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Phytochemical Analysis and Thin Layer Chromatography Profiling of Crude Extracts from *Senna Occidentalis* (Leaves)

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Abstract

Plants used for medicinal practices which were discovered since prehistoric stone ages are termed Medicinal plants, which are also referred to as medicinal herbs, since plants produces bioactive chemical compounds (phytochemicals), this research however, is concerned with the extraction using Soxhlet extraction technique, phytochemical screening using various test methods, which reveals the presence of anthraquinones (free anthraquinones and combined anthraquinones), carbohydrates, cardiac glycosides, glycosides, flavonoids, saponins, steroids/ terpenes, phenolic compounds and tannins, and absence of alkaloids for extracts of senna occidentalis and also, thin layer chromatography profiling which gives probable foundation for further structural elucidation amongst others. This research shows the presence of potent secondary metabolites present in the leaves of senna occidentalis (leaves).

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Introduction

A medicinal plant is any plant material be it seeds, root extracts or leaves extract that is used to cure or fight against infection or attempt to maintain health, which are to be administered for specific ailments which can either be in modern or traditional medicine, this definition of medicinal plant is also supported by Ahn, [1,2]. There are about 50,000 medicinal plants used across the world [3]. It is documented by Kew (2016) that there are conservatively about 18,000 plant species that are use either in modern or traditional ways which are conceived to have medicinal properties which are part of the about 30,000 species documented.

Medicinal plants provide various kinds of benefit which could either be economic, health or socio-cultural benefits as suggested by Schippmann *et al.*, [3]. However, development of plants or extracts and available expertise to prove that they have potential medicinal uses is blunted and weakened, upon which insufficient financing is a key player,[1].

Drug development researchers adopts ethno botany as a strategy to search for pharmacologically active substances in naturally occurring plants, using these strategies, they have discovered hundreds and thousands of useful compounds which can either be alkaloids, glycosides, polyphenols, or terpenes. Some of the drugs extracted from plants include aspirin, quinine, opium, etc.

Bioactive Chemical Compounds (Phytochemicals)

All plants produce chemical compounds which give them an evolutionary advantage, such as defense against herbivores or, in the example of salicylic acid, as a hormone in plant defenses, [4,5]. These phytochemicals have potential uses as drugs, and the content of known pharmacological activity of these substances in medicinal plants is the scientific basis for their use in modern medicine, if scientifically confirmed, [1].

Modern knowledge of medicinal plants is being systematized in the Medicinal Plant Transcriptomics Database, which by 2011 provided a sequence reference for the transcriptome of some thirty species, [6]. The major classes of pharmacologically active phytochemicals are described below, with examples of medicinal plants that contain them, [7].

Alkaloids

Alkaloids are bitter-tasting chemicals, very widespread in nature, and often toxic, found in many medicinal plants, [8]. There are several classes with different modes of action as drugs, both recreational and pharmaceutical. Medicines of different classes include atropine, scopolamine, and hyoscyamine (all from nightshade), [9,10].

Glycosides

Anthraquinone glycosides are found in medicinal plants such as rhubarb, cascara, Aloe and Alexandrian senna, [9,11,12,13]. The cardiac glycosides are powerful drugs from medicinal plants including foxglove and lily of the valley. They include digoxin and digitoxin which support the beating of the heart, and act as diuretics, [5].

Polyphenols

Polyphenols of several classes are widespread in plants, having diverse roles in defenses against plant diseases and predators, [5]. They include hormone-mimicking phytoestrogens and astringent tannins, [9,14]. Plants containing phytoestrogens have been administered for centuries for gynecological disorders, such as fertility, menstrual, and menopausal problems, [15,16,17,18,19].

Terpenes

Terpenes and terpenoids of many kinds are found in a variety of medicinal plants, [18] and in resinous plants such as the conifers. They are strongly aromatic and serve to repel herbivores. Their scent makes them useful in essential oils, whether for perfumes such as rose and lavender, or for aromatherapy, [9, 20,21]. Some have medicinal uses: for example, thymol is an antiseptic and was once used as a vermifuge (anti-worm medicine), [21].

Cassia Occidentalis

S. occidentalis is an annual too short-lived perennial herb to small shrub with a pantropical distribution. It is reported as invasive throughout Oceania, and various countries in Asia and Africa. Within its native range *S. occiden-alis* is listed as invasive for Cuba by Oviedo Prieto *et al.* [22]. The scientific name is *Senna occidentalis* with a common name as Coffee

Senna.

Taxonomy

Domain: Eukaryota

Kingdom: Plantae

Phylum: Spermatophyta

Subphylum: Angiospermae

Class: Dicotyledonae

Order: Fabales

Family: Fabaceae

Subfamily: Caesalpinioideae

Genus: *Senna*

Species: *Senna Occidentalis*

Description

The morphology of *S. occidentalis* as described by Parsons and Cuthbertson [23], closely matches that as described in other places in the world. It is a low growing, sparsely branching annual or short-lived perennial plant up to 0.5-2 m high and having a characteristic fetid odour. The stems are reddish purple, erect, 4-angled when young, becoming rounded with age. The plant has a robust primary root with several laterals. The leaves are pale green on reddish stalks; alternate, pinnate, with 3-5 (sometimes 6) pairs of opposite ovate to lanceolate-elliptic leaflets, 25-100 mm long, 20-30 mm wide, rounded at the base. A conspicuous, dark-coloured gland occurs at the base of the petiole (leaf stalk) but not on the stalks of the leaflets. The flowers are pale to bright yellow, 20-30 mm in diameter, in 2-6 flowered axils of the upper leaves; sepals are red veined; 5 petals per flower, the 2 anterior ones are smaller than the others; fertile stamens 6, the two basal ones longer than the rest, 4 infertile stamens are reduced to tiny petal-like staminodes. Further descriptions of the floral anatomy of *S. occidentalis* and nine other species of *Cassia* are given by Chhavi-Thakur and Thakur [24]. The fruit is a dark brown, flattened, sickle-shaped pod with paler stripes along the edges when mature. Pods are 75-130 mm long, 8-10 mm wide, containing a single row of 25-35 seeds. The seeds are dark brown, flattened, hard, 5 mm long and 3 mm wide. Plate 1

Phytochemistry

Phytochemical screening of the plant showed the presence of carbohydrates, saponins, sterols, flavonoids, resins, alkaloids, terpenes, anthraquinones, glycosides and balsam. Presence of these metabolites strongly concluded the great potential of the plant as a source of phytomedicines. As the flavonoids and resins are present, it might be responsible for its anti-inflammatory properties. Chinese folkloric medicine contains flavonoids which has anti-inflammatory effect on both acute and chronic inflammation, alkaloids for blood pressure decrease and nervous system balancing, Tannins for wound healing and terpenes with anti-viral properties, [25,26,27].

Eudesmane sesquiterpenes have been reported to contain antibacterial properties. Saponins are believed to have antioxidant, anti-cancer, anti-inflammatory, and anti-viral properties. The anthraquinones, emodin and chrysophanone have been reported to possess wound healing properties. Other compounds reported in literature include, 1,8-dihydroxyl-2-methyl anthraquinone, 1,4,5-trihydroxy-3-methyl-7-methoxy anthraquinone, cassiaoccidentalin A, B and C, which are C-glycosides, achrosine, anthrones, apigenin, aurantiobtusin, campesterol, cassiollin, chryso-obtusin, chrysophanic acid, chrysarobin, chrysoeriol, essential oils, funiculosin, galactopyranosyl, helminthosporin, islandicin, kaempferol, lignoceric acid, linoleic acid, linolenic acid, mannitol, mannopyranosyl, matteucinol, obtusifolin, obtusin, oleic acid, physcion, quercetin, rhamnosides, rhein, rubrofusarin, sitosterols, and xanthorin [28,29]. Figure 1

Methodology

The *Senna Occidentalis* plant leaves were collected from Federal University Dutse (F.U.D) Jigawa State, Nigeria on May 13th 2018. Below is the table 1 for the coordinates using Global positioning system device (GARMIN GPS 76).

The plant was authenticated by Mal. Namadi Sunusi at the herbarium unit in the Department of Plant Science, Ahmadu Bello University, Zaria, Nigeria. Where it was deposited and voucher numbers 01047 was assigned to *Cassia occidentalis*. The plant leaves were chopped into pieces using iron knife, oven dried at 80^{o}C for one (1) hour, pulverised using mortar and pestle, and sieved using a 600 MICS size sieve and then stored in a non-absorptive nylon for subsequent use. The plant materials were extracted using a soxhlet extractor

Plate 1. Close view of senna occidentalis in its natural habitat

Some Compounds Isolated from Senna Occidentalis

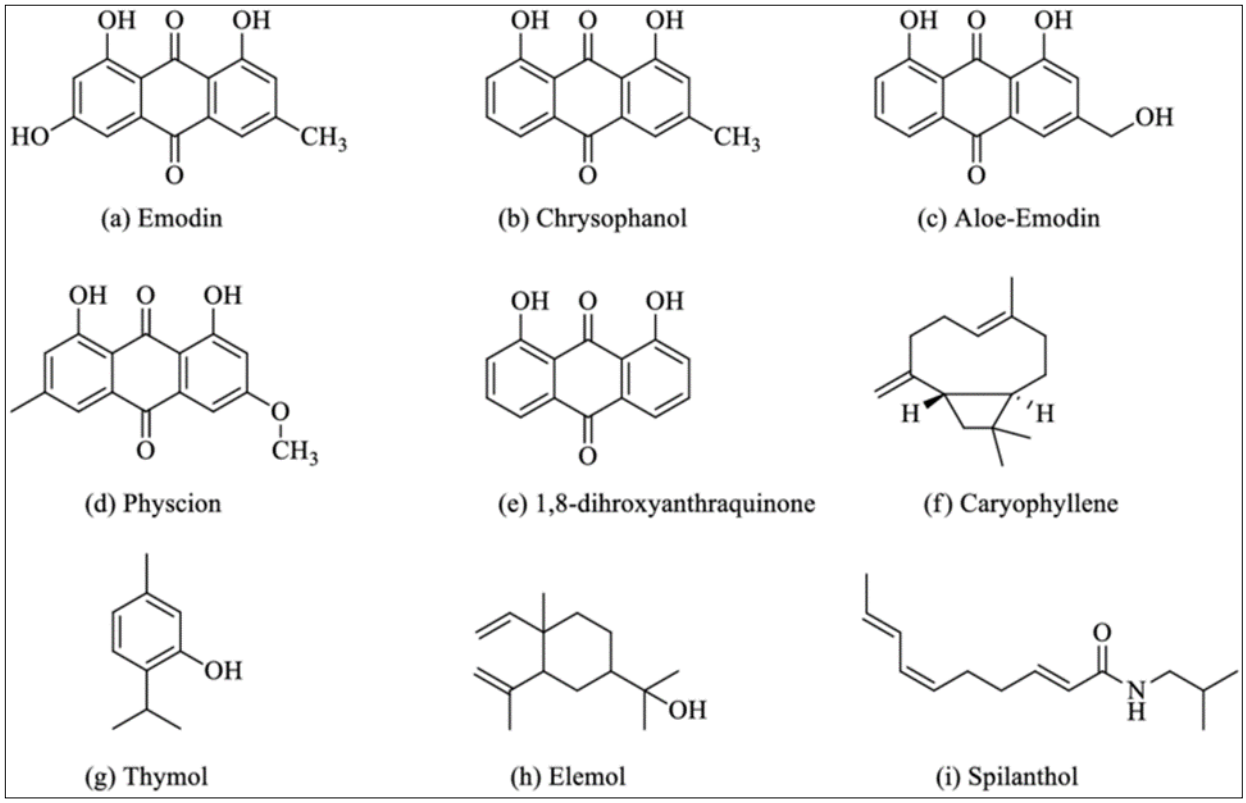

Figure 1. Structures of some compounds isolated from Senna occidentalis

Table 1. Coordinates of plants materials (leaves) collected

Plant's Name	Coordinate	Elevation (m)	Accuracy (m)
Senna occidentalis	N 11°42.074′ E009°22.357′	439.1	20.1

successively in n-hexane, diethyl ether, chloroform, ethyl acetate and methanol exhaustively for 12 hours; 1 hour; 8 hours; 10 hours; and 12 hours each for both plant materials until complete extraction. The solvents were removed and concentrated using a rotary evaporator and stored in a screw cap bottles at 0°C until usage then labelled. The extracts were subjected to various phytochemical tests to identify the constituent secondary metabolites using standard methods [30,31] with some modifications. The metabolites tested for includes: Anthraquinones, Alkaloids, Carbohydrates, Cardiac glycosides, Flavonoids, Saponnins, Steroids, Tannins and Terpenes.

Test for Steroids/Terpenes

Liebermann-Buchard Test

To each 0.5 g of sample in 20 mL test tube was added chloroform (1 mL) and also few drops of acetic anhydride were added followed by concentrated H_2SO_4. The mixture was mixed carefully and a blue colour that changed with time was observed in the resulting solution which indicated the presence of steroids/terpenes [32].

Salkowski Test

To each 0.5 g of sample in a 20 mL test tube chloroform (1 ml) was added and to it 1 mL of concentrated H_2SO_4 was added down the test tube to form two phases. Formation of yellow coloration was taken as that which indicates the presence of sterols [32].

Test for flavonoids

Shinoda Test

To 1 g of the sample in 20 mL test tube was added methanol (5 mL). Also, to the sample was added three pieces of magnesium chips followed by few drops of concentrated HCl. A purple colour was observed which indicated the presence of flavonoids [31].

Sulfuric Acid Test

Little quantity of the extract was dissolved in 1 mL concentrated sulfuric acid and a colour change was observed which indicated the presence of flavonoids [32].

Lead Acetate Test

A small quantity of the extract was dissolved in water and filtered. Few drops of 10 % lead acetate were added to 5 mL of the filtrate. A buff colored precipitate indicated the presence of flavonoids [33].

Sodium Hydroxide Test

To 1 g sample in 100 mL beaker was added 10% aqueous sodium hydroxide solution (5 mL) and filtered to give yellow color, a change in color from yellow to colorless on addition of dilute HCl was observed which indicated the presence of flavonoids [34].

Test for Alkaloids

To the sample (0.5 g) in 20 mL test tube was added 5ml of 1% aqueous hydrochloric acid then stirred on a water bath and filtered. The filtrate (3 mL) was divided into three. To the first portion, three drops of freshly prepared Dragendoff's reagent was added and an orange to brownish precipitate was observed. To the second portion 1 drop of Mayer's reagent was added and yellowish color precipitate was observed. To the third portion 1 drop of Wagner's reagent was added to give a reddish- brown precipitate which indicated the presence of alkaloids [32].

Test for Phenolic Compounds and Tannins (Ferric Chloride Test)

To 1 g of sample in 20 mL test tube was added 5 mL of distilled water and boiled and the mixture was filtered. Two drops of ferric chloride were added to the filtrate, formation of green precipitate was observed which indicated the presence of tannins [35].

Test for Anthraquinones (Free Anthraquinones)

Small quantity of the extract was shaken with 10 mL of benzene, the content was filtered, and 5ml of

10% ammonia solution was added to the filtrate then, the mixture was shaken. No color change was observed in the ammoniacal layer (Lower phase) which indicated the presence of free anthraquinones [34].

Test for Saponins (Frothing Test)

About 0.1g of the extract was shaken with water in a test tube. Frothing was observed which persisted for 1 minute that indicated the presence of Saponins [32].

Test for Glycosides (Ferric chloride Test)

To small quantity of the extract 5ml of conc. H_2SO_4 was added and boiled for 15 min. This was then cooled and neutralized with 20% KOH. The solution was divided into two portions. Three drops of ferric chloride solution were added to one of the portions, and a green to black precipitate indicated phenolic glycone as a result of hydrolysis of glycoside [32].

Test for Cardiac glycoside (Kella-Killani Test)

To each sample (0.5 g) in 20 mL test tube was added glacial acetic acid (5 mL) containing traces of ferric chloride. The test tube was held at an angle of 45° and concentrated sulphuric acid (1 mL) was added carefully down the side. A purple ring color at the interface was observed which indicated the presence of cardiac glycoside.

Thin Layer Chromatography Profiling of the Extracts

Thin layer chromatography was carried out on TLC plastic sheet of silica gel pre-coated with layer thickness of 0.2 mm using various solvent system comprising hexane/ethyl acetate mixtures (99, 98, 97, 96, 95, 93, 91, 90, %).

Spotting and Development

Spots were applied manually using capillary tube; plates were dried using air blower and developed at room temperature using Shandon chromate tank.

Detection of Spots

Spots on TLC plates were visualized using destructive method by spraying with 10% sulphuric acid in methanol, followed by heating at 110°C for 1-2 min.

Results and Discussion

Results of extraction, phytochemical screening, free radical scavenging and TLC profiling of the leaves of Senna Occidentalis. Table 2

Discussion

From the above results, the weight of the crude extracts was found to be 33.20, 3.00, 12.40, 3.80, 24.80, (g) for hexane, diethyl ether, chloroform, ethyl acetate and methanol extract of *senna occidentalis* leaves with hexane having the highest mass using the aforementioned successive soxhlet extraction technique.

The preliminary phytochemical screening reveals the presence of anthraquinones (free anthraquinones and combined anthraquinones), carbohydrates, cardiac glycosides, glycosides, flavonoids, saponnins, steroids/ terpenes, phenolic compounds and tannins, and absence of alkaloids for various extracts of *senna occidentalis* using various tests. (Table 3). This may account for their various uses in ethno-botanical and traditional medicines.

Also, the TLC profiling was carried to know the solvent system that could possibly be used in the further

Table 2. Result of extraction

SN	SAMPLE	WEIGHT USED (g)	SOLVENTS USED	WEIGHT OF CRUDE EXTRACT (g)
1	*Senna Occidentalis*/Leaves	400	Hexane	33.20
			Diethyl Ether	3.00
			Chloroform	12.40
			Ethyl Acetate	3.80
			Methanol	24.80

Result of Phytochemical Screening of leaves of Senna Occidentalis.

Table 3. Preliminary phytochemical analysis of leaves extract obtained from S. occidentalis

Metabolites	**Test Used**	**Leaves Extract**				
		N-HX	DE	CF	EA	ME
Anthraquinones Free anthraquinones	General test	-	-	-	+	+
Combined anthraquinones		-	-	-	+	+
Alkaloids	Dragendoff's test	-	-	-	-	-
	Mayer's test	-	-	-	-	-
	Wagner's test	-	-	-	---	-
Carbohydrates	Molisch's test	+	-	-	-	+
Cardiac glycosides	Kella-Killani test	+	+	+	-	-
Glycosides	$FeCl_3$ test	-	-	-	-	+
Flavonoids	Shinoda test	-	-	--	-	+
	NaOH test	-	-	---		+
	Sulphuric acid test	-	-	-	-	+
	Lead acetate test	-	-	-	-	+
Saponnins	Frothing test	-	-	-	+	+
Steroids/ Terpenes	Liebermann-Buchard test	+	+	+	+	+
	Salkowski test	+	+	+	+	+
Phenolic compounds and tannins	$FeCl_3$ test	-	-	-	-	+

n-HX= n-Hexane, DE= Diethyl Ether, CF= Chloroform, EA= Ethyl Acetate, ME= Methanol

Table 4. Result of Thin Layer Chromatography (TLC) Profiling using solvent system comprising hexane/ethyl acetate mixtures (99, 98, 97, 96, 95, 93, 91, 90, %)

SN	SOLVENT	PLANT	SPOT	COLOUR 10% H_2SO_4
1	Hexane	*Senna Occidentalis*	1,2 & 3	Purple, Yellow & Dark green
2	Diethyl ether	*Senna Occidentalis*	1 & 2	Orange & Yellow
3	Chloroform	*Senna Occidentalis*	1,2,3 & 4	Green, Light blue, Yellow & Black
4	Ethyl acetate	*Senna Occidentalis*	1,2 & 3	Black, Yellow & Dark green
5	Methanol	*Senna Occidentalis*	1,2,3 & 4	Purple, Yellow, Dark red & Blue

isolation of the crude extract from the plants. Although, an ascending chromatography it gives an idea about the possible compounds that can be present in the extract from the number of spots (Table 4), and also, the possible solvent mixture that could be used for further isolation.

Conclusion

From the above premises it can be concluded that, after the extraction of the crude extracts, the leaves of *senna occidentalis* were found to be rich in phytochemicals. Also from relevant literatures, it clearly indicates that, the leaves extracts of *Senna occidentalis* houses some pure compounds whose structures can be further elucidated and be used for drug design. This means that the leaves would be useful as an antioxidant and free radical scavenging agent and also aids in the treatment of many diseases mediated by reactive oxygen species amongst others.

Recommendation

From the results obtained in this research, the following recommendations were made;

It is recommended that further research should be carried out to elucidate the structures of bioactive compounds responsible for and also, mechanism of action through which the extract exert the antipyretic and antioxidant activity.

Also, government agencies should educate the populace on the use of medicinal plants which serves as a reservoir for many antioxidants either as vegetables, tea, dietary supplements, etc.

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