PHYTOCHEMISTRY AND BIOACTIVITY STUDIES OF *Cassia singueana* Del. AND *C. sieberiana* DC. (FABACEAE)

SAIDU JIBRIL

UNIVERSITI TEKNOLOGI MALAYSIA

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SAIDU JIBRIL

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To my beloved father Alhaji Jibril Usman and my beloved mother Hajiya Rahmatu Mohammad

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ABSTRACT

Cassia singueana Del. and *C. sieberiana* DC. are medicinal plants used for treating diabetes, ulcer, malaria, and wound healing and also used as poison by hunters in Africa. This study discuss the phytochemicals and bioactivity of the root and leaf of *C. singueana* Del. and *C. sieberiana* DC. Extraction of the plant samples by maceration in *n*-hexane, ethyl acetate, and methanol sequentially, followed by fractionation and purification using various chromatographic techniques led to the isolation of twenty compounds. Structural elucidation of these compounds using spectroscopic methods enabled the identification of seven anthraquinones, four flavonoids, three stilbenes, three terpenes, one bianthrone, one xanthone, and a benzoic acid derivative. *C. singueana* root extract afforded islandicin, xanthorin, monodictyxanthone, 3-hydroxy-5-methoxystilbene, and 4-hydroxybenzoic acid, which were reported for the first time from *Cassia* genus, while *C. sieberiana* root extract gave a new compound, cassiberianol A. The efficiency of ultrasonic assisted extraction (UAE) was optimised using response surface methodology (RSM) for high extraction yield from the root of *C. singueana.* The Box-Behnken design (BBD) was employed to propose optimised UAE conditions of time (25 min), temperature (50ºC), and solvent-sample ratio (10 mL/g) for high extraction yield. The ability of the BBD model equation for predicting the optimum yield was verified and the predicted yield (1.64%) was in good agreement with the experimental yield (1.65 ± 0.07%). This agreement indicated the suitability of the model and also the success of using RSM in optimising UAE conditions for root of *C. singueana.* The extraction yield (1.65 ± 0.07%) obtained under the optimised UAE conditions resulted in two-fold improvement when compared to yield obtained by maceration (0.71 ± 0.60%) or Soxhlet extraction (0.79 ± 0.40%) techniques. The RSM was successfully used to optimise UAE conditions for improved efficiency of UAE over maceration and Soxhlet extraction techniques. Bioactivity screenings which include antioxidant, α-glucosidase, acetylcholinesterase, tyrosinase, and 15-lipoxygenase inhibitory activities were conducted on the crude extracts and pure compounds. The results revealed that the ethyl acetate extract from the root of *C. sieberiana* was the most potent DPPH radical scavenger (1.88 µg/mL), and also gave the highest total phenolic (927 mg gallic acid equivalent/g) and total flavonoid content (346 mg quercetin equivalent/g). The ethyl acetate extract from the root of *C. singueana* also displayed the strongest ferric reducing power (2298 µmol Fe^{2+}/g dry weight). Meanwhile, the methanol extract from the leaf of *C. singueana* demonstrated the most effective *β*-carotene bleaching activity (87.05%). Quercetin and piceatannol showed broad spectrum of inhibitory activities against α-glucosidase (IC_{50} 5.73 and 7.37 µM, respectively), acetylcholinesterase (IC_{50} 2.89 and 10.57 µM, respectively), tyrosinase (IC_{50} 92.40 and 95.14%, respectively), and 15-lipoxygenase (IC_{50} 0.98 and 1.27 µM, respectively) compared to the positive controls used in these assays. However, physcion highly suppressed the activity of tyrosinase enzyme (79.66%), while cassiberianol A showed significant inhibition (IC_{50} 2.63 µM) towards 15-lipoxygenase enzyme. The significant bioactivities demonstrated by the polar extracts from *C. singueana* and *C. sieberiana* can be attributed to the presence of phytochemicals such as flavonoids and stilbenes isolated from these polar extracts.

ABSTRAK

Cassia singueana Del. dan *C. sieberiana* DC. adalah tumbuhan ubatan yang digunakan untuk merawat kencing manis, ulser, malaria, dan penyembuhan luka dan juga digunakan sebagai racun oleh pemburu di Afrika. Kajian ini membincangkan fitokimia dan bioaktiviti daripada akar dan daun *C. singueana* Del. dan *C. sieberiana* DC. Pengekstrakan sampel tumbuhan secara rendaman dalam *n*-heksana, etil asetat, dan metanol secara berurutan, diikuti dengan pemeringkatan dan penulenan menggunakan pelbagai teknik kromatografi telah berjaya mengasingkan dua puluh sebatian. Penentuan struktur sebatian ini menggunakan kaedah spektroskopi membolehkan mengenalpasti tujuh antrakuinon, empat flavonoid, tiga stilbena, tiga terpena, satu biantron, satu xanton, dan satu terbitan asid benzoik. Ekstrak akar *C. singuena* memberikan islandisin, xantorin, monodiktixanton, 3-hidroksi-5-metoksistilbena, dan asid 4-hidroksibenzoik yang dilaporkan buat kali pertama daripada genus *Cassia*, manakala ekstrak akar *C. sieberiana* menghasilkan sebatian baharu, kassiberianol A. Kecekapan pengekstrakan berbantukan ultrasonik (UAE) telah dioptimumkan menggunakan metodologi permukaan gerak balas (RSM) untuk hasil pengekstrakan yang tinggi daripada akar *C. singueana*. Rekabentuk Box-Behnken (BBD) telah digunakan untuk mencadangkan keadaan UAE optimum bagi masa (25 min), suhu (50ºC), dan nisbah pelarut-sampel (10 mL/g) untuk hasil pengekstrakan yang tinggi. Kebolehan persamaan model BBD untuk meramalkan hasil optimum telah disahkan dan hasil yang diramalkan (1.64%) adalah setara dengan hasil eksperimen (1.65 ± 0.07%). Kesetaraan ini menunjukkan kesesuaian model dan juga kejayaan menggunakan RSM dalam mengoptimumkan keadaan UAE bagi akar *C. singueana*. Hasil pengekstrakan (1.65 ± 0.07%) yang diperolehi di bawah keadaan UAE optimum telah menghasilkan penambahbaikan dua kali ganda berbanding hasil daripada teknik rendaman (0.71 ± 0.60%) atau pengekstrakan Soxhlet (0.79 ± 0.40%). RSM telah berjaya digunakan untuk mengoptimumkan keadaan UAE untuk menambahbaik kecekapan UAE mengatasi teknik rendaman dan pengekstrakan Soxhlet. Pemeriksaan bioaktiviti termasuk aktiviti antioksidan, perencatan enzim α-glukosidase, asetilkolinesterase, tirosinase, dan 15-lipoksigenase telah dijalankan ke atas ekstrak mentah dan sebatian tulen. Keputusan menunjukkan bahawa ekstrak etil asetat daripada akar *C. sieberiana* adalah pemerangkapan radikal DPPH yang paling berpotensi (1.88 µg/mL) dan juga menghasilkan nilai tertinggi fenolik jumlah (927 mg setara asid galik/g), dan kandungan flavonoid jumlah (346 mg setara kuersetin/g). Ekstrak etil asetat daripada akar *C. singueana* juga memberikan kuasa penurunan ferik tertinggi (2298 µmol Fe^{2+}/g berat kering). Sementara itu, ekstrak metanol daripada daun *C. singueana* menunjukkan aktiviti pelunturan *β*-karotena yang paling berkesan (87.05%). Kuersetin dan piketanol menunjukkan aktiviti yang baik terhadap α-glukosidase (masing-masing pada IC_{50} 5.73 dan 7.37 µM), asetilkolinesterase (masing-masing pada IC_{50} 2.89 dan 10.57 µM), tirosinase (masing-masing pada IC_{50} 92.40 dan 95.14% µM), dan 15-lipoksigenase (masing-masing pada IC_{50} 0.98 dan 1.27 µM) berbanding dengan kawalan positif yang digunakan dalam cerakin ini. Walau bagaimanapun, fision sangat menindas aktiviti enzim tirosinase (79.66%), manakala kasiberianol A menunjukkan perencatan (IC_{50} 2.63 µM) yang signifikan terhadap enzim 15-lipoksigenase. Bioaktiviti signifikan yang dipamerkan oleh ekstrak berkutub daripada *C. singueana* dan *C. sieberiana* berpunca daripada kehadiran fitokimia seperti flavonoid dan stilbena yang diasingkan daripada ekstrak berkutub ini.

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LIST OF ABBREVIATIONS

α	-	Alpha
AA	-	Ascorbic acid
Abs	-	Absorbance
AChE	-	Acetylcholinesterase
ATR	-	Attenuated Total Reflectance
β	-	Beta
BHT	-	Butylated hydroxytoluene
br	-	Broad
c	-	Concentration
^{13}C	-	Carbon-13
CC	-	Column Chromatography
$CDCl_3$	-	Deuterated chloroform
$CHCl_3$	-	Chloroform
cm	-	Centimeter
cm^{-1}	-	Per centimeter
COSY	-	Correlation spectroscopy
1D	-	1 Dimension
2D	-	2 Dimension
δ	-	chemical shift
d	-	doublet

dd	-	doublet of doublets
DEPT	-	Distortionless Enhancement by Polarization Transfer
DMSO	-	Dimethyl sulfoxide
DPPH	-	2,2̀-Diphenyl-1-picrylhydrazyl
EIMS	-	Electron Ionization Mass Spectrometry
Et_2O	-	Diethyl ether
EtOAc	-	Ethyl acetate
GA	-	Gallic acid
Glc	-	Glucose
h	-	Hour(s)
n-Hex	-	Hexane
^1H	-	Proton
H_2O	-	Water
H_2SO4	-	Sulfuric acid
HCl	-	Hydrochloric acid
HMBC	-	Heteronuclear Multiple Bond Correlation
HMQC	-	Heteronuclear Multiple Quantum Coherence
HRAPCIMS	-	High Resolution Atmospheric Pressure Chemical Ionization Mass Spectrometry
Hz	-	Hertz
IR	-	Infrared
IC	-	Inhibition concentration
J	-	Coupling constant
KBr	-	Potassium bromide
L	-	Liter

lit.	-	Literature
LOX	-	Lipoxygenase
λ	-	Lambda
m	-	multiplet
M^+	-	Molecular ion
MeOH	-	Methanol
MHz	-	Megahertz
min	-	Minute(s)
m/z	-	Mass to charge ion
mg	-	milligram
m.p.	-	Melting point
mL	-	milliliter
mm	-	millimeter
MS	-	Mass Spectrometer
NaOH	-	Sodium hydroxide
NMR	-	Nuclear Magnetic Resonance
nm	-	nanometer
PTLC	-	Preparative Thin Layer Chromatography
R_f	-	Retention factor
Rha	-	Rhamnose
s	-	singlet
SD	-	Standard deviation
SiO_2	-	Silica gel
t	-	triplet
TLC	-	Thin Layer Chromatography

TPC	-	Total phenolic content
TFC	-	Total flavonoid content
μM	-	Micro molar
UV	-	Ultraviolet
VLC	-	Vacuum Liquid Chromatography

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CHAPTER 1

INTRODUCTION

1.1 Preamble

Medicinal plants have provided the modern medicine with a lot of plant-derived therapeutic agents. For example, the drug morphine used as an analgesic agent was from *Papaver somniferum*; quinine as an antimalarial drug has its source from *Cinchona ledgeriana*, and atropine an anticholinergic was isolated from *Atropa belladonna* [1]. Similarly, special materials such as cosmetics, dyes, colorants and biocides have also been obtained from plant sources [2]. Furthermore, the industrial use of herbal plants has led to new concepts such as nutraceuticals, cosmeceuticals and phytopharmaceuticals, hence widening the scope of medicinal plants utilisation. Plants are known to contain phytochemicals which find great applications in the field of agriculture, human and animal medicine. Through natural product studies, it has been established that this phytochemicals are responsible for the therapeutic properties of plants [2]. Modern scientific techniques have led to isolation and identification of thousands of phytochemicals, many of which had served as chemical leads for the development of chemotherapeutic drugs against several diseases [3].

The outbreak of ebola and zika diseases in some parts of the world; the bird flu disease that suddenly attack epileptically and the current trend of microorganism resistance to antimicrobial drugs are some of the major challenging health hazards in humans and animals today. These diseases are caused by pathogenic bacteria, virus and fungi. However, in recent years, various human pathogens have been reported to acquire resistance towards the common drugs as a result of climate change [4] and also due to misuse of medication by some individuals, which has rendered several antibiotics and other life-saving drugs inefficient, hence there is an urgent need to search for new and effective drugs.

The issue of health care should be tackled from a holistic point of view. Going through the WHO statistics, 80% of the population of Asian and African are relying on traditional medicine [1]. It is obvious that one cannot separate an individual from his or her environment, tradition and culture, thus the use of medicinal plants as alternative or complementary medicine in health care system is of enormous importance [5]. Almost half of the flowering plant species in the world are habitant of tropical forest. The natural product chemists continue to identify the novel compounds which serves as a starting material for the development of new drugs from these plants [6]. However, with urbanization, many of these medicinal plants are gradually getting extinct in the wild hence, the urgent need to screen medicinal plants ethnopharmacologically for bioactive compounds which will serve as lead chemicals/drugs for immediate usage and future synthesis of potential drugs.

National health care system (primary and secondary) are recognised universally for effective health care delivery. Moreover, the importance of traditional medicine as an alternative, in the primary health care system cannot be overemphasised [2]. Health care practice involving herbal medicine has undergone radical transformations in most countries such as China, Japan, India, Thailand, and Korea [7]. Furthermore, plant based system is always playing a vital role in health care delivery all over the world [7]. Plants from the same family usually contain similar types of compounds and therefore, may possess similar beneficial or toxic effect [8]. Local usage of plants medicinally, can provide vital information for proper pharmacological investigation of a lesser-known plant [9].

1.2 Ethnopharmacological Study

Drug discovery through the methods of combinatorial chemistry, molecular modelling and synthetic chemistry have attracted attention [10]. However, natural product derived compounds as a source of medicine remains indispensable in the search for safe and effective drug. The use of plant secondary metabolites as main drugs, precursor, pharmacological probe and template for semisynthetic drug modification cannot be underestimated. The selection of plant material for its biological activity screening can be based on ethnopharmacology or chemotaxonomy of the plant. The ethnopharmacology information of the plant is obtained from existing knowledge of the particular healing properties of the plant. This existing knowledge is usually handed down from generation to generation among traditional herbalists. Hence, the desperate need to screen existing medicinal plants, due to the extinction of most medicinal plants, coupled with the ethical obligation of preserving and conservation of traditional medicine knowledge to avoid complete disappearance of indigenous knowledge. Almost 10,000 plants species are recognised for their traditional medicinal values among which *Cassia* species is one [11].

1.3 Extraction of Medicinal plants

Medicinal plants contain a wide range of bioactive compounds which include phytochemicals used in the pharmaceutical, cosmetics, and food industries [12]. The quality and quantity of these phytochemicals are subject to their extraction process. The conventional methods such as maceration and Soxhlet extraction of medicinal plants requires large volume of solvent, longer extraction time and lack proper agitation to enhance effective extraction process [13].

Alternative modern extraction techniques that uses ultrasound, microwave or supercritical fluids for effective extraction of phytochemicals from medicinal plants has been developed [13]. Ultrasonic assisted extraction (UAE) technique uses sound waves to create cavitation which can break the cell wall of plants through an increase in the kinetic energy of extraction solvent and sample. The microwave assisted extraction (MAE) technique uses microwave to deliver energy to the extraction solvent and matrix with subsequent heating of the solvent and sample. Supercritical fluid extraction (SFE) method is achieved when the pressure and temperature of the extraction solvent such as carbon (IV) oxide is raised above its critical value [12]. The UAE, MAE and SFE techniques requires less volume of extraction solvent compared to the conventional methods, maceration and Soxhlet extraction techniques. However, the efficiency of MAE and SFE is limited by the choice of extraction solvents. Meanwhile, the UAE apparatus is cheaper and easy to operate. Furthermore, the UAE like the maceration and Soxhlet extraction techniques can be used with variety of solvent suitable for the extraction of a wide range of phytochemicals from medicinal plants [12]. The time, energy and cost of getting the pure compounds for biological screening from medicinal plants are determined by the extraction and isolation procedure [14]. Therefore, the use of response surface methodology (RSM) technique to develop an effective UAE protocol for the extraction of medicinal plants such as *Cassia* species will reduce the number of years it will take for discovery of effective, safe and less costly drugs from medicinal plants.

1.4 The Fabaceae Family

Fabaceae, also known as the legume, pea or bean family, is the third largest of the angiosperm family after Orchidaceae (Orchids) and Asteraceae (Sunflowers). In terms of its importance in agriculture and economics, it is second to Poaceae, the family of grasses. Members of Fabaceae family, range from annual and perennial herbs to shrubs, vines, trees and few aquatic plants [15]. The distributions of Fabaceae cut across tropical and temperate regions of the world and even aquatic region. Fabaceae species usually have simple to compound leaves, regular to irregular flowers, bisexual, with fruit bearing typically one chamber pod. The family, Fabaceae consist of 39 tribes; 727 genera and 19,237 species. It is divided into three subfamilies, Caesalpinioideae, Mimosoideae and Papilionoideae. The subfamilies are further distributed within the 39 tribes; 30 tribes in Papilionoideae, 5 in Mimosoideae and 4 in Caesalpinioideae. These subfamilies are differentiated by their flowers. The

Papilionoideae which is the largest of the subfamily are mainly the beans, the Mimosoideae include the group of *Acacia*, while the Caesalpinioideae consist of diverse group with 162 genera and 3,000 species among which is *Cassia* [16].

Members of Fabaceae are source of food to both human and farm animals. The beans, peas, peanuts and soyabean serve as food to human. Some species such as clover (*Trifolium repens*) and lupin (*Lupinus* spp.) are grown for animal feed and fertilizer respectively. The Fabaceae also helps in improving soil fertility through nitrogen fixation with the help of some special nitrogen fixing bacteria that lives in their root nodules. Valuable products such as wattle bark which is used in tanning are produced from *Acacia* species. Some members of the Mimosoideae subfamily are source of timber, gums and resins. The dye, indigo is produced from the species of Indigofera (*Baptisia australis* and *Baptisia tinctoria*). Other members of Fabaceae (*Erythrina spp* and *Abrus precatorius*) are ornamental, hence they are planted for landscaping and beautification. The Fabaceae family has been found to produce secondary metabolites that can prevent against human cancer, reduce blood cholesterol and prevent rise in blood glucose level [17]. This activity was also related to the presence of flavonoids, terpenes, coumarins and other polyphenolic compounds present in these plants [18].

1.5 The genus *Cassia*

Cassia is a large genus of the Fabaceae family with about 600 species, which are usually trees, shrubs or herbs. The leaves are in pinnate with opposite paired leaflets [19]. They produce numerous flowers and are cultivated for ornament and shade. They are widely distributed in the tropics and sub-tropical region. It is found in Asia, Africa, America and Brazil [20]. There are about 22 species of *Cassia* originally found in West Africa [21]. *Cassia* species are often use as fish poison in Africa. *Cassia sieberiana* and *C. italic* are use as ingredient in arrow poison in Niger, while *C. singueana* and *C. occidentalis* are also use as ingredient in poison by hunters in Kenya and Cameroon [22]. *Cassia sieberiana*, *C. alata*, *C. glauca*, *C. fruticose*, *C. siamea*, *C. tomentosa*, *C. skinner*, *Senna obtusifolia* and *S. mellitu* are prominent plants traditionally used in the treatment of diabetes mellitus [23]. This genus is commonly known as *Cassia* or *Senna* among traditional medicine system and the taxonomical classification is shown below [19];

Kingdom:	Plantae
Order:	Fabales
Family:	Fabaceae
Subfamily:	Caesalpinioideae
Tribe:	Cassieae
Subtribe:	Cassiinae
Genus:	*Cassia*

The medicinal values of various parts of *Cassia* are widely recognised across different countries of the world. Many international pharmacopoeias such as Potter's new Encyclopaedia of Botanical Drugs and Preparations, Thai National List of Essential Drug, Indian Herbal Pharmacopoeia [24] and British Herbal Pharmacopoeia [25], have included *Cassia* in their archive. The pharmacopoeia of India has mentioned the leaves of *C. alata*, been used as an effective ointment [24]. The pod of some species enclosed a pulp which contain purgative glycoside and it is used as laxative. *Cassia* species have wide applications in traditional medicine. They are used in the treatment of skin infection, such as eczema, ringworm and scabies. Also in the treatment of wound, rheumatism, diabetes, jaundice, fever, ulcer, gonorrhoea and gastrointestinal disorder [26]. The medicinal uses of some species of *Cassia* from different parts of the world is shown in **Table 1.1**.

Table 1.1: Parts of the plant and its traditional uses

Name of the plant species	**Part of the plant used/ Country**	**Traditional uses**
C. tora	Seeds (China)	Used as a vision improving, aperient, antiasthenic and diuretic agent. Also used in reducing blood pressure and lowering cholesterol [27].
C. siamea	Whole plant (Sir Lanka)	Used to treat fever, diabetes, insomnia, hypertension and constipation [28].

C. sieberiana	Root (Sierra Leone)	Used in the treatment of gonorrhoea, fever, schistosomiasis, dysentery, diarrhoea, elephantiasis, intestinal parasite, tapeworm, haemorrhoids [29].
C. occidentalis	Leaf (Nigeria)	For treatment as antimalarial and antipyretic [30].
C. obtusifolia	Seeds (China)	Popular in the treatment of diabetes and also for reducing serum level of fat and cholesterol [31].
C. alata	Leaf (Malaysia)	Used in the treatment of skin infections [32].
S. italic	Roots and leaf (Namibia)	Fever, digestive disorders and to free the placenta [33].
S. hirsute	Leaf (Gabon)	Hepatic diseases, coughs, psoriasis, eczema, constipation, as sedatives and analgesics [33].
S. petersianna	Root (South Africa)	Used as a purgative for treating stomach-ache, gonorrhoea, epilepsy and intestinal worm [34].
C. singueana	Leaf and root (Nigeria)	Malaria, conjunctivitis, convulsions, gonorrhoea, bilharzias, stomach-aches, constipation, Epilepsy, syphilis, heartburn, purgative, stomach troubles, and fever [35].
C. sophera	Leaf (India)	As antidote for snake bite [36].
C. nigrican	Leaf (Senegal)	For treatment of malarial and also to protect grain from insects [37].
C. noname	Aerial part (Japan)	As a diuretic agent and tonic in traditional medicine preparation [38].
S. timoriensis	(Thailand)	For treatment of cough, blood stasis, menstrual disorder and as tonic [39].

1.5.1 *Cassia singueana*

Cassia singueana Delile syn *Cassia goratensis* (Fresen) is commonly known as winter *Cassia* [40]. It is a shrub or small tree of about 15 m high with conspicuous yellow flower and dark grey bark. The leaflets are 5-12 pairs and 13-30 cm long. The fruits are cylindrical with the beak sharp towards the apex. The seeds are flat and dark brown [22]. *C. singueana* has numerous medicinal values across Africa. The leaf juice is used to treat malaria, syphilis, ulcer, pneumonia, snake bite and eye infection. Decoction of the root bark is drunk against mental disorder, swollen breast, fever, hernia, abdominal pain, convulsion, and gonorrhoea, bilharziosis, and women infertility, painful uterus, constipation, anti-emetic, painful menstruation and to prevent still birth [41].

Previous studies have identified the antioxidant, anti-ulcer, antiplasmodial, antipyretic [42], and cytotoxic [43] property of *C. singueana*. The aqueous acidic extract of the whole plant showed presence of flavonoids. The root was reported to contain anthraquinones and terpenes [44].

Fig 1.1: *Cassia singueana* plant

1.5.2 *Cassia sieberiana*

Cassia sieberiana DC. syn *Cassia kotschyana* (Oliver), commonly known as West Africa laburnum, Africa laburnum, or drumstick tree [41]. It is a shrub or small tree of about 7-15 m high with bright yellow flowers, long cylindrical (75 cm), and narrow fruits of about 1-2 cm thick. The bark is blackish and fissure. The fruit contain light brown seeds. The leaves are 20-30 cm long with leaflets 4-9 pairs arranged opposite to each other [22]. The leaves, root and pods are widely utilised in traditional medicine. The whole plant is purgative and has diuretic property. *Cassia sieberiana* is used in the treatment of various diseases in children. The powdered form of different part of *C. sieberiana* is applied to affected site to cure toothache, burn and skin diseases. Decoction of various part of the plant is used to treat stomach ache, diabetes, head ache, ulcer, diarrhoea, gonorrhoea, haemorrhoids, leprosy, dysentery, sterility, malaria, rheumatism, general body pain, inflammation conditions, and venereal diseases [45, 46]. The seed and root are used as fish poison in Nigeria [22]. The leaves of *C. sieberiana* was reported to contain flavone, anthraquinone and tannins while the root contain anthraquinone, tannins and sterol [41].

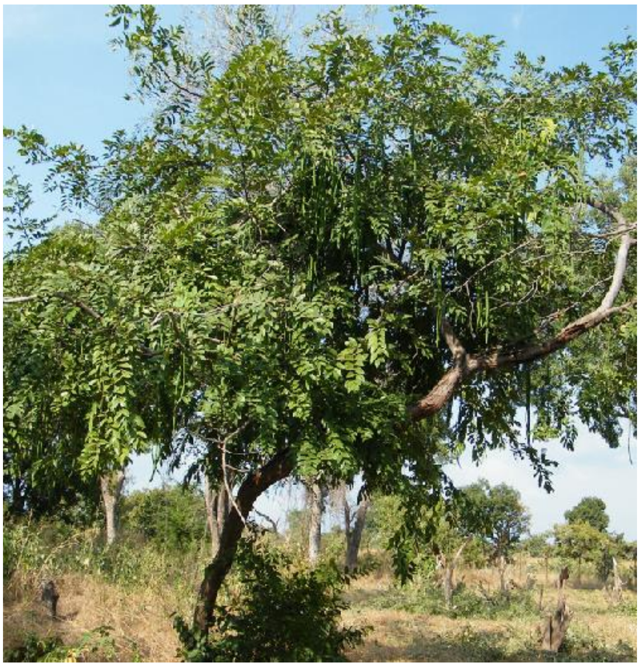

Fig. 1.2: *Cassia sieberiana* plant

1.6 Problem Statement

Cassia species has been mentioned officially in the Pharmacopoeia of many countries such as Philippines, India and Thailand. Some *Cassia* species (e.g. *C. alata*, *C. siamea*, and *C. auriculata*) which are introduced into many countries have been naturalised in these countries [24]. This naturalisation is as a result of the species numerous significance to man, animal and environment. *Cassia singueana* and *C. sieberiana* are used in the treatment of diabetes, ulcer, malaria and wound healing by traditional herbalist [22]. However, despite the numerous applications of *C. singueana* and *C. sieberiana* by traditional herbalist across Africa, the phytochemical constituents in these species are still less studied compared to their congeners.

The conventional plant extraction technique such as maceration and Soxhlet extraction consume large volume of solvent, require longer extraction time and lack effective agitation ability for efficient extraction of plant constituents [13]. Meanwhile, modern extraction technique such as ultrasonic assisted extraction (UAE), microwave assisted extraction (MAE) and supercritical fluid extraction (SFE) utilises less amount of solvent and extracts phytochemical constituents within a shorter time. However, variety of extraction solvents are suitable for UAE compared to MAE and SFE techniques. Furthermore, the one variable at-a-time (OVAT) method of optimising an extraction process can only investigate one extraction parameter at a time. Nevertheless, the response surface methodology (RSM) can be used to improve the extraction yield of constituents by systematically analysing individual extraction parameter and their interactions simultaneously to give the optimum extraction condition. The use of UAE technique has not been reported for the extraction of *C. singueana* and *C. sieberiana*.

The interrelationship between pharmacology and toxicology is very important because, therapeutic efficacy occur usually at a lower dose, whereas an overdose can cause severe side effect or induce poisoning. More so, toxic plants may contain some active compounds that display important pharmacological effects [47]. Furthermore, oxidative stress has been implicated in causing several diseases which include diabetes, ulcer and malaria. Although, *C. singueana* and *C. sieberiana,* are used in the treatment of various diseases and as poison by hunters, however scientific reports on the bioactivity of *C. singueana* and *C. sieberiana* is scanty, hence more attention on the extraction protocol, phytochemical and bioactivity screening of these two *Cassia* species are required.

1.7 Objectives of Research

The objectives of this study are;

1. To extract, isolate, purify and identify phytochemicals from *C. singueana* and *C. sieberiana.*
2. To develop and validate RSM model on ultrasonic assisted extraction (UAE) using RSM software to achieve high extraction yield from *C. singueana.*
3. To screen the crude extracts and pure compounds from *C. singueana* and *C. sieberiana* for bioactivity such as antioxidant, anti-tyrosinase, α-glucosidase, acetylcholinesterase, and anti-inflammatory activities.

1.8 Significance of Research

The outbreak of new diseases and the resistance by microorganisms to current drugs has led to a call by WHO into the investigation of medicinal plants. Also, the issue of climate change as it affects these medicinal plants, coupled with the obligation of conservation/preservation of the traditional medicine knowledge are clear facts that require urgent need to screen medicinal plants. The isolation and identification of potential bioactive compounds from *C. singueana* and C. *sieberiana* will add value to the drug discovery library. The comparative study between conventional (maceration, Soxhlet) extraction technique and ultrasonic extraction (UAE) technique, will add to the body of knowledge on the extraction protocol for *Cassia* species. Furthermore, application of response surface methodology (RSM) study will pave way for cost effective process, in extraction and isolation of bioactive compounds from *C. singueana* and *C. sieberiana*. These compounds might serve as probe, drug or semisynthetic drug for immediate or future use in developing an effective, safe and less costly drug.

Traditional medicine preparation in Africa uses parts of *C. singueana* and *C. sieberiana* for the treatment of diabetes, ulcer, malaria, skin cancer and for wound healing. Oxidative stress has been implicated in several human diseases such as diabetes, ulcer, malaria and cancer. Therefore, the bioactivity screening of the crude extracts and pure compounds from *C. singueana* and *C. sieberiana* will scientifically ascertain the medicinal uses of these two *Cassia* species as claimed by the traditional herbalists.

1.9 Scope of Research

This study investigates the phytochemicals in *C. singueana* and *C. sieberiana* plants. The air-dried and powdered root and leaf of both plant species were subjected to extraction with *n*-hexane, ethyl acetate (EtOAc) and methanol (MeOH) sequentially in the order of increasing polarity using maceration technique. The solvents were evaporated in *vacuo* to afford the respective crude extracts. The UAE conditions which include extraction time, temperature and solvent to sample ratio were optimized for high extraction yield using RSM technique. The crude extracts were fractionated and purified by chromatographic techniques such as vacuum liquid chromatography (VLC) over silica gel, column chromatography (CC) over silica gel, sephadex LH-20, MCI-gel and recycling preparative HPLC to yield pure compounds. The structure of all the pure compounds were elucidated using combined spectroscopic methods which include, NMR, IR, UV, MS and X-ray crystallography. The melting points of all the pure compounds were also determined. Bioactivity evaluation which include antioxidant properties, α-glucosidase, tyrosinase, acetylcholinesterase, 15-lipoxygenase enzyme inhibitory activities were carried out on the crude extracts and pure compounds.

1.10 Structure of the Thesis

This thesis has 7 chapters and the content of each chapter is described as follows:

Chapter 1	This chapter gives some background information on medicinal plants, drug discovery and drug resistance.
Chapter 2	A review on the phytochemicals from *Cassia* species and a skim through the bioactivity of *Cassia* genus is described in this chapter.
Chapter 3	Description of the RSM model for high extraction yield from *C. singueana* and comparison between conventional extraction techniques and UAE method were the highlights of this chapter.
Chapter 4	This chapter discuss findings from the investigation into phytochemicals from *C. singueana* and *C. sieberiana.*
Chapter 5	The bioactivity screening on crude extracts and pure compounds isolated from *C. singueana* and *C. sieberiana* are described.
Chapter 6	This chapter describes the materials and methods employed in this study.
Chapter 7	Synopsis on the findings from this study and recommendations are discussed in this chapter.

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