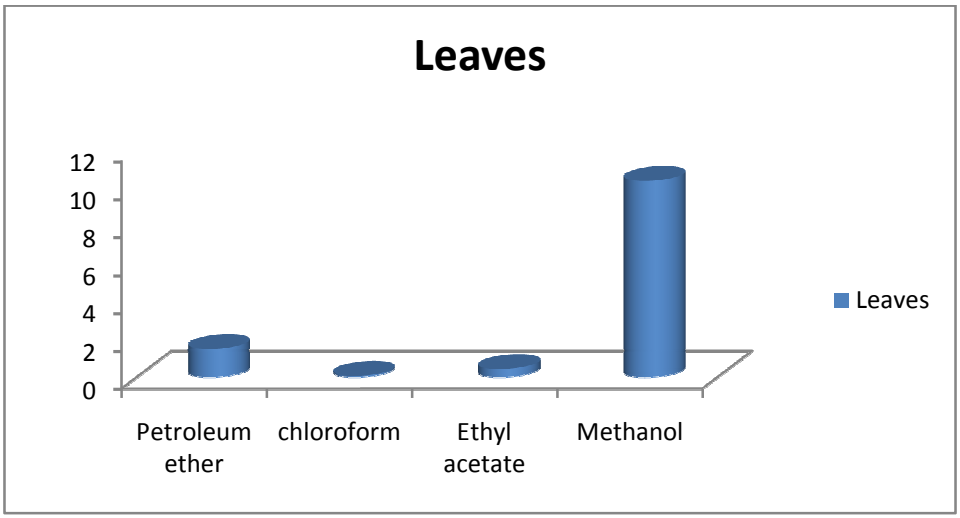


Fig (3.3): Yield (%) of different extracts from *Stylochiton Borumensis* leaves

3.2 Phytochemical analysis

3.2.1 Qualitative preliminary phytochemical analysis

Qualitative preliminary phytochemical analysis was performed initially with different chemical reagents to detect the nature of phytoconstituents and their presence in leaves and seeds extracts of *Senna Singueana*, *Chrozophora Plicata* and leaves extracts *Stylochiton Borumensis*. Results of the presence of secondary metabolites like sterols/triterpenoids, flavonoids, tannins, alkaloids, lignin, saponin and coumarin are presented in Table (3.1), (3.2), (3.3), (3.4) and (3.5) respectively.

Phytochemical screening of the *S.Singueana* leaves extracts indicated the presence of alkaloids and sterols. Glycosids and triterpences found in all leaves extracts expect chloroform extract as in table (3.1). Whereas analysis of seeds indicated the presence of sterols, flavonoids and tannins in all extracts except in the petroleum ether extract showed in Table (3.2). Phytochemical studies had been previously carried out on *Senna Singueana* only by Olusola *et al*. (2011) on the aqueous and ethanol leaves extracts, their results revealed the presence of

tannins, saponins, alkaloids, glycosides, flavonoids and terpenes. Luteolin isolated from methanol extract from leaves of the plant this reported by Ode and Asuzu. (2014).

Table (3.1): Preliminary phytochemical screening of leaves extracts of *S. Singueana*

Class of compound	Test Reagent	Extracts			
		PE	**$CHCl_3$**	**EtOAc**	**MeOH**
Alkaloids	Wagner	-	-ve	+ ++	++ +
	Mayer	+	++	+	+++
	Dragendrorff	-	+	+++	+ ++
Flavonoids	Lead acetate	-ve	-ve	+++	+++
	$FeCl_3$	-ve	-ve	+++	++
	KOH 1%	-ve	-ve	+++	+++
Sterols	Salkowski	+ +	+ +	+ + +	+ + +
	Liebermann	+	+ +	+ +	+ +
Triterpenes	Salkowski	+++	-ve	-ve	+++
	Liebermann	+++	-ve	+++	++
Tannins	$FeCl_3$	-ve	-ve	++	+++
	Lead acetate	-ve	-ve	++	+++
	Gelatin	-ve	+ + +	+ +	++
Glycosid	conc.H_2SO_4	+++	-ve	+++	+++
	Keller	+++	-ve	+++	+++
Lignin	Labat	-ve	+ +	-ve	++
Coumarin (powder of leaves)		+ +			
Saponin (powder of leaves)		+ +			

Table (3.2): Preliminary phytochemical screening of Seeds extracts of *S. Singueana*

Class of compound	Test Reagent	Extracts			
		PE	$CHCl_3$	EtOAc	MeOH
Alkaloids	Wagner	-ve	+++	+ +	-ve
	Mayer	-ve	+	+ +	-ve
	Dragendrorff	-ve	++	+ + +	-ve
Flavonoids	Lead acetate	-ve	++ +	+ ++	+++
	KOH 1%	-ve	++ +	+ ++	+++
	$FeCl_3$	-ve	+++	+++	+ +
Sterols	Salkowski	-ve	+ + +	+ + +	+ + +
	Liebermann	-ve	+ + +	+ + +	+ + +
Triterpenes	Salkowski	++	-ve	-ve	-ve
	Liebermann	++	-ve	+ + +	-ve
Tannins	$FeCl_3$	-ve	-ve	+++	+++
	Lead acetate	-ve	+++	++ +	+++
	Gelatin	-ve	++	+++	+++
Glycosids	conc. H_2SO_4	++	-ve	++	+++
	Keller	++	-ve	++	+++
Lignin	Labat	-	+ + +	+ +	+++
Saponin (powder of seeds)	++				
Coumarin (powder of seeds)	+ + +				

Note: "+" low, "++" average, "+++" high, "-" Not detected, P.E: petrolume ether

Table (3-3): Preliminary phytochemical screening of leaves extracts of *Chrozophora Plicata*

Class of compound	Test Reagent	Extracts			
		PE	**$CHCl_3$**	**EtOAc**	**MeOH**
Alkaloids	Wagner	-ve	+++	++	-ve
	Mayer	-ve	+++	++	+
	Dragendrorff	-ve	++	++	+
Flavonoids	Lead acetate	++ +	++ +	++ +	+++
	$FeCl_3$	++	++ +	+ ++	+++
	KOH 1%	+	+	+++	++ +
	$ALCl_3$	++	++	+++	++
Sterols	Salkowski	+ +	+ +	++	-ve
	Liebermann	+ ++	+ +	+ +	-ve
Triterpenes	Salkowski	-ve	-ve	-ve	-ve
	Liebermann	-ve	-ve	-ve	-ve
Glycosids	conc. H_2SO_4	+++	-ve	+++	+++
	keller	+++	-ve	+++	+++
Lignin	Labat	-ve	+ +	-ve	++
Saponin (powder of leaves)		+ +			
Coumarin (powder of leaves)		+ +			

Phytochemical screening of *Chrozophora Plicata* leaves extracts indicated the presence of flavonoids, alkaloids and tannins in methanol and ethyl acetate of leaves extracts in high concentration while the tannins and flavonoids were not found in petroleum ether and chloroform extracts as shown in Table (3.3). Whereas, analysis of seeds extracts of *C.Plicata* indicated the presence of

flavonoids in all seeds extracts while tannins and Triterpins were not found in petroleum ether and chloroform extracts also the sterols and alkaloids were not found in methanol and ethyl acetate extracts as shown in Table (3.4).

Table (3-4): Preliminary phytochemical screening of Seeds extracts of *Chrozophora Plicata*

Class of compound	Test Reagent	Extracts			
		P.E	$CHCl_3$	EtOAc	MeOH
Alkaloids	Wagner	++	+ +	-ve	-ve
	Mayer	-ve	+ +	+ +	-ve
	Dragendrorff	+++	+++	-ve	-ve
Flavonoids	Lead acetate	+++	+++	+++	-ve
	KOH 1%	++	++	+++	-ve
	$FeCl_3$	-ve	-ve	++	-ve
Sterols	Salkowski	++	+ +	-ve	-ve
	Lieberman	+++	+ + +	-ve	-ve
Triterpenes	Salkowski	-ve	-ve	-ve	+++
	Liebermann	-ve	-ve	+ + +	-ve
Tannins	$FeCl_3$	-ve	-ve	++	-ve
	Lead acetate	-ve	-ve	+++	+++
	Gelatin	-ve	Ve	+++	++
Glycosids	conc. H_2SO_4	+++	-ve	+++	+++
	keller	++	-ve	++	++
Lignin	Labat	-	+ + +	+ +	+ + +
Saponin (powder of seeds)	+				
Coumarin (powder of seeds)	++				

Note: "+" low, "++" average, "+++" high, "-" Not detected

From the preliminary previous studies, it was found that the leaves of *C. Plicata* contain triterpenoids and related compounds (sterols, alcohols and hydrocarbons),

phenolic compounds (flavonoids, lignans, coumarins, tannins, phenanthrenes, quinones, phenolic acids, etc.) only by (Kadiri and Avanapu, 2014).

Table (3.5): Preliminary phytochemical screening of leaves extracts of *Stylochiton Borumensis*

Class of compound	Test Reagent	Extracts			
		MeOH	**EtOAC**	**$CHCl_3$**	**P.E**
Alkaloids	Wagner	++	-ve	-ve	++
	Mayer	++	-ve	-ve	++
	Dragendrorff	++	-ve	-ve	++
Flavonoids	Lead acetate	++	++	++	++
	$FeCl_3$	+++	++	++	+++
	KOH 1%	+++	+++	++	+ +
	$ALCl_3$	+++	++	++	+
Sterol	Salkowski	+ +	+ +	+++	+
	Liebermann	+ ++	+ +	++ +	+
Tannins	$FeCl_3$	+++	++	++	++
	Lead acetate	++	+ +	++	+++
	Gelatin	+++	+++	++	+++
Triterpenes	Salkowski	-ve	-ve	-ve	-ve
	Liebermann	-ve	-ve	-ve	-ve
Glycosids	Conc. H_2SO_4	++	++	+++	+++
	Keller	++	++	+++	+++
Lignin	Labat	-ve	-ve	++	-ve
Saponin (powder of leaves)	-ve				
Coumarin (powder of leaves)	++				

Phytochemical screening of *Stylochiton Borumensis* leaves extracts indicated the presence of flavonoids, sterols, glycosids and tannins in methanol in all extracts in high concentration while Triterpenes was not found on all extracts. This result was presented in table (3.5).

3.2.2 Quantitative analysis for total phenols, flavonoids and tannins content

The total phenolic, flavonoid and tannin contents of leaves and seeds extracts of *Senna Singueana*, *Chrozophora Plicata* and leaves extracts for *Stylochiton Borumensis* were evaluated for total phenols, flavonoids and tannins content and results are presented in Tables (3.6), (3.7), (3.8) respectively. The total phenolic contents in the examined plant extracts using the Folin-Ciocalteu's reagent is expressed in terms of gallic acid equivalent. The values obtained for the concentration of total phenols are expressed as mg of GA/l of extract.

The concentration of flavonoids in various plant extracts was determined using spectrophotometric method with aluminum chloride. The content of flavonoids was expressed in terms of Querstin equivalent.

Tannin content of plants extracts was calculated as tannic acid equivalent.

3.2.2.1 Quantitative analysis for total phenols, flavonoids and tannins content in leaves -Seeds extracts of *Senna Singueana*

The total phenolic, flavonoid and tannin contents of leaves and seeds extracts of *S. Singueana* were evaluated and results are presented in table (3-6). The total phenolic contents in the examined leaves extracts ranged from 122.75 to 752.313 mg GA/l. The highest concentration of phenols was found in methanol and ethyl acetate extracts, Moreover the highest concentration of phenols in seeds part was found in methanol, ethyl acetate, chloroform and petroleum ether

extracts respectively. The total phenolic content in plant extracts of the species M. peregrinum depends on the type of extract, i.e. the polarity of solvent used in extraction. High solubility of phenols in polar solvents provides high concentration of these compounds in the extracts obtained using polar solvents for the extraction (Mohsen and Ammar, 2008; Zhou. 2004).

The concentration of flavonoids in plant extracts of *Senna* S*ingueana* ranged from 42.857 to 2361.857 mg/L. Ethyl acetate extract contained the highest flavonoid concentration whereas the lowest flavonoid concentration was found in petroleum ether extract. The concentration of flavonoids in plant extracts depends on the polarity of solvents used in the extract preparation (Min and Chun, 2005). The concentration of flavonoids in seeds part ranged from 275 to 1710.938 mg/l also the highest concentration of flavonoids was found in methanol, ethyl acetate, petroleum ether and chloroform extracts respectively.

Tannin content was calculated as tannic acid equivalent and values ranged between 126.21 mg/l to 1323.21 mg/l for leaves part. The highest tannin content (1323.2 mg/l) was observed in methanol extract followed by ethyl acetate (242.68 mg/l), chloroform (128.964 mg/l) and petroleum ether extracts (126.21 mg/l), respectively. On the seeds part the value ranged from 188.34 to 454.54 mg/l. The highest tannin content (453.54 mg/l) was observed in ethyl acetate followed by methanol (449.89 mg/l), chloroform (243.63 mg/l) and petroleum ether extracts (188.339 mg/l) respectively.

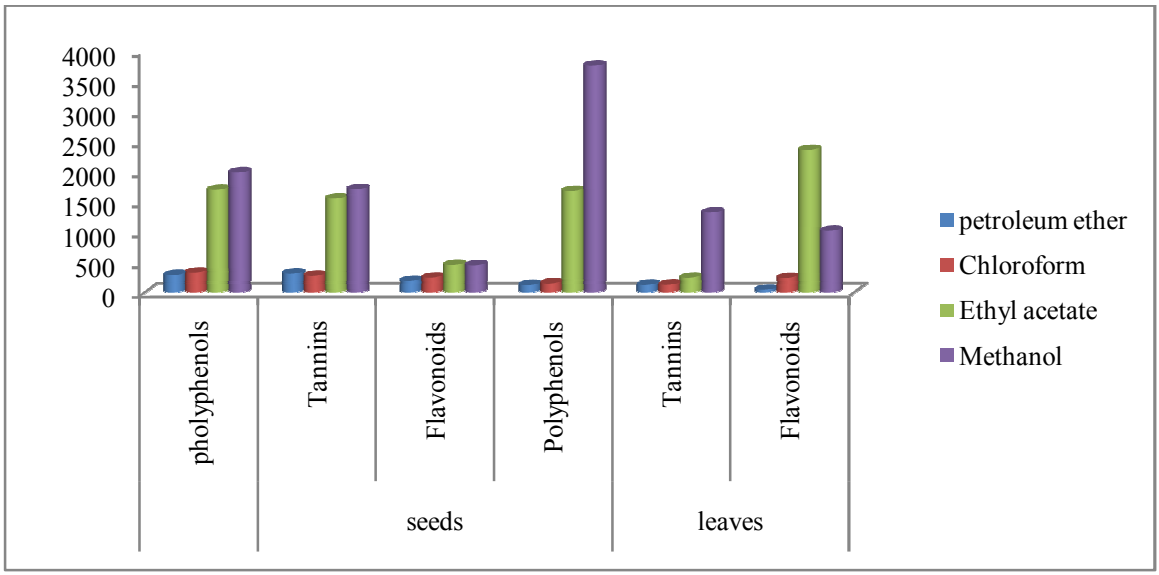


Fig (3.4): Total phenols, flavonoids and tannins contents in seeds and leaves extracts of *Senna Singueana*

Table (3.6): Concentration of total polyphenols, flavonoids and total tannins of the *Senna Singueana* leaves- seeds extracts

Phenolic	Part of plant	Petroleum ether Mean	Chloroform Mean	Ethyl acetate Mean	Methanol Mean
Total Polyphenol	Leaves	122.75	147.313	1683	3761
	Seeds	286.94	328.75	1700.1	1990.88
	P-value	0.170	0.224	0.498	0.132
Total Tannins	Leaves	126.21	128.96	242.6786	1323.214
	Seeds	309.88	275.25	1555.313	1710.94
	P-value	0.01	0.021	0.565	0.715
Total Flavonoids	Leaves	42.86	239.357	2361.86	1020.64
	Seeds	188.34	243.625	453.536	449.089
	P-value	0.533	0.369	0.802	0.126

Total polyphenol is expressed as mg Gallic acid/g of dry plant material. Total Flavonoids is expressed as mg quercetin/g of dry plant material. Total tannin is expressed as mg of tannic acid/g of dry plant material.* significantly different when compared the values of leaves and seeds at $P < 0.05$.

3.2.2.2 Quantitative analysis for total phenols, flavonoids and tannins content in leaves and seeds extract *Chrozophora Plicata*

The total phenolic, flavonoid and tannin contents of leaves and seeds extracts of *C. Plicata* were evaluated and results are presented in Table (3.7). The total phenolic contents in the examined plant extracts using the Folin-Ciocalteu's reagent is expressed in terms of gallic acid equivalent (the standard curve equation: y = 0.0008x + 0.0397, (R2 = 0.999). The values obtained for the concentration of total phenols are expressed as mg of GA/l of extract. The total phenolic contents in the examined leaves extracts ranged from 10.625 to 6107 mg GA/l. The highest concentration of phenols was measured in methanolic, ethyl acetate, chloroform and petroleum ether extracts of the leaves. The total phenolic content in the seeds was ranged from 21.625 to 2878.125 mg GA/l. The highest concentration of phenols was measured in methanolic, ethyl acetate, petroleum ether and chloroform extracts in the seeds part. The concentration of flavonoids in various plant extracts of the *Chrozophora Plicata* was determined using spectrophotometric method with aluminum chloride. The content of flavonoids was expressed in terms of Querestin equivalent. The standard curve equation that used in calculation was y = 0.0007x+ 0.0537. The concentration of flavonoids in plant extracts of *C. Plicata* leaves part ranged from 364 to 1697 mg/L. Ethyl acetate extract contains the highest flavonoid concentration whereas the lowest flavonoid concentration was measured in chloroform extract.The concentration of flavonoids in seeds part ranged from 25.28571 to 981.3571 mg/l also the highest concentration of flavonoids was found in ethyl acetate, methanol, chloroform and petroleum ether extracts respectively.

Tannin content values ranged between 64.57143 to 2533.213 mg/l for leaves part.

The highest tannin content (2533.213 mg/l) was observed in methanol followed by ethyl acetate (1826.665mg/l), petroleum ether extracts (137.23 mg/l) and chloroform (64.57143 mg/l) extracts respectively. On the seeds part the value ranged from 62.5714 to 28571 mg/l. The highest tannin content (28571 mg/l) was observed in ethyl acetate followed by methanol (1811.375 mg/l), chloroform (246.839mg/l) and petroleum ether extracts (62.5714 mg/l), respectively.

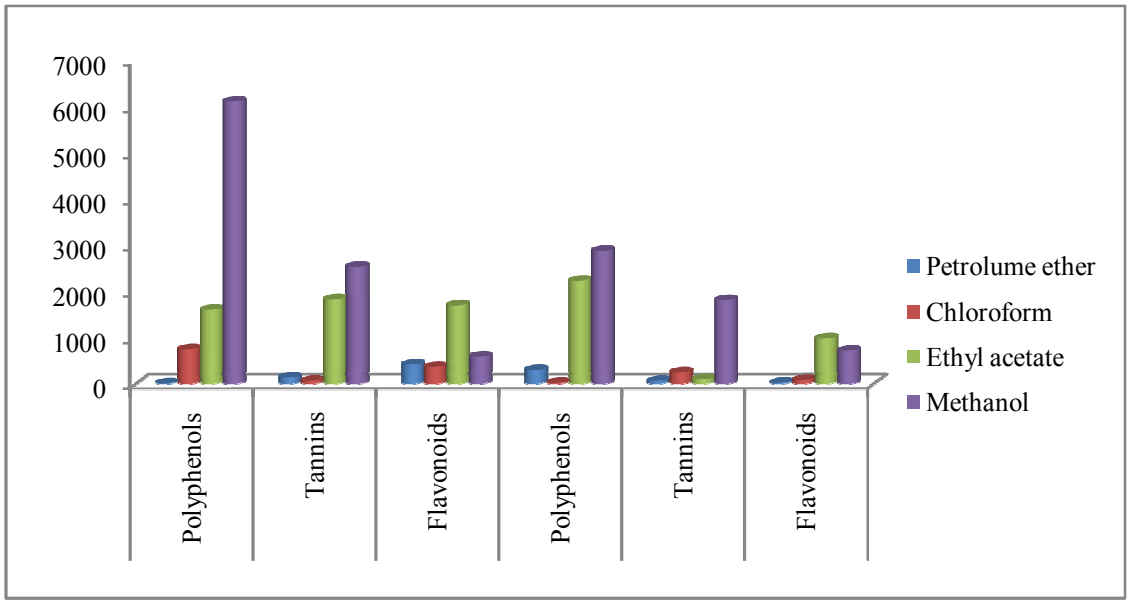


Fig (3.5): Total phenol, flavonoids and tannins contents in extracts of *Chrozophora Plicata*

Table (3.7): Concentration of total polyphenols, flavonoids and total tannins of *Chrozophora Plicata* leaves – Seeds extracts

Phenolic	Part of plant	Petroleum ether Mean	Chloroform Mean	Ethyl acetate Mean	Methanol Mean
Total Polyphenol	Leaves	10.625	745.937	1601.25	6107
	Seeds	296.4313	21.625	2233.25	2878.125
	P-value	0.112	0.001	0.091	0.012
Total Tannins	Leaves	137.23	64.57143	1826.665	2533.213
	Seeds	62.5714	246.839	99.28571	1811.375
	P-value	0.042	0.000	0.009	0.009
Total Flavonoids	Leaves	416.3571	364	1697	588.6429
	Seeds	25.28571	85.07143	981.3571	717.4286

Total polyphenol is expressed as mg Gallic acid/g of dry plant extract. Total Flavonoids is expressed as mg quercqaetin/g of dry plant extract. Total tannin is expressed as mg of tannic acid/g of plant extract.* significantly different from the other at $P< 0.05$.

3.2.2.3 Quantitative analysis for total phenols, flavonoids and tannins content in leaves extracts of *Stylochiton Borumensis*

The total phenolic, flavonoid and tannin contents of leaves extracts of *S. Borumensis* were evaluated and results are presented in table (3.8). The total phenolic content ranged from 752.8 to 864.5 mg/l gallic acid equivalent. The highest content was found in the ethyl acetate extract (864.5 mg/l) followed by the methanol (803.5 mg/l), chloroform (784.06 mg/l) and petroleum ether extracts (752.81 mg/l) respectively. Flavonoids content was calculated as quercetin equivalent and leaves extracts contained flavonoids in the range of 118.57 to 437.85 mg/l quercetin equivalent. The highest flavonoid content was ethyl acetate (437.85 mg/l), methanol (353.85 mg/l) chloroform (226.95mg/l) and

Petroleum ether (118.57 mg/l) extracts respectively. Tannin content was calculated as tannic acid equivalent and values ranged between 386 mg/l and 1083 mg/l. The highest tannin content (1083mg/l) was observed in chloroform followed by ethyl acetate (1019.286 mg/l), methanol (656.57 mg/l) and petroleum ether extract (386 mg/l) extracts respectively.

Table (3.8): Concentration of total polyphenols, flavonoids and total tannins of *Stylochiton Borumensis* Leaves extracts:

Phenolic	Part of plant	Petroleum ether Mean	$CHCl_3$ Mean	Ethyl acetate Mean	Methanol Mean
Total Polyphenol	Leaves	752.8125	784.062	864.5	803.5
Total Tannins	Leaves	386	1083	1019.286	656.57
Total Flavonoids	Leaves	118.575	226.95	437.85	353.85

Statistical analysis: Data are presented as the mean. Data were analyzed by SPSS statistical software (version 16). Values were considered significantly different when compared values of leaves and seeds of same plant at $p < 0.05$.

3.3 Proximate composition and nutritional value

3.3.1 Proximate composition and nutritional value of *Senna Singueana*

The proximate analysis of *S. Singueana* leaves and seeds are represented in Table (3.9).

Nutritional value of *Senna Singueana*

Seed of *legumes* family are generally considered to be rich in protein, energy, vitamin and minerals. In the present study, crude protein of *Senna Singueana*

seeds (14.88%) is slightly higher than the value (11.38%) of *Senna Singueana* leaves so both seeds- leaves can contribute to the formation of hormones which controls a variety of body functions, Mau *et al.* (1999). The values of crude protein of *S. Singueana* was higher than values (2.3%) of *S. Occidentalis* but low when compared with *S. Obtusifolia* (18.46%) reported by Ismaila *et al*. (2011).

The crude fiber value (15.85) of *S. Singueana* seeds was higher than *S. Singueana* leaves (1.36%).The comparison the crude fiber of this seeds was slightly higher than *S.Obtusifolia* (2.45%) and lower than *S. Alata* (25%), *S. Hirsute* (40 %) reported by Essiett *et al*. (2013).

The moisture content of *S.Singueana* seeds (4.07%) is high than then leaves (2.92%) the result of moisture content in two part of this plants is not high that was not high indicates less chances of microbial degradation of the drug during storage because excess moisture can result in the breakdown of important constituents by enzymatic activity and as a result may encourage the growth of yeast and fungi during storage African Pharmacopoeia.(1986), such as the moisture content of 12.5, 13.5, 13 % in *S. alata, S. hirsuta* and *S. obtusifolia* respectively. In general requirement for moisture content in crude drug was that, it should not be more than 14% since it was normal, and implies that the plants can be stored for longer period with lower change of microbial attack.

The total ash value of *S. Singueana* seed was (7.67%) and leaves was found to be (6.93%) this implies that plants have normal complexes of inorganic and organic compound (British Pharmacopoeia, 1980) as such as ash content of The total ash value was 6, 11, and 9 % in *S. alata*, *S. hirsuta* and *S. obtusifolia* respectively reported by Essiett *et al*. (2013).

Total carbohydrate of *S. Singueana* leaves (74.09 %) is higher than that found in seeds (55.68%) the two part of the plant have high carbohydrate when compared them with *S. alata* (53.7%), *S. Hirsuta* (42 %) and *S. Obtusifolia* 40

%). The relatively high carbohydrate content can be used as energy sources and also it is necessary in the digestion and assimilation of other food; however *S. Singueana* contains essential nutrients for good human and animal health saied by Essiett *et al*. (2013).

Table (3.9): Proximate composition and nutritional values of *S. Singueana*

Parameter	***Senna Singueana* (Leaves)**	***Senna Singueana* (seeds)**
Crude protein	11.38%	14.88%
Crude fiber	1.36%	15.85%
Ash	6.93%	4.07%
Ether extract	3.32%	1.85%
Moisture	2.92%	4.07%
Carbohydrate	74.09%	55.68%
Total Sugar	0.0800%	0.070%

3.3.2 Proximate composition and nutritional value of *Chrozophora Plicata*

The proximate analysis of *Chrozophora Plicata* leaves and seeds are represented in Table (3.10).

Table (3.10): Proximate composition and nutritional value of *Chrozophora Plicata* seeds and leaves

Parameter	*Chrozophora Plicata*	
	Leaves	**Seeds**
Crude protein	10.50 %	7.8 %
Crude fiber	0.61 %	31.01 %
Ash	12.62 %	6.99 %
Ether extract	2.88 %	5.12 %
Moisture	3.63 %	4.40 %
Carbohydrate	69.76 %	44.60 %
Total Sugar	0.11 %	0.17 %

The proximate analysis of *Chrozophora Plicata* as shown in table (3-10). It was observed that the moisture content of *Chrozophora Plicata* seeds (4.40 %) is high than the leaves (3.63%) the result of moisture content in two part of this plants was not high indicated less chances of microbial degradation of the drug during storage because excess moisture can result in the breakdown of important constituents by enzymatic activity. The results agree with Musharaf *et al.*(2014) who observed similar moisture contents in *Chrozophora Oblique* (3.16%). but Muibat *et al* .(2014) observed that the *Chrozophora Tinctoria* leaves had high moisture content (8.9%). The total ash content was a measure of the presence of inorganic compounds in drug, Mammen *et al.* (2010). Ash was highest (12.62%) in leaves and lowest (8.99%) in seeds of *Chrozophora Plicata* as preented in table (3-10). The results agree with Ghulam *et al.*(2014) who also reported high ash in the leaves of *Chrozophora Tinctoria* (13.5%), also the results disagree with Musharaf *et al.* (2014) who reported lowest ash (6.44%) observed in *Chrozophora Oblique.*

The protein content was highest (10.50 %) in leaves of *Chrozophora Plicata* and lowest in seeds (7.88%). Ghulam *et al.* (2014) who also reported that *Chrozophora tinctoria* had highest protein content in leaves (20%) but lowest in the roots (5.2%) also the result was gree with Muibat *et al*. (2014) who reported that (13.13%) protein content founded in leaves of Muibat *et al.* (2014) in *Chrozophora Zambesicu*, The importance of fiber in diet cannot be neglected as it decreases serum cholesterol levels, risk of coronary heart diseases, hypertension, diabetes and breast cancer. Ishida *et al*. (2000). The fiber was highest (31.01%) in seeds and lowest (0.62%) in leaves of *Chrozophora Plicata* in the present study. Ishida *et al*. (2000) also reported maximum fiber content (56.3%) in the leaves and minimum (10.3%) in roots of *Chrozophora Tinctoria*., when compared fiber content with *Chrozophora Oblique* leaves (2.78%) this was reported by Musharaf *et al.* (2014). The fat content was highest (13%) in leaves and lowest (8.5%) in stem and roots of *Chrozophora Tinictoria* this reported by Ghulam *et al.* (2014). Carbohydrates play several vital roles in living organisms They can be oxidized to yield energy, their polymers act as energy molecules and their derivatives are found in a number of biological molecules including coenzymes and the nucleic acids this reported by Hasan *et al*. (2011). Carbohydrates were highest (69.76%) in the leaves and lowest (44.6%) in the seeds of *Chrozophora Plicata* as shown in table (3.10), Musharaf *et al.* (2014) observed higher carbohydrate contents (76%) in *Chrozophora Oblique* leaves.

3.3.3 Proximate composition and nutritional value of *Stylochiton Borumensis*

The proximate analysis of *Stylochiton Borumensis* leaves as represented in Table (3.11). The Crude protein of *Stylochiton Borumensis* leaves (28.88%) is slightly higher; also the leaves showed high content of carbohydrate (29.21%).

Table (3.11): Proximate composition and nutritional value of *Stylochiton Borumensis*

Parameter	Percentage (Leaves)
Crude protein	28.88
Crude fiber	12.53
Ash	23.58
Ether extract	2.18
Moisture	3.62
Carbohydrate	29.21
Total Sugar	0.08

3.4 Determination of fatty acids by GC-MS

Fatty acid is a carboxylic acid with long aliphatic chain. Fatty acid is divided into saturated and unsaturated acid, depending on the presence of unsaturated double bond in the fatty acid chain. Essential fatty acids are polyunsaturated fatty acid. Linoleic acid (C18:2) and α-linolenic acid (C18:3) are the parent compounds of the omega-6(ω-6) and omega-3(ω-3) fatty acid series, respectively. They are essential in the human diet since they cannot be synthesized by the body. The essential fatty acids are very important to human immune system, to help regulate blood pressure. The ω-3 and ω-6 fatty acid are found in some food; fish, shellfish flaxseed, soya oil, canola (rape seed) oil, hemp oil, chia seed, pumkin Seed, sunflower seed, cotton seed oil, leafy vegetables and walnut. Wanna (2007).

3.4.1 Fatty acids Composition of *Senna Singueana* Seeds

Fatty acids were identified as their methyl esters by searching potential

structures Fatty acids from NIST MS Search 2.0 database.

Table (3.12): Fatty acids Composition of *Senna Singueana* seeds

Fatty acid	Area %	R.time	Simlarty %
Palmatic acid, methyl ester	45.13	24.27	99
Hexadecenoic acid, methyl ester	1.56	26.37	97
16-Octadecadienoic acid, methyl ester	26.01	28.61	98
9,11- Octadecadienoic acid, M.E	12.42	28.99	96
Behenic acid, M.E	3.06	36.66	98
Tetracosanoic acid, M.E	3.64	40.27	95
Pentacosanoic acid, M.E	0.97	41.98	99
Arachidnoic acid M.E	4.17	32.79	97

M.E: **Methyl Ester**

The seeds of *Senna Singueana* was found to contain Palmatic acid, methyl ester (45.13%), and 9,11- Octadecadienoic acid, M.E (12.4), 16-Octadecenoic acid, methyl ester (26.01) % the result represented in table (3-12) and when compared the result with other same plant species the results showed that The extracted seed oil of *Senna* species contained significant amount of linoleic acid (45.96% to 60.25%), which is the one of the most important unsaturated fatty acid. Oleic acid was second major unsaturated fatty acid (34.91% in *S. laevigata*, 34.80% in *Senna javanica* and 30.11% in *Senna alata* and 26.29 % in *Senna absus*) except in *Senna roxburghii* where the percentage of oleic acid and linoleic acid were 46.08% and 45.96% respectively. Palmitic acid and stearic acid exhibited the third and fourth highest fatty acids content and ranged between 16.41% to 2.61% and 1.62% to 8.10% respectively. Minor percentage of linolenic acid was observed in *Senna absus* (1.96%) and in *Senna roxburghii* (1.28%) this reported by Lalita and Shelley. (2010).

3.4.2 Fatty acids methyl esters of *C. Plicata* seeds by GC-MS

As in table (3.13) the seeds of *Chrozophora Plicata* contained high concentration of Stearic acids, methyl ester (63.13%), palmatic acid, M.E (16.73%) and Linoleic acid methyl ester (6.73%).

Table (3.13): fatty acids composition of *Chrozophora Plicata* seeds

Fatty acid	Area %	R.time	Simlarty %
10- Undecenoic acid, methyl ester	0.45	13.7	97
Nonanoic acid, methyl ester	4.82	14.92	99
Stearic acid M.E	63.13	28.6	99
Linoleic acid, M.E	6.73	29.00	98
Arachidnoic acid M.E	1.32	32.79	96
palmatic acid M.E	16.73	24.06	98
Heptadecanoic acid, methyl ester	0.55	26.36	96

3.5 Determination of amino acids concentration in tested plants raw materials

Amino acids are the basic constituents of proteins also are intermediates in metabolic pathways. Qualitative and quantitative analysis of the amino acid composition of hydrolyzed samples of pure proteins or peptides was used to identify and measured the concentration of amino acids.

Quantification of total amino acids through amino acid analyzer

The results of quantification of total amino acids were given in table (3.14) and (3.15). All considered tested plants material were screened for amino acids employing ninhydrin. They proved the presence of amino acids in *Chrozophora Plicata*, *Senna Singueana* and *Stylochiton Borumensis* leaves and seeds.

Table (3.14): determination of essential amino acids composition of tested plants

Amino acid	Part	S. *Singueana*	*C. Plicata*	*S. Stylichion*	P-value
		Mean±SE	Mean±SE	Mean±SE	
Aspartic	Leaves	653.3±1.4	230±1.4	2086.7±1.4	0.0001
	Seeds	974.02±23.2	576.21±11.0	-	0.028
Serine	Leaves	233±4.4	209.7±0.8	248.7±33.9	0.427
	Seeds	271.1±4.9	174.4±28	-	0.030
Glutamic	Leaves	835.9±56.4	922.4±19.5	1671.0 ±18.7	0.004
	Seeds	727.12±17.16	940.4±17.5	-	0.293
Glycine	Leaves	210.4±23.4	205.7±23.4	240.5 ±23.4	0.340
	Seeds	355..17±10.3	143.35±32.0	-	0.003
Alanine	Leaves	754.7±11.9	633.7±11.9	1350.0±11.9	0.002
	Seeds	583.01±11.2	434.9±80.1	-	0.141
Arginine	Leaves	588.7±41.6	468.2±41.6	740.5±41.6	0.002
	Seeds	595.0±10.3	279.4±57.4	-	0.006

A general pattern was found among the samples regarding the composition of essential amino acids, aspartic acid, glutamic acid, alanine, and arginine and non essential amino acids leucine, iso leucine and isoalanine were all presented in high concentrations in C. *Plicata* leaves.

Table (3.15): Determination of non essential amino acids concentration of tested plants

Amino acids	parts	*S. Singueana*	*C. Plicata*	*S. Stylichion*	P-value
		Mean±SE	Mean±SE	Mean±SE	
Leucine	Leaves	731.6±1.4	728.6±1.4	863.8±1.4	0.065
	Seeds	742.7±16.4	541.4±10.7	-	0.139
Isoluecine	Leaves	416.5±25.5	389.6±25.5	509.4±25.5	0.008
	Seeds	444.6±11.6	328.4±68.5	-	0.170
Tyrosine	Leaves	192.1±19.87	201.64±19.87	310.7±19.87	0.002
	Seeds	165.1±3.3	97.38±22.1	-	0.039
Phenyl alanine	Leaves	478.8±53.1	439.6±53.1	727.6 ±53.1	0.003
	Seeds	197.8±32.5	179.3±51.0	-	0.574
Histidine	Leaves	185.6±153.8	151.53±153.8	187.9±153.8	0.098
	Seeds	179.8±7.8	64.3±13.1	-	0.002
Methionine	Leaves	49.25±8.05	59.72±8.05	67.5±8.05	0.155
	Seeds	39.5±1.3	29.6±7.3	-	0.257
Threonine	Leaves	304.04±27.3	289.2±27.3	336.07±27.3	0.291
	Seeds	333.2±9.7	211.2±34	-	0.028

Out of fourteen amino acids investigated in leaves and seeds respectively of *S.Singueana* the highest essential amino acids observed on leaves part were aspartic (1078 - 974.02 ppm); glutamic (835.9 - 727.12 ppm) and alanine (587.7 - 583.01 ppm) while the highest concentration of non essential amino acids were found to be luecine (731.6 – 742.7 ppm); phenylalanine (478.8 – 197.8 ppm); Arginine (588.7 – 595 ppm); isoluecine (416.59 – 444.6 ppm), methionine found to be in low concentration in two part (49.25 – 39.5 ppm).

Amino acids contents of *Senna* species were previously reported by many researchers Gaikwad *et al*. (2010); Mbailao *et al*. (2005).

The highest concentration of essential amino acids of *C. Plicata* leaves and seeds respectively were observed by Glutamic (922.4 – 940.4 ppm); Alanine (633.7 – 434.9 ppm) and arginine (468.2 – 279.4 ppm), also contain high concentration of non essential amino acids like leucine (728.6 – 541.4 ppm); Isoluecine (389.6 – 328.4 ppm) and phenyl alanine which founds in leave part more than the seed part (439.6 – 179.3 ppm).

S. Borumensis leaves contain high concentration of aspartic 2086.7, Alanine 1350 ppm and arginine740.5 ppm, also contained high concentrateion of non essential amino acids like leucine 863.8, phenyl alanine 727 and isoluecine 509.4 ppm. When compeared the concentration of amino acids in tested plants, the highest concentration of essential amino acids in leaves part observed by *S. Borumensis* which contain the higest concentration of aspartic 2086.7 ppm, and glutamic acid 1671.0 ppm while the concentration of glutamic in *S.Singueana*, *C. Plicata* contain 835.9, 922.4 ppm respectively. The highest concentration of non essential amino acids observed by leucine at *S. Borumensis* leaves731.6 ppm.

Statistical analysis: Data are presented as the mean. Data were analyzed by SPSS statistical software (version 16). Values were considered significantly different when compared values of leaves and seeds of same plant at $p < 0.05$.

3.6 Determination of trace and heavy metal by ICP-ES

3.6.1 Determination of heavy metals level of tested plants material by ICP ES

Several metals including Pb, Cr, Ni, Cu, Zn, etc were analysed in *C. Plicata, S. Singueana and S. Borumensis* leaves and seeds by ICP-ES spectrometer. These

interactions would include antagonism and synergism. Absorption of heavy metal in medicinal plant is governed by soil characteristics such as pH, salinity, conductivity and organic matter content.

Table (3.16): Heavy metals concentration (mg kg-1) of tested raw plants material

Plants	Parts	metals concentration (ppm)									
		Ag	As	Cd	Co	Cr	Cu	Fe	Mn	Pb	Zn
C. plicata	Leaves	3.28	<0.028	0.002	0.045	5.4	1.09	81.4	11.04	0.19	0.64
	Seeds	0.67	<0.028	0.005	0.036	15.5	1.37	76.9	9.733	0.169	1.39
S. Singueana	Leaves	0.25	<0.028	0.053	0.701	264	36.0	1082	168.2	0.464	1.18
	Seeds	2.07	<0.028	0.027	0.351	13.1	17.5	588.6	81.4	0.276	1.44
S .Borumensis	Leaves	6.72	<0.028	0.004	0.046	3.46	0.65	65.12	4.630	0.141	1.10

Before using a plant for medicinal purpose one should know the level of heavy metals in that particular plant, because if the level of a particular heavy metal is exceeding its normal permissible limit, it may result in serious harms to the human health as reported by Tahir *et al*. (2014). Even though WHO has formulated guidelines for quality assurance and control of herbal medicine. Lead well known for its adverse effects on many parts of the body. Progressive exposure to lead results in a decrease in the performance of the nervous system and affects renal clearance Salawu (2009). Inorganic lead is carcinogenic and cause miscarriages in pregnant women. The results obtained in this study of leaves and seeds of *C. Plicata*, *S. Singueana* and leaves of *Stylochiton Borumensis* showed that all of them consist lead in a concentration range of 0.1493-0.4648 ppm which are within the permissible limits as laid down by guidelines for quality standardized herbal formulations (WHO Guidelines, 2004). Heavy metals that are essential for the health of the body include Cu, Ni, Zn and Fe and are required in negligible quantities for the proper functioning of

enzymes, hemoglobin formation and vitamin synthesis in men Sivapermal *et al*. (2007).

In *Chrozophora Plicata*, the iron content was the highest (81and 76 mg/kg plant) for leaves and seeds respectively. whereas, the concentrations of Cu, Zn, Ag, Cr and Mn were remarkably lower between (0.649 and 15.45) ppm while the quantity of cobalt was very low between (0.0455-0.0368) ppm and the concentration of As was remarkable low <0.028 ppm as shown in table (3.16).

The leaves material of *C. Plicata* was reported to have high concentration of different metals like Ca, Cu, Fe, Zn and Co) and non essential heavy metal (Cd, Pb and AS) Tahir *et al*. (2014).

For *Senna Singueana* leaves and seeds, the iron content was the highest (1082 and 588.6 mg/kg plant) for leaves and seeds respectively. Concentrations of Cd, Zn, Ag and Co were remarkably lower between (2.027 and 0.0275) ppm. Quantity of Mn and Cu were ranged between (168.2 - 81.39) and (36.64 – 17.48) ppm. The concentration of As was remarkable low <0.028 ppm for leaves and seeds of the plant as shown in table (3.16). Similar element contents of *Senna* species were previously reported Kistamma *et al*. (2014).

Stylochiton Borumensis leaves showed the iron content of (65.12 mg/kg plant). The Cd, Zn, Ag, Cu, Mn and Co were remarkably low between (0.004 and 6.729) ppm also the concentration of As was remarkable low <0.028 ppm as shown in table (3.16).

3.6.2 Determination of trace metals level of tested plants material by ICP ES

Several metals including Ca, K, Mg, P, Na, etc were analysed in *C. Plicata, S. Singueana and S. Borumensis* leaves and seeds by ICP-ES spectrometer. These interactions would include antagonism and synergism. Absorption of heavy metal

in medicinal plant is governed by soil characteristics such as pH, salinity, conductivity and organic matter content.

Table (3.17): Trace metals concentration (mg kg-1) of tested raw plants material

Plants	Parts	metals concentration (ppm)								
		AL	Ba	Ca	K	Li	Mg	Na	P	Si
Chrozophora plicata	Leaves	26.3	2.02	915.8	212	<0.042	94.5	15.96	54.46	8.51
	Seeds	2.97	0.167	327.6	195.4	<0.042	69.5	18.76	83.89	7.30
Senna Singueana	Leaves	4.71	1.123	295.1	527	<0.042	132.	5.679	101.7	11.5
	Seeds	2.96	0.229	116.4	861.1	<0.042	108	5.924	157.8	7.47
Stylochiton Borumensis	Leaves	21.2	1.248	539.2	2133	<0.042	133	22.84	181.3	18.1

In analyzed medicinal plants the Calcium contents (915.8 – 327.6) ppm for leaves and seeds respectively the Concentrations of essential metals Na, Ca, Mg, K, Li, Ba and Si ranged from (94.5, 54.46 and 15.96 ppm), concentration of Li is less than 0.0424 ppm.

The highest concentration of element on leaves and seeds part of *C. Plicata* was noticed by calcium 915.8, 327.6 ppm, while the concentration of potassium ranged from 212.2 to 195.8 ppm for leaves and seeds respectively. The concentration of trace element of *S. Singueana* leaves and seeds showed that the concentration of calcium was in the way (295.1 -116.4 ppm), the content of potassium was ranged from 527 -861.1 ppm for leaves and seeds respectively, moreover the content of Magnesium ranged from 132.3 to 108 ppm for leaves and seeds as shown in table (3.17).

The content of trace elements in *Stylochiton Borumensis* leaves was potassium 2133 ppm, calcium 539.2 ppm, magnesium 133.2 ppm and phosphorus 181.3 ppm as shown in table (3.17).

Table (3.18): Certified reference material concentrations (mg Kg-1)

Metals	Certified value (CRM1)	Certified value (CMR2)
Cu	120 ± 0.4	2.5 ± 0.03
Zn	260 ± 0.5	13.1 ± 0.5
Ni	1.2 ± 0.01	0.10 ± 0.00
Pb	73 ± 0.5	0.11 ± 0.01
As	1.6 ± 0.5	0.05 ± 0.00

All results above indicating that the tested plants materials are within the range heavy metals when compared with certified reference material this represented in table (3.18) Dzomba *et al*. (2012).

3.7 Column chromatography

Column chromatographic technique

Column chromatography is an isolation technique in which the phytoconstituents are being eluted by adsorption. The principle involved in this separation of constituents is adsorption at the interface between solid and liquid. The component must have various degree of affinity towards adsorbent and also reversible interaction to achieve successful separation. The bands formed during the elution process were localized in both visible and UV light. The fractions or sub fractions received were analyzed by PC or TLC and separately investigated.

Column Chromatography fractionation

In an attempt to simplify the isolation of compounds, methanol crude extract which showed broader spectrum of activity according to the bioautography results, was subjected to separation by normal phase silica gel column chromatography. In order to select the best mobile phase for eluting the methanol extract, 5 ml of 10 mg\ml solution was spotted on TLC and ran with combinations of solvents. In this way the solvent system that exhibited the most favorable separation of compounds was chosen.

Justification of choosen methanol leaves extract of *Chrozophora Plicata* for column chromatography.

1- From TLC it contained more compounds

2- It had high yield.

3- The extract has high antimicrobial activity, high antioxidant activity and showed antigarider activity.

4- The extract showed high phenolic compounds content**.**

Table (3.19): Preparative thin layer chromatographic studies of fractions of *Chrozophora Plicata* methanolic leaves extract

Fraction	Solvent system	Spray reagent	Color of spot	Rf values
20-38 (F1)	Ethyl acetate: hexane (1:1)	Vanillin	Purple Green	0.531 0.31
187-210 (F4)	Chloroform:hexane:Methanol (7: 2: 1)	Ceric acid	Brown Yellow	0.24 0.6
211-219 (F5)	Hexane :ethyl acetate (4:6)	Vanillin	Brown Yellow	0.46 0.76
304-315 (F8)	Hexane: Ethyl acetate (3:7)	Anise aldehyde	Orange Yellow	0.2 0.33
323- 329 (F10)	Hexane: ethyl acetate (3:7)	Vanillin	Yellow	0.23

Table (3.20): Preparative thin layer chromatography for sub-sub-fraction (18)

Fraction	Solvent system	Spray reagent	No of spot	Rf values
21-26 (F4)	Methanol:Chloroform (2:8)	Anise aldehyde	Yellow Pink	0.23 0.88
30 (F8)	Methanol:Chloroform (2:8)	Anise aldehyde	Pink	0.78

3.8 Identification of steroids in tested plants by GC-MS

The chloroform extracts of leaves and seeds contained steroids in different concentration, *S. Singueana* leaves contain Gamma sitosterol and lanosterol 3-with area percentage (16.2 %) and (22.1%) while seeds extract contain Stigmast-7-en-3-ol, (3.beta., 5., alpha (54.4 %) and Campesterol (14.4%). the highest steroid concentration foun on *C. plicata* chloroform leave extract was Gamma sitosterol (37.1%), Stigmasterol (19.9%), the chloroform extract of the seed extract contained Stigmast-7-en-3-ol, (3.beta., 5., alpha) with percentage (54.7%) and Campesterol, Stigmasterol (22.4%).

The S. *Borumensis* chloroform leaves extract contained Stigmast-7-en-3-ol, (3.beta, 5. alpha) in high concentration (49.3%) and Stigmasterol (21.2%). As shown in table (3.21).

All above compounds were searched in NIST- library (version 2015) with similarty percentage ranged (99-95 %).

Table (3-21) Identification of steroids in tested plants by GC-MS

Sample / Compund	*C. plicata* (L)		*C. plicata* (S)		*S. Singueana* (L)		*S. Singueana* (S)		*S. Borumensis* (L)	
	RT	Area%	RT	Area%	RT	Area%	RT	Area%	RT	Area%
Campesterol	**13.3**	**12.3**	**13.3**	**22.4**	**-**	**-**	**13.39**	**16.4**	**-**	**-**
Stigmasterol	**13.6**	**19.9**	**13.6**	**22.4**	**-**	**-**	**13.6**	**15.8**	**13.6**	**21.2**
Fucosterol	**-**	**-**	**-**	**-**	**-**	**-**	**14.2**	**2.7**	**-**	**-**
Stigmast-7-en-3-ol, (3.beta., 5., alpha)	**-**	**-**	**14.1**	**54.7**	**-**	**-**	**14.1**	**53.4**	**14.1**	**49.3**
Cholest-7-en-3-ol,4-methyl	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**13.3**	**21.7**
Gamma sitosterol	**22.9**	**37.1**	**-**	**-**	**22.9**	**16.2**	**-**	**-**	**-**	**-**
Lanosterol	**-**	**-**	**-**	**-**	**22.1**	**22.1**	**-**	**-**	**-**	**-**

3.9 Antimicrobial activity

The higher plants was rewarding as it will lead to the development of a phytomedicine to act against microbes, Plant based antimicrobials have enormous therapeutic potential as they can serve the purpose without any side effects that are often associated with synthetic compounds Hostettmann and Terreaux. (2000). The most active plant extracts was further subjected for the evaluation of antifungal potency. African medicinal plants have been screened for their *in vitro* antibacterial activities and many described antibacterial activities have been focused on phenolic compounds, terpenoids or essential oils Bassole *et al*. (2003; Viljoen *et al*., 2003). The plants have been found to exert good *in vitro* antimicrobial activities and some active principles have been isolated. Examples are muzigadial isolated from *Warburgia salutari*s Rabe and Van. (2000).

Antimicrobial Assay of plants extracts

The antibacterial and antifungal activity of the plant extracts (20, 10, 5 mg/ml) were investigated by the disk diffusion assay, 20 extracts were tested against 6 micro-organisms; two strains of gram-positive (*Staphylococcus aureus*, *Bacillus subtilis group A*), two strains of gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*) and two kinds of fungi (*Candida albicans, Aspergillus niger*). *The* results showed that for all micro organism the activity decreased by decreasing the concentration of the extracts.

3.9.1 Antibacterial activity of *Chrozophora Plicata* of leaves - seeds extracts

The antibacterial activity of the different extracts of *C. Plicata* leaves against *P. aeruginosa* was found to display an inhibition zones within the range between 8 and 19 mm. The highest activity observed in the methanol extracts (19 mm) followed by the chloroform (18 mm), ethyl acetate and petroleum ether (15mm) extracts respectively and the inhibition zone decreased with decreasing extracts concentration. While the seeds extracts against *P. aeruginosa* showed inhibition zones ranged between 8 and 22 mm. the highest activity was observed in chloroform extract (18mm) whereas, all other extracts gave activity with inhibition zone ranged (15-13mm) where the reference amoxicillin (10µg/disk) gave a zone of 9.00 mm result found in table (3.21) and (3.22).

Different extracts of *Chrozophora Plicata* leaves showed antibacterial activity against *E. coli* with inhibition zone ranged from 11 to 20 mm. The highest activity (20 mm) was obtained from the ethyl acetate extract followed by methanol (18 mm), petroleum ether (17mm) and chloroform with inhibition zone (15 mm) respectively. While the inhibition activity on the seeds extracts against *E.Coli* ranged from (11-18) mm, the chloroform and ethyl acetate extracts

showed the highest with inhibition zone 18 mm followed by petroleum ether (17 mm) and methanol (14 mm) extracts respectively. All extracts of leaves were active against *B. subtilis* with inhibition zones ranged (from11 to 17 mm), The ethyl acetate and methanol extracts showed the highest activity (17mm), whereas all other extracts gave activity with inhibition zone of 16 mm. but the inhibition activity on the seeds part against *B. subtilis* with inhibition zones ranged from (11-22 mm), the methanol extract showed the highest antibacterial activity 22 mm followed by ethyl acetate and chloroform extracts with inhibition activity 15 mm table (3.21) and (3.22).

The tested extracts of leaves were active against *S. aureus* with inhibition zones ranged from 10 to 20 mm. The methanol extract showed potent antibacterial activity against *S. aureus* with inhibition zone of 20 mm followed by ethyl acetate and chloroform extracts with inhibition activity (15mm) and and the petroleum ether extract gave the lowest inhibition activity (14mm).while for the same organism for seed extracts showed inhibition activity ranged from (12-19) mm. the highest activity obtained by ethyl acetate extract with inhibition zone (19 mm) followed by petroleum ether (17 mm), chloroform (15 mm) and methanol (12 mm) extracts.

Table (3.22): antimicrobial activity of *Chrozophora Plicata* leaves extracts against standard bacteria and fungal

Name of plant	part	Solvent	Con mg/l	Mean diameter of growth inhibition zone (mg\ml)					
				Bactria				Fungi	
				P.s	E.c	B.s	S.a	A.s	C.a
Chrozophora plicata	Leave	Petroleum ether	20	15±0	17±0	16±0	14±0	13±1.5	14±0
			10	14±0	14±0	13±0	13±0	10±2.5	8±0
			5	13±0	11±0	11±0	13±0	-	-
		Chloroform	20	15±0	15±0	16±0	15±0	19±1.7	10± 0.5
			10	14±0	13±0	13±0	13±0	11±2.5	9.6±0.5
			5	12±0	13±0	12±0	10±0	-	-
		Ethyl acetate	20	15±0	20±0	17±0	15±0	16±1	12±3
			10	12±0	17±0	15±0	13±0	15±0	12.6±0
			5	10±0	14±0	13±0	11±0	-	-
		Methanol	20	20±0	18±0	17±0	20±0	23±2.8	9±0
			10	15±0	15±0	15±0	15±1	14±1.1	8.6±0.5
			5	14±0	14±0	14±0	13±0	-	-

Different extracts of *Chrozophora Plicata* showed variable activity against the tested bacteria. Generally, the ethyl acetate and methanol extracts of leaves extracts showed higher antibacterial activity than the petroleum ether and chloroform extracts. The ethyl acetate and methanol leaves extracts displayed antibacterial activity compared to standard antibiotics; ampicillin (20 mcg/disc) and ciprofloxacin (5 mcg/disc). Moreover, the response of the bacteria to the tested extracts varied among the strains. The differences in susceptibility of the Gram positive and Gram negative bacteria may be due to the differences in cell wall composition and thicknesses or genetic content of their plasmids Yao (1995); Karaman *et al*. (2003). Gram positive bacteria are usually reported as being more affected by plant extracts Yani *et al*. (2005); Sofidiya *et al*. (2009); Afolayan *et al*. (2009). The petroleum ether, chloroform, ethyl acetate and

methanol extracts demonstrated antibacterial activity against the Gram negative bacteria

P. aeruginosa with inhibition zone (9-20 mm) higher than that obtained for Gintamicin at 10 mcg/disc (8mm) but lower than that displayed by ciprofloxacin at 5 mcg/disc (35 mm). Previous studies on the antibacterial activity of *C. borcchiana* stem and leaves extracts were carried out by (Manal *et al.,* 2014) reported that the methanol extract of the leaves and stem bark of the plant exhibited a significant antimicrobial activity against *B. subtilis* and *S. aureus*, and three gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa*, and *Salmonella*). (Usman *et al*., 2007) reported that the ethanol extract of the whole plant of *C.senegalensis* possessed antibacterial activity against *Bacillus subtilis, Escherichia coli Pseudomonas aeruginosa* and *Staphylococcus aureus*.The methanol and ethyl acetate extracts showed highest antibacterial activity against the two gram positive bacteria, the result found in tables (3.21) and (3.22) and plate (3.1) and (3.2).

3.9.2 Antifungal activity of *Chrozophora Plicata* seed and leave extracts

All leave extracts exhibited high antifungal activity against *C. albicans* with inhibition zone of 14 mm for the petroleum ether, 12 mm for the ethyl acetate, 10 mm for chloroform and 9 mm for the methanol extracts respectively at 20mg/ml. The seeds extracts exhibited high antifungal activity against *C. albicans* with inhition zone ranged from 15 to 9 mm at concentration 20 mg/ml. The high antifungal activity against *C. albicans* was obtained from the ethyl acetate extract 15 mm followed by petroleum ether 14 mm and methanol and chloroform extract with inhibition zone 13 mm.

All leave extracts possessed high antifungal activity against *A. niger* with inhibition zone ranged from 13 to 23 mm. The highest antifungal activity was

obtained from the methanol (23 mm) followed by the chloroform (19 mm), ethyl acetate (16 mm) and petroleum ether extracts with inhibition zone of 13 mm. The seeds extracts showed inhibition zone ranged from 10 to 23 mm against *A.niger*, the highest activity of the seeds extracts obtained from methanol 23 mm followed by chloroform 19 mm and ethyl acetate 16 mm extracts respectivelly. The methanol leaves and seeds extracts of the plant showed the highest antifungal activity. Previous studies on the antifungal activity of *C. borcchiana* stem and leaves extracts were carried out by (Manal *et al*., 2014) reported that the methanol, ethyl acetate and hexane extracts of the leaves and stem bark had appreciable activities against *Aspergillus flavus* and *Candida albicans*. Results are presented in Table (3.21), (3.22) and plate (3.7).

Table (3-23): antimicrobial activity of *Chrozophora Plicata* seeds extracts against standard bacterial and fungal

Name of plant	Part	Solvent	Con mg/l	Mean diameter of growth inhibition zone (mg\ml)					
				Bactria				Fungi	
				P.s	E.c	B.s	S.a	A.s	C.a
Chrozophora plicata	Seed	Petroleum ether	20	15±0	17±0	12±0	17±0	13±1.5	14±1.7
			10	15±0	15±0	11±0	14±0	10±1.6	9±0
			5	13±0	12±0	8±0	10±0	-	-
		chloroform	20	18±0	18±0	15±0	15±0	19±1.7	13± 1.5
			10	15±0	11±0	13±0	13±0	11.7±0.1	12±2.5
			5	12±0	10±0	13±0	11±0	-	-
		Ethyl acetate	20	15±0	18±0	15±0	19±0	16±0	15±0
			10	14±0	17±0	14±0	14±0	15±0	12±0
			5	10±0	12±0	11±0	12±0	-	-
		Methanol	20	13±0	14±0	22±3	12±0	23±0.9	13±1.3
			10	13±0	13±0	17±0	10±0	15±0	9.7±0.2
			5	9±0	11±0	15±0	9±0	-	-

3.9.3 Antibacterial of *Senna Singueana* leaves and seeds extracts

The results showed that for all micro organism the activity decreased by decreasing the concentration of the extracts as shown in table (3.23) and (3.24). The antibacterial activity of different extracts of *S. Singueana* leaves against *P. aeruginosa* was found to display an inhibition zones within the range between 6 and 12 mm. The highest activity was observed in the petroleum ether extract (12 mm), while there is no activity on the ethyl acetate and methanol extracts against *P. aeruginosa*. While the seeds extracts showed inhibition zones between 8 and 20 mm against *P. aeruginosa*, the highest activity was observed in chloroform extract (20 mm) whereas, all other extracts gave activity with inhibition zone of 11-14 mm where the reference amoxicillin (10µg/disk) gave zone of 9.00 mm.

Different extracts of *Senna Singueana* leaves and seeds showed antibacterial activity against *E. coli*, the leaves extracts showed inhibition zone ranged from 9 to 13 mm. The highest activity (13 mm) was obtained from the petroleum ether followed by ethyl acetate (10 mm) and methanol (9 mm) extracts but there is no activity on chloroform extract against *E.coli*. The inhibition activity on the seeds extracts against *E.Coli* ranged from (10-20) mm, the chloroform extract showed the highest activity with inhibition zone 20 mm whereas the ethyl acetate and methanol extracts gave activity with inhibition zone 11 mm, but there was no inhibition zone obtained by petroleum ether extract against *E. Coli.* All extracts of leaves extracts were inactive against *B. subtilis,* whereas the seeds part extracts gave low activity with inhibition zone ranged from (7-10), except petroleum ether was inactive against *B. subtilis* table (3.23), (3.24) and plate (3.3), (3.4).

The leave extracts of *Senna Singueana* were inactive against *S. aureus*. The petroleum ether and chloroform extracts of seeds were inactive against

S.aureus but the ethyl acetate and methanol seeds extracts gave inhibition zone ranged from (9-13) mm.

The petroleum ether, chloroform, ethyl acetate and methanol seeds extracts demonstrated antibacterial activity against *P. aeruginosa* with inhibition zone ranged (10-20 mm) more than that obtained for Gintamicin at 10 mcg/disc (8 mm) but lower than that obtained by ciprofloxacin at 5 mcg/disc (35 mm) table (3.23) and (3.24). Previous studies on the antibacterial activity of *Senna Singueana* leaves extracts were carried out by Olusola *et al*. (2011) they demonstrated that the ethanolic and aqueous leaves extracts of *S.Singueana* showed a broad spectrum of antimicrobial activity. Teklay *et al.* (2014) reported that the ethanolic root extract of *S.Singueana* demonstrated antibacterial activities.

Table (3.24): antimicrobial activity of leaves extracts of *Senna Singueana* against standard bacteria and fungal.

Name of plant	Part	Solvent	Con. mg/l	Mean diameter of growth inhibition zone (mg\ml)					
				Bactria				Fungi	
				P.s	E.c	B.s	S.a	A.s	C.a
Senna Singueana	Leaves	Petroleum ether	20	12±1.5	13±0	0	0	20±0	14±1.1
			10	12±2.08	12±0	0	0	11±1.1	13±1.7
			5	8±1.5	11±2	0	0	-	-
		Chloroform	20	8±1.1	0	0	0	12±0	18± 0
			10	6±0	0	0	0	0	10±1.5
			5	0	0	0	0	-	-
		Ethyl acetate	20	6±0	10±1.5	0	0	19±1	14±1.1
			10	0	7±0.5	0	0	18±0.5	12±0
			5	0	7±0.5	0	0	-	-
		Methanol	20	6±0	9±0.5	0	0	13±2	20±0
			10	0	6±0	0	0	12±0.5	13±1.7
			5	0	5±0.4	0	0	-	-

3.9.4 Antifungal activity of *Senna Singueana* leave and seed

All leaves extracts exhibited high antifungal activity against *C. albicans* with inhibition zone 20 mm for the methanol, 18 mm for the chloroform, 14 mm for the petroleum ether and ethyl acetate extracts. The seeds exhibited high antifungal activity against *C. albicans* with inhition zone ranged from 9 to 15 mm the high antifungal activity of *C. albicans* was obtained by ethyl acetate and methanol extracts with inhibition zone (24-17) mm followed by chloroform and petroleum ether extracts with inhibition zone 15-14 mm respectively.

Table (3-25): antimicrobial activity of seeds extracts of *Senna Singueana* against standard bacteria and fungal.

Name of plant	Part	Solvent	Con. mg/l	Mean diameter of growth inhibition zone (mg\ml)					
				Bactria				Fungi	
				P.s	E.c	B.s	S.a	A.s	C.a
Senna Singueana	Seeds	Petroleum ether	20 10 5	14±1.1 9±1.1 8±2	0 0 0	0 0 0	9.5±0.5 0 0	16±1 15±1.1 -	14±1.1 9±0.5 -
		Chloroform	20 10 5	20±0 19±0 10±0	20±0 16±2 12±0.5	7±0 7±0 0	0 0 0	23±0 7±0.5 -	15± 1 9±0.5 -
		Ethyl acetate	20 10 5	10±1.1 10±0.5 9±0.5	10±1.5 10±2 9±0	10±0 9±1.7 9±1.7	13±0 10±0 6±0	24±1 18±0.5 -	24±1.1 17±0 -
		Methanol	20 10 5	11±0.5 11±1.1 0	10±1 9.6±0.5 9.4±0.5	11±2 10±2 8.6±0.5	9±0.5 9±0.5 0	19±1.5 13±1.7 -	17±2 10±0 -

All leaves extracts possessed high antifungal activity against *A. niger* with inhibition zone ranged from 12 to 20 mm. The highest antifungal activity was obtained from the petroleum ether (20 mm) followed by ethyl acetate (19 mm),

methanol (13 mm) and chloroform extracts with inhibition zone (12 mm). The seeds extracts against *A.niger* showed inhibition zone ranged from (16 to 24 mm), the highest activity of the seeds obtained from ethyl acetate 24 mm followed by chloroform 23 mm and methanol extracts 19 mm. Petroleume ether extract showed the least inhibition zone (16 mm). All extracts of *S.Singueana* leaves and seeds possess antifungal activity more than antibacterial activity as in Tables (3.23), (3.24) and plate (3.9).

Previous studies on the antifungal activity of *Senna Singueana* was not found but on the other species. (Egharevba *et al*., 2010) the study reported that *Senna occidentalis* showed that the plant may be good as an antibacterial recipe but may not be very useful as an antifungus moreover (Odeja *et al*.**,** 2014) reported that the methanol extract exhibited a significant antifungal activity against *A. niger* and *C. albicans*.

3.9.5 Antibacterial activity of *Stylochiton Borumensis* leaves

The antibacterial activity of different extracts of *Stylochiton Borumensis* leaves the petroleum ether and chloroform extracts were found to be inactive against *P. aeruginosa*, the ethyl acetate and methanol extracts gave activity with inhibition zone (15-14) mm respectively.Where the reference amoxicillin (10µg/disk) gave a zone of 9.00 mm.

Different leaves extracts showed antibacterial activity against *E. coli*, all extracts gave activity with inhibition zone of 14 mm.

The extracts were susceptible against *B. subtilis* with inhibition zones ranged from (11 to 17) mm. The ethyl acetate extract showed the highest activity (17 mm) followed by the methanol (13 mm) and chloroform (11 mm) extracts respectively but the petroleum ether extract was inactive against *B. subtilis*. The four tested extracts were tested against *S.aureus* the results showed that

chloroform and petroleum ether extracts were inactive. The ethyl acetate showed potent antibacterial activity against *S. aureus* with inhibition zone of 16 mm followed by the methanol (15 mm).

Generally, the methanol and ethyl acetate extracts showed higher antibacterial activity than the petroleum ether and chloroform extracts. The petroleum ether of leaves extract was inactive against *Staphylococcus aureus*, *Bacillus subtilis* and *P. aeruginosa.* The chloroform extract was inactive against *P. aeruginosa* and *Bacillus subtilis*.

The ethyl acetate and methanol extracts of *S. Borumensis* showed activity ranged from (14-15 mm) higher than that obtained for Gintamicin at 10 mcg/disc (8 mm) but lower than that displayed by ciprofloxacin at 5 mcg/disc (35 mm). As showed in table (3.25) and plate (3.5).

Table (3-26): antimicrobial activity of leaves extracts of *Stylochiton Borumensis* against standard bacteria and fungal

Name of plant	Solvent	Con. mg/l	Mean diameter of growth inhibition zone (mg\ml)					
			Bactria				Fungi	
			P.s	E.c	B.s	S.a	A.s	C.a
Stylochiton Borumensis	Petroleum ether	20	0	14±0	0	0	18±0.5	15±4
		10	0	13±0	0	0	17±0	12±0.5
		5	0	12±0	0	0	-	-
	Chloroform	20	0	14±0	11±0	0	22±1	15± 1
		10	0	14±0	11±0	0	7±0.5	19±1.7
		5	0	13±0	11±0	0	-	-
	Ethyl acetate	20	15±0	14±1.	17±0	16±0	21±1.7	12±2.6
		10	14±0	13±2	15±0	15±0	14±1.1	10±0
		5	13±0	12±0	14±0	13±0	-	-
	Methanol	20	14±0	14±1.	13±0	15±0	12±0.5	16±3
		10	13±0	14±2	11±0	12±0	11±0.5	14±0
		5	11±0	13±0	10±0	10±0	-	-

3.9.6 Antifungal activity of *Stylochiton Borumensis*

All leaves extracts exhibited high antifungal activity against *C. albicans* inhibition zone value of 16 mm for the methanol and chloroform extracts, 15 mm for the petroleum ether and ethyl acetate extracts with inhibition zone 12 mm. All leaves extracts possessed high antifungal activity against *A. niger* with inhibition zone ranged from 22 to 12 mm, the highest antifungal activity was obtained from the chloroform (22 mm) followed by ethyl acetate (21 mm), petroleum ether (18 mm) and methanol (12 mm) extracts respectively. From the results all leaves extracts of the plant showed highest antifungal activity on the *A. niger* more than *C. albicans* also *Stylochiton Borumensis* leaves possess antifungal activity more than antibacterial activity, Results are presented in Table (3.25) and plate (3.8).

Table (3-27): Standard antibiotics against standard bacteria

Antibiotic	M. D. I. Z .(mm)**			
	E. coli	***P. aeruginosa***	***B. subtilis***	***S. aureus***
Ampicillin (20 mcg/disc)	20	-	20	11
Ciprofloxac (5 mcg/disc)	33	35	20	10
Gintamicin (10mcg/disc)	17	8	25	17

*M. D. I. Z., Mean diameter of growth inhibition zone

Table (3-28): Standard antibiotics against standard fungal

Antibiotic	M. D. I. Z .(mm)**		
	Concentration (µg/ml)	***C. albicans***	***A. niger***
Griseofulyin	5	**18**	19
	25	21	23
Nystatn	5	19	18
	25	21	19

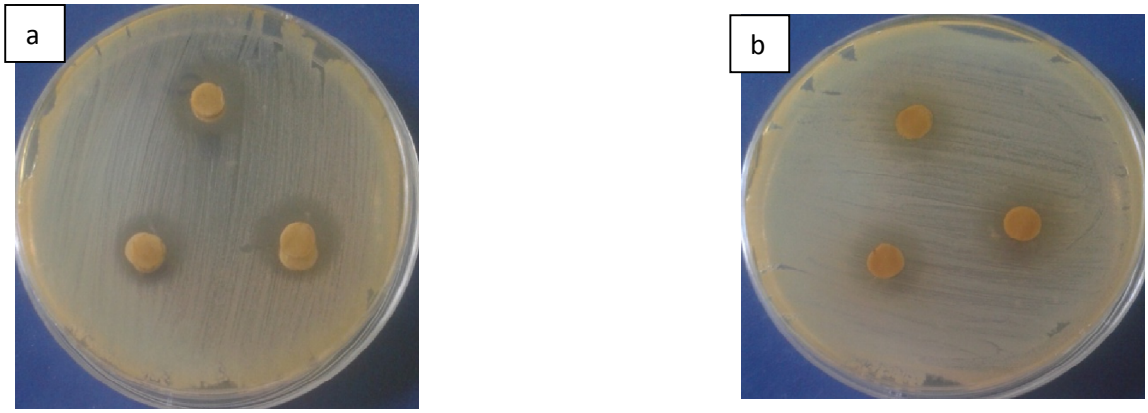


Plate (3-1): antibacterial activity of seed extract of *Chrozophora Plicata*.

a&b chloroform and methanol extracts against *Escherichia coli* and *Bacillus subtilis* respectively

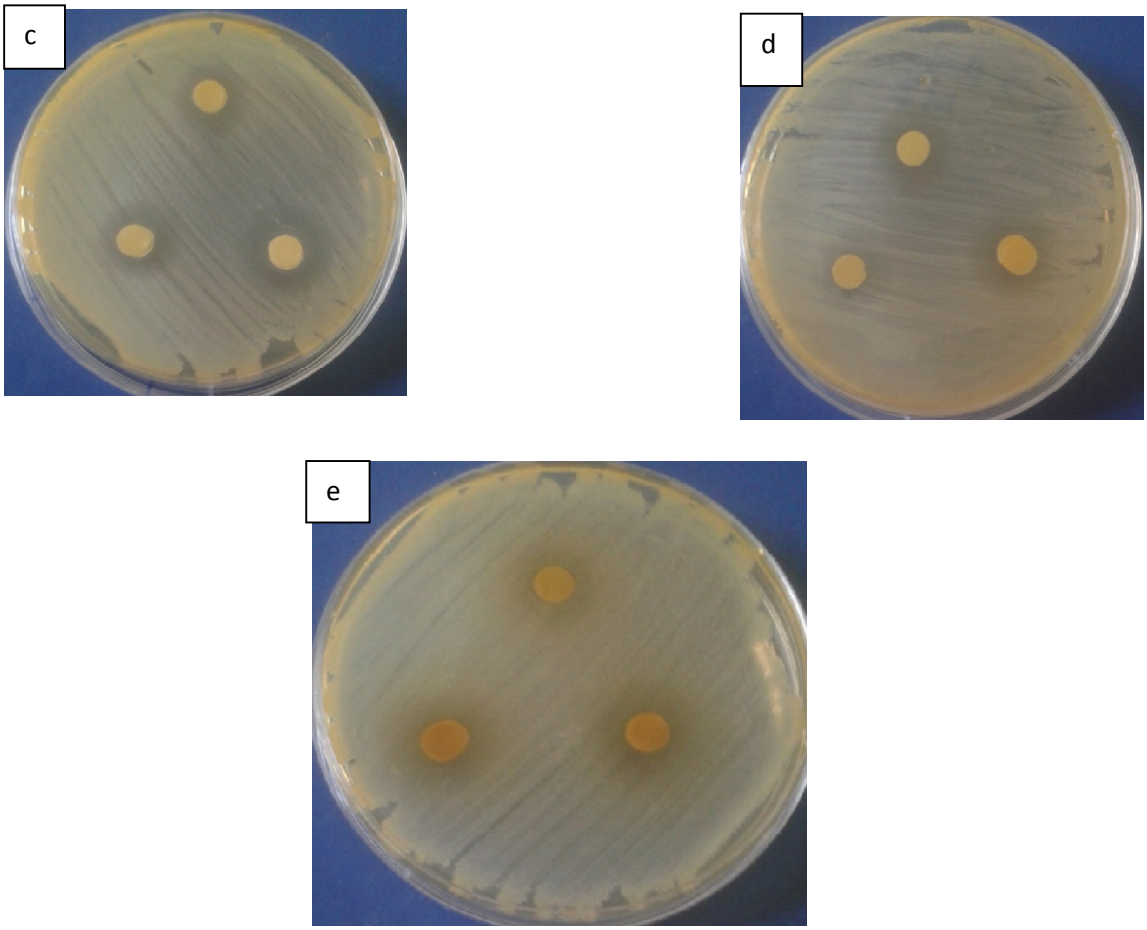


Plate (3-2) antibacterial activity of leaves extracts of *Chrozophora Plicata*

C&d&e methanol extract against *Bacillus subtilis* and *Pseudomonas aeruginosa* respectively

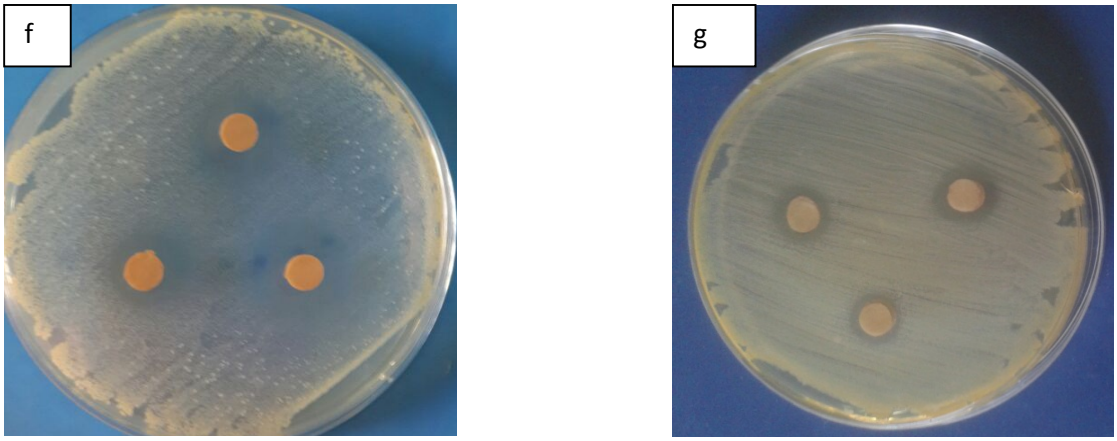


Plate (3-3): antibacterial activity of seed extracts of *Senna Singueana*

F & g, Ethyl acetate and chloroform extract against *Pseudomonas aeruginosa* and *Escherichia coli* respectively.

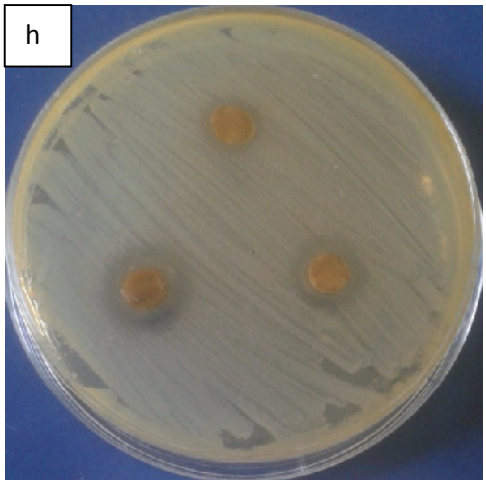


Plate (3-4): antibacterial activity of leaf extract of *Senna Singueana*

h. Petroleum ether extract against *Escherichia coli*.

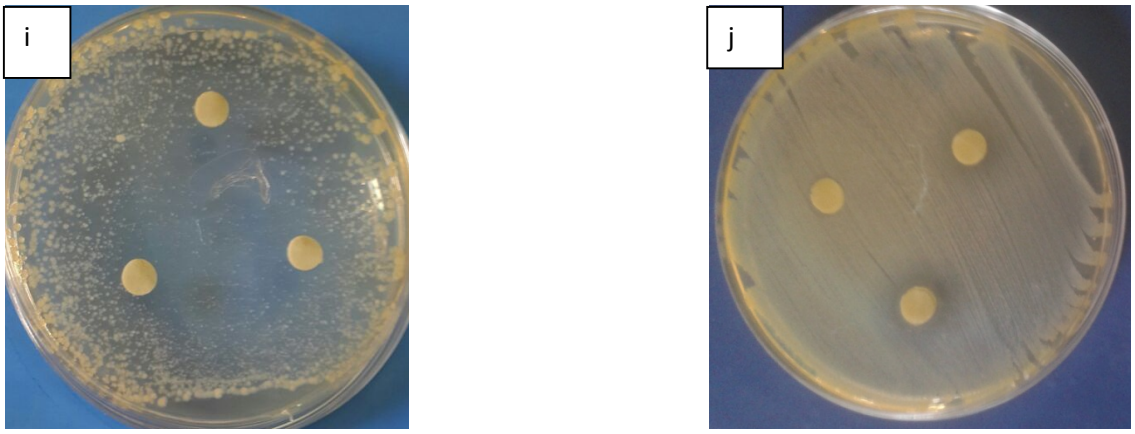


Plate (3-5): antibacterial activity of leaf extract of *Stylochiton Borumensis*

I &j, Ethyl acetate extract against *Staphylococcus aureus* and *Bacillus subtilis* respectively

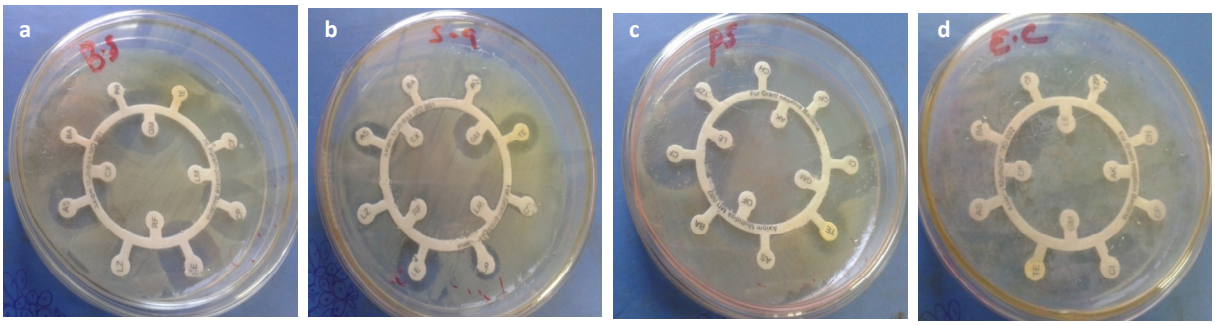


Plate (3-6): Antibacterial activity of standard antibiotics

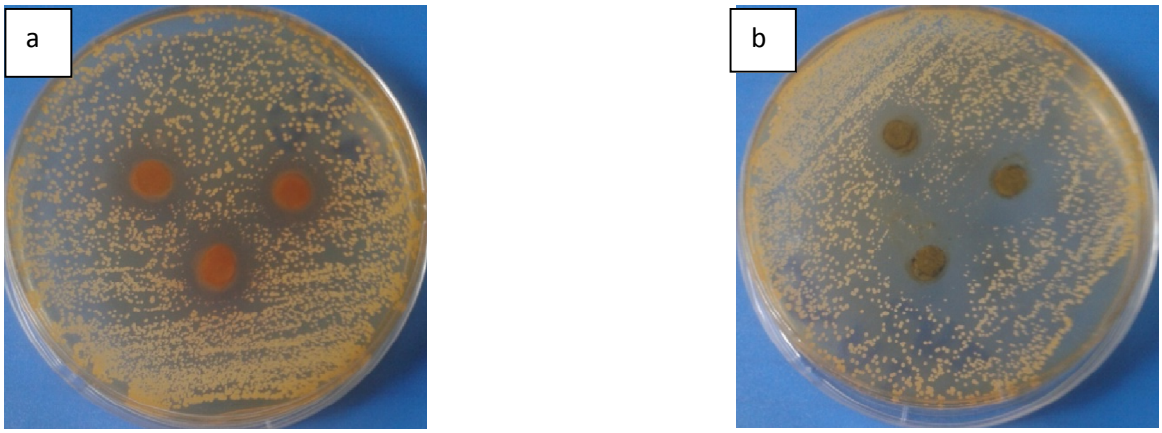


Plate (3-7): antifungal activity of seed and leave extracts of *Chrozophora Plicata*

a&b., Ethyl acetate seed extract and chloroform leave extract respectively against *Candida alibicans*

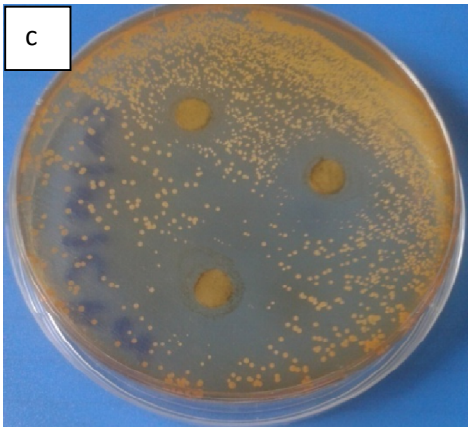
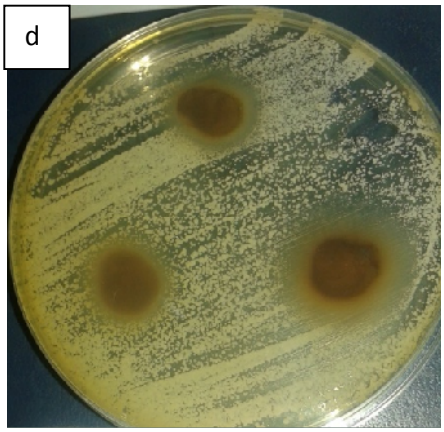


Plate (3-8): antifungal activity of leave extract of *Stylochiton Borumensis*

c., chloroform leave extract against *Candida alibicans*



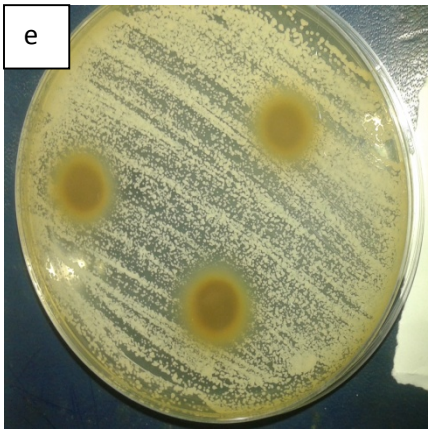
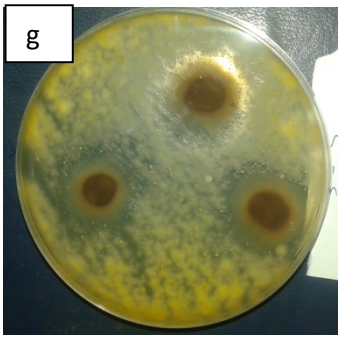


Plate (3-9): antifungal activity of seed and leave extracts of *Senna Singueana*

d,e, Ethyl acetate seed extract and methanol leave extract respectively against *Candida* alibicans.



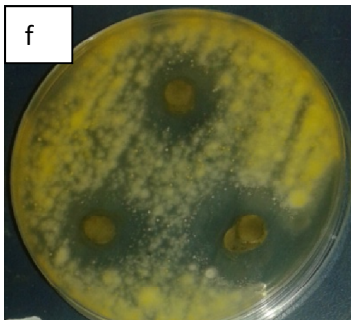


Plate (3-10): antifungal activity of leaves and seeds extract of *Senna Singueana*

f&g., Petroleum ether and ethyl acetate of leave and seeds extracts respectively against *Aspergillus niger.*

3.10 Antioxidant activity:

The antioxidant activity of different plant extracts was determined using a methanol solution of DPPH reagent. DPPH is very stable free radical. Unlike *in vitro* generated free radicals such as the hydroxyl radical and superoxide anion, DPPH has the advantage of being unaffected by certain side reactions, such as metal ion chelating and enzyme inhibition. A freshly prepared DPPH solution exhibits a deep purple colour with an absorption maximum at 517 nm. This purple colour generally fades when antioxidant molecule quench DPPH free radicals (i.e. by providing atoms or by electron donation, conceivably via a free-radical attack on the DPPH molecule) and convert them into a colourless-/bleached product (i.e. 2,2-diphenyl-1-hydrazine, or a substituted analogous hydrazine), resulting in a decrease in absorbance at 517 nm band (Amarowicz *et al*., 2003). The *in vitro* antioxidant activity of the petroleum ether, chloroform, ethyl acetate and methanol extracts from leaves and seeds of *C. Plicata*, *S Singueana* and leaves of *S. Borumensis* were evaluated using DPPH assays and the activity are expressed in terms of percentage of inhibition and IC_{50} values (µg/ml) was calculated. The values of the antioxidant activity of the plant extract are obtained and compared with the standard. The standard substance that used in the test was propyl gallate. The high DPPH radical scavenging activities of the various solvent extracts which are comparable to standard antioxidants used suggest that the extracts have compounds with high proton donating ability and could serve as free radical inhibitors. However, the organic solvent extract from the leaves and seeds demonstrated a more remarkable anti-radical activity with IC_{50} values lower than propyl gallate.The EtOH extracts of the stem bark and root had a consistently higher DPPH radical scavenging ability than other extracts in these parts. (Mohammed *et al*, 2013).

3.10.1 Antioxidant activity of *Chrozophora plicata* leaves – seeds extracts

In vitro antioxidant activity of the petroleum ether, chloroform, ethyl acetate and methanol extracts for leaves and seeds of *C. Plicata* was evaluated using DPPH assays table (3-30). The ethyl acetate and methanol of leaves extracts showed the highest activity 97% and 85% the IC_{50} value was found to be (0.078 – 0.062) µg/ml respectively while the ethyl acetate and methanol of seeds extracts showed also high inhibition activity 79 % and 67% with IC_{50} (0.239-0.119) µg/ml respectively but the petroleum ether and chloroform of the seeds extracts were inactive against DPPH scavenging activity. The chloroform leaves extract showed low DPPH scavenging activity with inhibition percentage 44%. Due to moderate and low activity of petroleum ether and chloroform extract of the leaves and seeds extracts, IC_{50} was not calculated..

Table (3-29): Antioxidant activity of seeds – leaves extracts of *Chrozophora Plicata*

Extract	%RSA±SD (DPPH) (leaves)	IC_{50} (µg/mL) (Leaves)	%RSA±SD (DPPH) (seeds)	IC_{50} (µg/mL) (seeds)
Petroleum ether	13 ± 0.12	-	Inactive	-
Chloroform	44 ± 0.32	-	9 ± 0.26	-
Ethyl acetate	97 ± 0.02	0.078 ± 0.00	79 ± 0.14	0.239 ± 0.00
Methanol	95 ± 0.02	0.062± 0.03	67 ± 0.14	0.119 ± 0.01
Propyl gallate	84 ± 0.02	0.055 ± 0.00	84 ± 0.02	0.055 ± 0.00

The high DPPH radical scavenging activities of the various solvent extracts which are comparable to standard antioxidants used suggest that the extracts have compounds with high proton donating ability and could serve as free radical

inhibitors. However, the organic solvent extract from the leaves and seeds demonstrated a more remarkable anti-radical activity (Kadiri *et al.*, 2013) reported that the Leaves part of *C. Plicata* was found to have potent antioxidant activity, reducing different types of radicals like Superoxide anion, Hydroxyl radical and Nitric oxide antioxidant power activity

3.10.2 Antioxidant activity of *Senna Singueana* leaves and seeds extracts

The ethyl acetate of leaves and seeds extracts showed the highest activity 91 and 84 % and the IC_{50} value was 0.032 and 0.046 µg/ml respectively. The methanol extracts of leaves and seeds showed high DPPH scavenging activity with inhibition percentage of 89 and 85 % with IC_{50} 0.118 and 0.119 µg/ml, respectively. The petroleum ether and chloroform leaves extracts showed moderate DPPH scavenging activity with inhibition percentage 56 and 51%, respectively. The petroleum ether and chloroform seeds extracts were found to have low DPPH scavenging activity with inhibition percentage 36 and 32 leaves and seeds extracts.

Table (3-30): Antioxidant activity of seeds – leaves extracts of *S. Singueana*

Extract	% RSA±SD (DPPH) (leaves)	IC_{50} (µg/mL) (Leaves)	% RSA±SD (DPPH) (seeds)	IC_{50} (µg/mL) (seeds)
Petroleum ether	56 ± 0.28	-	36 ± 0.22	-
Chloroform	51 ± 0.18	-	32 ± 0.31	-
Ethyl acetate	91 ± 0.02	0.032 ± 0.00	84 ± 0.05	0.046 ± 0.00
Methanol	89 ± 0.02	0.119 ± 0.07	85 ± 0.03	0.119 ± 0.01
Propyl gallate (positive control)	84 ± 0.02	0.055 ± 0.00	84 ± 0.02	0.055 ± 0.00

(Mohammmed *et al*., 2013) Reported that three different parts (roots, stem bark and leaves) of *S. Singueana* Aqueous and ethanol extracts were found to had potent antioxidant activity, reducing different types of radicals like DPPH, nitric oxide and as well as ferric reducing antioxidant power (FRAP).

3.10.3 Antioxidant activity of leaves extracts of *Stylochiton Borumensis*

In vitro antioxidant activity of methanol and chloroform leave extracts showed moderate inhibition activity 64 % and 55% respectively but the petroleum ether of leave extract showed low DPPH scavenging activity with inhibition percentage 31%. The ethyl acetate leaves extract showed the highest activity 94% and the IC_{50} value (0.0148 µg/ml) this result represented in table (3.30).

Table (3-31): Antioxidant activity of leaves extracts of *Stylochiton Borumensis*

Extract	%RSA±SD (DPPH) (leaves)	IC_{50} (µg/mL) (Leaves)
Petroleum ether	31± 0.08	-
Chloroform	55± 0.11	-
Ethyl acetate	94 ± 0.03	0.0148 ± 0.09
Methanol	64 ± 0.17	-
Propyl gallate	84 ± 0.02	0.055 ± 0.00

3.11 Cytotoxicity

3.11.1 Cytotoxicity of extracts by Brine shrimps lethality test

Several studies had shown that brine shrimp lethality is a general bioassay which is an excellent method for preliminary investigations of toxicity in order to screen medicinal plants popularly used for several purposes and for monitoring the isolation of biologically active compounds (Pisutthanan *et al.,* 2004). The technique is easily mastered, costs little and utilizes small amount of the test

material. The brine shrimp assay has been successively employed for bioassay-guided fractionation of active cytotoxicity and antitumor agents (Parra *et al*., 2001). They also reported that there is a positive correlation between the lethality to brine shrimp and the corresponding lethal oral dose in mice.

Cytotoxicity of petroleum ether, chloroform, ethyl acetate and methanol extracts of plants under study (of leaves and seeds of *C. Plicata* and *S. Singueana* and just seeds for *S. Borumensis*) at different concentration was evaluated by Brine shrimp (*Artemia salina*) test and against vero cell lines using MTT assay.

Preliminary screening of plants extracts through cytotoxicity provides helpful information about the antitumor activity of the extracts. Cytotoxic effects of various fractions of plants were measured against brine shrimps growth under controlled condition using normal control Table (3.31). After complete hatching shrimps was transferred into glass bottles already contained saline of sea salt and extracts of various fractions, after 24 h effects of fraction was noted and found that the brine shrimp survival is inversely proportional to the concentration of the various fraction of both plants. All tested extracts were moderately toxic to brine shrimps.

The LD_{50} value for *Chrozophora Plicata* leaves ranged from 173.714 to 303.404 ppm indicating that all extracts displayed moderate activity while the seeds part the petroleum ether and methanol extracts were not toxic the LD_{50} was >1000 ppm while the chloroform and ethyl acetate extracts showed moderate toxicity.

The LD_{50} value for *Senna Singueana* leaves showed that the petroleum ether and methanol extracts were not toxic whereas the chloroform and ethyl acetate extracts showed moderate activity but the seeds part all extracts showed

moderate activity and as shown in table (3.31). Methanol extract of *Senna Singueana* leaves induced no toxic damage in the liver, heart or bone tissues of the experimental rats (Okwoche *et al*., 2011)

The LD_{50} value for *Stylochiton Borumensis* leaves extracts ranged from 214 to 288 ppm which displayed moderate activity.

Table (3.32): LD_{50} value of brine shrimp lethality assay of leaves and seeds extracts of tested plants:

Species	Solvents	LD_{50} ppm		Remarks	
		Leaves	Seeds	Leaves	Seeds
Chrozophora Plicata	Petroleum ether	303.404	300	Moderate	Moderate
	Chloroform	287.209	199.16	Moderate	Moderate
	Ethyl acetate	214.9188	315.384	Moderate	Moderate
	Methanol	173.714	1010	Moderate	Non- toxic
Senna Singueana	Petroleum ether	>1000	161.676	Non- toxic	Moderate
	Chloroform	158.58	269.015	Moderate	Moderate
	Ethyl acetate	122.46	230.66	Moderate	Moderate
	Methanol	>1000	288.58	Non- toxic	Moderate
Stylochiton Borumensis	Petroleum ether	214.92	-	Moderate	-
	Chloroform	230.608	-	Moderate	-
	Ethyl acetate	248.55	-	Moderate	-
	Methanol	288.586	-	Moderate	-

Key: LD_{50} >1000 (non toxic), 500-1000 (weak), 100-500 (moderate), 0-100 (strong), <20 µg/ml (very active), (Mayer *et al*., 1982**).**

3.11.2 Cytotoxicity MTT assay

This colorimetric assay is based on the capacity of mitochondaria succinate dehydrongenase enzymes in living cells to reduce the yellow water soluble substrate (3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium-bromide) in to

an insoluble purple formazan product which is measured spectrophotometrically. The reduction of MTT can only occur in metabolically active cell, the level of activity is a measure of the viability of the cells (Patel *et al*., 2009).

Cytotoxicity of petroleum ether, chloroform, ethyl acetate and methanol extracts of *C. plicata* leaves and seeds parts of at different concentrations was evaluated against vero cell lines using MTT assay.

Cytotoxicity of extracts against vero cell lines using MTT assay were evaluated using concentrations of 125, 250 and 500 ppm. All leaves and seeds extracts of *C. Plicata* were virtually non-toxic against vero cell lines Table (3.32). The IC_{50} value for leaves extracts ranged from 92.6 to 229.7 ppm. While the seeds part the IC_{50} ranged from 247.7 to 118.05 ppm, Extracts revealed $IC_{50} < 90$ ppm were considered toxic (Sigaroodi *et al*., 2012).

Cytoxicity of *C. Plicata* leaves were previously reported by (May *et al*., 2013).

Table (3-33): Cytotoxicity of leaves - seeds extracts of *Chrozophora Plicata* against *vero cell lines*

Part	Name of Extracts	Concentration (µg/ml)			IC_{50} µg/ml
		Inhibition (%) ± SD			
		500	250	125	
Leaves	Petroleum ether	60.9 ± 0.06	52.7 ± 0.09	44.3 ± 0.01	200.6
	Chloroform	77.4 ± 0.03	65.5 ± 0.04	55.1± 0.03	92.6
	Ethyl acetate	70.1 ± 0.02	60.4 ± 0.06	55.7 ± 0.02	122.8
	Methanol	60.9 ± 0.06	52.5 ± 0.07	40.4 ± 0.06	229.7
Seeds	Petroleum ether	60.3 ± 0.05	54.7 ± 0.05	45.4 ± 0.08	182.3
	Chloroform	60.1 ± 0.04	50.4 ± 0.02	39.9 ± 0.03	247.7
	Ethyl acetate	68.2 ± 0.08	57.0 ± 0.01	45.9 ± 0.05	161.5
	Methanol	75.3 ± 0.05	67.5± 0.03	50.1± 0.02	118.05
	*Control	95.3 ± 0.00			< 30

Key: IC50 < 30 µg/ml high toxic, control= Tritonx100 was used as control positive at 0.2 µg/ml.

3.12 Antigiardial activity of the *Chrozophora Plicata*

Antigiardial activity against *Giardia lamblia*

The activity of different leaves extracts of *C. plicata* against *G. lamblia* was investigated using three different concentrations and results are presented in table (3-33) (3.6) and (3.7). Extract is considered active with mortality value ≥ 50%. In fact, *G. lamblia* is one of the most common intestinal pathogenic protozoan parasites (Newman *et al*., 2001). It is becoming increasingly important among HIV/AIDS patients. There are reports that some cases of acute and chronic diarrhea in AIDS patients may be associated with giardial infection (Merchant and Shroff, 1996). However, metronidazole, the common drug of choice, can cause mutagenicity in bacteria (Legator *et al.,* 1975) and is

carcinogenic in rodents (Rustia and Shubik, 1972). It also possesses undesirable side effects and treatment failures have been reported (Llibre *et al*., 1989).

Table (3-34) Antigiardial activity of the *Chrozophora Plicata*

Name of extract	Part	Concentration (µg/ml)		
		Mortality (%)		
		500	**250**	**125**
Petroleum ether	Leave	70.85	59.85	51.18
	Seed	72.51	64.67	59.70
Chloroform	Leave	87.44	84.34	71.60
	Seed	64.54	54.23	42.81
Ethyl acetate	Leave	80.44	75.13	61.70
	Seed	72.72	65.27	60.03
Methanol	Leave	74.99	66.29	55.31
	Seed	80.59	74.49	53.69
Control	90			

Metronidazole was used as the control positive at 312.5 µg/ml.

The petroleum ether of leaves extract was effective against *G. lambilia* and showed an increase in susceptibility period at all concentrations (125, 250 and 500 ppm) used. After 72 hours it gave 70.85% mortality for the highest concentration (500 ppm), while the other concentrations gave 59.85 and 51.18% mortalities at 125 and 250 ppm respectively, while the seeds at 500 ppm it gave 72.51% mortality and 64.67, 9.70 % mortalities at 250 and 125 ppm respectively compared with control positive which gave 90% mortality.

The antigiardial activity of the chloroform leaves extract was concentration dependant. after 72 hours the chloroform extract showed high activity with 87.44, 84.34 and 71.60 % mortality for concentration 500, 250 and

125 ppm respectively, comparable to seedS part which showed low activity ranged from

64.54, 54.23 and 42.81 % mortality for concentration 500, 250 and 125 ppm respectively.

The highest antigiardial activity of the ethyl acetate leaves extract after 24 hours was obtained at higher concentration whereas after 72 hours it gave mortality percentage of 80.44%, 75.13% and 61.70% mortality whereas the seeds showed low concentration ranged from 72.725%, 65.27% and 60.03% mortality for concentrations 500, 250 and 125 ppm respectively.

The highest antigiardial activity of the methanol leave extract after 24 hours was obtained at higher concentration whereas after 72 hours it gave mortality percentage of 74.99%, 66.29% and 55.31% mortality while the seed part gave high mortality percentage ranged from 80.59, 74.49 and 53.69 % mortality for concentrations 500, 250 and 125 ppm respectively. Compared with control positive which gave 90% mortality.

Antigiardial activity has been described for pentacyclic triterpenes, such as quinonamethides (Mena-Rejón *et al*., 2007). Plants from Sudan shown to possess antigiardial activity belonged mainly to the family Cucurbitaceae. (Elhadi *et al.* 2013) showed that seeds of *Cucurbita maxima* had potent antigiardial activity.

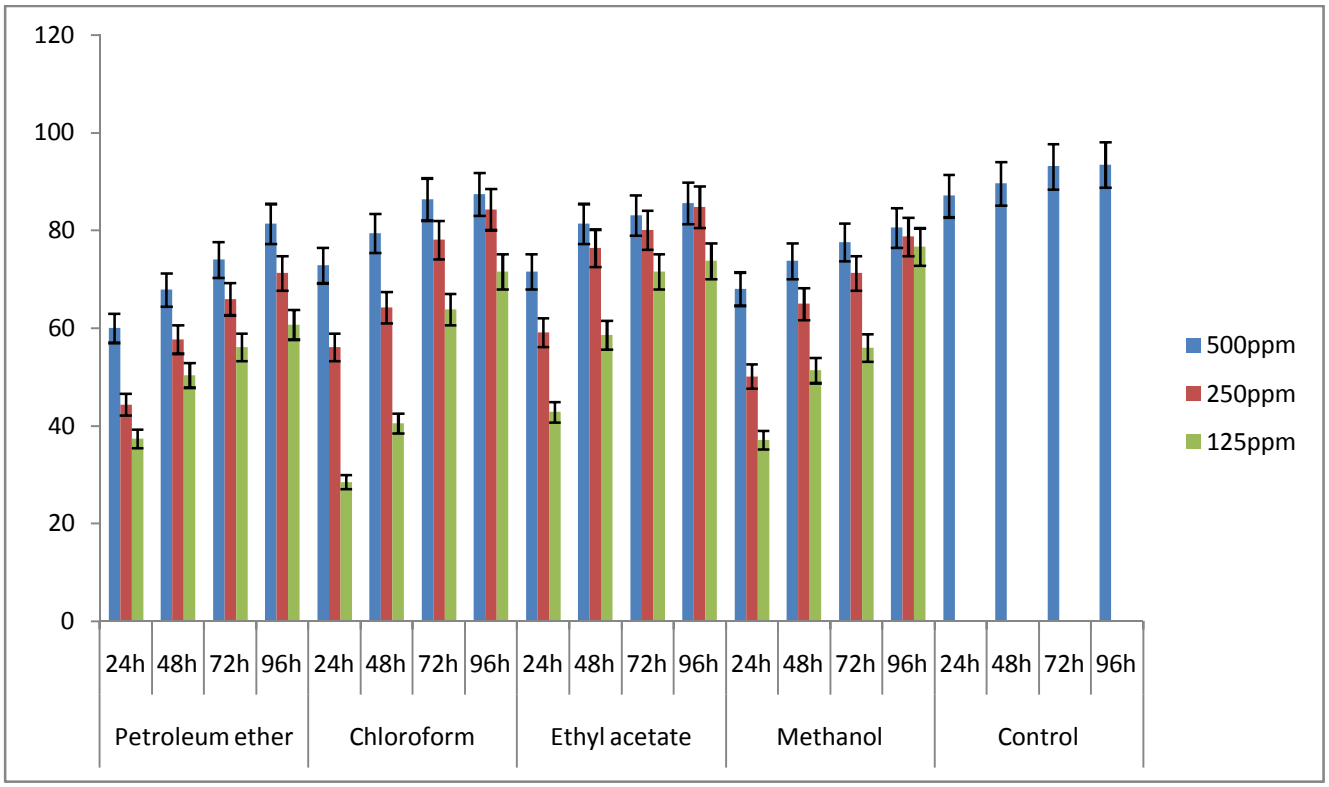


Figure (3.6): Atigiardial activity of leaves of *Chrozophora Plicata* against *Giardia Lamblia*

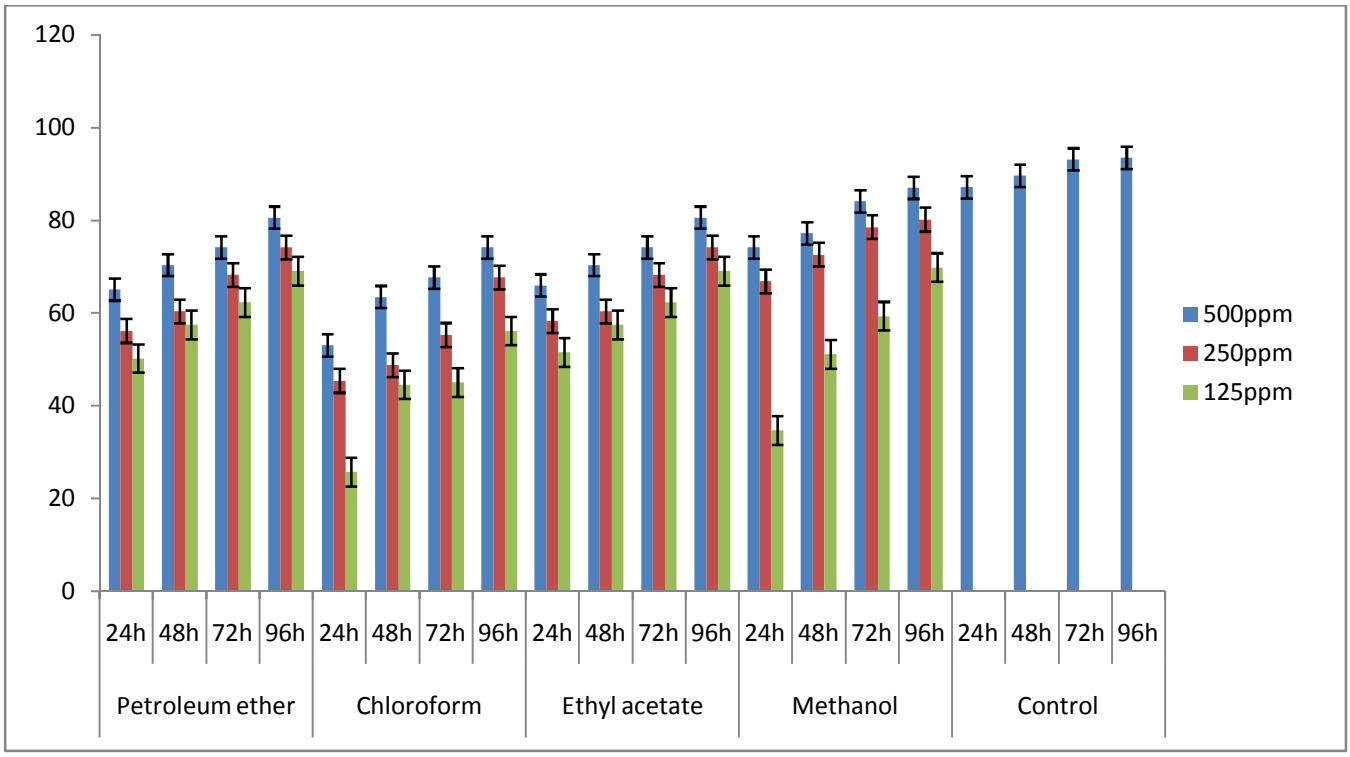


Figure (3.7): Atigiardial activity of seeds of *Chrozophora Plicata* against *Giardia Lamblia*

3.13 Characterization of compound 36

This compound isolated from fraction No (18)

Compound 36 (10 mg) was obtained as yellow powder eluted from column by used methanol: ethyl acetate (50:50, v/v). Rf value 0.375 (solvent system; methanol: chloroform (2:8 v/v) and developed yellow colour with ceric reagent. IR: 3433.08 (OH), 2358.85 (aliphatic C-H), 1652.88 (C=O), 1558, 1500 (C-C in ring stretch), 1298 (C-O stretch), 879, 798 (aromatic C-H, bending).

[M]+, 316, 315, 314, 269.02, 243.04, 213.98, 287.01, 180.01, 120.94, 91.99, 77.94, 42.91.

M/z [180.01] +: fragmentation at C-3 and C-1′

m/z [287] +: fragmentation at C-3.

Kaempferol -3-O-C1″ glucose:

^{1}H-NMR (CH_3OD, 400 MHz): Aglycone, δ: 7.7 (1H, d, J = 2.1 Hz, H-2′), 7.6 (3H, dd, J = 8.3, 2.1 Hz, H-4′), 6.92 (3H, s, J = 8.3 Hz, H-5′), 6.4 (1H, d, J = 2.2 Hz, H-6′, 6.20 (1H, d, J = 2.1 Hz, H-3′), glucose Hs, δ: ″), 4.5 (1H,dd, J = 3.3, 1.7 Hz, H-2″), 3.65 (1H, dd, J = 9.4, 3.4 Hz, H-3″), 3.36 (1H, s, J = 9.5 Hz, H-4″), 3.33 (1H, p, J = 9.6 Hz, H-5″), 1.13 (3H, d, J = 6.2 Hz, H-6″) 5.1 (d, J= 7.5 Hz, H-1″glucose), 3.88 (m, six sugar protons),.

^{13}C-NMR: Aglycone, δ: 157.9 (d, C-2), 144.4 (C-3), 178.04 (s, C-4), 164.6 (s, C-5), 121.7 (s, C-6), 161.6 (s, C-7), 104.2 (s, CH, C-8), 148.4 (s, C-9), 134.2 (s, C-10), 122.1 (s, C-1′), 114.6 (s, CH, C-2′), 116.2 (s, C-3′), 157.9 (s, C-4′), 104.2 (s, C- 5′), 101 (S, C-6′), glucose Cs, δ: 103.2 (s, CH, C-1″), 98.5 (s, CH, C-2″), 70.8 (d, CH, C-3″),74.3 (s, CH, C-4″), 72.1 (s, CH, C-5″),68.3 (s.CH, C-6″)

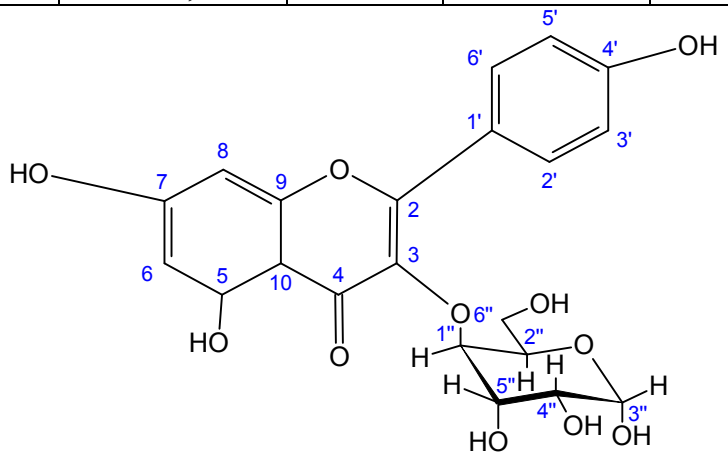
Summary of ^{1}H and C^{13} data is given in table (), moreover, structure of compound 36 was confirmed by COSY, HMBC and DEPT-135.

Previous study of compound found in this plant reported by (Naheed *et al*., 2013).

3.13.1 The NMR data of compound (36)

Position	1H-NMR	*J* (Hz)	13C -NMR	DEPT
2	-	-	157.1	C
3	-	-	144.4	C
4	-	-	178.04	C
5	-	-	164.6	CH
6	6.40, *s*	-	121.7	C
7	-	-	161.6	C
8	-	-	104.2	C
9	-	-	148.4	C
10	-	-	134.2	C
1′	-	-	122.1	C
2′	7.67, *d*	1.7	114.6	CH
3′	-	145.3 C	116.2	C
4′	-		157.9	C
5′	6.95, *d*	8.5	104.2	CH
6′	7.3, *dd*	-	101	CH

1″	5.15, *d*	7.5	103.2	CH
2″	3.2, *m*	-	98.5	CH
3″	3.2 – 3.5, *m*	-	70.8	CH
4″	3.2 – 3.5, *m*	-	74.3	CH
5″	3.2 – 3.5, *m*	-	72.1	CH
6″	3.65, *d*	11.5	68.3	CH_2



3.14 Characterization of compound 10 (sub- sub column) column (3)

This compound isolated from fraction F (23-33)

Compound 10 (12 mg) was obtained as black oily substance eluted from column by chloroform: ethyl acetate (30:70, v/v). Rf value 2 (solvent system; hexane: ethyl acetate (9.5:0.5 v/v) and developed dark blue colour with ceric reagent and black with anise aldehyde and vanillin.

IR: 3683.8.08 (OH), 3433.08, 2866.02 (aliphatic C-H), 1652.88 (C=O), 1730, 1635.52 (C-C in ring stretch), 1558.38 (C-C in aromatic ring), 1458 (C-H) bending, 1128.28 (=C-H bending) 750 (C-H), 698 (C-H bending).

[M]+, 187.09, 166.9, 148.9, 120.99, 95.05, 83.00, 80.98, 68.98, 43.02, 40.98.

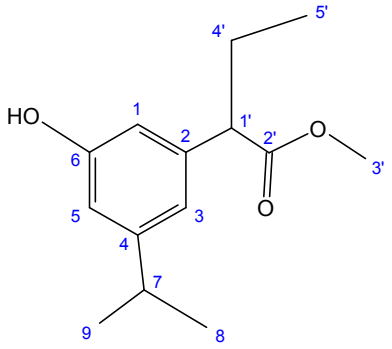
M/z [120] +: fragmentation at C-2

M/z [148.9] +: fragmentation at C-2′

M/z [43] +: fragmentation at C-7

The ^{1}H NMR (400MHz, $CHCl_3$-d) spectrum of compound 10 indicated the presence of hydroxyl group at 7.7), also ^{1}H NMR spectrum displayed two pairs of coupled doublets [q 7.6. (2H, d, J = 8.1 Hz), 7.5 (2H, d) and aromatic proton at 3.2 (1H, s) is identified as proton chemical shift of methoxy group. 3.2 (1H. triplet), 1.5 (1H quartered), 1.3 (3H, s), 0.8 (2H, s), 28.7 (2H, s).

The ^{13}C NMR spectrum showed signal at 167.93 (C- 2′, s), 132.19 (C-2, s), 67.7 (C-3′, s, methoxy group), 131 (C-3,S) 128.46 (C-1,3.s)′, 38.78 (C-4), 22.6,23.5 (C-8,9), 12 (C-5′),



3.14.1 The NMR data of compound (10)

Position	**^{1}H- NMR**	*J* (Hz)	^{13}C-NMR	DEPT
1	7.7	-	128.4	CH
2	-	-	132.1	C
3	7.6	-	131	CH
4	-	-	38	C
5	7.54	-	128.4	CH
6	7.52 *s*	-	121.7	CH
7	3.2	-	30.22	CH
8	1.3	-	23.5	CH
9	1.3	-	22.6	CH
1′	1.6	-	132.3	CH
2′	-	7,7 (J =2.1 Hz)	167.9	C
3′	3.2, *d*	3 (J=7.3Hz)	67.7	CH
4′	1.2	1.5 Hz	28.7	CH
5′	0.86	0.82	12.9	CH

CONCLUSION AND RECOMMEDATIONS

CONCLUSION

The dried seeds and leaves powder for *Chrozophora Plicata* and *Senna Singueana* and leaves for *Stylochiton borumensis* was subjected to hot continuous extraction (Soxhelt extraction) followed by phytochemical work of petrolume ether (40-60 ̊C), chloroform, ethyl acetate and methanol extracts. The results revealed the presence of fats, oils, flavonids, tannins, terpins, saponin, coumarins and glycosides in different concentration for each plant.

Antibacterial effects of leaves and seeds extracts of *C. Plicata* showed different degrees of inhibition profile against both Gram positive and Gram negative bacteria. The methanol and ethyl acetate extracts of leaves showed higher antibacterial activity than those of petroleum ether and chloroform extracts, while the seeds part the ethyl acetate and chloroform extracts of seeds showed higher antibacterial activity than those of methanol and ethyl acetate extracts. Moreover, all extracts displayed higher antifungal activity against *A.niger* than *C. albicans* in the two parts of the plant.

The petroleum ether and chloroform extracts of *S. borumensis* leaves showed no activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* while ethyl acetate and methanol extracts gave high antibacterial activity. Meanwhile all extracts of *S. borumensis* leaves showed higher antifungal activity against *A.niger* than against *C. albicans*.

Antibacterial effects of leaves and seeds extracts of *S. Singueana* showed that the seeds part extracts have higher antibacterial activity than the leaves part extracts. Moreover, all extracts for leaves and seeds showed higher antifungal activity against *A.niger* than aganist *C. albicans.*

All leaves and seeds extracts of *C. Plicata* displayed interesting antigirdial activity against *G.Lambelia* of which the chloroform leaves extract was the most effective extract; since 500 ppm of it displayed, 87.44 % mortality after 24 hours while the methanol of its seeds extracts was the most effective against

G.Lambelia; since it displayed 80.59% mortality when appling the same conditions, in comparison with metronidazole drug that exhibited 90% mortality. All extracts of tested plants material were rich in phenolic content especially those of methanol and ethyl acetate which showed also high antioxidant activity.

S. borumensis leaves contained high concentration of essential and non essential amino acids, while *S. Singueana* and *C. Plicata* leaves contained high concentration of Aspartic, Glutamic and Leucine.

The metal content in the extracts was lower than that in the plant. The contents of total forms of Pb, Cu and As of the studied plant samples above the MAC (maximum allowable concentration) were found to be 5.28%, 0.25%, and 1.51%, respectively. In the examined plant material, however toxic concentrations of arsenic and mercury were not detected.

All tested extracts of tested plants material were non- toxic to brine shrimps at concentrations of 10 and 100 ppm, but they were toxic at concentration of 1000 at which ppm 100 % mortality was obtained. Thus, all extracts displayed moderate toxicity except petroleum ether and methanol of *C. Plicata* seeds. Likewise, also petroleum ether and methanol of *S. Singueana* leaves extract revealed no toxic damage for brine shrimps. These obtained results confirmed the traditional medicinal practice of these plants. Even though, the present study on these crude extracts is an addition to the scientific literature, further detailed investigations on individual plants for pharmacological activities and active ingredients could provide leads to interesting pharmaceuticals of plant origin. As all leaves and seeds extracts of *C. Plicata* were virtually non-toxic against vero cell lines, it could be concluded that this extracts were safe.

Chromatography methods (TLC, CC and MS) were successfully used to isolate and identify compounds from the methanol extract of *C. Plicata*. *S.Borumensis* based on its biological potential and phytochemical analysis that had never been reported earlier.

RECOMMEDATIONS

Based on these results, the following studies could be recommended:-

- Further phytochemical studies on the crude extracts of the stems, flowers, root, wood and fruits of the three plants.
- Anti-microbial studies on use of other bacteria and fungi on the crude and pure compounds.
- Analytical fractionationstudies of the medicinal polar plant extracts, obtained traditionally by decoction and maceration, using ion- exchange and revesed-phase chromatography.
- Pharmacological studies on the extracts of *S.Singueana* and *S. borumensis*.
- Social-economic studies on the usage in herbal medicine of these plants by local communities where they grow.
- Further on the application of the tested plants as anticancer drug.
- In-vivo studies to determine the therapeutic potential of the isolated compounds prior to subsequent clinical evaluation.

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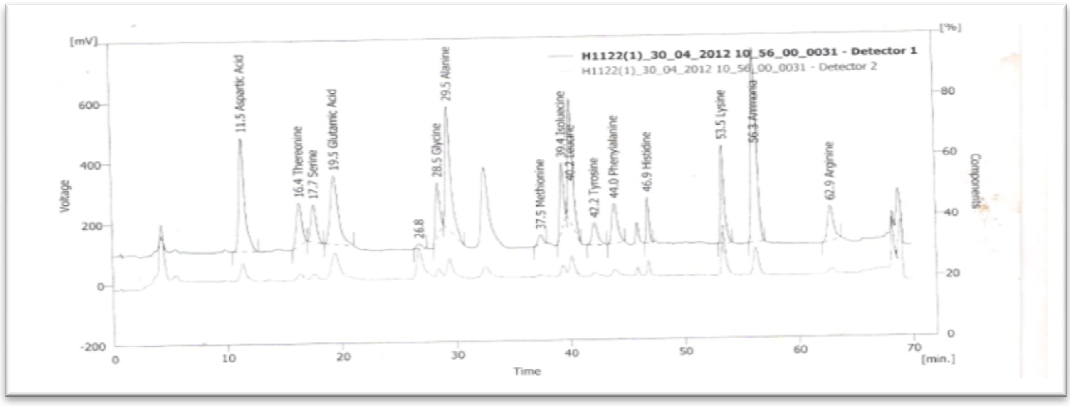
Amino acids chromatogram of *Chrozophora Plicata* leaves



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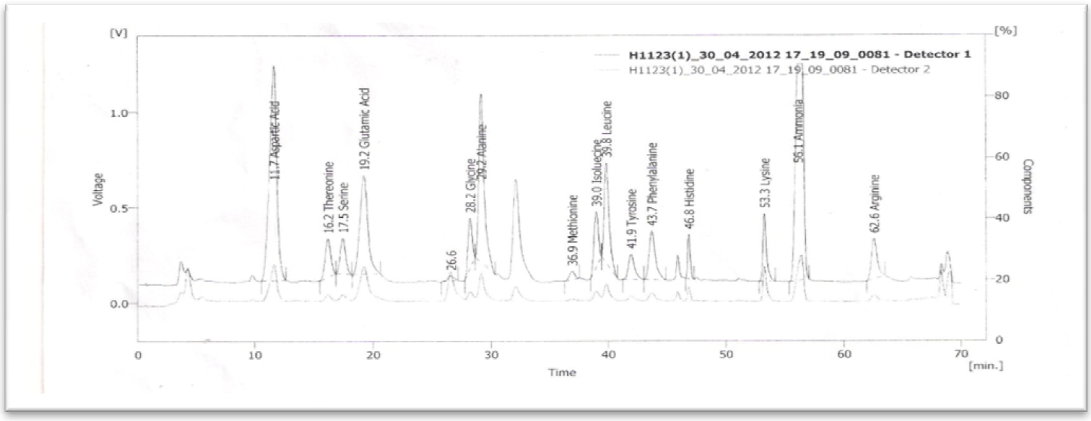

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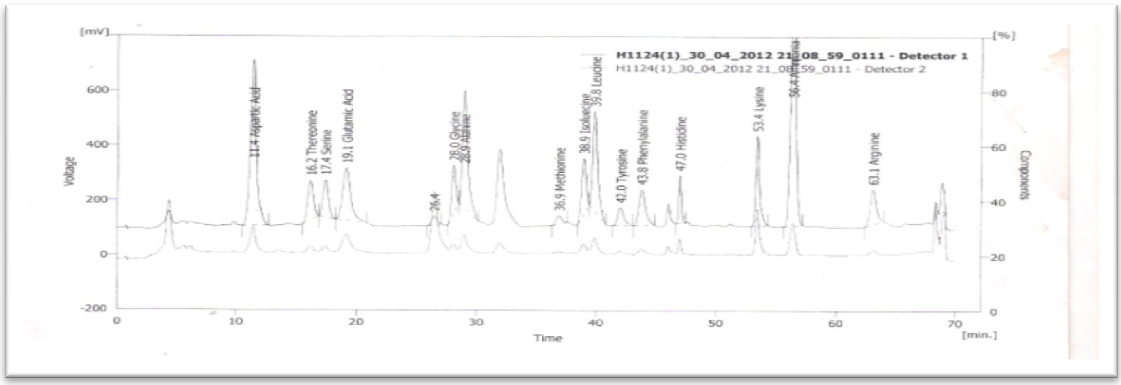

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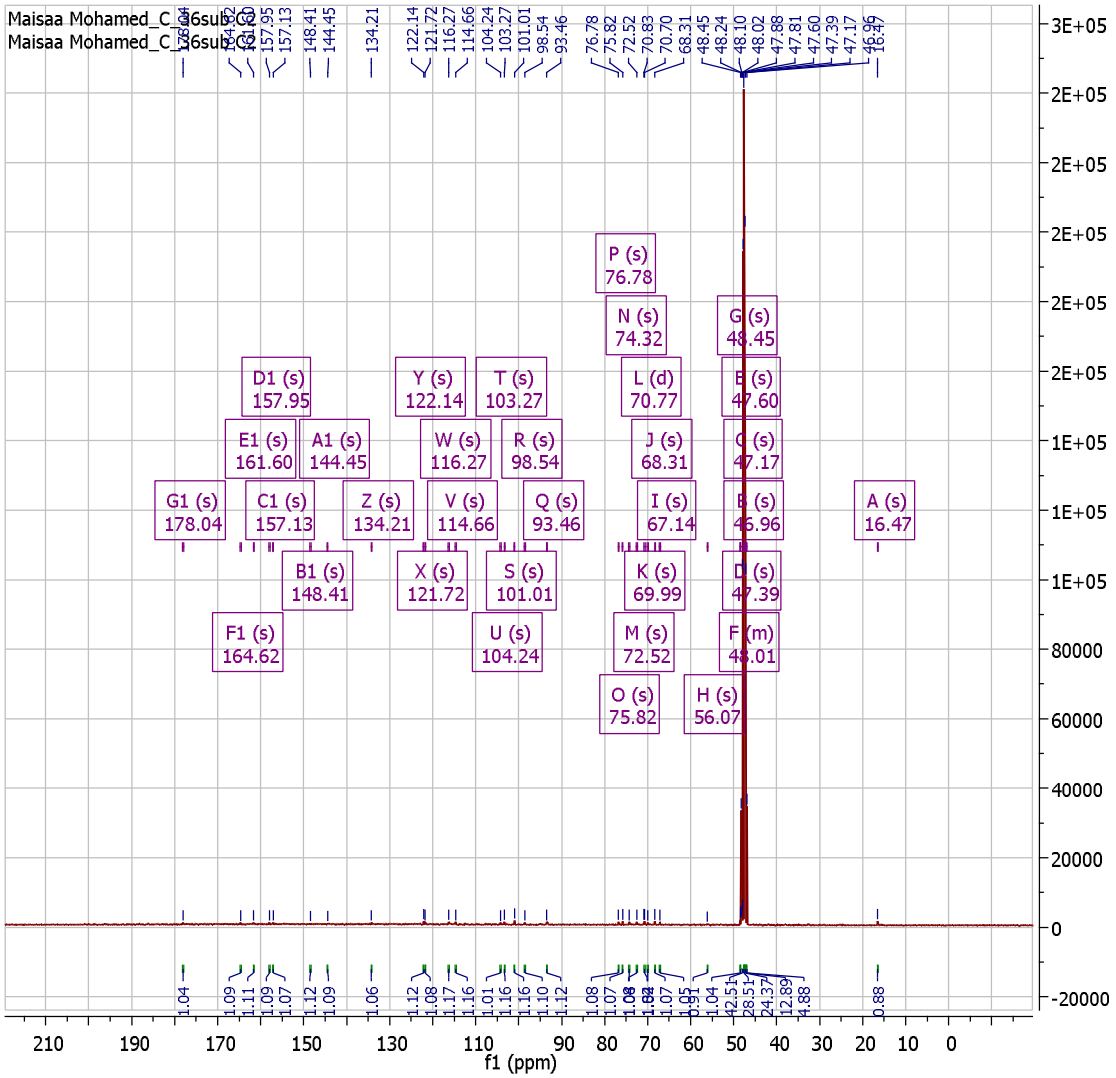

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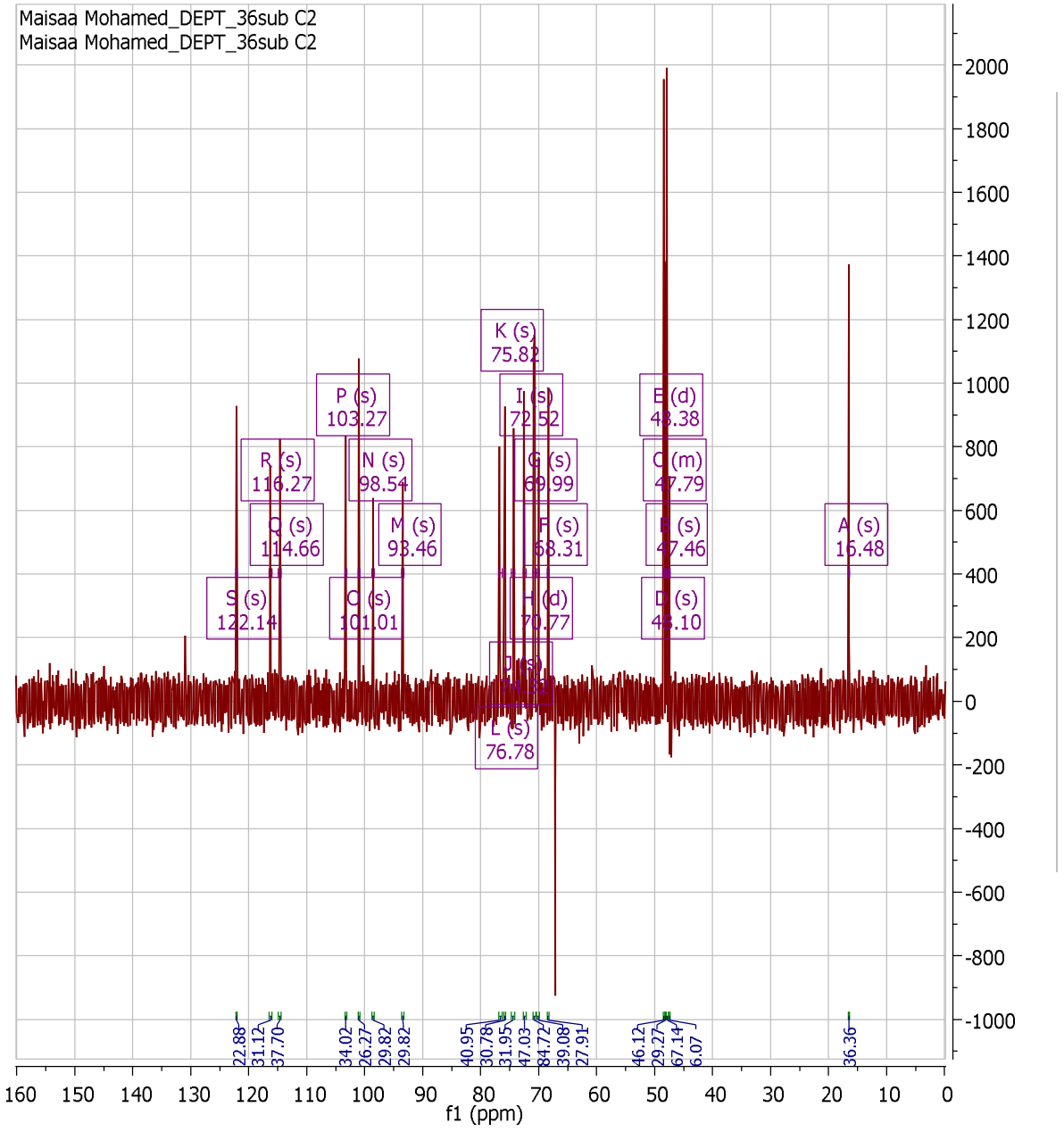

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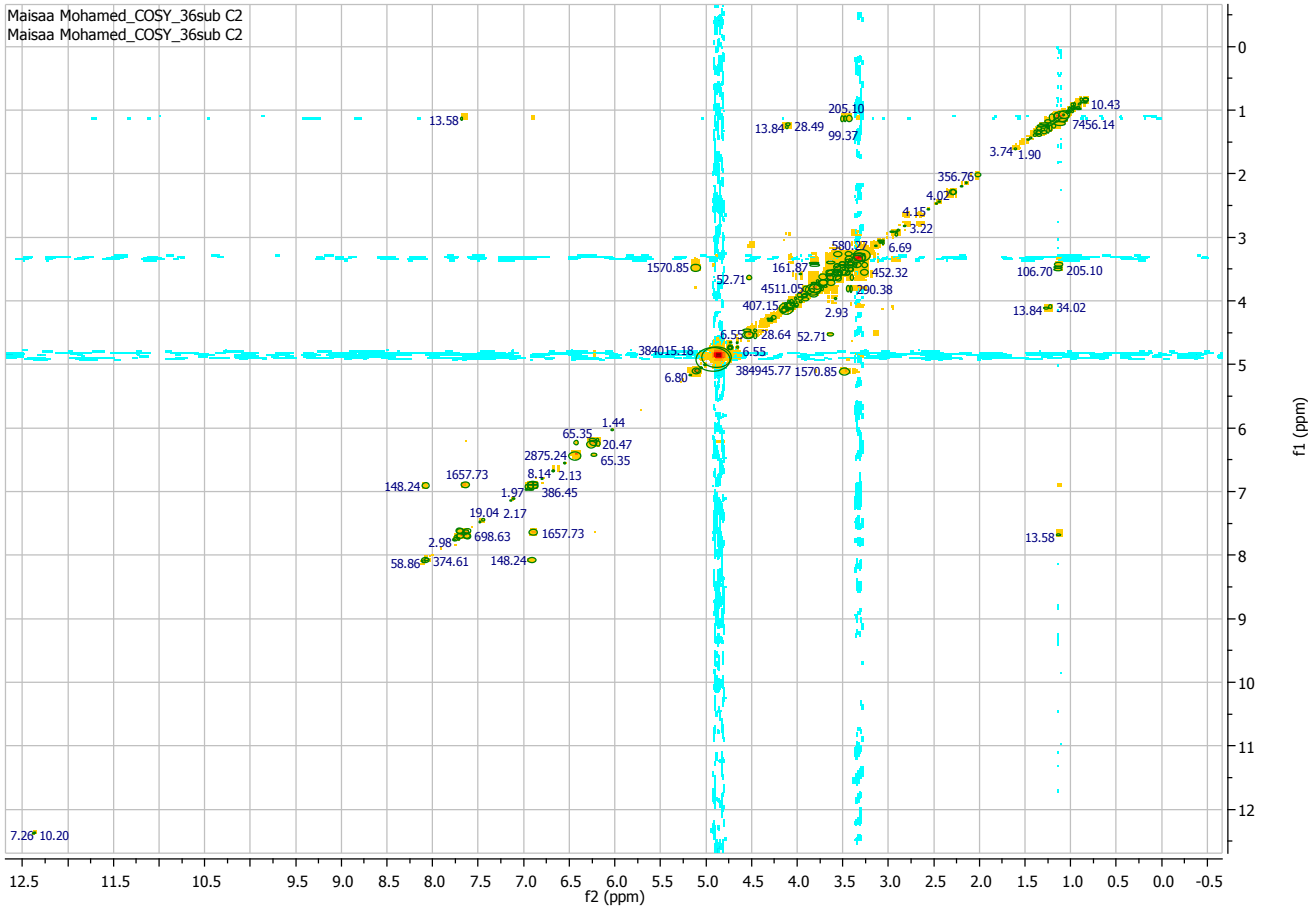

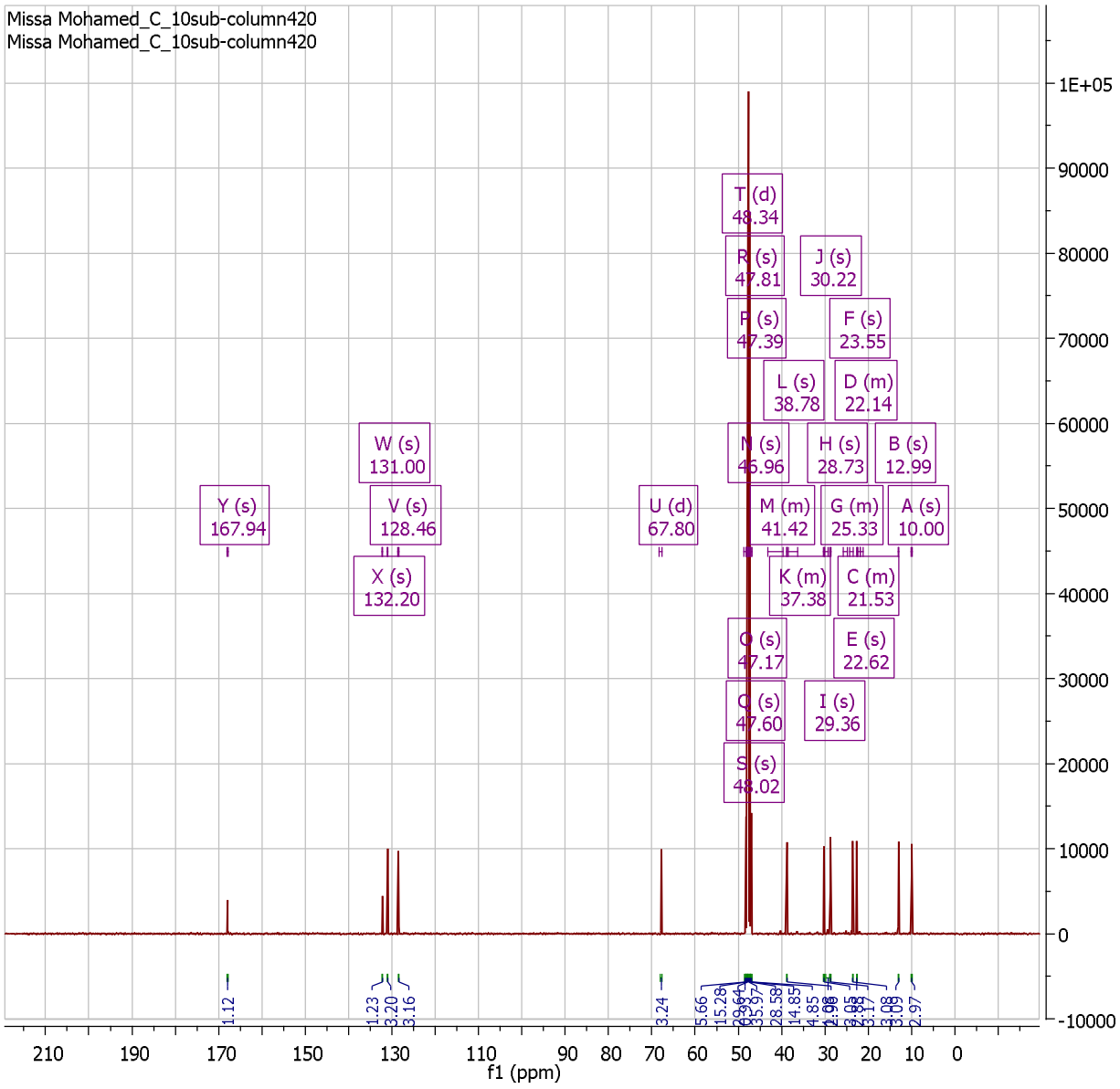
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Missa Mohamed_C_10sub-column420
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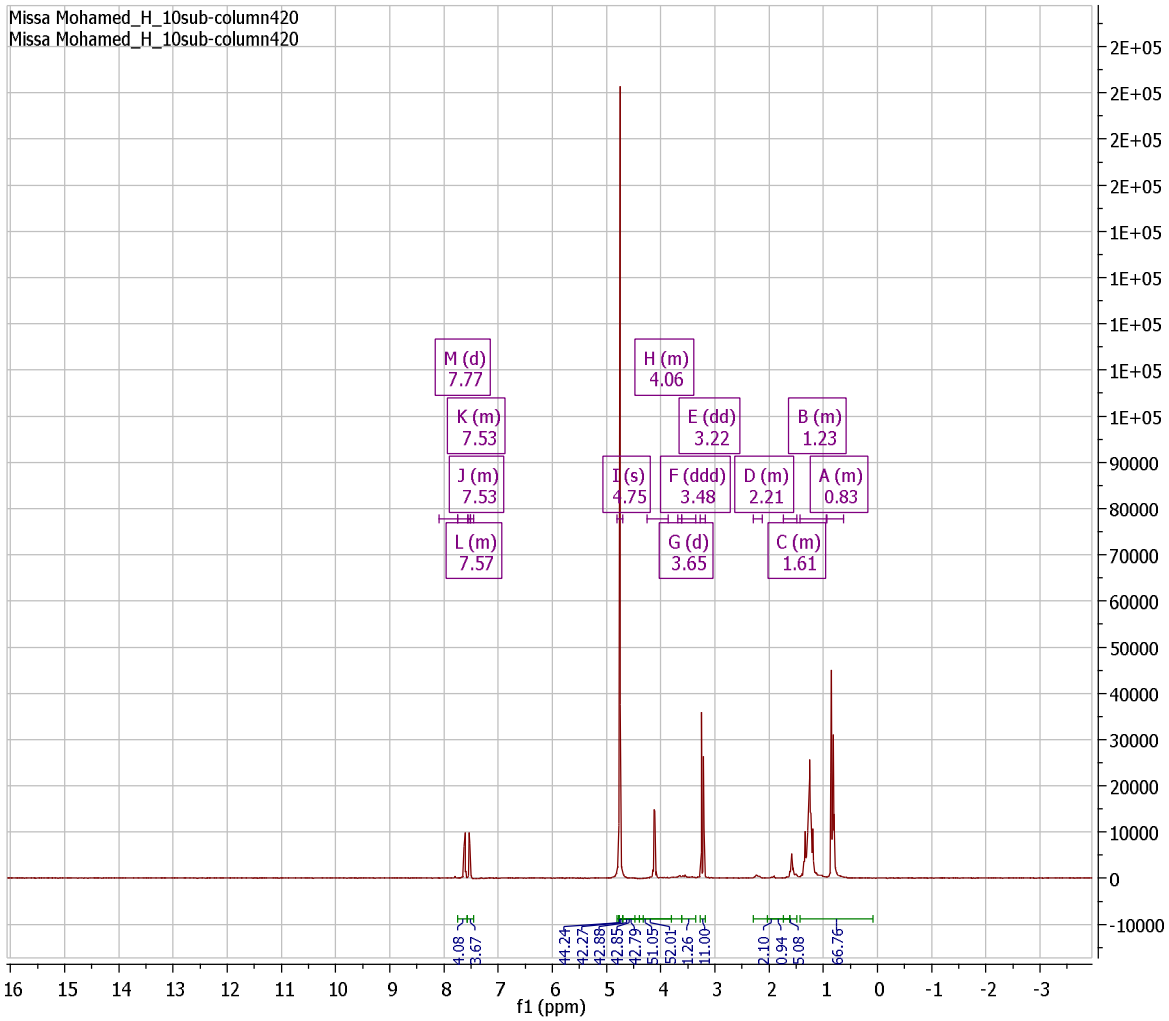
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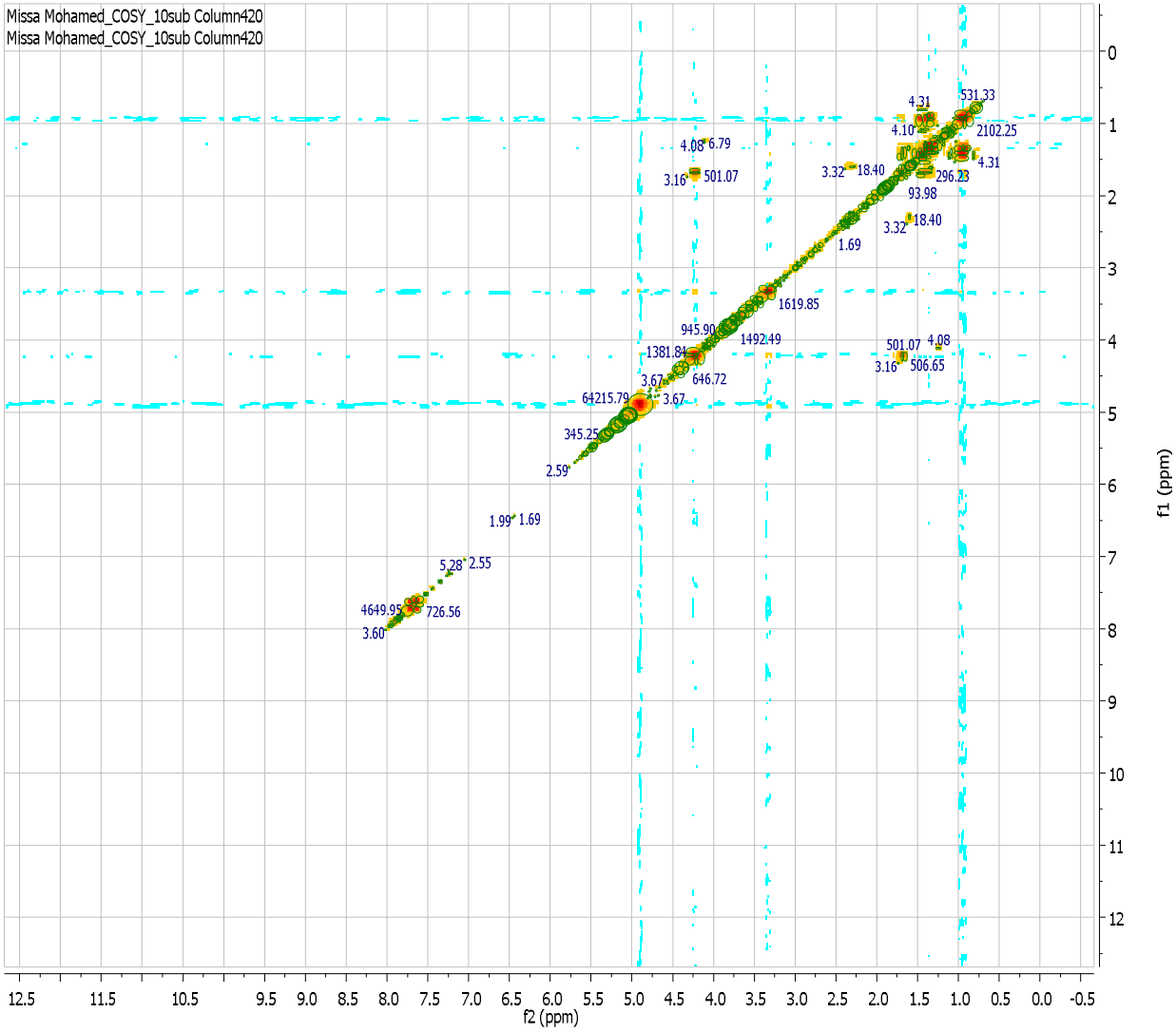
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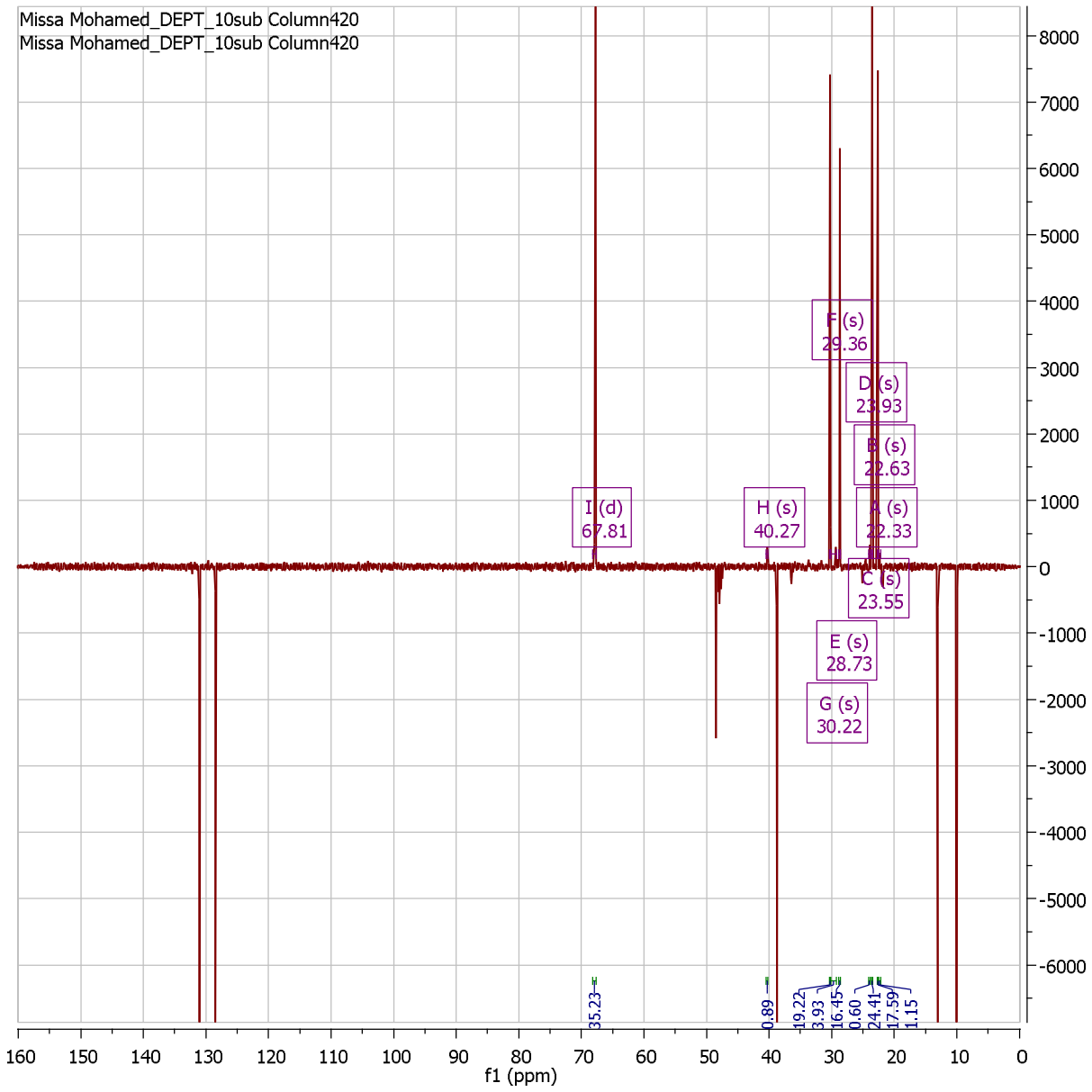
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f1 (ppm)

List of published papers:

1- Abde Alsiede Missa M.S.; Abddrahman, M.A.and Saeed, A.E.M (2015). Phytochemical screening, total phenolics content and antioxidant activity of *Senna Singueana*; Journal of Medicinal Plants Studies; 3(5): 160-165.

2- Abde Alsiede Missa M.S.; Abddrahman, M.A.and Saeed, A.E.M (2015). Nutritional Composition and Fatty acids analysis of *Senna Singueana* Leaves and Seeds; American Journal of Science and Technology; 2(6): 270-273.

3- Abde Alsiede Missa M.S.; Abddrahman, M.A.and Saeed, A.E.M (2015).Total phenolic content, flavonoid concentration and antioxidant activity of *Chrozophora Plicata* leaves and seeds extracts; International Journal of Advanced Research; 3(8): 986 – 993.

Submitted paper:

1- **Chemical composition and Biological activity of extracts of *Chrozophora plicata.* Submitted in (International Journal of Pharmaceutical and Biological Research).**

2- **Determination of metals, amino acids and Biological activity of extracts of *Senna Singueana* Submitted in (Journal of Pharmacognosy and Phytotherapy)**

3- **Total phenolic content, nutritional value, amino acids content, heavy metals analysis, antioxidant activity and biological activity of *Stylochiton borumensis* leaves. Submitted in Academic journal (Journal of Medicinal Plant Research).**