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Research Article

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Metabolomics characterization of *Senna tora* (L.) Roxb. using different approaches

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ABSTRACT

The present study aimed to investigate the variety of elements, chemical compounds and their corresponding functional groups in the whole plant, leaves, and seeds of *Senna tora*. A preliminary phytochemical analysis has revealed the presence of secondary metabolites including alkaloids, flavonoids, tannins, terpenoids, cardiac active glycosides, phenolics, *etc*. Gas Chromatography and Mass Spectrophotometry (GC-MS) analysis of leaves and seeds of *S. tora* has depicted 31 and 27 compounds, respectively. Fourier Transform Infrared (FT-IR) Spectroscopy has further unveiled the presence of different functional groups such as amines, aromatic compounds, carboxyl groups, ketones *etc.* associated with different metabolites. Wavelength Dispersive X-ray Fluorescence (WD-XRF) has revealed the presence of more than 20 elements (macro and micro) including Ca, Mg, Fe, K, *etc*. This study has highlighted the detailed account of the chemical compounds and elements present in the plant species under investigation and substantiated its medicinal importance in the traditional health care system.

KEYWORDS: Bioactivity, Species, Volatile Compounds, Elements, Phytoconstituents, Medicinal

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INTRODUCTION

India is one of the top 12 mega biodiversity countries of the world and possesses a unique plant diversity and variety of plants in general and medicinal plants in particular. The richness of Indian floristic diversity is due to altitudinal, climatic and ecosystems variations in different parts of the country. Because of the medicinal value, plant species have their mention in Ayurveda, Unani, Siddha, *etc*. (Singh & Chowdhery, 2002). Medicinal plants, therefore, are being indiscriminately since by nature for a long time. Certain weeds or Neglected Underutilized Species (NUS) have not received much attention because of crops, intensive agronomic practices, social and cultural reasons. Limited cultivation and increased demand for herbal species has threatened the existence of certain species of medicinal importance. Due to this apprehension, there is an urgent need to plan effective conservation strategies to conserve plant diversity. Large numbers of NUS species contain phytoconstituents of medicinal value (Ugbaja *et al*., 2017).

Different species of the genus *Cassia* L. are being used in various health care medicinal preparations. Different species genus are occurring throughout the world out of which nearly 49 (*Cassia, Senna, Chamaecrista*) are growing in India (Efloraofindia, 2007; Kabila *et al*., 2020). *Senna tora* (Syn *Cassia tora*) is a member of the family Fabaceae and subfamily Caesalpinioideae (Singh *et al*., 2013). It is useful in skin diseases such as ringworm, eczema, scabies, rheumatic, asthma and also has hepatoprotective, anti-helminthic and anti-inflammatory activities. Its medicinal activity is likely due to the presence of various chemical compounds and elements (Choudhary *et al*., 2011; Mate *et al*., 2013; Vijayalakshmi *et al*., 2015).

Senna tora is a small foetid, herb or undershrub weed species (Figure 1), growing in tropical and subtropical regions. It is a native of southeastern Asia and grows well in Malaysia, Japan, Burma, Bangladesh, India, *etc*. This species has also been referred to as a destructive weed throughout the country (Kabila *et al*., 2017). The plant body is spreading with branches and has a glabrous stem. Leaves are long stipulate, linear, membranous or lanceolate. Leaflets are in three pairs, obovate, nearly equal on both sides, both surfaces of the leaflets are pubescent, glabrous with entire margin. The yellow colored gland is located between the lowermost pair of the leaflets. The inflorescence is an axillary raceme with bright yellow flowers, long-stalked in pairs with a short peduncle, linear bracts and acute pubescent. Sepals are 5 (3 large, 2 small) ovate, glabrous, acute apex; petals are 5 (bright yellow), subequal, oblong and upper petal is truncate; stamens are 10 with 3 staminodes and 7 antheriferous (3 large

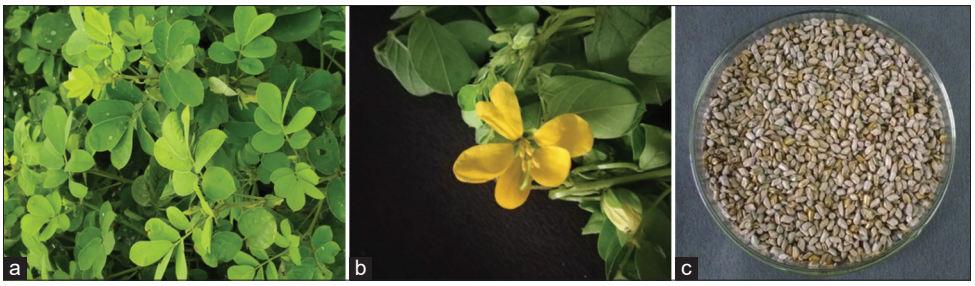

Figure 1: a) Whole Plant, b) Leaves, c) Seeds of *Senna tora*

anthers beak at apex and 4 with the rounded apex) (Ingle *et al.*, 2012; Dubey & Sawant, 2015).

Since the plant is profusely growing in the wild habitats and is traditionally used in various health care remedies, the present study has been planned to investigate the detailed account of the chemical compounds of medicinal importance.

MATERIALS AND METHODS

Collection and Processing of Plant Material

The plant material is collected from the natural habitats in district Mohali and Rupnagar, Punjab. The collected specimens were identified using the available literature, flora, and manuals (Bamber, 1916; Nair, 1978; Sidhu, 1991; Singh, 2001; Efloraofindia, 2007; The Plant list, 2013; Flowers of India, 2016) and by consulting the Herbarium, Department of Botany, Panjab University, Chandigarh, where the herbarium sheet of *S. tora* has been deposited (PAN No- 22179).

Preparation of Extracts

The fresh plant material was washed, first with tap water followed by distilled water. The material was then allowed to dry at room temperature. The completely dried material was powdered using an electric grinder. The powdered material was stored in air tight container for further use.

Phytochemical Analysis

Ethanol, hexane and aqueous extracts of *Senna tora* (whole plant, leaves and seeds) have been screened for alkaloids, flavonoids, saponins, coumarins, anthraquinones, terpenoids, steroids *etc.* The analysis was carried out using standard procedures (Trease & Evans, 1989; Sofowora, 1993; Kokate, 1994; Harborne, 1998; Kokate *et al.*, 2005; Roopashree *et al.*, 2008; Evans, 2009; Njoku & Obi, 2009; Sidhu & Thakur, 2016; Sidhu & Sharma, 2016).

GC-MS Analysis

GC-MS analysis was carried out using thermo trace 1300GC coupled with Thermo TSQ 8000 triple quadrupole MS (for GC–Thermo Trace 1300 GC; for MS-Thermo TSQ 8000) at Central Instrumentation Laboratory, Panjab University, Chandigarh. Column TG 5MS was composed of 5% Diphenyl; 95% dimethyl polysiloxane operating in electron impact mode and helium was used as carrier gas at a constant flow of 1.5 mL/min and an injection volume of 1µl was employed (Split ratio 33.3) at an injected temperature of 250 °C. The oven temperature was programmed for 60 °C with a hold time of one minute and an increase of 10 °C/min at 220 °C withhold time of four minute. Mass spectra transfer line temperature is 250 °C, Ion source temperature was 230 and Mass range from 50-700 °C. The compound identification was done by comparison of retention time and mass spectra of GC-MS. The compounds were identified using the Database of National Institute of Standards and Technology (NIST) Library 2.0.

FT-IR Spectroscopy

Fourier Transform Infrared (FTIR) Spectroscopy classify the chemical bonds/functional groups relevant to the phytochemicals. The chemical bonds in the spectrum absorb light of a particular wavelength. Thus, chemical bonds reported in the compounds can be resolved by interpreting the IR absorption spectrum (Visveshwari *et al.*, 2017). The test has been performed using Perkin Elmer Spectrum 400 FT-IR/FT-FIR spectrometer with a scan range from 400 to 4000 cm^{-1}. Plant samples in the form of powder were used for analysis.

WD-XRF Analysis

WD-XRF analysis was performed to detect the elements (atoms, ions) in the studied samples. The analysis was carried out by instrument, Wavelength Dispersive X-ray fluorescence (WD-XRF), Model: S8 TIGER, Bruker, Germany at Central Instrumentation Laboratory, Panjab University, Chandigarh. X-Ray Tube: Anode material 'Rhodium', A sample pellet was made using hydraulic press and a pressure of 10 tons. Approx. the sample used for pellet was 3-4 gms, sample thickness ~ 2 mm (minimum thickness required than 1.5 mm), Sample diameter - 34 mm, analysis time ~ 20 mins and software used for the analysis was SpectraPlus.

RESULTS AND DISCUSSION

Phytochemical Screening

The phytochemical screening of *Senna tora* (whole plant, leaves, and seeds) was carried out using aqueous, ethanol and hexane extracts. Most of the studied phytochemicals were present in aqueous and ethanol extracts whereas, hexane extract yielded the least (Table 1). Ethanol extracts of the whole plant, seeds,

and aqueous extract of seeds contained maximum number of phytoconstituents as compared to other extracts. Carbohydrates were observed in all the extracts. Similarly, cardiac active glycosides were also present in all the extracts except whole plant hexane extract. Alkaloids, anthraquinones, coumarins, diterpenoids, flavonoids, phenolics, quinones, reducing sugar, starch and tannins were present in all the extracts except hexane.

Alkaloids and tannins in methanol extract, steroid in petroleum ether and chloroform extracts of *C. tora* leaves were reported by Mazumder *et al*. (2005). Das *et al*. (2011) studied the phytoconstituents of *Cassia tora* stem bark in five different solvents and reported maximum phytochemicals including alkaloids, flavonoids, tannins, protein, steroids and glycosides in methanol extract followed by diethyl ether and ethyl acetate. Ethanol, methanol and ethyl acetate extracts of (*Cassia tora)* possess alkaloids, steroids, terpenoids, cardiac glycosides, carbohydrates and proteins whereas tannins and phlobatannins were reported only in methanol and ethanol extracts (Veerachari & Bopaiah, 2012). John *et al*. (2012) also reported the presence of tannins, anthraquinones, flavonoids, glycosides and coumarins in methanol and ethyl acetate leaves extracts of *C. tora* but methanol extract showed only steroids, cardiac glycosides, amino acids and saponins. The leaves of *C. tora* contain alkaloids, anthraquinones, flavonoids, phenolics and proteins in methanol and aqueous extracts (Khan *et al*., 2016). Among the leaves, bark, seeds and pods (containing seeds), ethyl acetate and methanol extracts of leaves showed all the studied phytoconstituents but hexane extract revealed the presence of only steroids (Khatak *et al*., 2014).

Screening of phytoconstituents in *Senna tora* had shown the presence of alkaloids, phenols, saponins, carbohydrates, glycosides and protein in aqueous, ethyl acetate and hexane extracts of leaf and seeds (Sabyasachi *et al*., 2016). Rao and Chattrerjee (2016) studied the aerial parts of *S. tora* and reported the presence of alkaloids, triterpenoids, steroids and tannins. Similarly, Asba and Meeta (2017) studied various phytoconstituents in roots, leaves, stem, flowers and pods of *C. tora* in different solvents (six solvents) and most of the phytochemicals were reported from the aqueous and methanol extract as compared to other solvent extracts. Suradkar *et al*. (2017) and Sahu *et al*. (2017) reported the presence of alkaloids, flavonoids, saponins, tannins, phenols, steroids *etc*. in ethanol, aqueous and methanol extracts of the leaves. These have also been reported during the present study in aqueous and ethanol extracts. According to the available literature, most of the studies have been conducted on the leaves and seeds of *S. tora* and these were the most preferred parts along with methanol as a solvent for phytochemical investigations. Phytochemical screening of the seed extract of *Cassia tora* revealed the presence of carbohydrates, glycosides, saponins and triterpenes in petroleum ether, ethanol and aqueous extracts (Roopashree *et al*., 2008).

It is pertinent to mention that none of the extracts contain amino acids and anthocyanin during the present investigation. However, some metabolites like anthraquinones, betaxanthin, cardiac-active glycosides, coumarins, diterpenoids, lignins, oxalate, phenolics, quinones and resin have been reported for the first time from this plant and its parts (leaves and seeds) in different solvents.

Table 1: Phytochemical account of *Senna tora* whole plant, leaves and seeds

S.No.	Phytochemicals	Whole Plant			Leaves			Seeds		
		Aq.	Eth.	Hex.	Aq.	Et.	Hex.	Aq.	Eth.	Hex.
1.	Alkaloids	+	++	-	++	++	-	+	+	-
2.	Amino acids	-	-	-	-	-	-	-	-	-
3.	Anthocyanin	-	-	-	-	-	-	-	-	-
4.	Anthraquinones	±	+	-	+	+	-	±	++	-
5.	Betaxanthin	++	+++	±	++	+	-	++	+	-
6.	Carbohydrates	+++	++	+	±	+	+	++	+	+
7.	Cardiac active glycosides	±	+	-	+	++	±	±	+	+
8.	Coumarins	++	++	-	++	++	-	+++	++	-
9.	Diterpenoids	++	+++	-	++	+	-	++	+	-
10.	Flavonoids a) NaOH Test	+	++	-	++	+	-	+	+	-
	b) H_2SO_4 Test	++	±	-	+	-	-	++	-	-
11.	Glycosides	+++	-	-	+	++	+	+	++	-
12.	Gum and mucilage	-	+	-	+++	-	±	+++	-	-
13.	Lignins	-	-	-	-	++	-	-	+	-
14.	Oxalate	+	++	-	-	-	-	-	-	-
15.	Phenolics	++	±	-	+++	++	-	+	++	-
16.	Phlobatannins	-	++	-	-	-	-	-	±	-
17.	Proteins	-	++	-	+	-	-	++	++	-
18.	Quinones	+	++	-	+	+	-	+	+	-
19.	Reducing sugars	±	++	-	++	+	-	+++	+	-
20.	Resin	±	-	-	+	+	-	+	+++	-
21.	Saponins	+	+	+	-	-	-	+	-	-
22.	Starch	++	++	-	++	+++	-	+++	++	-
23.	Steroids	+	+	-	++	+	-	++	+++	+
24.	Tannins a) $FeCl_3$ Test	+	++	-	+++	++	-	++	+	-
	b) KOH Test	+++	+	+	+	+	-	±	-	-
25.	Terpenoids	++	++	-	+++	+	+	+	++	±

Aq-Aqueous, Eth- Ethanol, Hex-Hexane, +++ = Abundant, ++ = Moderate, + = Less, ± = Traces, - = Absent

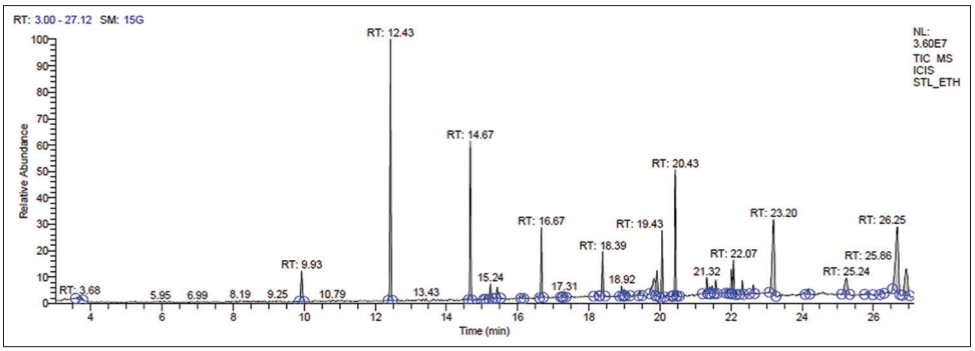

Figure 2: GC-MS Chromatogram of ethanol extract of leaves of *S. tora*

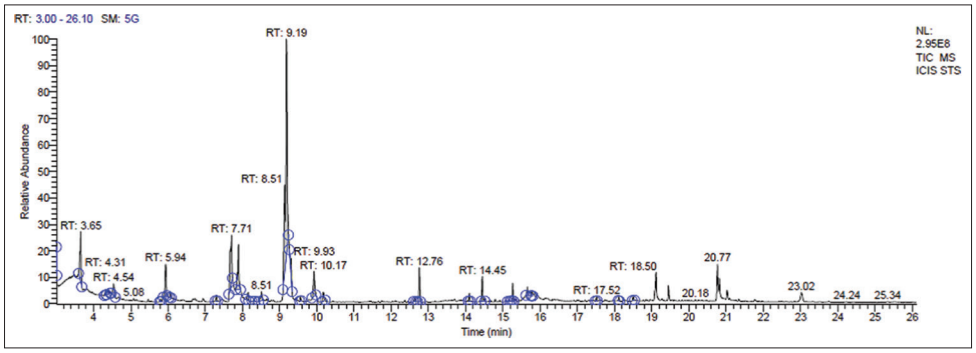

Figure 3: GC-MS Chromatogram of ethanol extract of seeds of *S. tora*

GC-MS Analysis

GC-MS chromatogram of ethanol extracts leaves and seeds have revealed the detail of compounds (Figures 2, 3 and Tables 2, 3). The presence of antimicrobial compounds such as 3,4-Dihydroxymandelic acid, ethyl ester, tri- TMS, 10-Heneicosene (c,t), Silicic acid, diethyl bis(trimethylsilyl) ester, Tetracosamethyl-cyclododecasiloxane, 1-Monolinoleoylglycerol trimethylsilyl ether, Phytol, Bis(cis-13-docosenamido) methane (in leaves) and N-Cbz-glycylglycine p-nitrophenyl ester, (+)-2-Bornanone, Hexadecen-1-ol, trans-9-, Phenol, 2,4-bis(1,1-dimethylethyl)-, Ethyl à-d-glucopyranoside, Phthalic acid and hex-3-yl isobutyl ester (in seeds) support the antibacterial activity of leaves and seeds. Anti-cancer, anti-tumour, antioxidant, anti-inflammatory, diuretic and acidifier activities are also attributed to other compounds of biological importance that have been reported during the present investigation. These observations are in favor of the traditional medicinal use of this species.

Fathalla *et al*. (2015) reported 27 fatty acids including hexadecanoic acid, octadecanoic acid and nonadecanoic acid in methyl extracts of seeds of *C. tora*. They also performed GC-MS of ethyl acetate extract of *C. tora* seeds and reported around 30 compounds such as Chrysophanol, Chrysarobin, Xanthone, Rubrofusarin, Parietin *etc* (Fathalla *et al*., 2018). *Senna tora* leaves and seeds have revealed the presence of various compounds as compared to earlier reports. A total of 67 compounds have been reported from *S. tora* seeds in the present study, out of which many have not been reported earlier, such as Hydrazine, methyl-, Tolycaine (Anesthetic), Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters, Phenol, 2,4-bis (1,1-dimethyl ethyl)-, Hexadecen-1-ol, trans-9-, Bicyclo[2.2.2]heptan-2-one, 1,7,7-trimethyl-,(1S)- and N-Cbz-glycylglycine p-nitrophenyl ester *etc*. Some of these compounds reported in leaves and seed extracts are of medicinal importance and can be explored further.

Fourier Transform Infrared (FT-IR) Spectroscopy

FT-IR analysis is an important tool for the identification and characterization of compounds and chemical bonds or functional groups (Eberhardt *et al*., 2007). The results of *S. tora* whole plant, leaves and seeds are summarized in Figure 4 and Table 4. The functional groups support the presence of metabolites in given sample. Various secondary metabolites contain functional groups in their structures such as aldehydes, epoxides, compounds having covalent bonds, compounds containing nitrogen (alkaloids, cyanogenic glycosides, glucosinolates, amines and lectins) and without nitrogen (such as phenols, flavonoids, coumarins, anthocyanins, tannins and terpenoids) (Dewick, 2001; Kabila *et al*., 2020). The exocyclic methylene groups indicate the presence of terpenes and phenylpropanoids, aliphatic hydrocarbons with C-C double and triple bonds shows polyacetylenes and monoterpenes (linalool, camphene and pinocarvone). Polyphenols have phenolic rings, phenolic OH groups and two aromatic rings with phenol hydroxyl or methyl groups (flavonoids and anthocyanins) (Duke, 1992; Wink, 2015; Pagare *et al*., 2015). The presence of S-S stretch at 466.08, 484.31 in leaves, C=C at 1595.16 (whole plant) and 1542.98 (seeds), transition metal carbonyl at 2029.83 and 1972.28 (seeds), C-O-H bending vibrations peaks in entire plant (1384.79, 1425.65), leaves (1409.30) and seeds (1399.75). Two different peaks in entire plant, leaves and seeds indicate methyl ester and C-H stretching in lipids and proteins.

Table 2: Importance of chemical compounds detected in the leaves of *S. tora*

S.No.	Compounds	Formulae	Mol Wt.	Importance
1.	1-Di (tert-butyl) silyloxy-2-phenylethane	$C_{16}H_{28}OSi$	264.48	Coronary dilator, antidote, Diuretic and digestive (Duke, 1992)
2.	Cyclopentasiloxane, decamthyl-Benzoic acid	$C_{10}H_{30}O_5Si_5$	370.77	Mostly used in cosmetic products, skin and hair care, deodorants and controlling hair fall (O'Neil, 2013; Dionisio *et al.,* 2018).
3.	3,4-Dihydroxymandelic acid, ethyl ester, tri- TMS	$C_{19}H_{36}O_5Si_3$	428.7	Antiprotozoals, acidifier, urine acidifier and antibacterial (Duke, 1992)
4.	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris (trimethylsiloxy) tetrasiloxane	$C_{18}H_{52}O_7Si_7$	577.2	Antidote (Theophylline), CNS toxic, Blood thinning, Endocrine tonic and anti-tumour (Duke, 1992)
5.	3-Butoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris (trimethylsiloxy) tetrasiloxane	$C_{19}H_{54}O_7Si_7$	591.2	Tranquilizer, thymostimulant and protective (Duke, 1992)
6.	Benzene acetic acid, 4-(1,1-dimethylethyl)-, methyl ester	$C_{13}H_{18}O_2$	206.28	Artificial flavouring agents, colouring spices (Monsalvo *et al.,* 2015)
7.	Ethyl 4-t-butylbenzoate	$C_{13}H_{18}O_2$	206.28	Antidote, CNS toxic, Blood thinning and anti-tumour (Duke, 1992)
8.	10-Heneicosene (c, t)	$C_{21}H_{42}$	294.6	Anti-tumour, antidote, CNS-toxic, Blood thinning, antibacterial, carminative, cardioprotective and cardioactive stimulant (Duke, 1992)
9.	Silicic acid, diethyl bis (trimethylsilyl) ester	$C_{10}H_{28}O_4Si_3$	296.58	Pathogens inhibitor and antibacterial (Musini *et al.,* 2013), Acidifier and Inhibit uric acid production (Duke, 1992)
10.	1,3-Benzenediol, 5-[1-hydroxy-2-[(1-methylethyl) amino] ethyl]	$C_{11}H_{17}NO_3$	211.26	One of the major compounds is used for asthma patients in Bronchiodilater and cardiovascular activity (Reynolds, 1982)
11.	Cyclononasiloxane, Octadecamethyl	$C_{18}H_{54}O_9Si_9$	667.4	Antioxidant activity (Ojekale *et al.,* 2013)
12.	Tetracosamethyl-cyclododecasiloxane	$C_{24}H_{72}O_{12}Si_{12}$	889.8	Antirheumatic, antispasmodic, hepato-protective, helpful in stomach pain and nausea (Babalola *et al.,* 2011; Jayashree *et al.,* 2015), anti-inflammatory, diuretic, rheumatoid arthritis and antimicrobial (Hultqvist *et al.,* 2006)
13.	Pentadecanoic acid, ethyl ester	$C_{17}H_{34}O_2$	270.5	Acidifier, urine acidifier, inhibit the production of uric acid and Hexokinase stimulator (Duke, 1992)
14.	Triacontane	$C_{30}H_{62}$	422.8	It inhibits uric acid production, aromatic compounds and increases decarboxylase activity and acidifier (Duke, 1992)
15.	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl	$C_{16}H_{50}O_7Si_8$	579.25	Antimicrobial (Rao *et al.,* 2018)
16.	(Z)-9-Hexadecenoic acid, methyl ester	$C_{17}H_{32}O_2$	268.4	Increases the zinc bioavailability, provide Zinc, Inhibit the production of uric acid and increases the aromatic amino acid decarboxylases activity (Duke, 1992)
17.	Methyl hexadec-9-enoate	$C_{17}H_{32}O_2$	268.4	Catechol-O-Methyl transferases inhibitor and Methyl donor (Duke, 1992)
18.	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284.5	Urine acidifier and increases aromatic amino acid decarboxylases activity (Duke, 1992)
19	Tetradecanoic acid, 2-methyl-, methyl ester	$C_{16}H_{32}O_2$	256.42	Urine acidifier and increases aromatic amino acid decarboxylases activity, and Catechol-O-Methyl transferases inhibitor (Duke, 1992)
20.	Theobromine, tert-butyldimethylsilyl deriv.	$C_{13}H_{22}N_4O_2Si$		Endothelium-derived relaxing factor promoter (Duke, 1992)
21.	Deoxycholic acid, di (trimethylsilyl) ester	$C_{30}H_{56}O_4Si_2$	536.93	Increases aromatic amino acid decarboxylases activity, antidote (Diazepam) and coronary dilator (Duke, 1992)
22.	1-Monolinoleoylglycerol trimethylsilyl ether	$C_{27}H_{54}O_4Si_2$		Antimicrobial, antioxidant, anti-inflammatory, antiarthritic, antiasthma, diuretic (Kala & Ammani, 2017)
23.	Phytol	$C_{20}H_{40}O$		The compound is produced from the hydrolysis of chlorophyll and it forms part of vitamin E and vitamin K (Finar, 2001) Anticancer, antioxidant, anti-inflammatory and diuretic (Kala & Ammani, 2017)
24.	n-propyl 9,12-octadecadienoate	$C_{21}H_{38}O_2$	322.52	Anaphylactic (antidote), anti-tumour, increases natural killing of cells activity, decreases norepinephrine production, narcotic, CNS depressant and neuro stimulant (Duke, 1992)
25.	(Z, Z, Z)- 9,12,15-Octadecatrienoic acid, ethyl ester	$C_{20}H_{34}O_2$	306.5	Provide zinc and increase zinc availability (Duke, 1992)

(Contd...)

Table 2: (*Continued*)

S.No.	Compounds	Formulae	Mol Wt.	Importance
26.	(-)-Octadecanoicacid, 2-methyl-methyl ester	$C_{20}H_{40}O_2$	312.5	Alcoholic beverages and flavouring agents (Dionisio *et al.,* 2018) and also used as pesticides, Hemolytic, 5-Alpha reductase inhibitors, antioxidants, hypocholesterolemic (Duke, 1992)
27.	Trans-13-Docosenamide	$C_{22}H_{43}NO$	337.6	Zinc bioavailability, transdermal, antioxidant and increases glyoxalate transaminases (Duke, 1992)
28.	Bis (cis-13-docosenamido) methane	$C_{45}H_{86}N_2O_2$	687.2	Antioxidant, anti-inflammatory, antimicrobial, antiarthritic, diuretic and antiasthma, (Duke, 1992; Parthipan *et al.,* 2015)
29.	Squalene	$C_{30}H_{50}$	410.7	Monoxygenases inhibitor (Duke, 1992), antioxidant, antibacterial, cancer preventive, immune-stimulant and anti-tumour (Beulah *et al.,* 2018)
30.	6,10,14,18,22-Tetracosapentaen-2-ol, 3-bromo-2,6,10,15,19,23-hexamethyl-,(all-E)	$C_{30}H_{51}BrO$		Allergic, anticancer, antitumour, decreases endothelial platelet adhesion, entero-stimulant, entero-relaxant, epileptogenic, increases citrate excretion, trypsin enhancer and fertility-enhancing (Duke, 1992)
31.	Trans-Geranylgeraniol	$C_{20}H_{34}O$	290.5	Reverse transcriptases inhibitor increases GST activity (Duke, 1992)

Table 3: Importance of chemical compounds detected in the seeds of *S. tora*

S.No.	Compound name	Formulae	Mol. Wt.	Importance
1.	Propane, 1,1-diethoxy-2-methyl-	$C_8H_{18}O_2$	146.23	Food additive and flavour includes spices, extracts, colourings, flavours, *etc.,* added to food for human consumption, flavouring agents used in foods (Kim *et al.,* 2019)
2.	Ethyl acetoacetate	$C_6H_{10}O_3$	130.14	Flavour and fragrance, it used as an intermediate in the production of pharmaceuticals, dyes and pigments (Kim *et al.,* 2019)
3.	Butane, 1,1-diethoxy-3-methyl-	$C_9H_{20}O_2$	160.25	Food flavouring, found in beverages and gives flavour (Masino *et al.,* 2009) Spasmolytic, coronary dilation, a local anaesthetic (Farheen & Ramesh, 2018)
4.	Benzyl alcohol	C_7H_8O	108.14	Pain reliever and local anaesthetic. It is used in cosmetic formulations as a fragrance component and viscosity-decreasing agent (Nair, 2001; Kim *et al.,* 2019)
5.	1,2-ethanediol, 1,2-diphenyl-, (R*, R*)-(ñ)-(Hydrobenzoin	$C_{14}H_{14}O_2$	214.26	Quinine reductases, induce radioprotective, Renotoxic, T-suppressor cell activity, Aldose reductase inhibitor, Dopamine receptor inhibitor, Anthocyanoside and carotenoid-rich (Duke, 1992)
6.	N-Cbz-glycylglycine p-nitrophenyl ester	$C_{18}H_{17}N_3O_7$	387.3	Antimicrobial (Vijayan, 2017), antitumour, narcotic, anti-NK cell activity, neuro depressant, neuro myostimulant, anticancer, antidote, increases alkaline phosphates, asthma and fistula preventive. (Duke, 1992)
7.	Acetaldehyde, (3,3-dimethylcyclohexylidene)-, (E)-	$C_{10}H_{16}O$	152.23	Common aggregation pheromone components in *Anthonomus* spp. Weevils (Coleoptera: Curculionidae) (Szendrei *et al.,* 2011), Anticancer, decreases endothelial platelet adhesion, endoanesthetic, entero stimulant and enterotoxic (Duke, 1992)
8.	á-Campholenal	$C_{10}H_{16}O$	152.23	Inhibitor in various cases such as Reductase, Glucosidase, alpha-reductase inhibitor, increase B-alpha-mannosidase activity, Interleukin-1-alpha inhibitor and keeps the homeostasis of the system (Mohammad *et al.,* 2019).
9.	(+)-2-Bornanone	$C_{10}H_{16}O$	152.23	Analgesic, sedative, fungicide, anti-tumour, antibacterial, anti-inflammatory (Pakkirisamy *et al.,* 2017)
10.	Bicyclo[2.2.2]heptan-2-one, 1,7,7-trimethyl-,(1S)-	$C_{10}H_{16}O$	152.23	Immune enhancement and antimicrobial (Duke, 1992)
11.	Camphor	$C_{10}H_{16}O$	152.23	The compound has a counter-irritant mild analgesic action, fibrositis, neuralgia and carminative properties. (Zuccarini, 2009), Antidote (Duke, 1992)
12.	endo-Borneol	$C_{10}H_{18}O$	154.25	Anti-inflammatory and antinociceptive (Shareef *et al.,* 2016), endothelium-derived relaxing factor promoter, endocrine protective, endo anesthetic (Duke, 1992)

(Contd...)

Table 3: (*Continued*)

S.No.	Compound name	Formulae	Mol. Wt.	Importance
13.	Hydroxylamine, O-decyl-	$C_{10}H_{23}NO$	173.3	Anticancer, antidote, decreases oxalate excretion, anti-tumours, osteolytic and increases osteocalcin (Duke, 1992)
14.	5-Hydroxymethylfurfural	$C_6H_6O_3$	126.11	The compound is formed when sugars are acidified or heated as a product of Maillard reaction and used for the food flavourings (Kim *et al.,* 2019)
15.	Furan, 2,3-dihydro-4-(1-methylpropyl)-, (S)-	$C_8H_{14}O$	126.2	Antifungal (Mallaiah *et al.,* 2016), anticancer, antidiabetic, anti-metastatic, antitumour, CNS Stimulant and sedative (Duke, 1992)
16.	Hexadecen-1-ol, trans-9-	$C_{16}H_{32}O$	240.42	Antimicrobial (Kubo *et al.*, 1993; Wijekoon *et al.,* 2013), Catechol-O-methyl transferases inhibitor and increases GST activity (Duke, 1992)
17.	Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters	$C_{19}H_{30}O_3$	306.4	17-betahydroxy steroid dehydrogenases inhibitor, acidifier and antidote (Duke, 1992)
18.	Phenol, 2,4-bis (1,1-dimethylethyl)-	$C_{14}H_{22}O$	206.32	Antifungal (Rangel-Sánchez *et al.,* 2014).
19.	Pentanedioic acid, (2,4-di-t-butylphenyl) mono-ester	$C_{19}H_{28}O_4$	320.4	Increases aromatic amino acid dehydrogenases activity, diaphoretic, antidote (Digoxin), increases SOD activity, Tranquilizer, Tyrosinase inhibitor and Anti-tumour (Duke, 1992)
20.	Ethyl à-d-glucopyranoside	$C_8H_{16}O_6$	208.21	Antimicrobial, antioxidant, anti-inflammatory, hypocholesterolemic and anti-cancer, (Chidambaram *et al.,* 2015), anticancer, anti-leukotriene, decreases epinephrine production, Dopaminergic and RNA depressant (Duke, 1992)
21.	5-Thio-D-glucose	$C_6H_{12}O_5S$	196.22	Antispermatogenic and radioprotective (Homm *et al.,* 1977; Schuman *et al.,* 1982), antidote, anticancer, CNS depressant, Decongestant, diuretic and detoxicant (Duke, 1992)
22.	3-O-Methyl-d-glucose	$C_7H_{14}O_6$	194.18	Antitumour, decalcifier, depressant and systolic depressant (Duke, 1992)
23.	Phthalic acid, hex-3-yl isobutyl ester	$C_{18}H_{26}O_4$	306.39	Antimicrobial and antifungal (Dulara *et al.,* 2019), Hexokinase stimulator and acidifier (Duke, 1992)
24.	Tolycaine	$C_{15}H_{22}N_2O_3$	278.35	Anesthetic (Blum *et al.,* 2018)
25.	1-Propanol, 3-(diethylamino)-2,2-dimethyl-, p-aminobenzoate (ester)	$C_{16}H_{26}N_2O_2$	278.38	Anticancer, antitumor, parkinsongenic, anti-inflammatory, ulcer protective and provide B, Ca, Mg, Mn, Vit D, K, Zn and Si (Duke, 1992)

Earlier, Vats and Kamal (2014) studied *C. tora* methanol extract and reported phenolic content, C=O carbonyl group stretching, some peaks of flavonoid compounds, OH groups and alkyl and aryl ethers. Screening of different functional groups from leaves of *C. tora* has revealed aromatic amines, aldehydes, amines (aliphatic and aromatic), alkanes, ethers, phenols, ketones, carboxylic acid and nitro compounds (Khyade *et al.*, 2015). Similarly, Kumar and Roy (2018) studied the different populations of *Cassia tora* from different geographical regions.The FTIR spectrum has yielded functional groups like carbonyl group, C-H, C=C, C-N and C-O-C (ethers), aldehydes and carbohydrates. Polysulphides, aryl sulphides, aliphatic iodine are reported exclusively during the present study. Leaves are the most preferred part for FTIR studies and it contains more phytoconstituents in comparison to whole plant and seeds. Some functional groups such as transition metal carbonyl, alkyl carbonyl, alcohols, esters and anhydrides are present only in whole plant and seeds.

Elemental Analysis

Maximum numbers of elements have been reported from the leaves followed by whole plant and seeds (Figure 5, Table 5). The amount of calcium was highest in leaves (48.9 mg/g), potassium in the whole plant (23.5 mg/g) and phosphorus in seeds (10.8 mg/g). The sodium, titanium and bromine were present in the whole plant and leaves, whereas molybdenum and zirconium were present in seeds and leaves respectively. Some of the reported elements are important for human health care system but their number and concentration vary in different species which may also be altered by the geographical and climatic conditions, soil nature and accumulation capacity of the plant species. Elements like sodium, potassium, calcium, magnesium *etc.* play a vital role in the regulation of human physiological processes (Silva *et al.*, 2016).

Kubmarawa *et al.* (2011) reported calcium (3.52 mg/g) and magnesium (0.86 mg/g) which is lesser than the observed 48.9 mg/g and 3.7 mg/g in the present study. Other elements like phosphorus, iron, sodium, zinc, manganese, cobalt and potassium were also present in different concentrations. The elemental details of *C. tora* leaves are comprised of magnesium, calcium, sulphur, iron, sodium and chlorine (Shaikh & Sayed, 2015). In addition to the previous reports, elements like silicon, aluminium, copper, lead, strontium *etc* have been reported in leaves of *S. tora* for the first time. Similarly, the number of essential minerals and trace elements, such as potassium, chlorine, phosphorus, sulphur,

Table 4: FTIR peak values and functional groups of *Senna tora*

IR Frequencies (Wavenumber cm^{-1})			IR frequencies range for the respective functionality (Wavenumber cm^{-1})	Functional Groups (Coates, 2000; Pavia *et al*., 2006)
Whole plant	Leaves	Seeds		
-------	3480.61	-------	3650-3300	O-H Stretching group
-------	3274.82	3271.14	3400-3200 3300-3030	Normal ''polymeric'' OH stretch, O-H stretching in alcohols, phenols and carboxylic acids, Sp, C- H Stretch Ammonium Ion
3178.66	-------	-------	3300-3030	Ammonium Ion
2918.21	2917.42	2922.49	3000-2800	O-CH_3, C-H Stretch, Methyl Ester,
2859.93	2849.21	2852.91		Alkanes, Sp^3 C- H Stretch
-------	-------	2029.83	2100-1800	Transition metal Carbonyls
-------	-------	1972.28		
-------	-------	1744.48	1760-1740	Alkyl Carbonate C=O carbonyl group
1728.04	-------	-------	1740-1705	C=O stretch carbonyl group in aldehydes, ketones
-------	1601.57	1632.97	1650-1550	N-H Bend, Secondary amine
1595.16	-------	1542.98	1650-1550 1600-1500	N-H Bend, Secondary amine C=C Stretch
1425.65	1409.30	1399.75	1475-1350	CH_2/CH_3 Bending Vibration
1384.79	-------	-------	1440-1200	C-O-H Bending Vibrations
1323.50	-------	1238.98	1440-1200	C-O-H Bending Vibrations
1258.12	-------	-------	1350-1000	C-O Alcohols, Ethers, Esters, Carboxylic acid, Anhydrides
1055.31	1013.62	1031.32	1350-1000	C-O Alcohols, Ethers, Esters, Carboxylic
1007.78	-------	-------		acid, Anhydrides
524.53	526.86	522.66	600-500	C-I Stretch, Aliphatic Iodine Compounds
-------	466.08	-------	500-470	S-S Stretch, Polysulphide
-------	484.31	-------		
-------	439.06	457.15	500-430	S-S Stretch, Aryl Sulphide

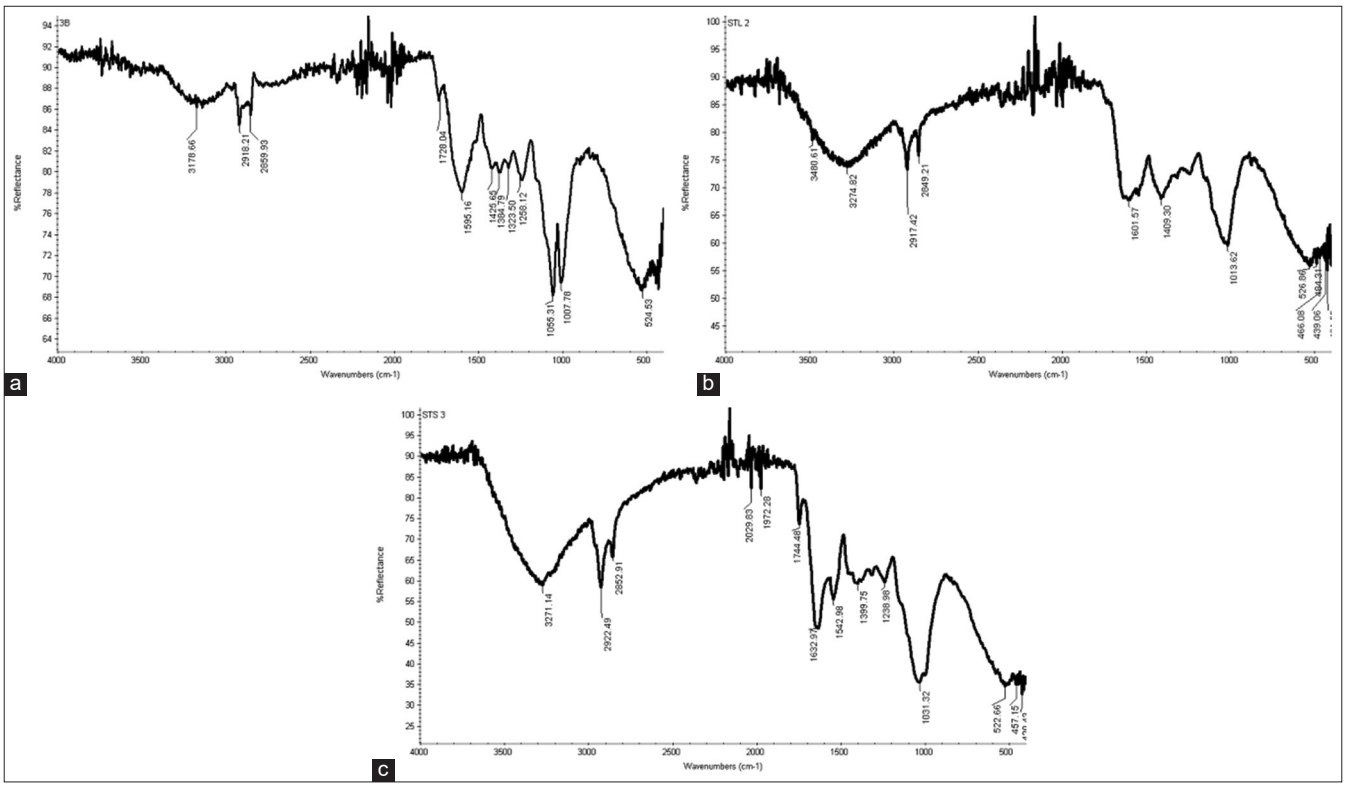

Figure 4: FT-IR Spectrum of (a) whole plant, (b) leaves and (c) seeds of *Senna tora*

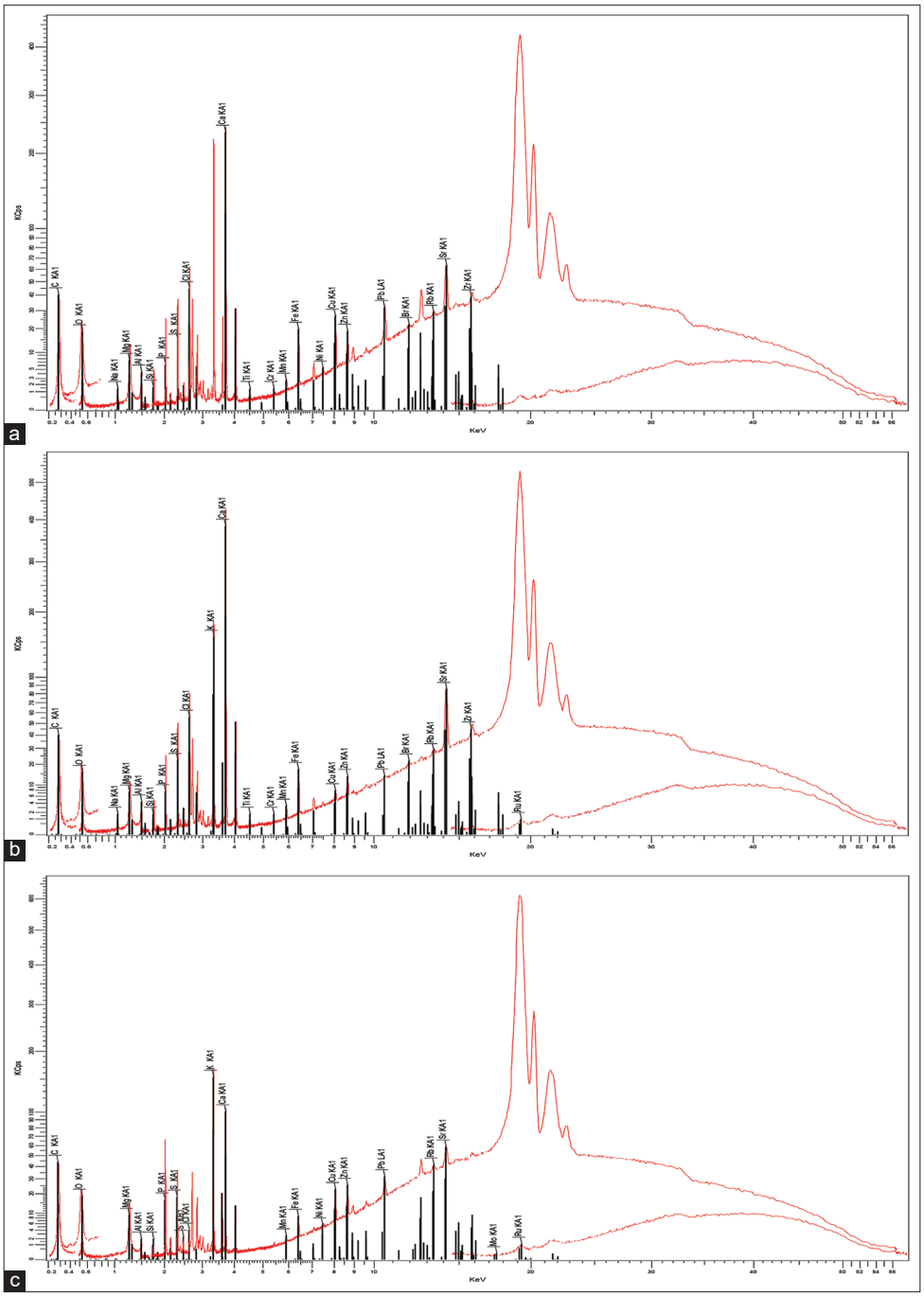

Figure 5: (a-c) WD-XRF Spectra of (a) Whole plant, (b) Leaves and (c) Seeds of *Senna tora*

aluminium, iron, sodium, nickel, chromium and bromine were also studied in the whole plant and seeds for the first time during this study.

Calcium is good for bones, teeth and muscles whereas magnesium helps in the management of heart diseases and repair damaged cells. Similarly, iron prevents anaemia and boost immunity and chromium control sugar levels, lipid homeostasis and diabetes (Balk *et al*., 2007; Larsson & Wolk, 2007; Agunbiade *et al*., 2012). Sulphur is active against acne, eczema, psoriasis, skin irritation, microbes, involved in proteins synthesis, cell regeneration, blood purification, insolubility of keratin and provide strength to skin hair due to disulfide bonds (Afolayan & Otunola, 2014; Haque *et al*., 2015). Researchers have added silicon, arsenic, chromium, molybdenum and vanadium to the list of essential elements. The presence of these elements (macro and trace) in *S. tora* is an indicator of its medicinal potential.

Table 5: WD-XRF analysis of whole plant, leaves and seeds of *S. tora*

S. N.	Elements	Symbol	Atomic No.	W.P (mg/g)	Le (mg/g)	Se (mg/g)
1.	Cellulose	$C_6H_{10}O_5$		926.6	904.2	952.7
2.	Calcium	Ca	20	26.6	48.9	10.1
3.	Potassium	K	19	23.5	20.3	16.6
4.	Chlorine	Cl	17	8.6	11.4	0.6
5.	Magnesium	Mg	12	4.3	3.7	5.1
6.	Phosphorus	P	15	4.2	4.2	10.8
7.	Sulphur	S	16	3.2	4.4	3.2
8.	Silicon	Si	14	1.3	1.3	0.2
9.	Aluminium	Al	13	0.6	0.6	0.094
10.	Iron	Fe	26	0.3	0.3	0.083
11.	Sodium	Na	11	0.2	0.3	-----
12.	Copper	Cu	29	0.2	0.026	0.092
13.	Lead	Pb	82	0.1	0.015	0.081
14.	Zinc	Zn	30	0.07	0.051	0.076
15.	Strontium	Sr	38	0.065	0.1	0.027
16.	Manganese	Mn	25	0.042	0.068	0.023
17.	Titanium	Ti	22	0.021	0.024	-----
18.	Nickel	Ni	28	0.016	-----	0.005
19.	Chromium	Cr	24	0.014	0.01	0.01
20.	Bromine	Br	35	0.006	0.008	-----
21.	Ruthenium	Ru	44	-----	0.023	0.018
22.	Rubidium	Rb	37	-----	0.005	0.007
23.	Zirconium	Zr	40	-----	0.002	-----
24.	Molybdenum	Mo	42	-----	-----	0.01

*W.P- Whole Plant, Le- Leaves and Se- Seeds

CONCLUSION

Senna tora, a traditional medicinal plant, has been evaluated for the presence of phytoconstituents, elements, chemical compounds and their related functional groups. The ethanol extract has shown the presence of maximum phytochemicals followed by aqueous and hexane extracts. The WD-XRF analysis has revealed the presence of various elements including calcium, potassium, magnesium, sulphur, iron, zinc, manganese *etc*. GC-MS of leaves and seeds has depicted various chemical compounds of medicinal importance including anticancer, antibacterial, antitumor, antioxidant, diuretic *etc*. The ethanol extract of leaves and seeds had shown the presence of compounds like Silicic acid, diethyl bis(trimethylsilyl) ester, 1-Monolinoleoylglycerol trimethylsilyl ether, Hexadecen-1-ol, trans-9-, Ethyl à-d-glucopyranoside and 10-Heneicosene (c,t) *etc*. Some of these are precursors of the modified derivatives for the compound drug analysis. FTIR spectroscopy has shown various functional groups supporting the presence of important phytochemicals like alcohols, amines, carbonyls, alkyl carbonate, ethers and esters. Thus, the observations of the present study have explained the potentiality of *Senna tora* in the pharmaceutical sector. This species can provide raw materials for the preparation of new or alternate medicine. Further studies are required for the isolation and characterization of anticancer, antitumour, antidiabetic and antibacterial compounds.

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