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Taxonomical, Chemical Characterization and Biological Activity of *Cassia* and *Senna* Species

التصنيف والتوصيف الكيميائي والنشاط البيولوجي لأنواع الكاسيا والسنا

A Thesis Submitted in Fulfilment of the Requirements for M.Sc Degree in (Botany)

By

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Dedication

To my parents,

Brothers and sisters,

Nephews,

Teachers and

Friends

Acknowledgement

Braise to Allah Almighty who guided me to the straight path in my life.

I would like to express my appreciation to my supervisor Professor/ Sakina Mohammed Ahmed Yagi- Department of Botany, Faculty of Science, University of Khartoum, who has cheerfully answered my queries, provided me with materials, checked my experiments, assisted me in a myriad ways with writing and helpfully commented on earlier draft of project.

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ABSTRACT

Several species within the genera *Cassia* or *Senna* have a treasure of traditional medicines worldwide. The objective of the present study was to study the morphological characteristics of *C. fistula, C. grandis, S. alexandrina* and *S. italic* and evaluate, *in vitro,* antimicrobial and antioxidant activities of their crude leaf extracts. In addition, phytochemical screening of secondary metabolites and the total polyphenolic, flavonoids and tannins contents of these extracts were determined. Extracts from each plant were prepared by sequential maceration of dried leaf powder in solvents of increasing polarity. The antimicrobial activity was evaluated against Gram positive and Gram negative as well as two fungi by disc diffusion method. Antioxidant activity was assessed based on the scavenging activity of the stable 2,2-Diphenyl- 1-picrylhydrazyl free radical. Phytochemical screening was performed by thin layer chromatography technique. Total polyphenolic, flavonoids and tannins contents were determined by spectrophotometric methods. Taxonomically the *Cassia* spp. and *Senna* spp were separated from each other by the filaments form and the presence or absence of bracteoles.

Results of antimicrobial activity showed that extracts of the four plants exhibited better antifungal activity than antibacterial one with highest antifungal activity against *C. albicans* (28 mm) and *A. niger* (27 mm) was recorded from the ethyl acetate extract of *C. fistula*. The highest antibacterial activity against *Staphylococcus aureus* (18 mm) and *Pseudomonas aeruginosa* (18 mm) was exerted by the methanolic extract of *C. grandis* and chloroform extract of *S. alexandrina* respectively. *Bacillus subtiles* and *Escherichia coli* were less sensitive to all tested extracts. The highest radical scavenging activity was obtained from the ethyl acetate extract of *C. fistula* (77%) and two polar extracts of *C. grandis* (71%). Phytochemical screening showed that extracts were mainly rich in phenolic compounds. Total polyphenolic were mainly accumulated in the

ethyl acetate (136.8 – 277 mg gallic acid equivalent (GAE)/g) and methanolic (20.8 - 108.8 mg GAE/g) extracts while the majority of extracts had higher flavonoids content (17.66 - 618.66 mg quercetin equivalent/g) than their respective polyphenolic content. All extracts except methanolic extract of *C. grandis* and *S. alexandrina* were devoid of tannins. In conclusion, these plants could be a very beneficial source of natural bioactive agents.

المستخلص

العديد من االنواع داخل أجناس الكاسيا أو السنا لديها ذخيره من الطب التقليدي في جميع انحاء العالم.الهدف من هذه الدراسة هو دراسة الخصائص المورفولوجيه لكل من

Senna alexandrina ,Cassia grandis ,Cassia fistula و *Senna italica*. وتقييم االنشطه المضاده للميكروبات ومضادات أألكسده في المختبر لخام مستخلصات االوراق. باإلضافه إلى ذلك تم تحديد الفحص الكيميائي النباتي للمنتجات الثانويه والفالفونويد والتانينات لهذه المستخلصات. المستخلصات من أي نبات تم تحضيرها عن طريق النقع المتسلسل لمسحوق أألوراق المجفف في المذيبات ذات القطبيه المتزايده. النشاط المضاد للميكروبات تم تقييمه مقابل بكتريا موجبة غرام وبكتريا سالبة غرام وكذلك إثنين من الفطريات من قبل طريقة نشر القرص. نشاط مضادات أألكسده تم تقييمه بناء على الكسح للمستقر 2,2-Diphenyl- 1-picrylhydrazyl. الفحص الكيميائي تم إجراءه بواسطة تقنية كروماتوغرافيا الطبقه الرقيقه. تم تحديدمجموع محتويات البولیفینول والفالفونويد والتانينات بواسطة الطرق الطيفيه.تصنيفيا" ال *Cassi*a وال *Senna* فصلت عن بعضها البعض عن طريق شكل الخيوط ووجود أو عدم وجود الفقاعات. نتائج النشاط المضاد للميكروبات عرضت أن المستخلصات من االربعه نباتات

اظهرت نشاطا" مضادا" للفطريات أفضل من النشاط المضاد للبكتريا واحد مع أعلى نشاط مضاد للبكتريا ضد*Candida albicans* (28mm) وضد ال *Aspergills niger* سجل في مستخلص اإليثايل اسيتيت لنبات C. fistula (72 مم).أعلى نشاط مضاد للبكتريا ضد *Staphylococcus aureus* (18mm) و *Pseudomonas aeruginosa* (18mm) ظهر في مستخلص الميثانول ل *C. grandis* ومستخلص الكلورفورم ل *S. alexandrina* على التوالي. ال*Bacillus subtiles* وال*Escherichia coli* كانت أقل حساسيه لكل إختبارات المستخلصات. أعلى نشاط كسح جذري تم الحصول عليه فيه مستخلص اإليثايل أسيتيت ل*C. fistula* (77%) وفي المستخلصات القطبيه ل *C. grandis* (71%). الفحص الكيميائي أظهر ان المستخلصات غنيه بصوره رئيسيه بالمركبات الفينوليه. مجموع البولیفینوالت مقاسا" في مستخلص اإليثايل أسيتيت (136.8 - 277 mg gallic acid) مكافئ ل (GAE)/g) و) 108.8 mg gallic acid -20.8 (لمستخلصات الميثانول بينما غالبية المستخلصات لها محتوى فالفونويد أعلى) 66

-618.mg quercetin 17.66(من محتوى الفينوالت .كل المستخلصات عدا مستخلص الميثانول ل *C. grandis* و *S. Alexandrina* خالية من التانينات. في الختام هذه النباتات يمكن أن تكون مصدرا" مفيدا" جدا" للعوامل الحيويه النشطه الطبيعيه.

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Chapter One

1. Introduction and Literature Review

1.1 Introduction

1.1.1 General introduction

Fabaceae or Leguminosae, also called as bean, pea, or legume family is the major family of genus *Cassia* and the third largest family of flowering plants on earth. This family comprises many shrubs, trees, and other herbaceous plants, which are primarily differentiated by their stipulate, compound leaves along with their fruit (legumes). It includes almost 751 genera and about 19,000 different species, which are widely distributed in the dry forests of Africa, America, and tropical rainforests (Christenhusz and Byng, 2016). Furthermore, the Fabaceae family has three sub-families, namely Caesalpinioideae, Faboideae or Papilionaceae, and Mimosoideae. Caesalpinioideae is the sub-family of genus *Cassia* which is also called the peacock sub-family. It comprises about 150 genera and 2500 species, which are mainly tropical in distribution and contain a variety of ornamental plants (Doyle, 2001). The presence of extra floral nectaries on the upper side of petiole, mainly between the leaflet pairs and pinnae is one of the distinguishable characteristics of this sub-family species (Azani *et al*., 2017).

Cassia, with approximately 500 species, is a large genus of the family Fabaceae (Lodha et al., 2010). *Cassia* species are used enormously for the treatment of many diseases in the traditional system of medicines. The synergistic mechanism behind the chemical substances of *Cassia* species makes them more beneficial. In the folk medicinal history, these plants are used as a laxative and purgative agent (Deshpande and Bhalsing, 2013). They exhibited pharmacological activities such as antiinflammatory, antitumor, antiplasmodial, antioxidant, hypoglycemic, hyperglycemic and antimutagenic. They are also used to treat wounds, skin infections including eczema, scabies and ringworm, jaundice, anorexia,

rheumatism, and gastrointestinal problems. According to the "The Plant List" (TPL, 2013), about 1854 names of various species are recorded for genus *Cassia*. It has been found that some species of *Cassia* are difficult to determine due to the presence of morphological complexes. Accordingly, this genus was segregated by some workers into three allied genera, although some authors still place all species in a single genus, i.e. *Cassia* L. sensu lato.

In recent years, the extensive uses of antibiotics in agriculture, veterinary, and clinical medicines lead to the development of multidrug-resistant bacterial and fungal strains and ultimately contribute to the recurrence of infectious maladies. This situation has created a need to find more effective antimicrobial agents. Natural products from microorganisms and plants have been the primary source of antibiotics, and with the increasing acceptance of herbal medicines, the screening of medicinal plants for new active compounds has become a very important source to discover new lead antibiotic molecules (Nkuete and Kuete, 2013).

Oxidative stress, the foremost reason for the majority of disorders and diseases are due to free radicals. Antioxidants are the substances which provide a defense mechanism against the damaging effects of these free radicals and serve as radical scavengers, suppressing lipid peroxidation and preventing human organs from multiple degenerative pathologies attributed to radial reactions such as cancer, cardiovascular, atherosclerosis, aging and diabetes among others. It was also reported that synthetic antioxidants were the cause of carcinogenesis and liver damage in laboratory animals. Thus there is a need to explore and develop antioxidants of natural origin with greater efficacy and fewer side effects. Extensive researches on medicinal plants have also indicated that they are good sources of natural biologically active molecules (Roy and Dutta, 2021).

1.1.2 Research problem

Over the past few decades, the misuse and overuse of antibiotics has contributed to the development and rapid spread of multidrug-resistant bacteria, leading to global threat due to the increased therapeutic failures (Nkuete and Kuete, 2013). Synthetic antioxidants are largely employed as preservatives by pharmaceutical, cosmetic and food industries, even if they are suspected of being responsible for liver damage and carcinogenesis in laboratory animals. There is a need to replace synthetic antioxidants with natural and safe ones.

1.1.3 Research hypothesis

Cassia and *Senna* species grown in Sudan, possess promising phytochemical constituents with interesting biological activities.

1.1.4 Objectives of the study

The objective of the present study was:

- To study the morphological characteristics of *Cassia fistula, C. grandis, Senna alexandrina* and *S. italica.*
- To evaluate the *in vitro* antimicrobial activity of their crude leaf extracts.
- To evaluate their antioxidant activity.
- To screen the presence of major secondary metabolites of these extracts.
- To determine the total polyphenolic, flavonoids and tannins contents in the different extracts.

1.2 Literature review

Cassia species have been well known for their laxative and purgative purposes. *Cassia* invites attention of researches worldwide for its phytochemistry and pharmacological activities ranging from anti-diabetic, antioxidant, antibacterial, antifungal, to antiviral. *Cassia* is a large genus of around 5000 species of flowering plants in the family Leguminaceae/ Fabaceae (Lavanya *et al.*, 2018).

Taxonomically, it has been found that some species are difficult to determine due to the presence of morphological complexes. Accordingly, this genus was segregated by some workers into three allied genera, namely *Cassia* L. Sensu stricto, *Chamaecrista* Moench and *Senna* miller. However, some authors still place all species in a single genus, i.e. *Cassia* L. Sensu lato (pechsri and boonkerd, 2003). General classification of Cassia is as follows:

Kingdom: Plantae

Class: Eudicots

Order: Fabales

Family: Fabaceae

Subfamily: Caesalpinioideae

Genus: *Cassia*.L/ *Senna* or *Chamaecrista* (Sundaramoorthy *et al.*, 2016).

1.2.1 Revision of the taxonomy of genus *Cassia*

An outstanding revision to the genus *Cassia* L. was made by Bentham (1871). Subsequently, De Wit (1955), Brenan (1958a, 1958b and 1967), Symon (1966), Ali and Quraishi (1967) and Isely (1974) revised the genus *Cassia* L. as according in Malaysia, Africa, Australia, Pakistan, and United states of America, respectively. Irwin and Barneby (1978) have also added to the taxonomy of *Cassia* L. found in America. Then, Irwin and Barneby (1981 and 1982) have

raised the genus *Cassia* L. to the level of subtribe and elevate previous subgenera to generic rank, viz. *Senna* Mill. and *Chamaecrista* Moench, in addition to *Cassia*, under the tribe Cassieae Bronn ex Irwin and Barneby of Caeslpinioideae. Their work was bases mainly on the characteristics of filaments and the presence or absence of bracteoles. They recognized the suits of characters successfully for the delimitation of subgroups which persist all over the world. Their concept has also been followed by lock (1988 and 1989), Randell (1988, 1989 and 1990) and Larsen and Hou (1996) in their works on the revision of subtribe Cassiinae Irwin and Barneby from Africa, Australia and Malaysia, respectively (Singh, 2001).

Many subsequent authors support this separation of genera with additional attributes, for example, Tucker (1996) found that distinctions in floral ontogeny (floral position in the inflorescence), presence of bracteoles, position of the first sepal initiation, order of petal initiation, asymmetric initiation, anther morphology, and time of carpel initiation support the segregation of genera.

Pechsri and Boonkerd (2003) made a numerical taxonomic study in *Cassia* species growing in Thailand. Their findings support the segregation of genera proposed earlier by Irwin and Barneby (1981). The most important characters they used to separate the three groups are filaments length, fruit length, and ovary stalk length.

1.2.2 A review of *Cassia* species in the Sudan

In Sudan the genus *Cassia* L. is well reported in the main floras of the country. Broun and Massey (1929) recoded 13 *Cassia* L. species. There are 23 species belonging to the genus *Cassia* in the Sudan, 16 of which are indigenous and 7 are exotic. They are distributed between the genera *Senna* (12 species), *Cassia* sensu stricto (8 species) and *chamaecrista* (3 species) (Andrews, 1952; El Amin, 1990). The names of all members of the genus *Cassia* sensu present in the Sudan are

updated by Abdalla *et al.* (2016) and a list of their synonyms is recorded in Table 1.

Table 1 : Species check-list for members of the genus *Cassia* L. sensu lato in the Sudan

No	Updated name	Previously used name(s)
1	*Cassia arereh Del*	Accepted name
2	*C. fistula L.*	-
3	*C. grandis L.*	-
4	C. javanica L. subsp. nodosa (Roxb.)	*C. nodosa* Roxb
5	*C. mannii Oliver*	-
6	*C. sieberiana DC.*	*C. kotschyana* Oliver
		C. sieberana DC.
7	*C. thyrsoidea Brenan*	Accepted name
8	*Chamaecrista absus (L.)*	*Cassia absus* L.
9	*C. mimosoides (L.) Greene*	*Cassia mimosoides* L.
10	*C. nigricans (Vahl) Greene*	*Cassia nigricans* Vahl
11	*Senna alata (L.) Roxb.*	*Cassia alata* L.
12	*S. alexandrina Mil*	*Cassia senna* L. *C. acutifolia* Del.
13	*S. auriculata (L.) Roxb.*	*Cassia auriculata* L.
14	*S. bicapsularis (L.) Roxb*	*Cassia bicapsularis* L.
15	*S. didymobotrya (Fresen.)*	*Cassia didymobotrya* Fresen.
16	*S. holosericea (Fresen.) Greuter*	*Cassia holosericea* Fresen.
17	*S. italica Mill.*	*Cassia obovata* Collad.
		C. italica (Mill.) Lam. ex Andrews
18	*S. obtusifolia (L.)*	*Cassia obtusifolia* L.
		C. tora sensu Oliver
19	*S. occidentalis (L.)*	*Cassia occidentalis* L.
20	*S. petersiana*	*Cassia petersiana* Bolle
21	*S. siamea (Lam.)*	*Cassia siamea* Lam
22	*S. singueana (Del.)*	*Cassia singueana* Del.
		C. goratensis Fresen
23	*S. surattensis (Burm.f.)*	*Cassia surattensis* Burm.f.

1.3 Traditional uses of the investigated plants

The seeds of *Cassia fistula* are slightly sweet, improve the appetite and possess laxative, carminative, cooling and antipyretic activities (Kirtikar *et al*., 2007). Seed powder is used as amoebiasis (Khare *et al*., 2007). They are also useful to treat jaundice, biliousness, skin disease, swollen throat and produce marked hypoglycaemic activity (Anonymous, 2007). The roots are used to cure chest pain, joint pain, migraine and blood dysentery. Also the root is used for the treatment of cardiac disorders biliousness, rheumatic condition, haemorrhages, fever wounds, ulcers and boils and various skin diseases (Anonymous, 2009; Nadkarni, 2009). The stem bark is used against amenorrhea, chest pain and swellings (Ayurvedic Pharmacopoeia of India, 2001). The leaves are laxative and used externally as emollient, a poultice is used for chilblains, in insect bites, swelling, rheumatism and facial paralysis (Gupta, 2010). Also the leaves are used against jaundice, piles, rheumatism, ulcers, ring worms and also, externally, to cure skin eruptions, eczema and other skin diseases. The leaves and bark mixed with oil are applied to pustules, insect bites (Kirtikar, 2006). Also the juice of leaves is useful as dressing for ringworm, relieving irritation and relief of dropsical swelling (Mohamed *et al*., 2011). The pulp of the fruit around the seeds is a mild purgative (Gupta, 2010). Also the fruit pulp is used for constipation, colic, chlorosis and urinary disorders (Khare, 2007). Leaves and flowers are both purgative like the pulp (Gupta 2010).

As a medicinal plant, *Cassia grandis* is used against worms and intestinal parasites, to treat stomach and respiratory problems, infected wounds, blood diseases and insulin resistance (Parra and Sardiñas, 2000; Meena *et al*., 2009; Lodha *et al*., 2010). Decoction of the leaves, fruit and bark is used orally to treat anemias, nosebleeds, liver disease, urinary tract infections, hysteria, colds and coughs. Topically applied ointment from leaves is used to treat dermato-mucosal conditions (herpes, sores, tinea and vitiligo). From root extracts, a liquid antiseptic

is obtained which is used for healing wounds. Also, the bark is used for healing. The juice of the leaves is used to combat ringworm. Decoction of the leaves is used as a laxative and for lumbago. Root preparations are attributed febrifuge, laxative and tonic effects. The bark of the trunk and large branches is believed to have antirheumatic properties and is used to treat skin conditions. The leaves are used for fungal skin infections (Prada *et al*., 2014).

Senna alexanderina pods and leaves are used as a laxative for centuries. It was considered as a "cleansing" herb because of its cathartic effect. In addition, the leaves are used for treating anemia, anorexia, biliousness, bronchosis, burns, cancer, cholera, constipation, cramp, dermatosis, dysentery, dyspepsia, enterosis, fever, fungal infections, gastrosis, gonorrhea, gout, halitosis, hemorrhoid, hepatosis, herpes, hiccups, infection, jaundice, leprosy, leukemia, mycosis, nausea, neural disorders, pimple, ringworm, splenosis, syphilis,typhoid, venereal disease, viral diseases, antihelmenthic and wound healing (El-Morsy, 2013).

Leaves, pods and immtured seeds of *S. italica* are used as purgative, decoction and maceration are used to cure stomach complaints, fever, jaundice, veneral diseases and biliousness. This plant is also used as abortifacient and against intestinal worms. Leaves fresh or dried or pulverized used to dress skin problems, burns and ulcers. Flowers are made into tea and used as purgative and to induce labour. Maceration of root is used to cure colic and influenza and boiled roots are used to dress wounds. Root infusion is used as eye drops for sore eyes and for the treatment of indigestion, liver complaints, gall bladder, nausea, vomiting and dysmenorrhea. Young seeds are eaten as snacks or as vegetable. Leaves are also used as neutral henna, hair conditioner which imparts yellow color (Bharathi *et al*., (2018).

1.4 Phytochemical constituents and pharmacological of the studied plants

A summary of the phytochemical constituents and pharmacological of studied plants are shown in Table 2. Photos of plants are given in Appendex 1.

Table 2: Phytochemical Constituents and pharmacological of the studied *Cassia* and *Senna* spp.

Plant name	Chemical constituents	Biological activity
Cassia fistula	3-Formyl-1-hydroxy-8-methoxy-anthraquinone, F/M (Agrawal *et al.*, 2012) Emodin, S/M Lee *et al*., 2001) Ziganein, S/M (Lee *et al*., 2001) 1,4,5-Trihydroxyanthraquinone, S/M (Lee *et al*. (2001) 1, 8-Dihydroxy-3-methylanthraquinone, SM (Rastogi and Mehrotra, 1993) Fistulic acid, F/M (Vaishnav and Gupta (1996) Barbaloin, F/M (Agarwal, 2005; Khare, 2007) Sennoside B, L/Aq (Habib and El-Sebakhy (1980) Rhamnetin 3-O-gentiobioside, R/M (Vaishnav and Gupta, 1996) 5,7,3',4'-Tetrahydroxy-6, 8-dimethoxyflavone-3-Oα-arabinopyranoside, B/M (Danish *et al*., 2011) Biochanin A, F/M (Sartorelli *et al*.,2009)	Antibacterial, L/E (Kumar *et al*., 2006) Antifungal, L/M & Aq (Ali *et al*., 1999; Phongpaichit *et al*., 2004 ; Panda *et al*., 2010) Antiviral, L/M(Li *et al*., 2014) Antioxidant, F,Fl,S, L/E (Siddhurajua *et al*., 2002) Anticancer, F/EA (Hsia *et al*., 2009; Ip *et al*., 2007) Hepatoprotective, L/H (Molander *et al*., 1957; (Thabrew *et al*., 1987) Hypolipidemic, S/E & B/H (Gupta & Jain, 2009; Nirmala *et al*., Eliza *et al*., 2008) Antidiabetic, L & B/M (Einstein *et al*., 2012) Laxative, F/W (Agrawal *et al.*, 2012). Wound healing, L/E (Kumar *et al.*, 2006).

Plant name	Chemical constituents	Biological activity
	(+) Catechin, F/E (Rastogi and Mehrotra,1999) Epi-afzelechin, F/E (Rastogi and Mehrotra, 1999) Kaempferol, F/E (Rastogi and Mehrotra, 1999) Dihydrokaempferol, F/E (Rastogi and Mehrotra,1999) Fistulaflavonoid B, S/M (Zhao *et al*., 2013) Licoisoflavone, S/M (Zhao *et al*., 2013) (3S)-3',7-dihydroxy-2',4',5',8-tetramethoxyisoflavan, S/M (Zhao *et al.*, 2013) (3S)-7-hydroxyl - 2',3',4',5',8-pentamethoxyisoflavan, S/M (Zhao *et al*., 2013) Morusyunnansins F, S/M (Zhao *et al.*, 2013) (2S)-2',4'-dihydroxy-7- methoxy-8-prenylflavan S/M (Zhao *et al.*, 2013) 5,7,3',4'-tetrahydroxy-6-methoxyflavone, S/E (Yadava and Verma, 2003)	Miscellaneous, L/M (Mazumder *et al*., 1998) Xanthine oxidase inhibition, S/M (Jothy *et al.*, 2011).
Cassia grandis	Aloe-emodin, L/M (Gritsanapan *et al*., 1984) Emodin-9-anthrone, S/M (Kalidhar, 1998) 1,3,4-Trihydroxy-6,7,8-trimethoxy-2-methyl anthraquinone-3-O-β-D-Glucopyranoside, P/EA (Verma and Sinha,1996)	Antiviral, L/E (Hernández-Castro *et al*., 2015). Antibacterial, S/M (Magalhães *et al*., 2020) Cytotoxic, S/M (Magalhães *et al*., 2020)

Plant name	Chemical constituents	Biological activity
		Schistosomicidal, S/M (Magalhães *et al*., 2020) Antioxidant, L/M (Meena *et al.*, 2009) Antidiabetic, S/W&E (Lodha *et al.,* 2010).
Senna alexanderina (*C. acutifolia*)	Glucorhein, R/M (Nazif *et al*., 2000) Anthraquinone glycosides, P&L/M (Singh *et al*., 1990) Rhein, emodine, physion, chrysophanol (marker), Obtusin, chrysoobtusin, chryso-obtusin-2-O-β-D-glucoside, obtusifolin and chryso-obtusifolin-2-O-β –Dglucoside, P&L/M (Singh *et al*., 1990). Crysophanic acid- 9- anthrone, P&L/M (Mukharjee *et al.*, 1996) Sennosides (sennoside A, B, C &D) P&L/M (Ganapaty *et al*., (2002) Rhein-8-diglucoside, Rhein-8glucoside, aloe-emodin, Anthrone diglucoside, Tinnevellin glycoside, 6-hydroxy musizin glycoside, Kaempferol, P/M (Khan. 2020)	Laxative Anthelmintic,Antidysenteric, Antihepatotoxic, Antiherpetic, Antileukemic, Antispasmodic, Antiviral, Antibacterial (El-Morsy, 2013), Antifungal, Hepatoprotective and Neuroprotectiveproperties, Carminative, Cathartic, Expectorant, Mutagenic, Trypsin Inhibition, Purgative, Vermifuge, Diuretic, Colon Cleansing Body detoxifing properties (Leelavathi and Udayasri 2018).
Senna italica (*C. italica*)	Tamarixetin 3-rutinoside-7-rhamnoside, P/E (El-Sayed *et al*., 1992). Apigenin 7-glucoside, P/E (El-Sayed *et al*., 1992) Kaempferol 7-glucoside, P/E (El-Sayed *et al*., 1992)	Antiinflammatory activity P/E (Jain *et al.,* 1997) Antiemetic L/M (Ahmed *et al.*, 2012).

Plant name	Chemical constituents	Biological activity
	Quercetin 7-glucoside, P/E (El-Sayed *et al*., 1992) 3-rutinoside-7-rhamnoside isorhamnetin, P/E (El-Sayed *et al*., 1992) Apigenin, P/E (El-Sayed *et al*., 1992) Physcion, Chrysophanol, Chrysophanol-10,10′-bianthrone, Chrysophanol-physcion bianthrone and Chrysophanolisophyscion bianthrone, P/M (Yagi *et al.*, 2013).	

L, leaf; B, bark; R, root; F, fruit; Fl, flower, P, pod; S, stem; E, ethanol extract, M, methanol extract; Aq, aqueous extract, W, water extract; H, hexane extract. EA, ethyl acetate extract.

1.5 Biological activity

Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents. Natural products from plants have been the primary source of antibiotics, and with the increasing acceptance of herbal medicines, the screening of medicinal plants for new active compounds has become a very important source to discover new lead antibiotic molecules (Roy and Dutta, 2021).

1.5.1 General characteristics of standard microorganisms used in the study

- ***Bacillus subtilis***

Gram-positive, spore-forming bacilli, aerobic, saprophytic prevalent in soil, water and air. It utilizes simple sources of nitrogen and carbon for energy and growth. The spores withstand heat and certain chemical disinfectants, sterilized by autoclaveing and it is non-pathogenic (Cheesbrough, 2000).

- ***Staphylococcus aureus***

Gram positive; aerobic and also grows an aerobically but less well. Temperature range for growth is 10-42ºC with an optimum of 35-37ºC. It grows in blood agar and produces yellow to cream or occasionally white 1-2 mm in diameter colonies, in MacConkey agar produces smaller colonies (0.1-0.5mm). *S. aureus* ferment mannitol (give yellow color), it is coagulase, catalase and DNase positive. *S. aureus* causes boils, styes. Pustules, imprtigo, infection of wound, ulcer and burns, osteomyelitis, mastitis, septicaemia, meningitis, pneumonia and pleural empyema. It is carried in the nose of 40% or more of healthy people (Cheesbrough, 2000).

- ***Escherichia coli***

Gram negative rod, coliform, motile, aerobic and facultative anaerobic. *E. coli* is catalase positive, oxidase negative, attack sugars fermentatively; gas normal produces, ferment lactose with production of acid and gas, produces indole, give positives methyl red reaction and negative Vogues-Proskaour and decomposes urea. The organism gives colorless colonies, 2-3 mm in diameter in 18 hours in nutrient agar and red large colonies in MacConkey agar and may be haemolytic on blood agar, *E.coli* commonly causes the urinary tract infections and diarrhea in infant and travelers. Also it causes meningitis, septicemia as well as sepsis in operating wounds and abscess (Jawetz *et al.*, 2001).

- ***Pseudomonas aeruginosa***

It is motile and Gram-negative rod shaped, obligate aerobe, and grow readily on many types of media at 37-42ºC sometimes producing sweet or grape-like odour due to 2-amino acetophenone production. It forms smooth round colonies with fluorescent greenish color (pyoverdin fluorescent pigment). It is oxidase positive; produce acid from carbohydrate due to oxidation not fermentation, catalase positive, citrate positive. It is pathogenic only when introduced into areas devoid of normal defenses. It can cause purulent infections of wounds, burns, external ear, urinary tract and contamination of traumatic lesions in the eye leads to opthlamitis (Jawetz *et al*., 1995).

- ***Aspergillus niger***

There are more than 100 species of *Aspergillus* but only a few have been implicated in human disease: the most important are *A. fumigayes*, *A. niger, A. flavus*, *A. terreus* and *A. nidulons*. All grow in nature and in culture as mycelia fungi ith septate hyphae and distinctive sporing structures; the spore bearing

hypha (conidiophores) terminates in a swollen cell (vesicle) surrounded by one or two rows of cells (sterrigmata) from which chains of asexual conidia are roduced. Aspergillosis most frequently affects the lungs, but infections at other sites such as the nasal sinuses and superficial tissues may also occur. The disease is usually caused by *A. fumigayes.* Inhalation of *Aspergillus* spores may lead to colonization of existing lung cavities (aspergilloma form) or a hypersensitivity reaction (allergic- aspergillosis). Rarely, *Aspergillus* may cause invasive disease of the lung and may disseminate to other organs; this form is seen in severely immune-compromised patients (Jawetz *et al*., 1995).

- ***Candida albicans***

Budding yeast that produces pseudohyphal both in culture and in tissue appears as a Gram-positive. On potato dextrose agar at room temperature, incubation gives cream- colored l of human serum incubated at 37ºC for 1.5-2 showed the formats of Germ-tube by some of the yeast cells.i.e Germ tube test (GTT) positive. It ferments glucose and maltose, producing both acid and gas and does not attack lactose. It is opportunistic fungi, is a member of the normal flora of the mucous membranes in the respiratory, gastrointestinal and female genital tract. In such location it may gain dominance and be associated with pathologic conditions (Jawetz *et al*., 1995).

1.6 Antioxidant activity

Antioxidants are the molecules that prevent cellular damage caused by oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from one molecule to an oxidizing agent. Oxidation reactions are known to produce free radicals. These free radicals are highly reactive species which contains one or more unpaired electrons in their outermost shell. Once they are formed, the chain reaction starts. Antioxidant reacts with these free radicals and terminates

this chain reaction by removing free radical intermediates and inhibits other oxidation reactions by oxidizing themselves (Hamid *et al*., 2010).

Though oxidation reactions are crucial for life, they can also be damaging. Plants and animals have a complex system of multiple types of antioxidants, such as vitamin C and vitamin E, as well as enzymes, such as catalase (CAT), superoxide dismutase (SOD), and various peroxidases (Hamid *et al*., 2010). Oxidative stress plays a key role in causing various human diseases, such as cellular necrosis, cardiovascular disease, cancer, neurological disorder, Parkinson's dementia, Alzheimer's disease, inflammatory disease, muscular dystrophy, liver disorder, and even aging (Amit and Priyadarsini 2011). Besides, there are some antioxidants in the form of micronutrients which cannot be manufactured by the body itself such as vitamin E, β-carotene, and vitamin C, and hence these must be supplemented in the normal diet. Antioxidants can also act as prooxidants when these are not present at the right place at the right concentration at the right time (Touriño *et al*. 2008). The relative importance of the antioxidant and prooxidant activities is not yet explored fully and needs further research.

Chapter Two

2. Materials and Methods

2.1 Materials

2.1.1 Plant materials

Fresh leaves of *Cassia fistula* and *C. grandis* were obtained from the Botanical Garden in Khartoum (Mugran), while those of *Senna alexandrina* and *S. italica* were collected from the southern Khartoum on October, 2019. The identity of each plant was authenticated by a taxonomist at the Botany department of the Sudan University of Sciences and Technology. Leaves of each plant were washed and dried under the shade to avoid possible damage to phytochemical constituents. They were stored in air-tight containers at room temperature until required for use (Onoruvwe and Olorunfemi, 1998).

2.1.2 Taxonomical studies

All collected specimens were identified using keys of written floras such as Abdalla *et al*. (2016).

2.1.3 Chemicals and drugs

All chemicals reagents and chemotherapeutic agents were obtained from Loba chemie PVT ltd, company, Equipment and instruments were obtained from Baird and tatlock ltd, company, Antibacterial and antifungal drugs and the culture media were obtained from Sigma Chemical Company USA.

2.2 Methods

2.2.1 Preparation of crude extracts

Extraction was carried out according to method described by sukhdev *et al*., (2008). Five hundred grams of leaves from each plant were coarsely powdered

using mortar and pestil. Samples were successively extracted with N-hexane, chloroform, ethyl acetate, and methanol. Extraction was carried out for about three days for every solvent. Solvents were evaporated under reduced pressure using rotatory evaporator. Finally, extracts were allowed to air in petri dishes till complete dryness and the percentages yield were then calculated as follows:

Weight of extract obtained / weight of plant sample X 100

2.2.2 Antimicrobial activity

2.2.2.1Test organisms

The test organisms, four bacteria and two fungal isolates, were obtained from Medicinal and Aromatic Institute of Research, National Research Center, Khartoum. The bacterial species used were the Gram-positive *Bacillus subtillis* (ATCC19430) and *Staphylococcus aureus* (ATCC 25923) and Gram-negative *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC27853). Fungal species were, *Candida albicans* (ATCC7596) and *Aspergillus niger* (ATCC9763).

2.2.2.2 Growth and maintenance of microorganisms

Bacteria were maintained on Müller-Hinton agar (MHA) at 4°C and were cultured in peptone broth before use in the bioassays. Fungi were maintained on Potato Dextrose Agar (PDA) at 4° C and were cultured in peptone broth and incubated overnight at 27°C prior conducting the bioassay.

2.2.2.3 Preparation of inocula

The concentration of bacterial suspension was adjusted to 1.5×10^8 colony forming units per milliliter (CFU/ml) as described by McFarland (1907). A loopful of isolated colonies was inoculated into 4 ml peptone water and incubated at 37° C for 24 hours. The turbidity of actively growing bacterial suspension was adjusted to match the turbidity standard of 0.5 McFarland units prepared by mixing 0.5 ml

of 1.75% (w/v) barium chloride dehydrate with 99.5 ml of 1% (v/v) sulphuric acid. This turbidity was approximately equivalent 1.5×10^8 CFU/ml.

For fungal suspensions, one Petri dish with 7-days culture of each fungal isolate was suspended in 1 ml of normal saline and were diluted 1/10 with sterile peptone broth.

Twenty-five ml per plate of pre-autoclaved media (MHA for bacteria and PDA for fungi) were poured into 90 mm diameter pre sterilized Petri plates. These plates were allowed to solidify at room temperature.

2.2.2.4 Antibacterial assay

Antibacterial activity of crude N- hexane, chloroform, ethyl acetate and methanol extracts of leaves extracts of the investigated plants was evaluated by the agar disc diffusion method (Mbavenge *et al*., 2008). Sterilized filter paper discs with a diameter of 6 mm were impregnated with 1ml of 20 mg of crude extracts dissolved in 1 ml of 5% dimethyl sulfoxide (DMSO) and left to dry. After the plates were solidified, the freshly prepared bacterial broth culture suspension was spread over the MHA media using sterilized swaps under aseptic conditions using laminar air flow. After 5 min, the extracts - impregnated discs were dispensed onto the surface of the inoculated agar plates. Discs impregnated with dimethyl sulfoxide (DMSO) were also used as a negative control, while Gentamicin (10 mg/disc) was used as a positive control. Three replicates were carried out for each extract against each of the test organisms. The Petri plates were incubated for 18-24 hours at room temperature. After incubation, the diameters of clear zone of inhibition produced around the discs were measured in mm and the plates were photographed.

2.2.2.5 Antifungal assay

Antifungal activity was also evaluated by the disc diffusion method as described above for antibacterial activity but instead PDA medium was used (Mothana and Lindequist, 2005). Plates were incubated at room temperature for 24 hours for *C. albicans* and 48 hours for *A. niger*. DMSO was used as a negative control, while Nystatin (100 000IU/ml) was used as a positive control.

2.2.2.5.6 Minimum inhibitory concentrations

The minimum inhibitory concentration (MIC) of the isolated compounds were evaluated by a modified resazurin microtiter-plate assay as reported by Sarker *et al*., (2007) with some modification. Briefly, a volume of 100 µl of 5.0 mg/ml^{-1} (w/v) extract in 10% dimethyl sulfoxide (DMSO, v/v) and 1 mg/ml^{-1} of standard antibiotic in 10% DMSO was transferred into the first row of the 96 well plates. To all other wells, 50 µl of nutrient broth were added. Two-fold serial dilutions were performed using a multichannel pipette such that each well had 50 µl of the test material in serially descending concentrations. Thirty microliter of 3.3 time stronger isosensitised broth (3.3 x) and 10 µL of resazurin indicator solution (prepared by dissolving 270 mg resazurin tablet in 40 ml of sterile distilled water) were added to each well. Finally, 10 µl of bacterial suspension were added to each well to achieve a concentration of approx. 5×10^5 CFU/ml. Each plate was wrapped loosely with cling film to ensure that the fungi did not become dehydrated. Each plate had a set of controls: a column with a Gentamicin as positive control, a column with all solutions with the exception of the test compound, a column with all solutions with the exception of the bacterial solution adding 10 µl of nutrient broth instead and a column with 10% DMSO (v/v) solution as a negative control. The plates were prepared in triplicate, and incubated at 37 °C for 24 h. The color change was then assessed visually. The growth was indicated by color changes from purple to pink or colorless. The lowest concentration at which color change appeared was taken as the MIC value.

2.2.3 Antioxidant activity

2.2.3.1 DPPH radical scavenging assay

The DPPH (2.2Di (4-tert-octylphenyl)-1-picryl-hydazyl) radical scavenging was determined according to the method of Shimada *et al.*, (1992). Test samples were dissolved separately in 5% DMSO to get test solution of 1 mg/ml. Series of extract solutions of different concentrations (1, 5, 10, 20, 40, 60, 80 and 100 µg/ml) were prepared by diluting with methanol. Assay was performed in 96-well, microtiter plates. 140 µl of 0.6×10^{-6} mol/DPPH were added to each well containing 70 µl of sample. The mixture was shaken gently and left to stand for 30 min in dark at room temperature. The absorbance was measured spectrophotometrically at 517 nm using a microtiter plate reader. Propyl gallate was used as positive control. The ability to scavenge DPPH radical was calculated by the following equation:

DPPH radical-scavenging (%) $=1-[(A_{blank} - A_{sample})/A_{blank}]\times100$

Where;

A_{blank} is the absorbance of the control reaction (containing all reagents except the test sample), and A_{sample} is the absorbance of the extracts/reference.

2.2.4 Phytochemistry

2.2.4.1 Thin Layer Chromatography (TLC)

Readymade plates of TLC were used.

- Stationary phase

Silica gel for thin layer chromatography type 60 GF_{254} with fluorescent indicator (BDH); U.K

- Mobile phase

The following mobile phase was tested:

Hexane: Ethyl acetate: Methanol (8: 2: 0.5, v/v/v) or (5:3:2, v/v/v).

2.2.4.1.1 Application of sample

The N-hexane, chloroform, ethyl acetate, and methanol extracts (0.5 mg) were dissolved in small amount of their respective solvents. The sample was spotted at a level about 1 cm from the bottom of the plate, kept to dry in air and placed in a tank containing the selected solvent system. After reaching the height of 15 to 20 cm, the plate was removed from the tank and allowed to air dry until the solvent was completely evaporated. The plate was inspected in day light, then examined under UV and finally sprayed with a reagent. R_f values of separated spots were calculated as follows:

Distant crossed by spot / distant crossed by solvent front.

2.2.4.1.2 Preparation of reagents

- Vanillin reagent:

About 0.5 g of vanillin was added to 20 ml of H_2SO_4con and 80 ml of ethanol.

- Dragendorff's reagent:

Dragendorff's reagent was prepared by mixing a concentrated solution of potassium iodide with a solution of bismuth subnitrate in a diluted acid.

- Aluminum chloride

One gram of $AlCl_3$ was dissolved in 100 ml water.

- Potassium hydroxide

One gram of KOH was dissolved in 100 ml ethanol.

- Ferric chloride

Five grams of $Fecl_3$ were dissolved in 100 ml water.

- Anisaldehyde

About 1 ml of anisaldehyde was added to 10 ml of acetic acid and 5ml of Sulphuric acid (conc).

2.2.4.2 Determination of total polyphenols content

The concentration of polyphenolics in plant extracts was determined using Folin-Ciocalteu method (Singleton *et al.*, 1999). Solution of each extract in the concentration of 1 mg/ml was used in the analysis. The Reaction mixture was prepared by mixing 0.5 ml of extract with 2.5 ml of 10% Folin-Ciocalteu reagent (dissolved in water) then the content was mixed. After 3 min, 2.5 ml 7.5 % $NaHCO_3$ solution was added. The samples were incubated at 45°C for 45 min. The absorbance was determined using spectrophotometer at 765nm against the blank using Shimadzu model 1800 double beam spectrophotometer. A calibration curve was constructed using gallic acid standard solutions (0-100 mg/L) (Figure 1). The content of polyphenolics in extract was expressed in terms of gallic acid equivalent (GAE) (mg of GAE/g of extract).

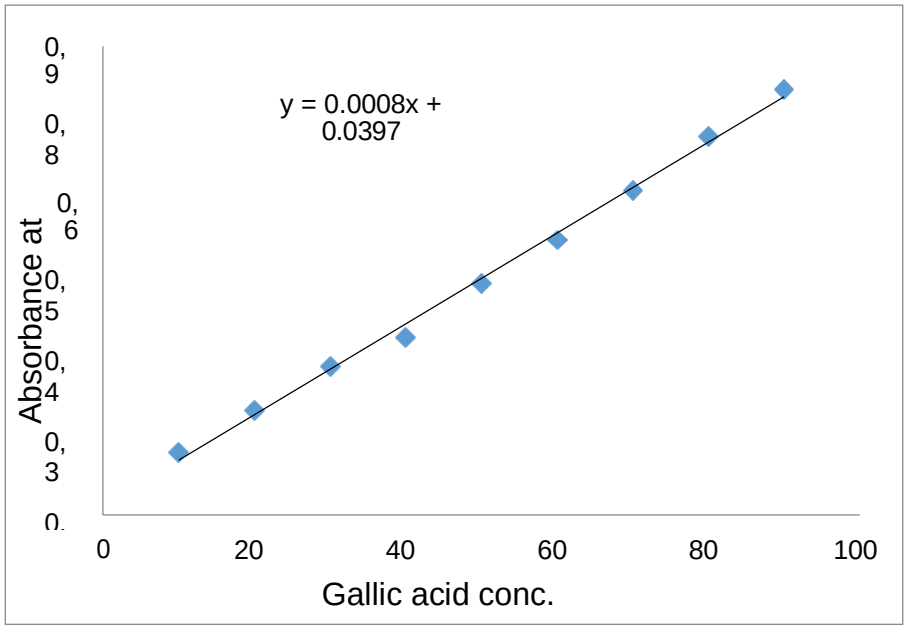

Figure 1: Standard curve of Gallic acid (mg/l).

2.2.4.3 Determination of total flavonoids content

The content of flavonoids in the examined plant extracts were determined according to a modified colorimetric assay with Aluminum chloride (Quettier *et al.*, 2000).

One ml of the extract (1 mg/l) was added to a test-tube, followed by addition of 0.3 ml of solution of $NaNO_2$ (0.05 g/l). After 5 min, 0.3 ml of a 0.1 g/l solution of $ALCl_3$ was added and 5 min later, 2 ml of NaOH (1 mol/l) was added to the mixture. The solution was mixed and the absorbance was measured at 415 nm against a blank. Quercetin was used as the standard for the construction of a calibration curve in different concentrations (0-100 mg/L) (Fig. 2). Flavonoids content was expressed in terms of quercetin equivalents (QE) (mg of QE/g of extract).

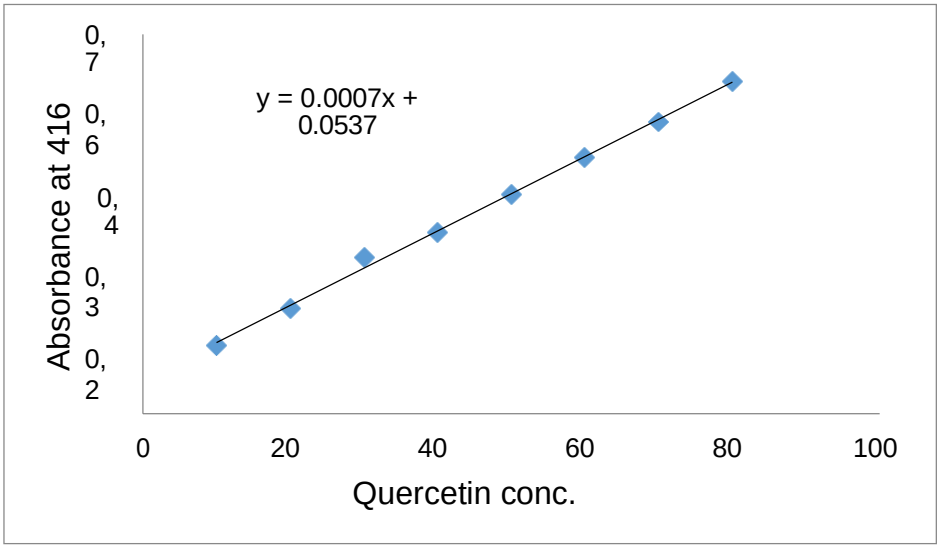

Figure 2: Standard curve of quercetin (mg/l).

2.2.4.4 Total tannins content

Tannins content was determined by using $FeCL_3$ and gelatin test (Sivakumar *et al.*, 2012) with some modification. One ml of extract (1 mg/ml) was transferred to vials, 1 ml 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. A calibration curve (Fig. 3) was constructed using tannic acid (100-800 mg/l) as standard and total tannins content of the extracts (mg/g) expressed as tannic acid equivalents (TAE).

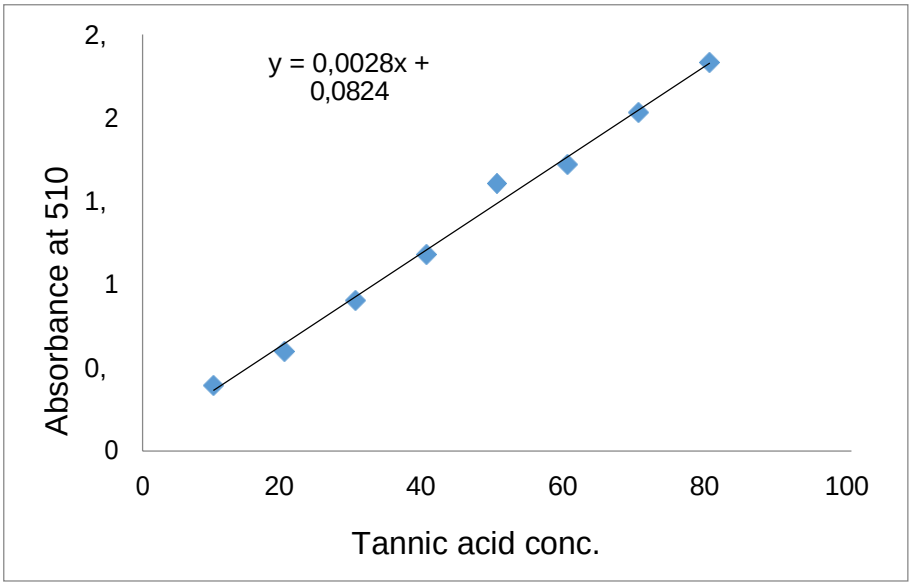

Figure 3: Standard curve of tannic acid (mg/l).

2.2.5 Statistical analysis

The samples were prepared in triplicate for each analysis. Values expressed as mean ±S.D.

Chapter Three

3. Results and Discussion

3.1 Taxonomy

3.1.1 Morphological description of the investigated plants

The morphological characteristics of *C. fistula, C. grandis, S. alexandrina,* and *S. italica* were summarized in Table 3. According to the different morphological description of the stem, leaf, flower, fruit and seed a taxonomical key was also presented.

Table 3: Taxonomical characteristics of the investigated *Cassia* and *Senna* species.

Plant name	Fruit	seed	Stem	leaves	flower
Cassia fistula	Large glossy Brown Cylindrical Many seeded	Brown Small Circular Smooth	Woody Smooth grey bark	Unipinnate,paripinn Petiole stipulated Leaflets in 4-5 pairs	Bright yellow Filaments of the 3 abaxial stamens curved 1 or 2 bracteoles Stamens 10
Cassia grandis	Large Brown Smooth Tube shape Many seeded	Black Small Circular Smooth	Woody Smooth	Unipinnate,paripinn Petiole Stipulated Leaflets in 9-13 pairs	Pink with orange Filaments of the 3 abaxial stamens curved 1 or 2 bracteoles Stamens 10
Senna alexandrina	Small Brown Flattened Straight	Yellow Very Small Un regular Smooth	Herbaceous Straight Smooth dark green	Leaflets in 2-7 pairs Unipinnate, paripinnate Petiole stipulated	Yellow Filaments of all stamens straight or slightly curved Bracteoles absent Stamens 10

Plant name	Fruit	seed	Stem	leaves	flower
Senna italic	Small Dark green Flattened Curved	Brown Very Small Unregularly Smooth	Herbaceous Straight	Leaflet in 3-6 pairs Unipinnate, paripinnate Petiole stipulated	Yellow Filaments of all stamens straight or slightly curved Bracteoles absent Stamens 9-10

3.1.2 Key to the species under study

A. Filaments of the 3 abaxial stamens distinctly curved, 1 or 2 bracteoles ……..

…….. ……... ***Cassia spp.***

B. Fruit large brown cylindrical, leaflets in 4-5 pairs, flower yellow ….***C. fistula***

BB. Fruit large brown tube, leaflets in 9-13 pairs, flower red or pink ...***C.grandis***

AA Filaments of all stamens straight or slightly curved, bracteoles absent

……..... ……..…. ***Senna spp***

C. Fruit small straight, leaflets in 2-7pairs, Stamens10.....***S. alexandrina***

CC. Fruit small curved, leaflets in 3-6 pairs, Stamens 9-10.....***S. italica***

3.2 Yields of crude extracts

The yield of hexane, chloroform, ethyl acetate and methanolic extracts of leaves of *C. fistula, C. grandis, S. alexandrina* and *S. italica* was determined. Results are depicted in Table 4. Generally, methanol extracts of the four studied plants gave highest yield and in the following descending order *C. fistula* (6.78%) > *S. italic* (6.44%) > *S. alexandrina* (%) > *C. grandis* (5.43%). Also the hexane extract of *C. fistula* gave high percentage yield (6.43%). Chloroform extracts (except of *C.*

grandis) donated also considerable extracts yield in range of 3.43% to 3.54% while all ethyl acetate extract had low yield (0.49% - 0.73%).

Table 4: Extracts yield of the investigated *Cassia* and *Senna* species.

Plants	Yield (%)			
	N-Hexane	Chloroform	Ethyl acetate	Methanol
Cassia fistula	6.43	3.54	0.49	6.78
Cassia grandis	2	1.63	0.49	5.43
Senna alexandrina	1.77	3.43	0.49	6.39
Senna italica	1.43	3.47	0.73	6.44

3.3 Biological activity

3.3.1 Antimicrobial activity

The antimicrobial activity of the hexane, chloroform, ethyl acetate and methanol extracts from the leaves of *C. fistula*, *C. grandis*, *S. alexandrina* and *S. italica* was performed against the bacteria; *Bacillus subtiles*, *Staphylococcus aureus*, *Escherichia coli and Pseudomonas aeruginosa* as well as the two fungi *Aspergillus niger* and *Candida albicans* using the disc diffusion method. Results are presented in Table 5 and figure 4.

3.3.1.1 Antimicrobial of *Cassia fistula* leaves

Hexane, chloroform, ethyl acetate and methanolic extracts from the leaves of *C. fistula* possessed variable antimicrobial activity. The highest antibacterial activity against *B. subtiles* was recorded from the chloroform extract with inhibition zone of 12mm, this followed by the ethyl acetate extract (10 mm). The other two extracts (hexane and methanol) were not active. Only the two polar solvents extracts; methanol and ethyl acetate exerted antibacterial activity against *S. aureus*

(13 and 11 mm respectively) and *P. aeruginosa* (15 and 9 mm respectively). However, *E. coli* was only sensitive towards the methanolic extract (11 mm).

All the four extract of *C. fistula* leaves were effective against the fungus *C. albicans* with highest antifungal activity was displayed from the ethyl acetate extract (27 mm) followed by the hexane (15 mm), chloroform (14 mm) and methanolic (11 mm) extracts respectively. Only the ethyl acetate and methanolic extracts showed antifungal activity against *A. niger* with inhibition zone values of (27 and 12 mm respectively).

Table 5: Antimicrobial activity of extracts of the investigated *Cassia* and *Senna* species.

Plant	Extract	Inhibition zone (mm)									
		Bacillus subtiles	AI	*Staphylococcus aureus*	AI	*Escherichia coli*	AI	*Pseudomonas aeruginosa*	AI	*Aspergillus niger*	*Candida albicans*
Cassia fistula	Hexane	NA	NA	NA	NA	NA	NA	NA	NA	NA	15 ± 1.4
	Chloroform	12 ± 0.5	0.521	NA	NA	NA	NA	NA	NA	NA	14 ± 1.5
	Ethyl acetate	10 ± 0.4	0.434	11 ± 0.7	0.478	NA	NA	9 ± 1.4	0.36	27 ± 1.7	28 ± 2.0
	Methanol	NA	NA	13 ± 1.5	0.565	11 ± 0.0	0.392	15 ± 1.0	0.6	12 ± 1.3	11 ± 0.0
Cassia grandis	Hexane	NA	NA	NA	NA	NA	NA	NA	NA	10 ± 0.0	10 ± 0.0
	Chloroform	NA	NA	NA	NA	NA	NA	12 ± 0.7	0.48	16 ± 0.8	16 ± 0.3
	Ethyl acetate	NA	NA	NA	NA	NA	NA	NA	NA	18 ± 0.7	20 ± 1.9
	Methanol	NA	NA	18 ± 2.0	0.782	9 ± 1.1	0.321	11 ± 0.5	0.44	22 ± 1.2	17 ± 0.5
Senna alexandrina	Hexane	NA	NA	13 ± 0.1	0.565	11 ± 0.1	0.392	11 ± 0.4	0.44	17 ± 0.6	12 ± 0.8
	Chloroform	11 ± 0.3	0.478	NA	NA	NA	NA	18 ± 0.6	0.72	NA	NA
	Ethyl acetate	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Methanol	NA	NA	11 ± 0.1	0.478	13 ± 0.8	0.464	11 ± 0.4	0.44	17 ± 0.8	25 ± 1.4
Senna italica	Hexane	10 ± 0.0	0.434	10 ± 0.0	0.434	10 ± 0.1	0.357	NA	NA	13 ± 0.3	11 ± 0.4
	Chloroform	11 ± 0.4	0.478	NA	NA	NA	NA	NA	NA	11 ± 0.4	13 ± 0.5

Plant	Extract	Inhibition zone (mm)									
		Bacillus subtiles	AI	*Staphylococcus aureus*	AI	*Escherichia coli*	AI	*Pseudomonas aeruginosa*	AI	*Aspergillus niger*	*Candida albicans*
	Ethyl acetate	12 ± 0.3	0.521	NA	NA	NA	NA	NA	NA	12 ± 0.0	14 ± 0.2
	Methanol	12 ± 0.1	0.521	10 ± 0.0	0.434	13 ± 0.4	0.464	12 ± 0.3	0.48	22 ± 1.4	20 ± 1.1
Gentamicin	10^5 IU/ml	23	23	28	25	-	-	Gentamicin	10^5 IU/ml	23	23
Nystatin	10^5 IU/ml	-	-	-	-	22	20	Nystatin	10^5 IU/ml	-	-

NA, not active; -, not determined. AI, Activity index.

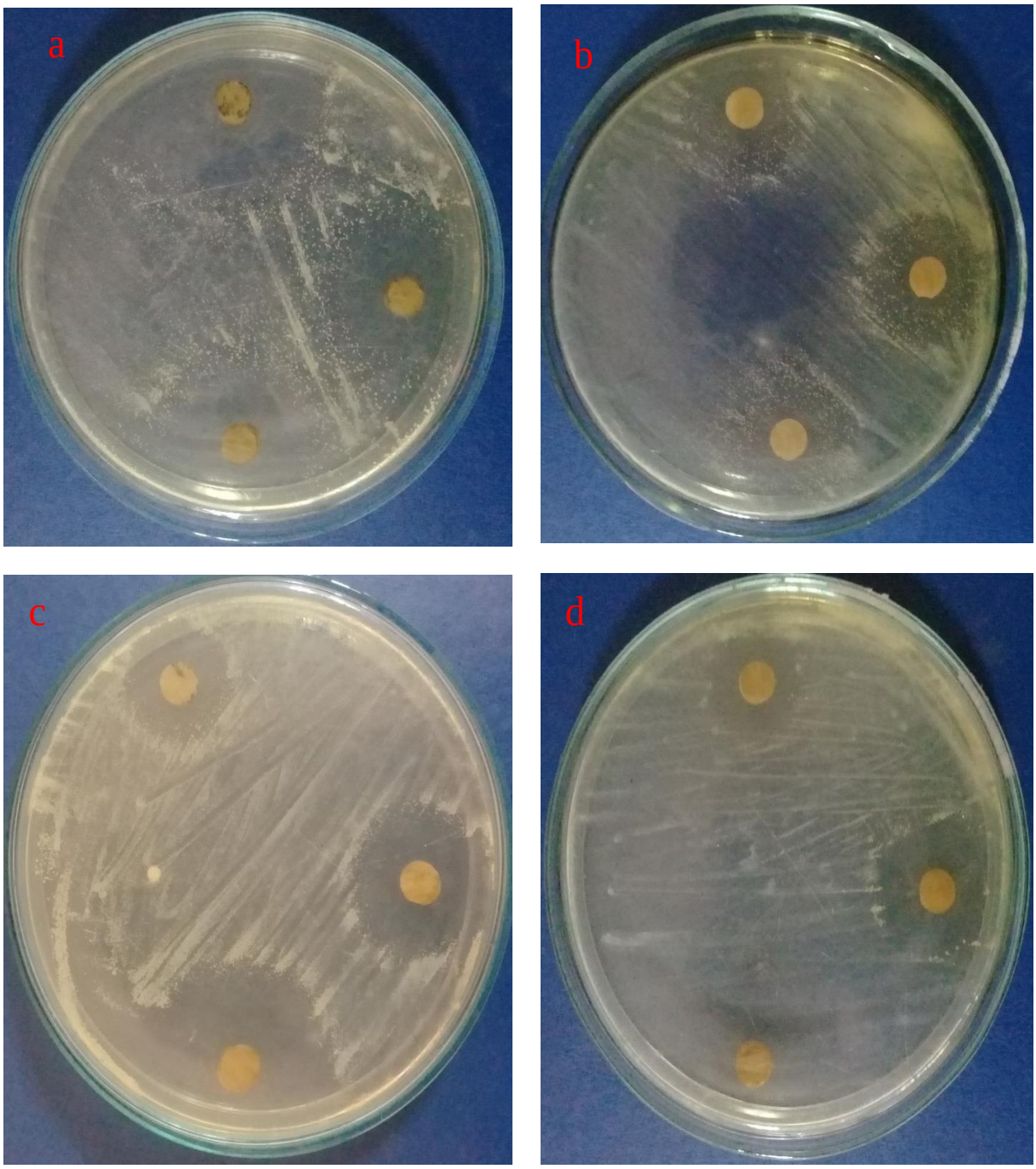

Figure 4: Antifungal activity of the investigated *Cassia* and *Senna* species.

a= Ethyl acetate extract of *Cassia fistula* against *Candida albicans*

b= Methanolic extract of *Cassia grandis* against *Aspergillus niger*

c= Methanolic extract of *Senna alexandrina* against *Candida albicans*

d= Methanolic extract of *Senna italica* against *Aspergillus niger*

3.3.1.2 Antimicrobial activity of *Cassia grandis* leaves

All four extracts of *C. grandis* leaves did not exhibit any activity against *B. subtilis* and only the methanolic extract reveled antibacterial activity against *S. aureus* (18 mm) and *E. coli* (9 mm). It was also active besides the chloroform extract against *P. aeruginosa* with inhibition values of (11 and 12 mm respectively). All extracts showed antifungal activity against the two tested fungi. The highest antifungal activity against *A. niger* was recorded from the methanolic extract (22 mm) followed by the ethyl acetate (18 mm), chloroform (16mm) and hexane (10 mm) extracts respectively. The ethyl acetate extract exerted the highest activity against *C. albicans* (20 mm) followed by the methanolic (17 mm), chloroform (16 mm) and hexane (10 mm) extracts respectively.

3.3.1.3 Antimicrobial activity of *Senna alexandrina* leaves

From all four extracts of *S. alexandrina* leaves, the ethyl acetate extract did not display any antibacterial and antifungal activity. The chloroform extract recorded highest antibacterial activity with inhibition zone (18 mm) against *P. aeruginosa*, it was also the only extract that showed antibacterial activity against *B. subtiles* with inhibition zone value (11 mm). Hexane and methanolic extracts revealed antibacterial activity against *S. aureus, E. coli* and *P. aeruginosa*, and the former exerted an inhibition zone values of 13mm for *S. aureus* and 11mm *E. coli* and *P. aeruginosa,* and the latter with inhibition zone values of 13 mm for *E. coli* and 11mm for *S. aureus* and *P. aeruginosa*.

Antifungal activity was only recorded from the hexane and methanolic extracts. The methanolic extract revealed highest antifungal activity with inhibition zone value of 25 mm against *C. albicans* while the hexane extract recorded an inhibition zone value of 12 mm. Both extracts exerted the same inhibition value against *A. niger* (17 mm).

3.3.1.4 Antimicrobial activity of *Senna italica* leaves

The four extracts of *S. italica* leaves possessed variable antimicrobial activity. The highest antibacterial activity against *B. subtiles* was recorded from the two polar solvents (methanol and ethyl acetate) with inhibition zone value of 12 mm, followed by the chloroform (11 mm) and hexane (10 mm) extracts respectively. The chloroform and ethyl acetate extracts were not active against *S. aureus*, *E. coli* and *P. aeruginosa*. Only the methanolic extract revealed antibacterial activity against *P. aeruginosa*, while the hexane and methanolic extracts exerted antibacterial activity against *E. coli* with inhibition zone values of 10 and 13 mm respectively.

Hexane, chloroform, ethyl acetate and methanolic extracts from the leaves of S. italica displayed variable antifungal activity. The highest antifungal activity against *A. niger* and *C. albicans* was recorded from the methanolic extract with inhibition zone of 22 and 20 mm respectively. The chloroform, ethyl acetate and hexane extracts recorded inhibition zone values of 11, 12 and 13 mm respectively against *A. niger*. Ethyl acetate showed antifungal activity against *C. albicans* with inhibition zone value of 14 mm followed by the chloroform extract (13 mm) while the hexane extract recorded the lowest value (11mm).

3.3.2 Determination of the minimum inhibitory concentration (MIC)

According to the results from the disc diffusion away, the MIC of the most active extracts (ethyl acetate and methanol extracts) against the tested fungi was determined. Results are presented in Table 6. The lowest MIC value (6.25 mg/ml), indicative to highest activity, was recorded against *C. albicans* by the ethyl acetate extract of *C. fistula* and methanolic extract of *S. italica*. The latter in addition to the methanolic extract of *C. grandis* exhibited the lowest MIC value (12.5 mg/ml) against *A. niger*.

Table 6: Minimum inhibitory concentration of the investigated *Cassia* and *Senna* species against tested pathogenic fungi.

Plant	Extract	MIC (mg/mL)	
		Aspergillus niger	*Candida albicans*
Cassia fistula	Ethyl acetate	25	6.25
Cassia grandis	Methanol	12.5	-
	Ethyl acetate	-	25
Senna alexandrina	Methanol	-	12.5
Senna italica	Methanol	12.5	6.25

-, not determined.

3.3.3 Antioxidant activity

The antioxidant activity of the different leaf extracts of the investigated plants was evaluated by their capacity to scavenge the DPPH free radicals. Results are presented in Figure 5.

3.3.3.1 Antiradical activity of *Cassia fistula* leaves

The hexane, chloroform, ethyl acetate, and methanolic extracts from the leaves of *C. fistula* were evaluated for their scavenging activity of the free DPPH radicals. Results showed that the antiradical activity of the extracts was in the range of 0-77%. The highest anti-DPPH radical activity was obtained from the ethyl acetate extracts and the other three extracts were either not active or weakly active.

3.3.3.2 Antiradical activity of *Cassia grandis* leaves

Results of the scavenging DPPH radicals activity of the four extracts from the leaves of *C. grandis* was in the range of 0- 71 %. The highest scavenging activity of free DPPH radical was exerted by the ethyl acetate and methanolic extracts

with percentage value of 71%. The other two extracts (hexane and chloroform) were not active.

3.3.3.3 Antiradical activity of *Senna alexandrina* leaves

The DPPH anti-radical activity of the *S. alexandrina* leaves extracts was in the range of 3%-56% where the highest value was obtained from the methanolic extract. The chloroform extract gave weak activity (25%) while the hexane and ethyl acetate extracts were not active.

3.3.3.4 Antiradical activity of *Senna italica* leaves

The anti-DPPH radical activity of *S. italica* leaves extracts was in the range of 0 to 24% indicating that the four extracts were either had weak activity or not active at all.

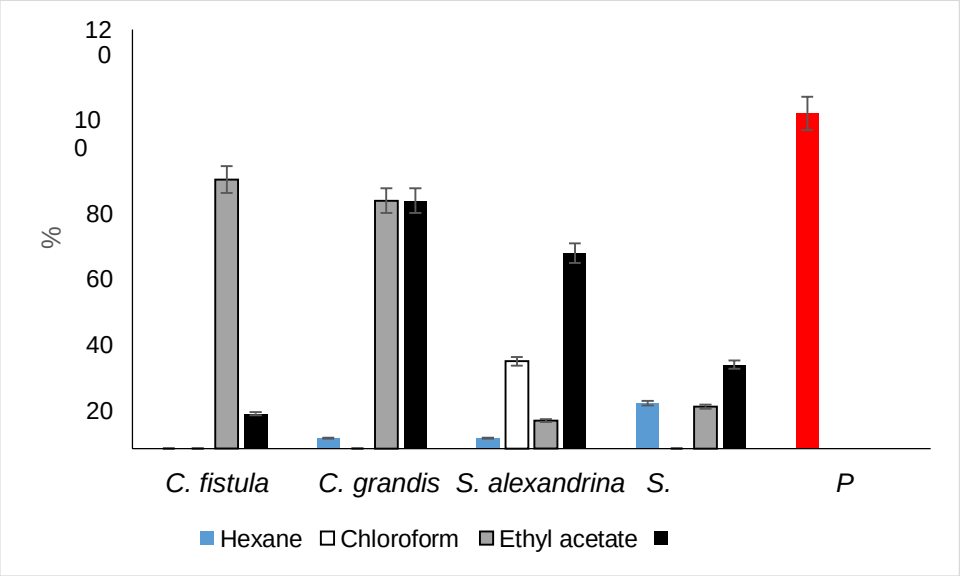

Figure 5: Antioxidant activity of extracts of the investigated *Cassia* and *Senna* species.

PG: Propyl gallate.

3.4 Phytochemistrey

3.4.1 Screening for the presence of major secondary metabolites

Preliminary screening for the presence of major secondary metabolites in the hexane, chloroform, ethyl acetate and methanolic extract of the investigated plants was carried out using thin layer chromatograohy (TLC). Chromatograms were developed in the solvent system; hexane: ethyl acetate: methanol (8:2:1, v/v) for the hexane, chloroform extracts and in hexane: ethyl acetate: methanol (5: 3:2, v/v) for the ethyl acetate and methanolic extracts.

All extracts displayed several spots with different polarities and colour characteristic. Many spots were visible under day light (Figures 6-8-a) and some showed were capable to reflect UV light and appeared as fluorescent violet spots while other absorbed the UV light and appeared as quenching spots (Figures 6-8-b). Generally all extracts were rich in secondary metabolite as shown upon spraying the chromatograms with the universal reagent vanillin/H_2SO_4 reagent (Figures 6-8-c).

Spraying chromatograms with different reagents indicated the presence of steroids (pink to reddish-coloured spots) and terpenes (violet-coloured spots) when sprayed with Anisealdehyde (Figures 6-8-d). *Senna italic* extracts displayed yellow-coloured spots when sprayed with $AlCl_3$ reagent indicated the presence of flavonoids, (Figures 6-8-e), while all extracts contained phenols as well as tannins as they gave violet- or grey-coloured spots when sprayed with $FeCl_3$ reagent (Figures 6-8-g). Anthraquinones were detected mainly in *C. fistula* extracts as they developed characteristic pink-coloured spots when sprayed with KOH reagent (Figures 6-8-f). All extracts were devoid of alkaloids as they did not show orange-coloured spots.

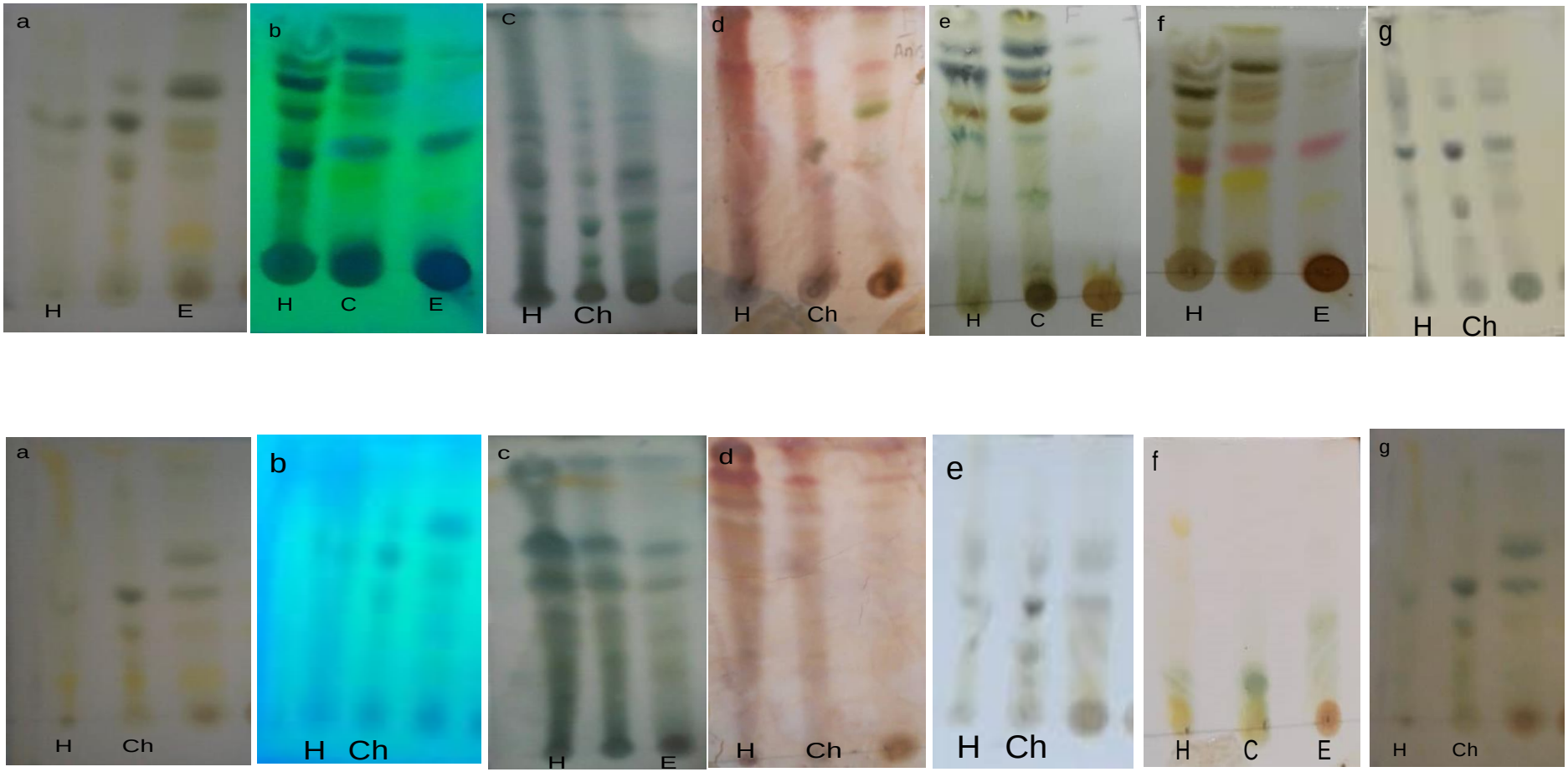

Figure 6: Chromatograms of the investigated *Cassia fistula* (upper) and *C. grandis* (lower) leaves extracts.

a, visible light; b, UV 254nm; c, Vanillin reagent; d, Anisealdehyde reagent; e, $AlCl_3$ reagent; f, KOH reagent; g, $FeCl_3$ reagent.

H, hexane extract; Ch, chloroform extract; EA, ethyl acetate extract,

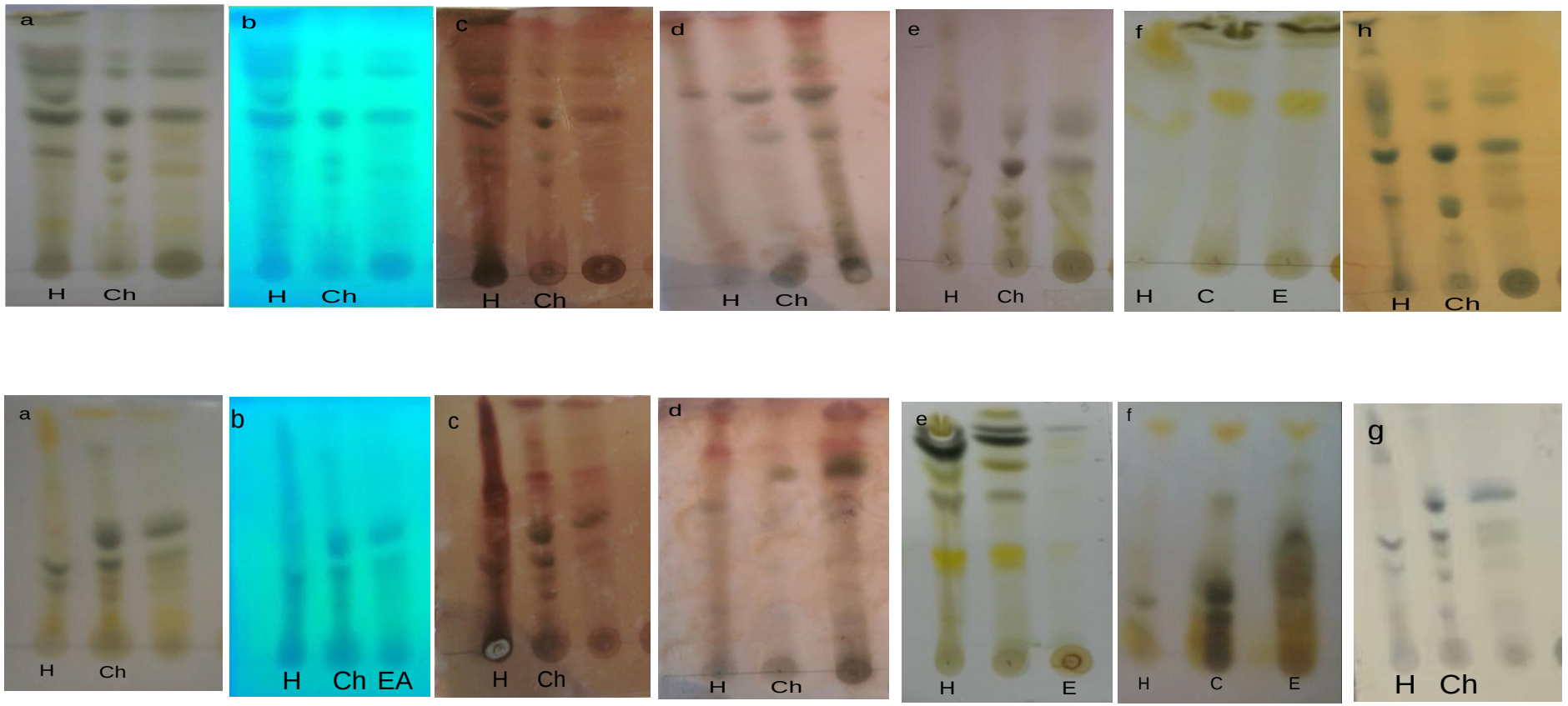

Figure 7: Chromatograms of the investigated *Senna alexandrina* (upper) and *S. italica* (lower) leaves extracts.

a, visible light; b, UV 254nm; c, Vanillin reagent; d, Anisealdehyde reagent; e, $AlCl_3$ reagent; f, KOH reagent; g, $FeCl_3$ reagent.

H, hexane extract; Ch, chloroform extract; EA, ethyl acetate extract.

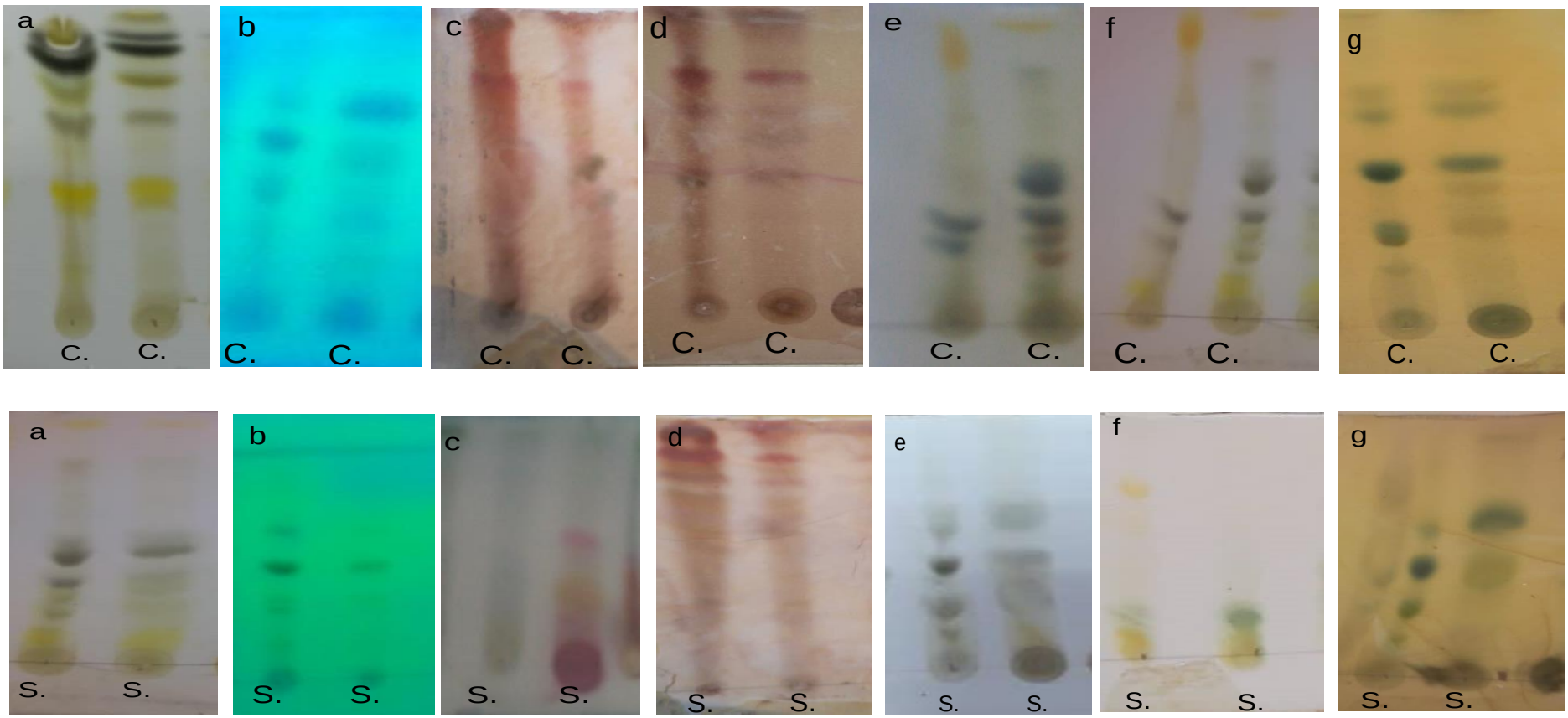

Figure 8: Chromatograms of the investigated *Cassia* (upper) and *Senna* (lower) species leaves methanolic extracts.

a, visible light; b, UV 254nm; c, Vanillin reagent; d, Anisealdehyde reagent; e, $AlCl_3$ reagent; f, KOH reagent; g, $FeCl_3$ reagent.

C.f, *Cassia fistula;* C.g, *Cassia grandis;* S.a, *Senna alexandrina;* S.i, *Senna italica*

3.4.2 Determination of total polyphenolic content

Total polyphenolic contents of the hexane, chloroform, ethyl acetate, and methanolic extracts of the investigated *Cassia* and *Senna* species were determined. Results were expressed as mg gallic acid equivalent (GAE)/ g dry weight and presented in Table 7.

3.4.2.1 *Cassia fistula* leaves

The total polyphenolic content of the four extracts of *C. fistula* leaves was in the range of 0- 277 mg GAE/g. The highest content was found in the ethyl acetate extract followed by the methanolic (20 mg GAE/g). The chloroform extract contained low amount (9.8 mg GAE/g) while the hexane extract was completely devoid from polyphenols.

3.4.2.2 *Cassia grandis* leaves

The total polyphenolic content of the four extracts of *C. grandis* leaves was in the range of 0- 261.2 mg GAE/g. The highest content was found in the ethyl acetate extract followed by the methanolic (108.8 mg GAE/g) and chloroform (57.8 mg GAE/g) extracts respectively. The hexane extract was completely devoid from polyphenols.

3.4.2.3 Senna alexandrina leaves

The total polyphenolic content of the four extracts of *S. alexandrina* leaves was in the range of 0-136.8 mg GAE/g. The highest content was found in the ethyl acetate extract followed by the methanolic extract (78 mg GAE/g). However, the chloroform and the hexane extracts did not show the presence of polyphenols.

3.4.2.4 *Senna italica* leaves

Extracts of *S. italica* leaves contained total polyphenolic in the range of 0-188.6 mg GAE/g. The highest content was found in the ethyl acetate extract followed

by methanolic extract (70.6 mg GAE/g) while the chloroform and hexane extracts were completely devoid from polyphenols.

Table 7: Total polyphenolic, flavonoids and tannins contents in extracts of the investigated *Cassia* and *Senna* species.

Plant	Extract	Total polyphenolic (mg GAE/g)	Total flavonoids (mg QE/g)	Total tannins (mg TAE/g)
Cassia fistula	Hexane	0.00	0.00	0.00
	Chloroform	9.8 ± 0.02	159.0 ± 0.01	0.00
	Ethyl acetate	277 ± 0.32	300.66 ± 0.03	0.00
	Methanol	20.8 ± 0.03	160.33 ± 0.02	0.00
Cassia grandis	Hexane	0.00	23.0 ± 0.00	0.00
	Chloroform	57.8 ± 0.07	223.0 ± 0.02	0.00
	Ethyl acetate	261.2 ± 0.16	618.66 ± 0.58	0.00
	Methanol	108.8 ± 0.04	416.00 ± 0.05	40 ± 0.03
Senna alexandrina	Hexane	0.00	262.33 ± 0.01	0.00
	Chloroform	0.00	152.66 ± 0.11	0.00
	Ethyl acetate	136.8 ± 0.03	499.33 ± 0.34	0.00
	Methanol	78.0 ± 0.03	431.33 ± 0.06	14 ± 0.03
Senna italica	Hexane	0.00	17.66 ± 0.01	0.00
	Chloroform	0.00	356.66 ± 0.43	0.00
	Ethyl acetate	188.6 ± 0.07	219.66 ± 0.10	0.00
	Methanol	70.6 ± 0.04	269.33 ± 0.01	38 ± 0.03

GAE: Gallic acid equivalent; QE: Quercitinutin equivalent; TAA: Tannic acid equivalent.

3.4.3 Determination of total flavonoids content

Total flavonoids content of the hexane, chloroform, ethyl acetate and methanolic extracts of the investigated *Cassia* and *Senna* species were also determined. Results, expressed as mg quercetin equivalents (QE))/ g dry weight and are presented in Table 7.

3.4.3.1 *Cassia fistula* leaves

The total flavonoids content on the four extracts of *C. fistula* leaves was in the range of 0 - 300.66 mg QE/g. The highest content was found in the ethyl acetate extract followed by the methanolic extract (160.3 mg QE/g). The chloroform extract contained low amount (159.0 mg QE/g) while flavonoids were not detected in the hexane extract.

3.4.3.2 *Cassia grandis* leaves

The total flavonoids content of the four extracts of *C. grandis* leaves was in the range of 23- 618.6 mg QE/g. The highest content was found in the ethyl acetate extract followed by the methanolic extract (416.0 mg QE/g). The chloroform extract contained 223.0 mg QE/g while the hexane extract contained the lowest amount (23.0 mg QE/g).

3.4.3.3 Senna alexandrina leaves

The total flavonoids content of *S. alexandrina* leaves extracts was in the range of 152.6-499.3 mg QE/g. The highest content was found in the ethyl acetate extract followed by the methanolic (431.3 mg QE/g) and hexane (262.3 mg QE/g) extracts respectively. The chloroform extract had the least content (152.6 mg QE/g).

3.4.3.4 *Senna italica* leaves

The total flavonoids content of the four extracts of *S. italica* leaves was in the range of 17.6-356.6 mg QE/g. The highest content was found in the chloroform

extract followed by the methanolic (269.3 mg QE/g), ethyl acetate (219.6 mg QE/g) and hexane (17.6 mg QE/g) extracts respectively.

3.4.4 Determination total tannins contents

Total tannins contents of the hexane, chloroform, ethyl acetate and methanolic extracts of the investigated *Cassia* and *Senna* species were determined. Results, expressed as mg tannic acid (TAE)/g dry weight and are depicted in Table 7.

3.4.4.1 *Cassia fistula* leaves

The hexane, chloroform, ethyl acetate and methanolic extracts of *C. fistula* leaves were completely devoid from tannins.

3.4.4.2 *Cassia grandis* leaves

The total tannins content of the four extracts of *C. grandis* leaves was only found in low content (40 mg TAE/g) in the methanolic extract while all other extracts (hexane, chloroform and ethyl acetate) did not contain tannins.

3.4.4.3 Senna alexandrina leaves

The total tannins content of the four extracts of *S. alexandrina* leaves was only recorded in low content (14 mg TAE/g) from the methanolic extract, it was not detected in the other three extracts.

3.4.4.4 *Senna italica* leaves

The total tannins content on the four extracts of *S. italica* leaves was only detected in the methanolic extract (38 mg TAE/g).

3.1 Discussion

The objective of the present study was to study the morphological characteristics of *C. fistula*, *C. grandis*, *S. alexandrina* and *S. italica* and evaluate the *in vitro* antimicrobial and antioxidant activities of their crude leaf extracts. In addition, phytochemical screening of secondary metabolites and the total polyphenolic, flavonoids and tannins contents in different extracts were performed. Morphological characteristics of the investigated *Cassia* and *Senna* species well separated the four species from each other. First the two *Cassia* species were separated from the *Senna* species by the filaments form and presence or absence of bracteoles. *Cassia* species have filaments of the 3 abaxial stamens distinctly curved and have 1 or 2 bracteoles while *Senna* spp. have filaments of all stamens straight or slightly curved and no bracteoles. Then *C. fistula* is separated from *C. grandis* by having cylindrical fruit shape, leaflets in 4-5 pairs and yellow petals while *C. grandis* possessed tube-shaped fruit, leaflets in 9-13 pairs and red- or pink-coloured petals. The characteristic straight pods and 2-7 pairs leaflets separated *S. alexandrina* from *S. italica* which has curved pods and 3-6 pairs leaflets. Irwin and Barneby (1982) were the first to segregate the genus *Cassia* sensu lato into the three allied genera (*Cassia* sensu stricto, *Chamaecrista* and *Senna*). Their work was based mainly on the characteristics of filaments and the presence or absence of bracteoles.

Extraction is the key process for phytochemicals recovery and isolation from plant materials in which extraction yield is directly affected by the chemical nature of phytoconstituents, processed extraction method, sample particle size, extraction solvent used, and presence of interfering compounds (Do *et al*., 2014). In this study, powder of the dried leaves of the four investigated plants was subjected separately to sequential maceration with hexane, chloroform, ethyl acetate and methanol. Generally, it was observed that the highest yield percentage was obtained from the methanolic extracts (Table 3). Feudjio *et al*. (2020) explained

that methanol has the ability to easily penetrate into the plant cell to solubilize a wide range of bioactive molecules (from polar to large portion of nonpolar) from the plant cells. This might be the possible reason that methanolic extract provided better extraction yields for the investigated plants. In addition, Stalikas (2007), demonstrated that types of plant parts, storage times, and temperature also affects the yield percentage.

Hexane, chloroform, ethyl acetate and methanol leaf extracts of *C. fistula*, *C. grandis*, *S. alexandrina* and *S. italica* were evaluated for their antimicrobial activity. Results are depicted in Table 5. Extracts from the four studied plants displayed variable antimicrobial activity. The highest antibacterial activity was recorded against *P. aeruginosa* and *S. aureus* exerted by chloroform extract of *S. alexandrina* and methanolic extract of *C. grandis* respectively with inhibition zone of 18 mm. Extracts were found less effective against *E. coli* (13 mm) and *B. subtiles* (12 mm) with highest activity against the former displayed by methanolic extract of *S. alexandrina* and *S. italic* and against the latter by chloroform extract of *C. fistula* and ethyl acetate and methanolic extracts of *S. italic*. Generally, extracts of the four plants exhibited better antifungal activity than antibacterial one. The highest antifungal activity against *C. albicans* was recorded from the ethyl acetate extract of *C. fistula* (28 mm) followed by methanolic extract of *S. alexandrina* (25 mm) while both the ethyl acetate extract of *C. grandis* and methanolic extract of *S. italica* exerted similar effect (20 mm). Also the ethyl acetate extract of *C. fistula* (27 mm) gave the highest antifungal activity against *A. niger* followed by the methanolic extract (22 mm) of *C. grandis* and *S. italica*. Furthermore, these extracts showed either higher or comparable inhibition values to that exerted by the standard drug nystatin which had inhibition zones of 22 and 20 mm against *A. niger* and *C. albicans* respectively. The minmum inhibitory concentration (MIC) was determined for the extracts showed highest antifungal activity. Results are presented in Table 6. The lowest MIC value (6.25 mg/mL), indicative to highest activity, was recorded against *C. albicans* by the ethyl acetate

extract of *C. fistula* leaf and methanolic extract of *S. italica* leaf. The latter in addition to the methanolic extract of *C. grandis* exhibited the lowest MIC value (12.5 mg/mL) against *A. niger*.

Comparing the antimicrobial activity of the investigated species with previous studies in the literature showed that in a study carried out by Kumar *et al*. (2006) on the antibacterial activity of *C. fistula* leaves, revealed that the ethanol extract recorded higher antibacterial activity against *S. aureus* (21 mm) but was less effective against *P. aeruginosa* (13 mm) than the results of methanolic extract obtained in the present study. Moreover, results of antifungal activity of methanolic extract of the leaf against *A. niger* (12 mm) was comparable to that obtained by Panda *et al*. (2010) against *A. flavus* (12.3 mm) suggesting the antifungal effect of *C. fistula* on different pathogens of the genus *Aspergillus* Phongpaichit *et al*. (2004) stated that the antifungal property of *C. fistula* might be due to the presence of a flavone glycoside. Magalhães *et al*. (2020) evaluated the antibacterial activity of *C. grandis* stem bark and their results revealed lower effect against *S. aureus* (15 mm) but higher activity against *E. coli* (15 mm) than that obtained in the present study. Comparing the antimicrobial activity of *S. alexandrina* with previous studies in the literature showed that the results obtained in the present study were in agreement with those performed by VijayaSekhar *et al*. (2016) who found that the methanolic extract showed potent antifungal activity. However, the same authors found that the hexane extract was not active which was in contrast to the results obtained in the present study where all tested microorganisims except *B. subtilus* were sensitive to the hexane extract. Moreover, the current study revealed higher antifungal activity than that recorded by Sood *et al*. (2012). Previous study on the antimicrobial activity of *S. italica* performed by Khalaf *et al*. (2019) on the aerial part revealed that the ethyl acetate extract showed considerable activity against *E. coli* (19 mm) and weak activity against *C. albicans*. Their results were not in agreement with those in the present study where the ethyl acetate was not active against *E. coli* and was effective

against *C. albicans* (14 mm). Overall, these variations could be attributed to different growing habitat of the plant, tested plant's part and solvent used for extraction in addition to strain type of microorganisms and culture media used. Moreover, many authors associated the antimicrobial activities of *Cassia* and *Senna* spp. to the presence of anthraquinones and flavonoids (Agarwal *et al.*, 2000; Wuthi-Udomlert *et al.*, 2010). In addition, the antimicrobial mechanistic aspects of extracts from species of these two genera were suggested to be attributed to their capacity to cause a leakage of appropriate ions from the cell wall of microorganisms (Oladunmoye *et al*., 2007).

Antioxidant activity of extracts from the four studied plants was determined by evaluating their capacity to scavenge the DPPH free radicals and results are presented in Figure 5. The highest scavenging radical activity was exerted by the two *Cassia* species with the ethyl acetate extract of *C. fistula* gave highest activity (77%) followed by the ethyl acetate and methanolic extracts of *C. grandis* (71%). The methanolic extract of *S. alexandrina* revealed moderate antiradical activity (56%) while all other extracts from the two *Senna* species in addition to non-polar extracts of *Cassia* species were either not active or exerted weak scavenging activity. The percentage inhibition value of antiradical activity of ethyl acetate extract from *C. fistula* and *C. grandis* was slightly higher for the former than that obtained by Siddhuraju *et al*. (2002) and lower for the latter than that recorded by Meena *et al.* (2009). Low antioxidant activity of other extracts could be due to the presence of some prooxidants, such as chrysophanol (rich in many *Senna* spp.) and reducing sugars which could dominate the antioxidant compounds present in the extracts (Siddhuraju *et al*., 2002).

Phytochemical screening of different extracts (Fig. 6-8) revealed that extracts were rich in phenolic compounds of different polarities and alkaloids were not detected in the four species extracts. Anthraquinones were detected mainly in *C. fistula* extracts. Results of total polyphenolic, flavonoids and tannins contents of different extracts from the four studied plants are presented in Table 7. Leaf ethyl

acetate extracts from the four species accumulated the highest total polyphenolic content with extract of *C. fistula* contained the highest amount (277 mg GAE/g) followed by that of *C. grandis* (261.2 mg GAE/g), *S. italica* (188.6 mg GAE/g) and *S. alexandrina* (136.8 mg GAE/g) respectively. The leaf methanolic extract also had relatively considerable amount of polyphenolic content with highest content obtained from *C. grandis* (108.8 mg GAE/g). On the other hand, all extracts of the four plants accumulated higher amount of total flavonoids than their respective polyphenolic content. The highest total flavonoids content was obtained from the methanolic extract of *C. grandis* (618.66 mg QE/g) followed by ethyl acetate extract of *S. alexandrina* (499.33 mg QE/g), methanolic extract of *S. alexandrina* (431.33 mg QE/g), methanolic extract of *C. grandis* (416 mg QE/g), chloroform extract of *S. italica* (356.66 mg QE/g), and ethyl acetate of *C. fistula* (300.66 mg QE/g) respectively. Other extracts contained values ≤ 269.33 mg QE/g. All extracts except methanolic extract of *C. grandis* and *S. alexandrina* were devoid of tannins. Total tannins content in these two species was also not in high abundance (40 and 14 mg TAE/g respectively) when compared to total polyphenolic and flavonoids contents. Variation in polyphenolic and falvonoids contents of the studied species from values reported for the same studied species in the literature could be attributed to different factors like geographical areas and climatic conditions for the growth of the plant (Khurm *et al.*, 2020).

Several researchers reported significant correlation between the phenolic content and antioxidant activity of extracts (Roy and Dutta, 2021; Sanoria *et al.*, 2020). Thus the highest content of polyphenolic and flavonoids in the polar extracts of *C. fistula* and *C. grandis* supported their contribution in their high antiradical activity. However, although the extracts of the two *Senna* species contained high phenolic content, they exhibited no or weak antiradical activity suggesting that the nature of their phytoconstituents do not possess potent antiradicale property. Nevertheless, it would be necessary in the future to carry out more complementary

assays to all extracts in order to have a comprehensive understanding about their antioxidant properties.

Chapter Four

4. Conclusion and Recommendations

4.1 Conclusion

The present study described different morphological characteristics of the two *Cassia* spp. and two *Senna* spp where they were separated from each other by the filaments form and the presence or absence of bracteoles.

Extracts of different polarity from the four studied *Cassia* and *Senna* species showed variable antimicrobial and antioxidant activities. The inhibitory zones of different extracts varied with the type of microorganism tested. Generally, extracts of the four plants exhibited better antifungal activity than antibacterial one with highest antifungal activity against *C. albicans* and *A. niger* which was recorded from the ethyl acetate extract of *C. fistula*. The highest scavenging radical activity was exerted by the two *Cassia* species. The majority of extracts were rich in flavonoids while the polyphenols were mainly accumulated in the two polar extracts. Therefore, these plants could be a very beneficial source of natural bioactive agents.

4.2 Recommendations

Further studies should be undertaken to elucidate the particular phytochemicals responsible of the observed antimicrobial and antiradicals activities and their pharmacological mechanism.

In vivo and clinical studies as well as toxicological parameters should be estimated to examine in future for suitable drug leads from these plants.

Other biological activities like anticancer, antimalarial, antiviral, anti-inflammatory should be evaluated.

Detailed phytochemical study could be performed to determine the chemotaxonomical markers that distinguished the four studied species in addition to other *Cassia* and *Senna* species indigenous to Sudan.

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Appendex 1

Cassis fistula

Cassia grandis

Senna alexandrina

Senna italica