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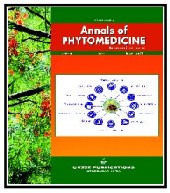

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Morphological, anatomical characterization and profiling of laxative principles sennosides in fifteen species from genus *Cassia, Chamaecrista and Senna*

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Abstract

Cassia, *Chamaecrista*, and *Senna* species are potential sources of laxative principle sennosides. About 45 species belonging to the family are reported from India. It is often essential to confirm the identity of official drug material as mentioned in the Pharmacopoeia. In the present investigation, 15 species were selected for morphological and anatomical characterization. Profiling of sennosides A and B in extracts of leaves of selected species was also carried out using a RP-HPLC method. Selected species could be differentiated on the basis of their habit, morphological and anatomical characters. *S. alata, S. auriculata, S. polyphylla, S. surattensis* are shrub or small tree whereas *S. alexandrina, C. pumila, S. tora, C. mimosoides, C. absus, S. occidentalis, S. uniflora* are herb and *C. fistula, C. javanica, C. renigera* and *S. siamea* are tree. These species can be differentiated by the shape of the leaflets, *viz.*, oblong, obovate-oblong in *S. alata, S. auriculata, C. javanica, S. surattensis, S. tora* and *S. uniflora*; elliptic-lanceolate in *S. alexandrina*; ovate-elliptical in *C. fistula*; Ovate-lanceolate in *S. occidentalis*; oblong to suborbicular in *Chamaecrista absus*; oblong-elliptic in *S. polyphylla* and linear-oblong in *Chamaecrista mimosoides* and *Chamaecrista pumila*. The length of the petiole in the selected species was in the range of 0.1 to 0.9 mm. The length of the leaves also varied in these species such as the leaflets were very small in size in *S. auriculata* , *Chamaecrista mimosoides, S. polyphylla* and *Chamaecrista pumila*. Medium size leaflets were present in *S. alexandrina, C. javanica, Chamaecrista absus, C. renigera, S. tora* and *S. uniflora*. The leaflets size were comparatively larger in *S. alata, S. occidentalis* , *C. fistula* and *S. surattensis*. The weight of the leaves was directly proportional to the size of the leaves. The anatomical characterstics showed that *C. fistula, S. polyphylla, Chamaecrista pumila, S. surattensis* and *S. siamea* had amphicribal type of vascular bundle and xylem was surrounded by phloem whereas in *S. alata, S. alexandrina, Chamaecrista absus, Chamaecrista mimosoides, S. auriculata, C. javanica, C. renigera, S. tora* and *S. uniflora* had collateral type of vascular bundle in which phloem was towards abaxial side and xylem towards adaxial side. Based on the sennoside content, three species, *i.e., S. tora* , *C. javanica* and *S. occidentalis* could be considered as potential alternative sources of sennoside A and B.

1. Introduction

Species from *Senna* were previously incorporated among about 600 species of *Cassia* (Irwin and Turner, 1960). On subsequent taxonomic classifications, this large genus was divided into three smaller genera (*Cassia*, *Chamaecrista* and *Senna*) and these three genera were ascribed to subtribe Cassiinae (Irwin and Barneby, 1982). However, there has been considerable diversions of opinion concerning the limitations and taxonomic status of its three constituents subgenera (Kumar *et al*., 2007). It is an economically as well as medicinally important genus (Mondal and Mandal, 1997). Great diversity in habit, ranging from tall trees to delicate prostrate, annual herbs may be seen within its bound (Irwin and Turner, 1960). About 45 species of *Cassia* are reported from India. *Cassia* species have been of keen interest in phytochemical and pharmacological research due to their excellent medicinal values.Their laxative and purgative uses are well known in folk medicine (Dalziel, 1948; Abo *et al*., 1999 ; Hennebelle *et al*., 2009). A wide range of biological activities including cytotoxic activity were reported from the different species of these genus. Anthraquinone compounds from species of genus *Cassia*, *Chamaecrista* and *Senna* are well established for their laxative property and also stimulating laxative activity of anthraquinone derivatives are well characterized (Sakulpanich and Gritsanapan, 2009). The laxative activity is dependent on the degree of content of total anthraquinone glycosides. Glycosides of anthraquinones are hydrolyzed by β-glucosides of the intestinal flora to free anthraquinones. Anthranones, reduced form of anthraquinones are active form of the laxative effect (Bennett, 1975 ; Bruneton 1995). It was reported that glycosides of anthraquinone have stronger activity than free aglycones (Thomson, 1971; Moreau *et al*., 1985; De Witte and Lemil, 1990) .

Constipation represents a major healthcare problem. About 25 per cent of the western population is affected by its acute or chronic forms. About fourteen per cent urban Indian population suffer

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from chronic constipation. The incidence of chronic constipation is more than 10 per cent worldwide. Also, 20-30% people over the age of 60 years in the UK are reported to take laxative once a week (Mukhopadhyay *et al*., 1998). Constipation is a symptomatic state than a proper disease; however, it is a considerable source of inconvenience. Piles or haemorrhoids, ulcers, abdominal pain, anal fissures and fistula are some of the known medical conditions spawned by constipation. Heart attack and strokes are also common among sufferers. Sennosides are listed as one of the most important pharmaceutical preparation of the plant origin (Morinaga *et al*., 2000). Despite the availability of a large number of synthetic purgatives, sennosides containing prescriptions are still widely used and their importance is increasing. Food habits including low water intake have been suggested as the main factor causing constipation. However, a major concern surrounding constipation is lack of awareness about the seriousness of the disease and patients' hesitation to approach specialists for related problems. Also, home remedies is the most preferred treatment option for relief from constipation. A safer over the counter laxative plant preparations requires a better knowledge of the chemical compositions of the various utilized extracts. Different species of *Cassia*, *Chamaecrista* and *Senna* have been used in traditional medicines as laxative by local people for a long time.

S. alata is a shrub. Pharmacological evaluation on rat showed that *S. alata* had a similar laxative activity as that of senna up to 200 mg/kg (leaf infusion in both cases). *S. alexandrina* is widely used for the relief of constipation. Sennosides are mainly present in the leaves and pods of *S. alexandrina*. It is a branching shrub with a height up to 1.80 m. *S. auriculata*, commonly known as Tanners's cassia, is a common plant in Asia. It has been widely used in traditional medicines as a cure for rheumatism, conjunctivitis and diabetes (Pari and Latha, 2002). Presence of anthraquinones was reported in heartwood and pod husk of *S. auriculata* (Rai *et al*., 1997). *C. absus* grows as a sticky plant in almost all the states of India particularly in North-West India (Nadkarni, 1976). The plant is mainly useful in the skin diseases and eye ailments. Its seed is used as astringent and also for cathartic properties (Pandya *et al*., 2010). *C. fistula* is cultivated widely throughout India as an ornamental and deciduous plant. It is also cultivated in the tropics including West Indies, Ceylon, China, Egypt and many other countries. In Ayurvedic medicine, this plant is used for treatment of heamatemesis, pruritus, leucoderma and diabetes. Its leaf juice is given for erysipelas and skin disease (Chopra *et al.*,1992). Urban people of North-Eastern part of India use pods and leaves of this plant as antiallergic and also as hepatoprotective agents (Bhakta *et al*., 2001). It has also been described as a cathartic agent due to anthraquinone derivatives present in the pulp of fruits (Iyengar *et al.*, 1966). Its pods are traditionally used as a mild laxative as are the leaves and flowers but to a minor degree. It is widely used in traditional medicine as mild laxative and also as purgative for children and pregnant women (Bahorun *et al*., 2005; Iyengar *et al*., 1966). *C. javanica* is a medium size tree. Presence of usual and novel anthraquinones were reported from various parts of this plant (Tiwari and Singh, 1979; Chaudhari and Chawla, 1987; Singh and Singh, 1988 ; Singh *et al*., 1999). Seed extract of *C. javanica* exhibited purgative and haemagglutinating activity (Rastogi and Mehrotra, 1995). *C. mimosoides* is a low, diffuse shrub up to 1.5 m in height, found in open grasslands at low and medium altitudes. Its all parts were reported to contain anthraquinones. The dried young leaves and stems are used as a substitute for tea in Japan. Root is used as cure for diarrhoea (Mukherjee *et al*., 1987). *S. occidentalis* is a common weed found throughout in India. It is used in indigenous and folk medicine for a variety of purposes. *C. occidentalis* is one of the ingredients of several polyherbal formulations available in India for liver disorders (Saraf *et al*., 1994). *S. polypylla* also known as dessert senna, is a shrub or occasionally a small tree having height up to 2.00 to 3.00 m. *Chamaecrista pumila* is a diffuse terrestrial and strout annual herb and usually found in shades of trees, crevices of rocks and also in the open gravelly substratum. Sennosides were reported from petroleum ether, benzene, acetone, chloroform and alcohol extracts of *C. pumila* (Sharma *et al*., 2012). *C. renigera* is a typical tropical tree and it is known as rich source of anthraquinones and flavonoids (The Wealth of India, 1992). *S. surattensis* is a flowering plant widely grown as an ornamental plants in tropical and sub-tropical areas. It has been traditionally used in many countries for food and medicinal use (Uthaya Kumar *et al*., 2014). *S. siamea* is a very widespread medicinal and food plant cultivated in Southeast Asia and sub-Saharan Africa. Its leaves and stem bark is used in constipation (Ahn *et al*., 1978). *S. tora* is a small annual legume shrub that grows as a common weed in Asian countries and cultivated as a traditional medicinal herb for multiple therapies. It is traditionally used as laxative for the treatment of leprosy and various skin disorders (Rejiya *et al*., 2009). *S. uniflora* is closely allied to *S. obtusifolia* but hairiness is the main characteristic of this species (Singh, 2001). It grows intermingled with *S. tora* and has the similar appearance (Meena and Yadav, 2009).

Authentication at different stages of harvesting of the plant materials as well as preparation of the final product is required in order to ensure efficacy and safety of herbal products. Misidentification of plants could be intentional or non-intentional (Kiran *et al*., 2010). Also, adulteration can take place because of ignorance or intentional substitution with cheaper plants which may affect efficacy and safety of the drug. Therefore, proper authentication of plant material is important. This would avoid the acceptance of wrong plant materials for drug uses. Characterization of morphological and anatomical parameters are essential for correct identification. These parameters could be used as reference for correct identification. Morphological and anatomical characterization are the general approaches for identification of medicinal plants. Literature survey revealed that information about these traits and comparative profiling of laxative principles sennosides (sennoside A, SA, $C_{42}H_{38}O_{20}$, MW=862 and sennoside B, SB, $C_{42}H_{38}O_{20}$, MW=862) in selected samples belonging to three genera (*Cassia*, *Chamaecrista* and *Senna*) is lacking (Sihanat *et al*., 2016). This information would be of immense utility as a number of *Cassia*, *Chamaecrista* and *Senna* species are widely used in many traditional systems of medicine including Ayurveda and Unani systems of medicine. The present investigation was conceived with the following objectives a. Characterization of morphological and anatomical traits of leaves of fifteen plants belonging to three genera and b. Comparative profiling of SA and SB in leaves extracts using a HPLC-PDA method.

2. Materials and Methods

2.1 Plant material and chemicals

The samples of *S. alata, S. alexandrina, S. auriculata* , *Chamaecrista absus*, *C. fistula, C. javanica*, *Chamaecrista mimosoides*, *S. occidentalis*, *S. polyphylla* , *Chamaecrista pumila*, *C. renigera* ,

S. surattensis, *S. siamea*, *S. tora* and *S. uniflora* were collected from Botanical Garden, Botany Department, Faculty of Science, The M.S. University of Baroda, Vadodara (latitude : 22.309°, longitude : 73.187° and altitude : 39.00 m) . The collected samples of plants were confirmed with the monograph by a taxonomist and specimen of samples were deposited in herbarium. Leaves, stems and roots (where applicable) of collected samples were separated. These samples were air dried in shade at ambient temperature (25-30ºC), followed by hot air oven drying (55-60ºC) for 8-10 h. The dried samples of leaves were made into fine powder (100 mesh) using an electric grinder. HPLC grade solvents methanol, acetonitrile and analytical grade trifluroacetic acid (TFA) were purchased from Merck, Mumbai, India. Deionized water obtained from a Millipore water purification system (Millipore, Milli Q gradient A10, France) was used throughout the experiment. Standard SA (purity ≥ 96.0%) and SB (purity ≥ 94.5%) were purchased from Sigma Aldrich, Mumbai, India.

2.2 Morphological and anatomical characterization

Leaves of 15 selected species belonging to three genera were used for morphological and anatomical characterization. The vegetative characters of the plants such as leaf length (cm), leaf width (cm), petiole length (mm), fresh weight of the leaves (g) and dry weight of the leaves (g) were recorded for morphological characterization. For anatomical characterization, mature leaf samples were freshly collected and immediately fixed in FAA (formalin : acetic acid: alcohol (70%), 10:5:85 v/v/v). After 72 h of fixation, samples were transferred to alcohol (70%) for further processing and storage. Leaf samples were trimmed into 1-2 mm long pieces and dehydrated through tertiary butyl alcohol series and infiltrated in paraffin (Berlyn and Miksche, 1976; Johansen,1940). Transverse sections of leaves were directly sectioned on rotary microtome (Leica 2010R). Sections of 15-20 µm thickness were obtained in transverse planes and stained with safranin-astra blue combination (Srebotnik and Messener, 1994). After dehydration through ethanol-xylene series, sections were mounted in dibutyl phthalate xylene (DPX). Important results were microphotographed using trinocular research microscope (Leica DM 2000) attached with fire wire digital camera (Leica DFC295).

2.3 Extracts preparation

The powdered dried samples of leaves (5 g) was refluxed with 100 ml of aqueous alcoholic solvent (water : methanol, 20 : 80, v/v) for five hours on a water bath (60ºC). After that, flask was cooled at room temperature and content was filtered using vacuum filtration. Supernatant obtained was concentrated using vacuum rotary evaporator at 60°C. Stock solution (1 mg/ml) of extract samples and standards (SA, and SB) were prepared by dissolving in aqueous alcoholic solvent (water: methanol, 2: 8, v/v). Extracts and standard samples were filtered through 0.45 µm membrane filters before HPLC-PDA analysis (Dhanani *et al*., 2017).

2.4 HPLC-PDA analysis

Chromatographic analysis was carried out using a HPLC system consisting of a quaternary pump, an in-line vacuum degasser, a PDA detector (Waters 2996) and Empower software (Waters). The chromatographic separation was carried out in linear gradient elution mode on a RP-18 column (250 x 4.6 mm, 5 µm, x-Bridge, Waters) at 25ºC. The mobile phase was a mixture of TFA in water (pH 2.25, 0.05 %, v/v, solvent A) and acetonitrile (solvent B). Gradient elution mode was used for separation of SA and SB in samples. The injection volume was 20 µl. The PDA detector wavelength was set at 272 nm for the identification and quantification of SB and SA in different extracts of samples. Concentration of SB and SA in extract samples were calculated by the comparison of the integrated peak area of the individual compounds with those of standard curve prepared from the corresponding standards (Dhanani *et al*., 2017).

3. Results

For development of more reliable quality control methodology, correct identfication of medicinal plants is important. A conventional method for identification of plant structural is microscopic evaluation and it is a simple, rapid and inexpensive method (Sihanat *et al*., 2016). The taxonomy of plants in genus *Cassia*, *Chamaecrista* and *Senna* has several synonyms at species level and also the morphological features of many species are often similar.

3.1 Characterization of morphological and anatomical parameters

Morphological and anatomical characters are useful diagonostic features of the leaf for correct identification.The first objective of the present investigation was collection of natural populations of different species of *Cassia*, *Chamaecrista* and *Senna* for evaluating them on morphological basis. In the present study, plants with different habits were taken into consideration and included seven herbs, four shrubs and four trees (Table 1). Leaves of samples were paripinnate; leaflets among the species studied were between two to twelve pairs of various shapes (ovate, obvate, lanceolate) and texture being glabrous to pubescent (Table 1). Data collected on various morphological parameters, namely; leaf length, leaf width, petiole length, fresh and dry weight exhibited significant variations amongst the selected species (Table 2). The leaf/leaflets were dorsiventral and showed the presence of midrib and lamina. The upper epidermis was single layered composed of somewhat squarish or rectangular having thin layer of cuticle. Its outer surface possessed unicellular or uniserate and multicelluar septate trichomes. Stomata were paracytic or anomocytic or anisocytic type (Table 3, Figure 1). Palisade tissue composed of single to many layered, elongated cells, compactly or loosely arranged chloroplast with spaces or no space. Palisade layer was usually single layered with expception of *Chamaecrista absus*, *C. renigera*, *C. javanica* and *S. auriculata*. It was two to three layered in *S. alexandrina* and palisade layer was present on both the sides. Spongy parenchyma in most of the species was 4 to 5 layered with exception of 2 to 3 layer in *S. alata*, *S. auriculata*, *Chamaecrista mimosoides* and *S. polyphylla* were single to many layered loosely arranged with air cavities. The midrib was hemispherical on abaxial side and short lump on adaxial side with collenchymatous cells however *Chamaecrista pumila* midrib was parallel on both abaxial and adaxial side. The pericycle was parenchymatous in nature. The vascular bundle was amphicribal in *C. fistula* , *Chamaecrista pumila*, *S. polyphylla* , *S. siamea and S. saurattensis*, while, it was collateral in *S. alata*, *S. alexandrina*, *S. auriculata*, *Chamaecrista absus*, *Chamaecrista mimosoides*, *S. occidentalis*, *C. javanica*, *C. renigera*, *S. tora* and *S. uniflora*.

Table 1: Morphological characteristics of selected species from genus *Cassia, Chamaecrista* and *Senna*

Species	Habit	Leaf shape	No. of leaflet	Leaf surface
Senna alata (L.) Roxb(CA)	Shrub or small tree	Oblong	8-12	Sparsely pubescent or glabrous
Senna alexandrina Mill. (CA1)	Herb or shrub	Elliptic *-lanceolate*	4-6	Pubescent
Senna auriculata (L.) Roxb. (CA2)	Shrub	Oblong or obovate-oblong	6-12	Pubescent
Chamaecrista absus (L.) H. (S. Irwin & Barneby) *(*CA3)	Herb	Obovate to suborbicular	2	Pubescent
Cassia fistula L. (CF)	Tree	Ovate-elliptic	5-8	Glabrous
Cassia javanica L. (CJ)	Medium size tree	Oblong or oval	6-18	Glabrous
Chamaecrista mimosoides (L.) Greene (CM)	Herb	Linear-oblong	7-60	Glabrous
Senna occidentalis (L.) Link (CO)	Herbs or undershrub	Ovate-lanceolate *to elliptic*	3-5	Sparsely pubescent or glabrous
Senna polyphylla (Jacq.) H.S. Irwin and Barneby (CP)	Shrub	Ovate-elliptic	5-12	Glabrous
Chamaecrista pumila (Lam.) K.Larsen (CP1)	Herb	Linear-oblong	10-18	Glabrous
Cassia renigera Benth. (CR)	Tree	Oblong-elliptic	10-20	Sparsely pubescent to glabrous
Senna surattensis (Burm.f.) H.S. Irwin and Barneby (CS)	Shrub or small tree	Elliptic or oblong –obovate	6-9	Glabrous or pubescent beneath
Senna siamea (Lam.) H.S. Irwin and Barneby (CS1)	Tree	Elliptic or oblong –obovate	8-15	Pubescent
Senna tora (L.) Roxb (CT)	Herb	Obovate or obovate -oblong	3	Sparsely pubescent
Senna uniflora (Mill.) H.S. Irwin and Barneby(CU)	Herb	Obovate or obovate -oblong	3-5	Pubescent

Table 2: Variability for morphological and chemotype attributes amongst the diversified ecotypes of selected species from genus *Cassia, Chamaecrista* and *Senna*

Name	Length of leaves (cm)		Width of leaves (cm)		Fresh weight of leaves (g)		Dry weight of leaves (g)		Petiole (mm)	
	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD
CA	5.000 - 5.500	5.267 ± 0.252	2.000 - 2.200	2.100 ± 0.100	6.000 - 6.440	6.187 ± 0.227	2.260 - 2.380	2.447 ± 0.227	0.100 - 0.200	0.167 ± 0.058
CA1	2.500 - 3.000	2.733 ± 0.252	1.000 - 1.200	1.067 ± 0.115	0.220 - 0.247	0.235 ± 0.014	0.067 - 0.093	0.082 ± 0.013	0.100 - 0.100	0.100 ± 0.000
CA2	1.700 - 2.000	1.867 ± 0.153	0.070 - 1.000	0.387 ± 0.531	0.099 - 0.190	0.159 ± 0.052	0.007 - 0.082	0.032 ± 0.043	0.100 - 0.200	0.167 ± 0.058
CA3	2.100 - 3.700	2.833 ± 0.808	1.600 - 2.000	1.800 ± 0.200	0.109 - 0.127	0.121 ± 0.010	0.001 - 0.017	0.006 ± 0.009	0.200 - 0.200	0.200 ± 0.000
CF	11.500 - 12.500	11.967 ± 0.503	5.500 - 8.500	6.967 ± 1.501	9.908 - 10.209	10.075 ± 0.153	4.499 - 4.800	4.666 ± 0.153	0.500 - 0.900	0.733 ± 0.208
CJ	3.200 - 4.400	3.833 ± 0.603	1.000 - 1.500	1.267 ± 0.252	2.390 - 2.450	2.430 ± 0.035	0.886 - 0.946	0.926 ± 0.035	0.100 - 0.200	0.167 ± 0.058
CM	0.900 - 1.200	1.033 ± 0.153	0.200 - 0.300	0.233 ± 0.058	0.055 - 0.051	0.054 ± 0.002	0.007 - 0.011	0.010 ± 0.002	0.100 - 0.100	0.100 ± 0.000
CO	5.000 - 10.000	7.933 ± 2.610	1.500 - 4.000	2.800 ± 1.253	1.167 - 1.186	1.175 ± 0.010	0.624 - 0.643	0.632 ± 0.010	0.200 - 0.300	0.267 ± 0.058
CP	0.600 - 0.700	0.633 ± 0.058	0.200 - 0.400	0.267 ± 0.115	0.103 - 0.126	0.117 ± 0.012	0.009 - 0.013	0.011 ± 0.002	0.100 - 0.100	0.100 ± 0.000
CP1	0.050 - 1.000	0.373 ± 0.543	0.010 - 0.020	0.017 ± 0.006	0.175 - 0.188	0.183 ± 0.007	0.010 - 0.023	0.018 ± 0.007	0.100 - 0.100	0.100 ± 0.000
CR	2.800 - 3.100	2.967 ± 0.153	2.000 - 2.100	2.033 ± 0.058	2.300 - 2.380	2.343 ± 0.040	0.983 - 1.063	1.026 ± 0.040	0.100 - 0.200	0.167 ± 0.058
CS	4.000 - 8.000	6.500 ± 2.179	2.500 - 3.500	2.967 ± 0.503	3.250 - 3.290	3.273 ± 0.021	0.490 - 0.530	0.513 ± 0.021	0.200 - 0.200	0.200 ± 0.000
CS1	3.500 - 5.500	4.400 ± 1.015	1.800 - 2.000	1.900 ± 0.100	7.200 - 7.280	7.250 ± 0.044	2.900 - 2.910	2.903 ± 0.006	0.400 - 0.500	0.467 ± 0.058
CT	2.300 - 4.500	3.500 ± 1.114	1.000 - 2.500	1.833 ± 0.764	0.330 - 0.400	0.360 ± 0.036	0.050 - 0.120	0.080 ± 0.036	0.100 - 0.100	0.100 ± 0.000
CU	2.000 - 5.000	3.333±1.528	1.000 - 2.500	1.867 ± 0.777	2.185 - 2.188	2.187 ± 0.002	0.303 - 0.306	0.305 ± 0.002	0.100 - 0.100	0.100 ± 0.000

CA = *Senna alata*, CA1 = *Senna alexandrina*, CA2 = *Senna auriculata*, CA3 = *Chamaecrista absus*, CF = *Cassia fistula*, CJ = *Cassia javanica*, CM = *Chamaecrista mimosoides*, CO = *Senna occidentalis*, CP = *Senna polyphylla*, CP1 = *Chamaecrista pumila*, CR = *Cassia renigera*, CS = *Senna surattensis*, CS1 = *Senna siamea*, CT = *Senna tora*, CU = *Senna uniflora*, SD = Standard Deviation.

Table 3: Anatomy description of selected species from genus *Cassia, Chamaecrista* and *Senna*

Species	Epidermis	Trichome	Stomata	Palisade tissue	Spongy tissue	Midrib	Pericycle	Vascular bundle
CA	Wavy in outline, single layered, rectangular shape and cells with cuticle and lower epidermis is papillose	Posses few unicellular uniserate multicellular trichomes	paracytic type and anisocytic type	Single layered, elongated cells, compactly arranged chloroplast	Few layered, loosely arranged	Hemispherical on abaxial side and short lump on adaxial side with collench-ymatous cells	Composed of parenchy-matous cells	Collateral type-phloem towards abaxial side and xylem towards adaxial side
CA1	Wavy in outline, single layered, square shape cells with cuticle	Very few unicellular trichomes	paracytic type and anomocytic type	Present on both the side of the leaves, elongated, loosely arranged cells with air spaces	4-5 layered loosely arranged cells	Hemispherical on abaxial side and short lump on adaxial side with collen-chymatous cells	Composed of parenchyma-tous cells	Collateral type-phloem towards side and xylem towards abaxial adaxial side
CA2	Wavy in outline, single layered, square shape cells with cuticle	Possess few unicellular and uniserate	paracytic type and anisocytic type	Double layered, elongated, loosely arran-ged cells	Few layered, loosely arranged cells with large spaces	Hemispherical on abaxial side and short lump on adaxial side with collen-chymatous cells	Composed of parenchyma-tous cells	Collateral type-phloem towards abaxial side and xylem towards adaxial side
CA3	Wavy in outline, single layered, square shape cells with cuticle	multicellular trichomes Possess few unicellular and uniserate multicellular trichomes	paracytic type and anisocytic type	Single layered, elongated cells, compactly arranged chloroplast	4-5 layered loosely arranged cells	Hemispherical on abaxial side and short lump on adaxial side with collen-chymatous cells	Composed of parenchy-matous cells	Collateral type-phloem towards abaxial side and xylem to wards adaxial side
CF	Wavy in outline, single layered, square shape cells with cuticle	Possess few unicellular trichomes	Paracytic type	Single layer present on both the side of the leaves, elongated, compactly arranged cells	4-5 layered loosely arranged cells	Hemispherical on abaxial side and little depression on adaxial side with collenc-hymatous cells	Composed of parenchy-matous cells	Amphic-ribal type-xylem surroun-ded by phloem
CJ	Wavy in outline, single layered, square shape cells with cuticle	unicellular trichomes throughout the epidermis	anisocytic, anomocytic type	2-3 layered elongated compactly arranged cells with no spaces	single or double layered compactly arranged cells	Hemispherical on abaxial side and short lump on adaxial side with collen-chymatous cells	Composed of parenc-hymatous cells	Collateral type-phloem towards abaxial side and xylem towards adaxial side

CM	Wavy in outline, single layered, square shape cells with cuticle	Very few unicellular trichomes	Paracytic and anisocytic type	Single layered, elongated cells, compactly arranged chloroplast	2-3 layered loosely arranged cells	Hemispherical on abaxial side and short lump on adaxial side	Composed of parenchy-matous cells	Collateral type-phloem towards abaxial side and xylem towards adaxial side
CO	Wavy in outline, single layered, square shape cells with cuticle	Very few unicellular trichomes	Paracytic and anisocytic type	1-2 layered elongated loosely arranged cells	4-5 layered loosely arranged chlorenchy-matous cells	Hemispherical on abaxial side and short lump on adaxial side with collen-chymatous cells	Composed of parenchy-matous cells	Collateral type-phloem towards abaxial side and xylem towards adaxial side
CP	Wavy in outline, single layered, square shape cells with cuticle	Possess few unicellular trichomes	Paracytic type	Single layered, elongated cells, compactly arranged chloroplast	2-3 layered compactly arranged with no spaces	Hemispherical on abaxial side and little depression on adaxial side with collenc-hymatous cells	Composed of parenchy-matous cells	Amphicri-bal type-xylem surroun-ded by phloem
CP1	Wavy in outline, single layered, square shape cells with cuticle	Very few unicellular trichomes	anisocytic , anomocytic type	Single layered elongated loosely arranged cells	3-4 layered loosely arranged cells	Parallel on both abaxial and adaxial side	Composed of parenchy-matous cells	Amphicri-bal type-xylem surroun-ded by phloem
CR	Wavy in outline, single layered, square shape cells with cuticle	Possess few unicellular and uniserate multicellular trichomes	anisocytic, anomocytic type	1-2 layered elongated compactly arranged cells with no spaces	3-4 layered loosely arranged cells	Hemispherical on abaxial side and little depression on adaxial side with collenc-hymatous cells	Composed of parench-ymatous cells	Collateral type-phloem towards abaxial side and xylem towards adaxial side
CS	Wavy in outline, single layered, square shape cells with cuticle	Possess no trichomes	paracytic type	Single layered elongated loo-sely arranged cells with large air spaces	3-4 layered loosely arranged cells with large air spaces	Hemispherical on abaxial side and little depression on adaxial side with collench-ymatous cells	Composed of parench-ymatous cells	Amphicri-bal type-xylem surroun-ded by phloem
CS1	Wavy in outline, single layered, square shape cells with cuticle	Possess few unicellular and uniserate multicellular trichomes	paracytic type	Single layer present on both the side elongated, compactly arranged cells	4-5 layered loosely arranged cells	Hemispherical on abaxial side and little depression on adaxial side with collench-ymatous cells	Composed of parench-ymatous cells	Amphicri-bal type-xylem surroun-ded by phloem

CT	Wavy in outline, single layered, square shape cells with cuticle	Possess few unicellular and uniserate multicellular trichomes	paracytic type anisocytic type	1-2 layered loosely arranged cells	4-5 layered loosely arranged cells	Hemispherical on abaxial side and short lump on adaxial side with colenc-ymatous cells	Composed of parench-ymatous cells	Collateral type-phloem towards abaxial side and xylem towards adaxial side
CU	Wavy in outline, single layered, square shape cells with cuticle	Possess few unicellular and uniserate multicellular trichomes	anisocytic, anomocytic type	Single layered very elonga-ted loosely arranged cells	4-5 layered loosely arranged cells	Hemispherical on abaxial side and little depression on adaxial side with collenchy matous cells	Composed of parenchy-matous cells	Collateral type-phloem towards abaxial side and xylem towards adaxial side

CA = *Senna alata*, CA1 = *Senna alexandrina*, CA2 = *Senna auriculata*, CA3 = *Chamaecrista absus*, CF = *Cassia fistula*, CJ = *Cassia javanica*, CM = *Chamaecrista mimosoides*, CO = *Senna occidentalis*, CP = *Senna polyphylla*, CP1 = *Chamaecrista pumila*, CR = *Cassia renigera*, CS = *Senna surattensis*, CS1 = *Senna siamea*, CT = *Senna tora*, CU = *Senna uniflora*.

Table 4: Extract yield and sennosides (SB and SA) content in extracts of selected species from genus *Cassia*, *Chamaecrista* and *Senna*

Species	Extract yield (%)	SB (%)	SA (%)	Total (SB + SA, %)
CA	38.05	0.1900	0.2050	0.3950
CA1	30.09	0.7100	0.4560	1.1660
CA2	29.79	0.2946	0.0993	0.3939
CA3	30.49	nd	nd	-
CF	23.62	0.1700	0.2050	0.3750
CJ	36.99	0.6272	0.0124	0.6396
CM	19.68	nd	nd	-
CO	30.73	0.4623	0.1499	0.6122
CP	19.92	nd	nd	-
CP1	44.44	nd	nd	-
CR	30.80	nd	nd	-
CS	56.20	0.000445	0.000187	0.000632
CS1	25.42	nd	nd	-
CT	11.56	0.6312	0.0364	0.6676
CU	31.12	nd	nd	-

CA = *Senna alata*, CA1 = *Senna alexandrina*, CA2 = *Senna auriculata*, CA3 = *Chamaecrista absus*, CF = *Cassia fistula*, CJ = *Cassia javanica*, CM = *Chamaecrista mimosoides*, CO = *Senna occidentalis*, CP = *Senna polyphylla*, CP1 = *Chamaecrista pumila*, CR = *Cassia renigera*, CS = *Senna surattensis*, CS1 = *Senna siamea*, CT = *Senna tora*, CU = *Senna uniflora*, nd = Not detected.

3.2 Profiling of sennosides A and B in leaves extracts

Most of phytochemical work on different species of *Cassia*, *Chamaecrista* and *Senna* species are restricted to isolation, characterization and evaluation of biological activities. Senna extracts are complex mixture with several active constituents such as dianthrone glycosides (sennosides A, B, C and D), free anthraquinone (aloe-emodin, chrysophenol, rhein) and anthraquinone glycosides. Among these constituents, sennoside A and B are present in higher concentration than the other constituents. Senna extracts are widely used in the treatment of intestinal constipation and their strong laxative effects are attributed to sennosides A and B. In addition to that, tissue containing sennosides are efficient sources of health teas (Kojima *et al*., 2001). Sennosides A and B (Figure 2) are unique anthraquinones having double carboxylic acid-, hydroxyl-, carbonyl-and O-glucosyl-groups at the C-3, C-1,C-9 and C-8 possible and possessing *threo*- and *erythro*-configurations between C-10 and C-10, respectively (Putalun *et al*., 2004).

In the present investigation SB and SA were identified and quantified in 15 species. Sennosides A and B were identified and quantified only in *S. alexandrina* (CA1), *S. tora* (CT), *C. javanica* (CJ), *S. occidentalis* (CO), *S. alata* (CA), *S. auriculata* (CA2), *C. fistula* (CF), *S. surattensis* (CS) (Table 4, Figure 3). SB and SA were not detected in species namely *Chamaecrista absus* (CA3), *Chamaecrista mimosoides* (CM), *Senna polyphylla* (CP) , *Chamaecrista pumila* (CP1), *Cassia renigera* (CR), *Senna siamea* (CS1) and *Senna uniflora* (CU). The content of SB varied in the following order: CA1 > CT > CJ > CO > CA2 > CA > CF > CS. Similarly, SA content was maximum in CA1 followed by CF and CA, CO, CA2, CT, CJ, CS. The total sennoside (SB+SA) varied in the following order : CA1 > CT > CJ > CO > CA > CA2 > CF > CS. Based on the sennoside content, it could be possible to select alternative source of laxative drug as the total anthraquninone content should not be less than 0.5 % of dried leaf raw materials (Sakulpanich and Gritsanapan, 2009).

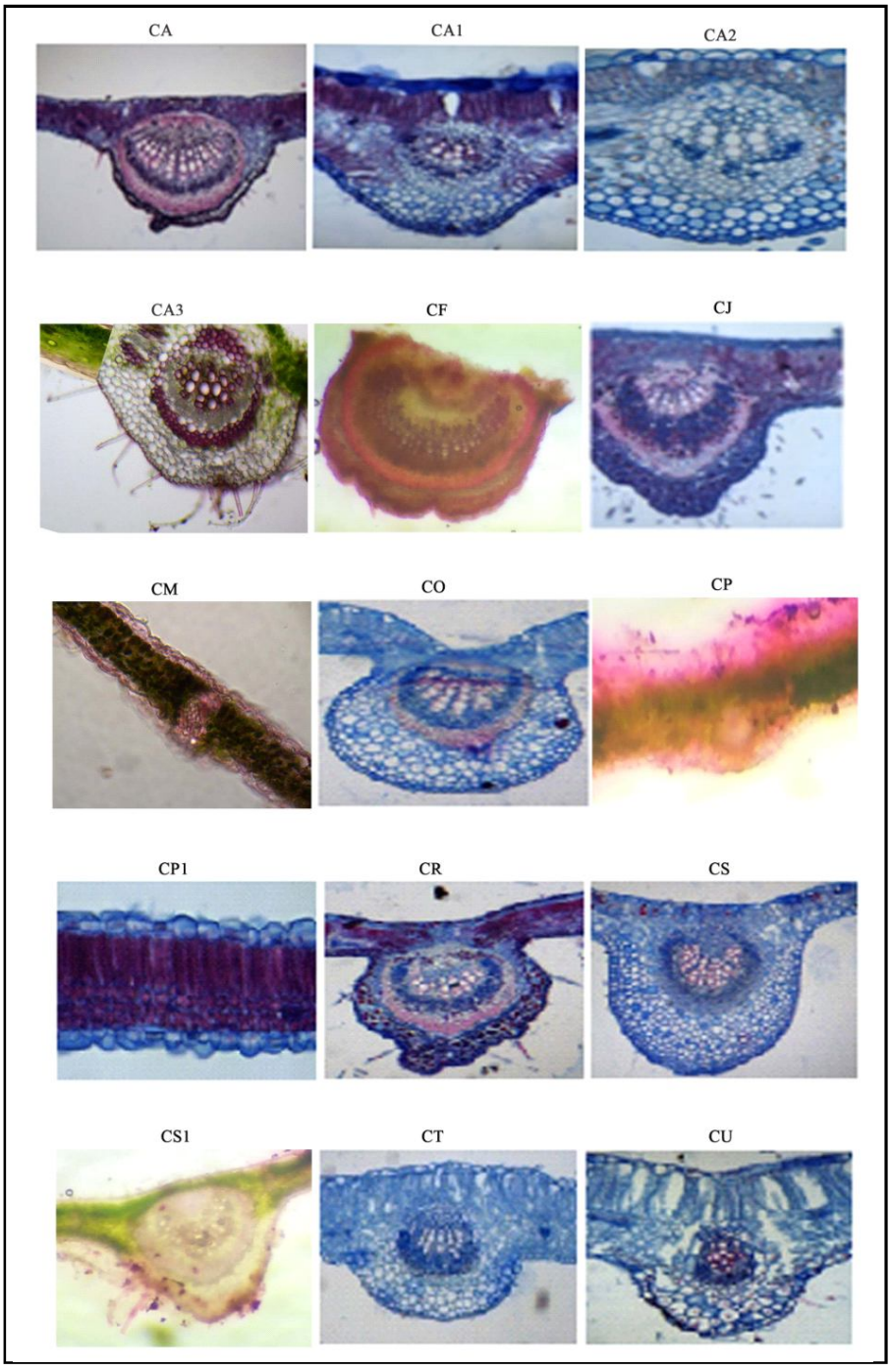


Figure 1: Anatomical characteristic of selected species from genus *Cassia, Chamaecrista* and *Senna*.

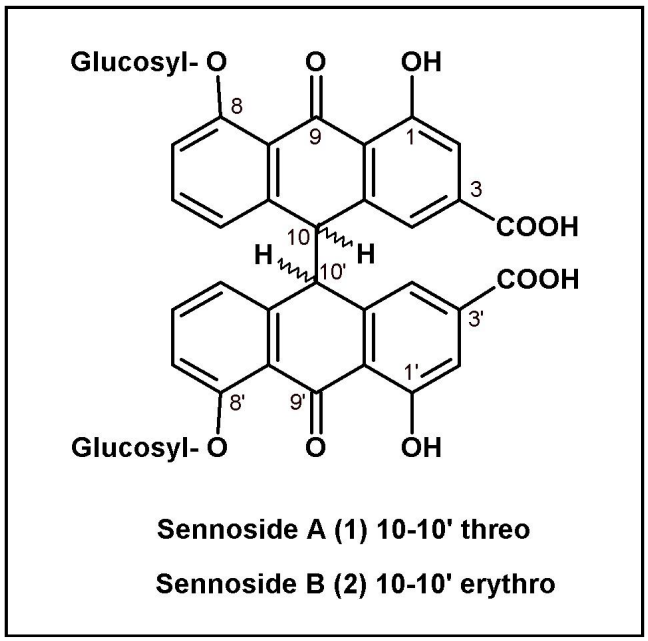
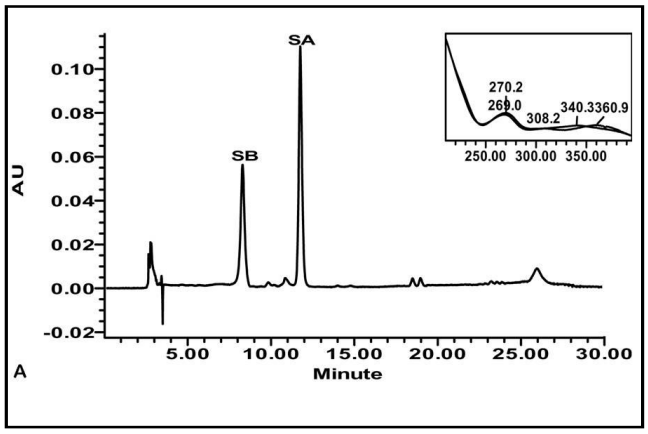
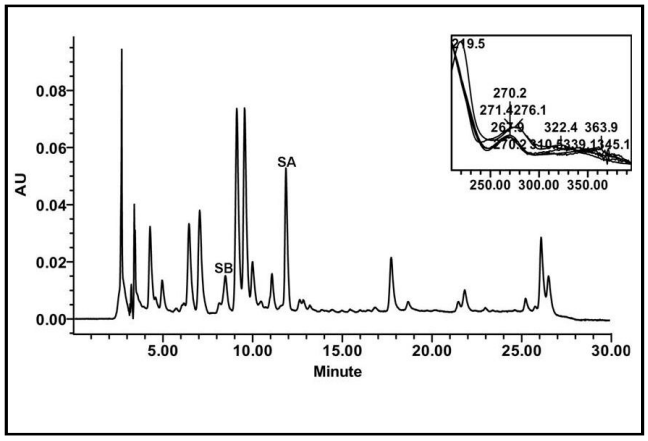


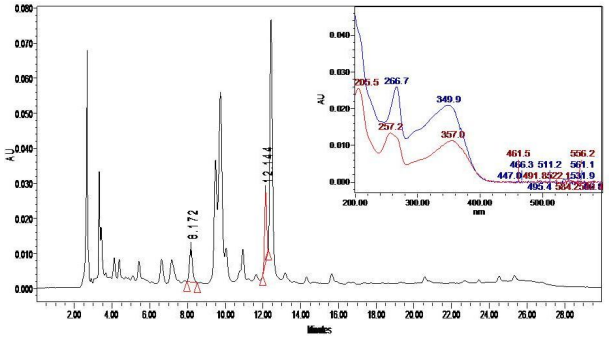
Figure 2: Chemical structure of sennosides A and B.



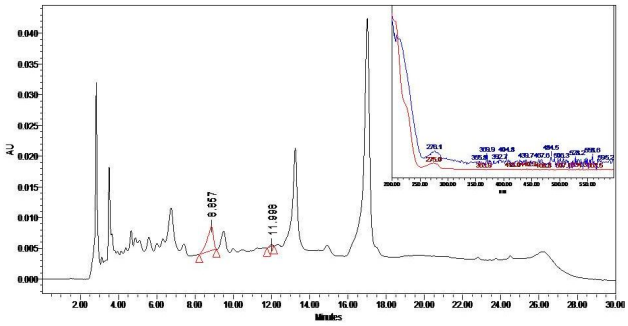
(A) Mixtute of Standard SB and SA



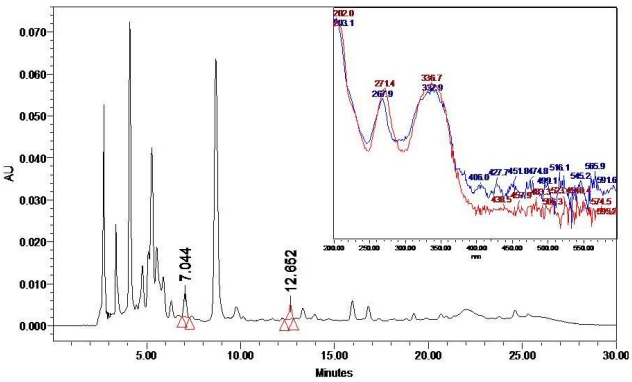
(B) *S. alexandrina*



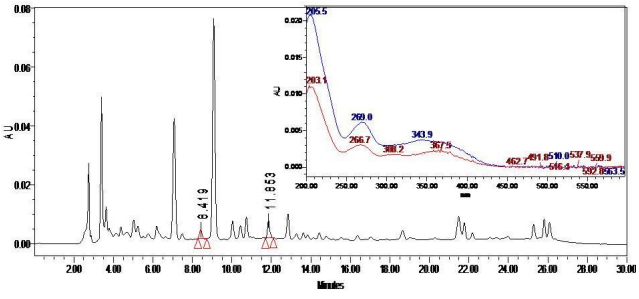
(C) *S. tora*



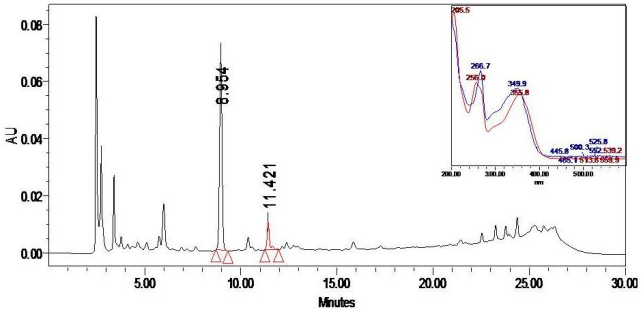
(D) *S. javanica*



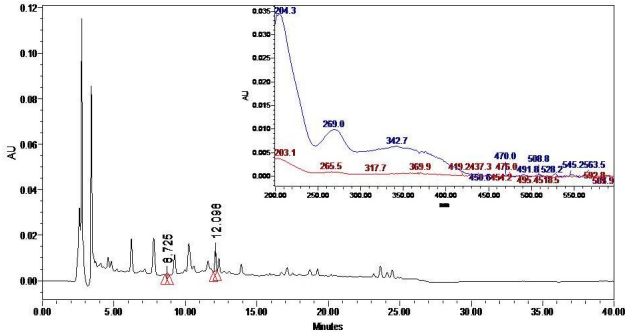
(E) *S. occidentalis*



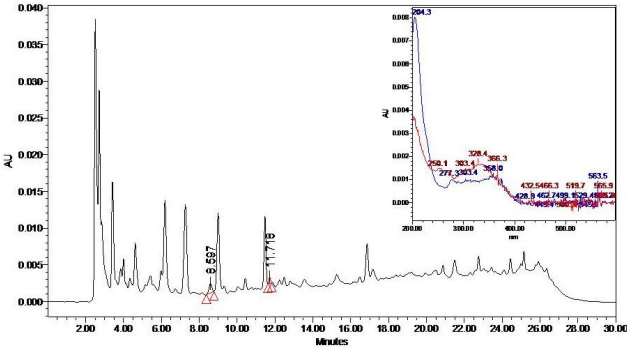
(F) *S. alata*



(G) *S. auriculata*



(H) *C. fistula*



(I). *S. surattensis*

Figure 3: HPLC-PDA chromatogram: (A).Mixture of standard of SB and SA, (B) *S. alexandrina*, (C) *S. tora*, (D) *S. javanica*, (E) *S. occidentalis*, (F) *S. alata*, (G) *S. auriculata*, (H) *C. fistula*, (I) *S. surattensis.*

4. Discussion

Earlier, Sihanat *et al*. (2016) reported characteristics and number of trichome of leaves, from sixteen species of *Cassia* found in Thailand. The trichome characteristics of investigated *Cassia* spp. were uniseriate, uni-or multicellular non-glandular and multicellular grandular types. However, trichome in some *Cassia* spp. were absent. *C. javanica* L. had the highest trichome number in both dorsal (78.94 ± 2.86) and ventral (127.39 ± 2.46) surfaces of the leaf whereas *C. surattensis* Burm.f had the lowest trichome numbers only on ventral (3.46 ± 0.80) surface. Based on the presence of trichomes on leaf surface, *Cassia* spp. were classified into three major groups. The trichome was present on both dorsal and ventral surfaces in 10 species (*C. bakeriana, C. fistula, C. grandis, C. javanica, C. alata, C. angustifolia, C. siamea, C. spectabilis, C. timoriensis* and *C. hirsuta*). In other three species, namely; *C. sulfurea, C. surrattensis* and *C. tora*, trichome was present on ventral surfaces. Trichome was absent in rest three species (*C. garrenttiana, C. occidentalis* and *C. sophera*).

Khan *et al*. (2011) reported that cluster analysis exhibited genetic diversity among four species of *Senna*. These four species were clustered into two groups: first group comprised of *S. aungustifolia*, which had high similarity (72.73%) to each other as compared to the second group which had *S. sophera* and *S. tora*. The authors also reported that the *S. aunguistifolia* and *S. acutifolia* had morphological similarities in leaves to great extent and in dried state, it was very difficult to differentiate to each other. However, in RAPD analysis both species showed more genetic divergence. These two species are so closely related that their status has been variously interpreted. The results demonstrated the ability of RAPD markers to reliably differentiate between *S. surattensis* and *S. sulfurea*.

Morinaga *et al*. (2009) reported SB and SA in leaves of nine *Cassia* species using eastern blotting technique with anti-sennoside A and anti-sennoside B monoclonal antibodies. The total sennoside (SB+SA, µg/mg dry wt powder) varied in the following order : *C. angustifolia* > *C. alata* > *C. fistula* > *C. bakeriana* > *C. mimosoides* > *C. siamea* > *C. floribunda* > *C. tora* > *C. surattensis*. Lohar *et al*. (1975) reported phytochemical studies on seven *Cassia* species of India namely *C. angustifolia, C. fistula, C. javanica, C. siamea, C. tora, C. sophera and C. auriculata* collected from Western Rajasthan, India. Total sennosides content in the leaves were estimated using spectrophotometric method as reported in British Pharmacopoeia (Anonymous, 1968). Total sennoside (%) was highest in *C. angustifolia* followed by *C. fistula*. The following order was observed for total sennosides content: *C. angustifolia* (4.23) > *C. fistula* (1.80) > *C. javanica* (0.20) > *C. auriculata* (0.15) > *C. tora* (0.14) > *C. siamea* (0.07) > *C. sophera* (0.07). Earlier, the sennoside contents in the leaves and seeds of wild *C. angustifolia* were reported to be 3.0 - 5.0 and 2.4- 3.0 %, respectively (Stoll *et al*., 1949; Pendse *et al*. 1973).

Asseleih *et al*. (1990) reported seasonal variation in the content of sennosides in leaves and pods of two *C. fistula* populations. The highest sennoside contents detected in *C. fistula* were 1.00 -1.50 % in leaves and 1.00-1.90% in pods as compared to senna species in which sennosides contents (%) were reported to be in the range of 2.00-3.00 in leaves and 2.50-4.50 in pods of *C. acuitifolia* and 2.00-3.00 in leaves and 1.20-2.50 in pods of *C. anguistifolia*. Elujoba *et al*.(1989) reported combined anthraquinone content and laxative properties of leaves of 10 *Cassia* species cultivated in Nigeria with *C. acuitifolia* as the reference standard. The results of both chemical and biological experiments suggested that *C. alata* and *C. podocarpa* were the most likely candidates for drug development.

5. Conclusion

Although, the quantitative method for the chemical markers confirm the presence of compounds, it does not confirm the presence of plant material which contains the chemical markers. Wide diversity was recorded for the studied morphological and anatomical characteritcics among the selected species. Based on the sennoside content, three species, namely; *S. tora*, *C. javanica* and *S. occidentalis* could be considered as potential alternative sources of sennosides A and B for laxative drug.

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Conflicts of interest

The authors declare that there are no conflicts of interest relevant to this article.

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