Separation, Isolation and Evaluation of Pharmacognostical And Phytochemical Parameters of Ethanol Extract of *Senna Uniflora* Seeds

Short version of the title : *Senna uniflora* seeds isolation and characterization phyto constituents

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Abstract

Senna uniflora plant has been reported to possess various pharmacological activities such as anti-inflammatory, antimicrobial and anti-rheumatic. The pharmacognostical and physico-chemical characterization of *Senna uniflora* seed is the objective of this research work. Physico- chemical parameters like ash value, extractive values, LOD were determined. The LOD was found to be 4.8%w/w. The different ash values like total ash content, acid insoluble ash, water soluble ash and sulphated ash was determined to be 3.4% w/w, 0.5 %w/w, 1.67 %w/w and 3.67%w/w respectively. The water soluble extractive and alcohol soluble extractive was found to be 24.8 % w/w and 9.6 % w/w respectively. *Senna uniflora* seeds were found to contain numerous phytochemicals like alkaloids, tannins, steroids, fixed oils, fats, mucilage and flavonoids. Transverse section of *Senna uniflora* seed shows the presence of testa, epidermis, parenchymatous cells, plumule, radical, tigellum, endosperm etc. Powder characters show the presence of mucilage containing cells, prismatic crystals, oil droplets, palisade cell fragments etc. The ethanol extract of the seeds was separated into its constituents by column chromatography technique followed by TLC. The isolated compound was analysed by IR, ^{1}HNMR, ^{13}C NMR and MS spectral analysis for molecular characterization. The isolated compound was determined and characterized to be fisetin. The chemical compositions of the seeds are going to play an essential role in the medical field, especially in the treatment of variety of diseases. The results of the current study may be used as standard parameters for the standardization of this medicinal seeds in future.

Keywords: *Senna uniflora*, pharmacognostical, phytochemical screening, standardization, molecular characterization.

1. Introduction

The use of plant as medicine dates long back as long as evolution and civilization of humans. Each civilization had their own practice of medicines which is now reflected as traditional system of medicines followed in different countries like the Ayurveda & Siddha medicine followed in India, Traditional Chinese Medicine (TCM) in China. Sowa-Rigpa is a traditional system of medicine that originated in India and is practiced in the Himalayas, China, Mongolia, Nepal, Russia, and Bhutan. Unani system of medicines is followed in Arabic countries. Japanese traditional medicine, also known as Kampo, is a well-known herbal medicinal system that is a part of Japan's healthcare system. Kampo is derived from ancient Chinese medicine and has been developed over centuries under the influence of Japanese culture and nature. Each and every traditional system of medicines from various civilizations relies on plants as medicines. All the synthetic medicines which are used in allopathic system of medicines today, directly or indirectly have the basis from the plant. In this modern world of developing and developed countries, still man relies on plants for the healing of his ailments. Now, when the population of the world has increased tremendously, the quantity of medicines requirement has also greatly increased. So there are chances of decrease in the quality of the medicines. As a

result, standardization of herbal medicines has become essential in order to completely experience the potency and efficacy of medicinal plants. Therefore an effort has been made to characterize one such medicinal plant and become a part of the scientists who are involved in the standardization of herbal drugs. In traditional system of medicine, seeds of many plants are utilised in many ways to decrease the level of disease condition or maintain a normal health in mankind. The seed extracts have been widely used in folk medicine for the treatment of large number of ailments especially in rural communities.

Senna uniflora is a woody, annual erect herb, commonly known as One Leaf Senna. Botanically the plant is known as *Cassia uniflora* Mill [1, 2]. The genus Senna can be classified under the Family Fabaceae, sub family Caesalpinioideae. This genus contains 350 species comprising of trees, shrubs and herbs. Due to the attractive yellow inflorescence, some species are used as ornamental plants[3]. The *Senna* species finds use as laxative, anti-malarial, relaxant, anti-inflammatory and anti-diabetic as given in ancient ayurvedic literatures. Many or almost all the species of the genus *Senna* are reported to be rich in numerous phytoconstituents namely glycosides, anthocyanosides, alkaloids, flavonoids, saponins, tannins, terpenoids. To date numerous *Senna sp* are used in the treatment of many disease and ailments of human. Most parts of the plant like the leaves, pods, roots, fruits, barks and seeds are used for their wide variety of pharmacological activities to aid in the diseases of mankind.

Senna uniflora is locally known as Kattu Thagarai in tamil. It is a herb that grows to a height 15 to 20 cm. This plant is native to Mexico, Caribbean Island and tropical South America and in India it can be seen in Andhra, Karnataka, Kerela Tamil Nadu and Maharashtra. *Senna uniflora* is a common weed found in tropics and poultice area of Tamil NSadu. *Senna spp* is traditionally used as wound healer, to treat eczema and also to combat dropsy [4]. The ability of *Senna uniflora* to minimize the risk of inflammation [5] and microbial infection has been proven. Mucilaginous polysaccharide present in *Senna uniflora* seed has a promising gelling property and finds wide industrial application. The whole plant of S*enna uniflora* has been found to possess pharmacological activities such as anti-inflammatory, analgesic, antioxidant, anti-bacterial, anti-microbial and anti rheumatic [6-8] etc. A wide-ranging literature survey revealed that no work has been reported on pharmacognostic studies of *Senna uniflora* seed. Therefore, in the present research study, pharmacognostic evaluation of the seeds of *Senna uniflora* is undertaken in order to standardize the seed drug.

2. Materials and Methods

2.1. Chemicals: All the chemicals like toluidine blue, chloral hydrate, glycerol ethanol used were of analytical grade obtained from Merck India, Qualigens, Loba chemie etc.

Equipments: Soxhlet apparatus set up, FTIR spectrophotometer 1650, JEOL NMR spectrometer, JEOL-Accu TOF JMS-T 100 LC Mass spectrometer.

2.2 Collection and authentication of *Senna uniflora* seeds.

Seeds of *Senna uniflora* were collected from Coimbatore, Tamil Nadu in the month of March. The seeds were identified and authenticated by Dr.M.U.Sharief 'E'& Head of office, Botanical Survey of India, Southern Regional Centre, Coimbatore. Seeds were shade dried powdered and stored in organic bag and labelled. Figure 1 shows the picture of the whole plant. *Senna uniflora* will be referred as *S. uniflora.*

2.3 Pharmacognostical Evaluation

Pharmacognostical evaluation involves macroscopical, microscopical analysis, maceration and extraction techniqes, organoleptic studies and fluorescence analysis of plant powder. The macroscopical

evaluation of the external features of the seeds of *S. uniflora* was observed documented using Nikon D-5600 Digital camera and shown in figure 2. Microscopical evaluation involves study of transverse section, longitudinal section of the plant parts and also powder microscopical studies. Microscopical evaluation of the seeds of *S.uniflora* was performed by soaking the seeds in water for more than 48 hrs. The soaked specimens were cut into several thin transverse section using a sharp blade, stained with toluidine blue and observed under microscope. A very good section shows all parts of the section under study clearly. Transverse sections were photographed using Nikon ECLIPSE E200 triangular microscope attached with Zeiss Axio Cam Erc 5s digital camera under bright field light. The pictures were captured by observing under both normal light and under polarized light. Magnifications were indicated by scale bar.

Figure 1. Whole plant of *Senna uniflora* with pods

Powder microscopical characters of the powdered seeds were evaluated. A pinch of the powder of *S.uniflora* seeds was treated with chloral hydrate and then placed with a drop of 50% glycerol on a microscopic slide. The powder characters were observed using potassium iodide solution for testing starch grains. The powder microscopy was observed through a Nikon ECLIPSE E200 trinocular microscope. The microscopical characters were photographed with Zeiss ERc5s digital camera under bright field light which was attached to the trinocular microscope. Photomicrographs of diagnostic characters were captured and well documented for further study.

2.4 Preparation of Seed Extract

Around 200 gm. of the seed powder was mixed in 400 ml of the solvent. Extracts were prepared using solvents of least polarity to solvents of high polarity (pet ether, ethyl Acetate, and ethanol) [9]. Hot continuous percolation method was adopted for extraction [10]. At first petroleum ether was used followed by ethyl acetate and finally by ethanol. The seed powder was extracted for 8 to 10 hours in Soxhlet apparatus in each solvent then dried and extracted with next solvent. The temperature was maintained (50 - 60° C) based on the solvent used, on an electric heating mantle with thermostat control. Each extracts thus collected was concentrated by heating on rotary evaporator under 50°C and low pressure. The concentrated extract was finally dried to a constant weight. Then the extract was used for further study like preliminary examinations as per standard methods.The extract will be referred as EtOH extract.

2.5 Physicochemical & Phytochemical Evaluation

The powder of the seeds of *S.uniflora* were evaluated for various physicochemical parameters such as various ash values, moisture content, various extractive values and LOD(loss on drying).The above parameters were analysed as per the procedures mentioned in WHO quality control methods for herbal materials [11]. The petroleum ether extract, ethyl acetate and ethanol extracts of the powdered seeds of *S. uniflora* were subjected to phytochemical analysis to screen the presence of various types of secondary metabolites like alkaloids, glycosides, amino acids, proteins, phenolic compounds, tannins, carbohydrates, terpenoids, flavonoids, gums and mucilages etc.

2.6 Isolation of active constituents

The concentrated EtOH extract of the powdered seeds of *S. uniflora* was dissolved in alcohol and fed in to the column for separation in to its components. Polarity gradient technique was adopted, to separate the phytochemicals present in the extract [12]. Hexane was used as first solvent and allowed pass through the column loaded with the EtOH extract. The polarity of the solvent was gradually increased by mixing hexane with ethyl acetate in the ratios of 90:10; 80:20; 70:30; 60:40; 50:50; 40:60; 30:70; 20:80; 10:90 and concluded with 100% ethyl acetate. Subsequent elution was continued with ethyl acetate: chloroform in the ratio of 95:05; 90:10; 80:20; 70:30; 60:40;65:35; 50:50; 45:35;40:60; 30:70; 20:80 and finally with 100% chloroform. The column was further eluted with chloroform: methanol mixture in the ratios of 98:2; 96:4; 94:6; 92:8; 90:10; 85:15; 80:20. A total of 78 fractions were collected and the extracts were assessed using thin-layer chromatography (TLC) technique. The separated components on the TLC plate were observed under UV light and also stained with iodine for spotting the separated constituents. Fractions numbered from 49 to 56 exhibited similar Rf values in TLC. These fractions were combined and the solvent was evaporated under reduced pressure. The resultant crude material was purified using activated charcoal in hot ethanol. The isolated compound was cooled in a refrigerator for crystallization. A pale yellow solid was obtained which was further analysed by Mass spectra, FT-IR, ^{13}C-NMR, and ^{1}H-NMR analysis.

3. Results

3.1 Macroscopical Evalution

The macroscopical evaluation of the *Senna uniflora* seeds **(**Figure 2) featured the following:

Colour : Pale Brown

Odour : None

Taste : Sweet

Shape : Flattened, rhomboid or sub quadrangular and glabrous

Size : 3 to 4 mm in length, 2 to 3 mm in width and 0.5 to 1 mm in thickness

Appearance : Hilum is present as a small dark brown colour dot.

Figure 2. *Senna uniflora* seed-Macroscopy

3.2 Microscopical Evalution [13-16]

T S of *Senna uniflora* seed

The transverse sections of the seed show an outer covering called testa. Testa is composed of single layer of thick walled, compactly arranged palisade cells characterized by flat ends with highly refractive narrow lumen. The testa was found to be covered with cuticle could be observed in figure 3a. A light line called linea lucida crossing across the upper surface of the palisade layer could be seen in figure 3a. Below the palisade layer, 6 to 7 rows of tangentially elongated parenchyma cells with brownish contents and prismatic crystals can be seen. Inner to this layer, is a layer of parenchymatous cells resembling spoon shaped cells as shown in figure 3b. To get a clear picture of the TS, the seed TS is viewed under a polarizer in which different layer of cells could be seen in figure 4. Tegument forms the collapsed layer of cells after testa followed by thick walled cells of endosperm with reserved carbohydrate embedded with aleurone grains. Cotyledon is composed of upper and lower epidermis encircling 4 to 6 rows of mesophyll cells as shown in figure 5. Just beneath epidermis the palisade layer is present. Mesophyll cells are embedded with aleurone grains, oil globules and clusters and few rosette crystals. TS of radical show an outer single layered epidermis covered by cuticle. About 5 to 6 layers of parenchymatous tissue are found beneath the epidermis.

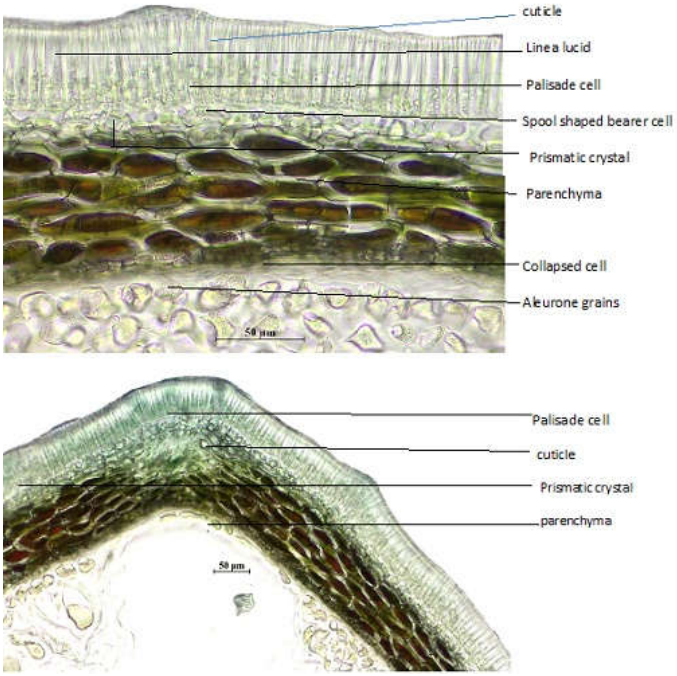

Figure 3a & 3b.TS of *Senna uniflora* seeds showing testa and enlarged endosperm

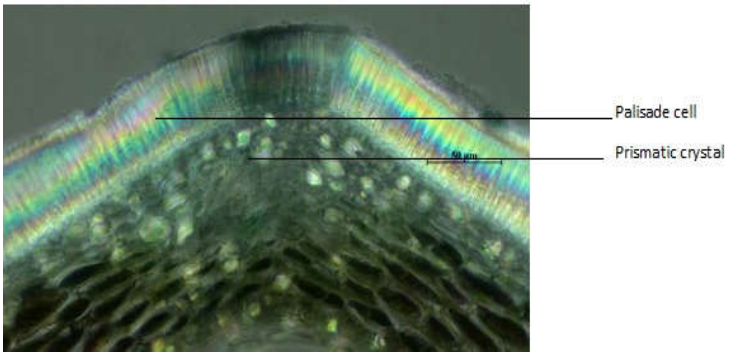

Figure 4. Testa of the seeds of *S.uniflora* viewed under polarizer

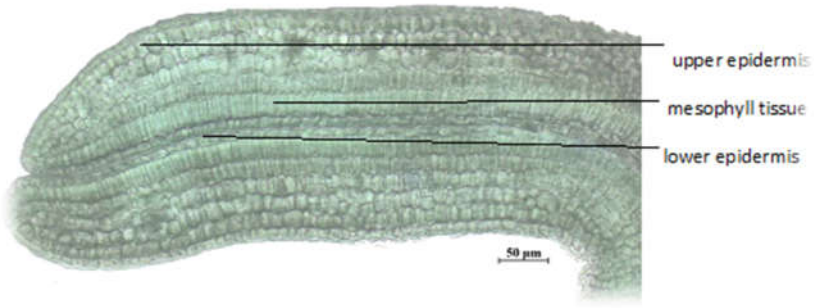

Figure 5. LS of Cotyledons of the seeds of *S.uniflora*

Powder Microscopy

The seed powder of *S.uniflora* is creamy yellow in colour with a characteristic odour and slightly sweet to taste. The powder microscopical studies shows sectional view of testa, fragment of palisade layer, thick walled parenchymatous cells with brown content, parenchyma cells from radical. Cells with mucilage, tracheal tissue from hilum region, sectional view of endosperm and cotyledons, oil droplets and prismatic crystals are also observed as shown in figure 6.

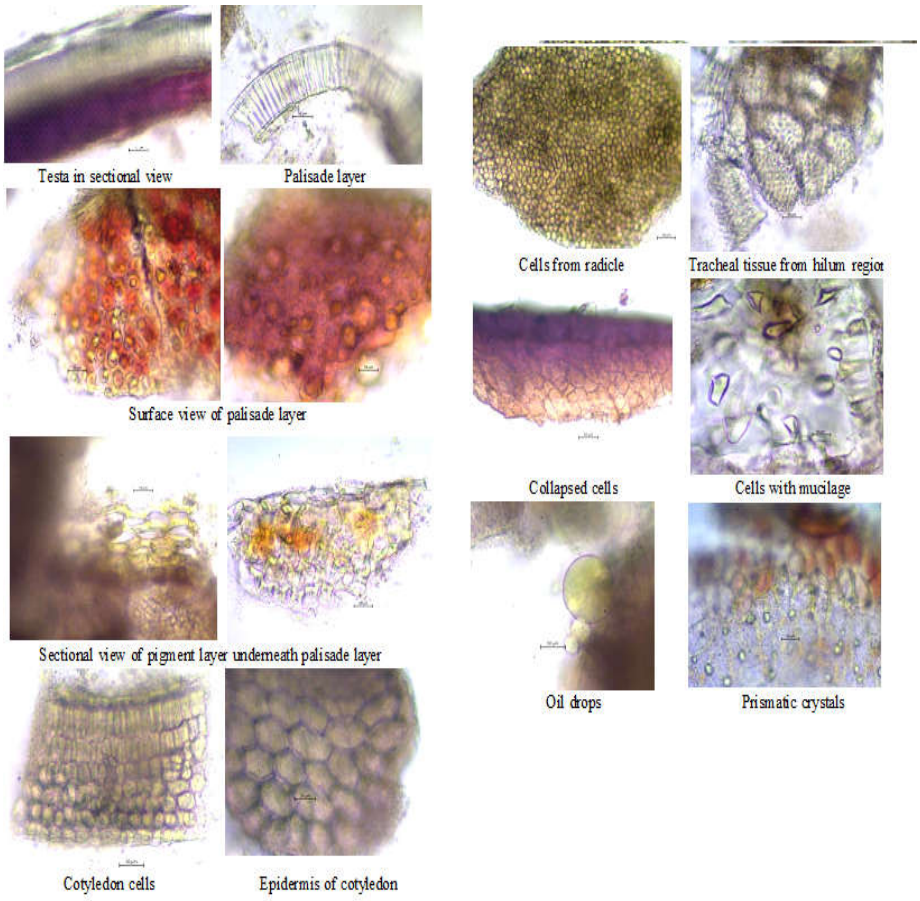

Figure 6. Powder microscopical characters of *Senna uniflora* Seeds

3.3 Physico-Chemical Studies

The Physicochemical parameters of the seed, viz; total ash, acid insoluble ash, water soluble ash sulphated ash were found to be 3.4 %, 0.5 %, 1.66 % and 3.366 %w/w respectively. Alcohol soluble extractive content in the seeds of *S.uniflora* was found to be 9.6% w/w. While water soluble extractive value was 24.8 % and it was the highest. The other extractives like chloroform soluble extractive, petroleum ether soluble extractive & ethyl acetate soluble extractive value was determined to be 8.24 %, 7.2 % & 8 % w/w respectively. LOD was determined to be 4.8 %w/w in the dried seeds. The physico chemical parameters seed powder of *S.uniflora* are presented in table 1.

3.4 Flourescence Analysis of seed powder *S.uniflora*

The identification of the powder characters of the seeds of *S.uniflora* was studied in various solvents and viewed under ordinary day light, UV-254 nm and at UV – 366 nm. The results are tabulated in the table 2. This can help in the identification of the seed powder to some extent.

3.5 Qualitative Phytochemical analysis of EtOH extract of *S. uniflora* seeds

The preliminary phytochemical screening of *Senna uniflora* seed powder extracts by chemical tests shows the presence of alkaloids, steroids, flavonoids, tannins, fixed oils and fats, gums and mucilages. The results are tabulated in table 3

3.6 Isolation of active constituents of EtOH extract of *S. uniflora* seeds

The product of ethanol extract of the *Senna uniflora* seed powder was subjected to chromatographic separation by column chromatographic technique. Gradient elution technique was adopted by starting the elution using the mobile phase combination of Hexane: ethyl acetate in the ratio of 90: 10. Slowly the polarity was increased by decreasing the concentration of hexane and increasing the proportion of ethyl acetate. The elution was continued by changing the solvent with ethyl acetate: chloroform and again eluted with chloroform: methanol mixture. About 78 fractions were obtained. The fractions were subjected to TLC for determining the Rf value. It was found that fraction 49 to 56 exhibited similar Rf value. These extracts were combined, concentrated. A pale yellow was solid obtained. This compound was characterized in molecular level by NMR both ^{1}H and ^{13}C, IR and MS methods.

Table 1. Physico chemical parameter of seed powder of *S.uniflora*

S.No	Parameters	%W/W
01	**Ashvalues**	
	(a)Totalash	3.4
	(b)Acid-insolubleash	0.5
	(c)Watersolubleash	1.66
	(d)Sulphateash	3.366
02	**Extractivevalues**	
	(a)Alcohol solubleextractivevalue	9.6
	(b)Water solubleextractivevalue	24.8
	(c)$CHCl_3$ solubleextractivevalue	8.24
	(d)Petroleumetherextractivevalue	7.2
	(e)Ethylacetateextractivevalue	8
03	**Loss ondrying (LOD)**	4.8

Table 2. *S.uniflora* seed powder characteristics in various solvent medium

S.No	Sample treatment with various solvents	Daylight	UV 254	UV 366
1	Powder (P) as such	Yellow	Fluorescent yellow	Fluorescent yellow
2	P+nitro cellulose in amyl acetate	Yellow	Fluorescent yellow	Fluorescent green
3	P+1NNaOH inWater	Fluorescent yellow	Dark Fluorescent yellow	Fluorescent Green
4	P+1NNaOH in nitro cellulose in amyl acetate	Fluorescent yellow	Fluorescent yellow	Fluorescent Yellow
5	P+ 1NHCl	Creamish yellow	Light fluorescent	Brown
6	P+1NHCl+ nitro cellulose in amyl acetate	Orangish yellow	Fluorescent creamish	Fluorescent green
7	P+1NNaOH in methanol	Fluorescent yellow	Fluorescent yellow	Fluorescent green
8	P+50%KOH	Creamish yellow	Dark fluorescent yellow	Greenish fluorescence
9	P+50%H_2SO_4	Fluorescent yellow	Light Fluorescent yellow	Fluorescent green
10	P+50%HNO_3	Creamish yellow	Fluorescent yellow	Dark-brown Greenish fluorescence
11	P+ Conc HNO_3	Orangish brown	Fluorescent green dots	Fluorescent green
12	P+Aceticacid	Light brown	Fluorescent green	Fluorescent green
13	P+I_2water	Cream	Fluorescent green	Green

Table 3. Phytochemical analysis of *Senna uniflora* seeds

Phytoconstituents	Pet Ether	Ethyl acetate	Ethanol
Alkaloids	+	+	+
Saponins	-	-	-
Glycosides	-	-	-
Carbohydrates	+	-	+
Phenolic compounds and tannin	-	+	+
Steriods	+	+	+
Proteins and amino acid	-	+	-
Terpenoids	-	-	-
Fixed oils and fats	+	+	+
Gums and Mucillage	-	-	+
Flavonoids	+	+	+

3.7 Characterization of isolated compound from ETOH extract of *S. uniflora* seeds

The isolated compound from the ETOH extract of the seed powder of *S.uniflora*were characterized by NMR both ^{1}H and ^{13}C, IR and MS methods [17].

The ^{13}C-NMR spectrum of the isolated compound is shown in figure7. The compound exhibited resonances at 126.92(C-5), 116.01(C-6), 102.28(C-8), 114.64(C-5′), 120.12 ppm, (C-6′), indicating the presence of the aromatic ring structure. The chemical shift at 172.43 ppm (C-4) ppm corresponds to the keto group at the C-4 position. The peaks of hydroxyl groups were evident at 162.69 (C-7), 137.60 (C-3), 145.47 (C-4′), 145.52 (C-3′).

The [1]H-NMR spectrum of the isolated compound exhibited various resonances in the aromatic region 7.91 - 7.93δ (1H, t, Ar-H); 7.67- 7.68 δ (1H, d, Ar-H); 7.53-7.55 δ (1H, m, Ar-H); 6.87- 6.92 δ (3H, m, Ar-H). A distinctive signal at 10.81 δ (1H, s, -OH); 9.52 δ (1H, s, -OH); 9.31 δ (1H, s, -OH); 9.03 δ (1H, s, -OH); indicated the presence of a hydroxyl protons. The [1]H-NMR spectrum is represented in figure 8.

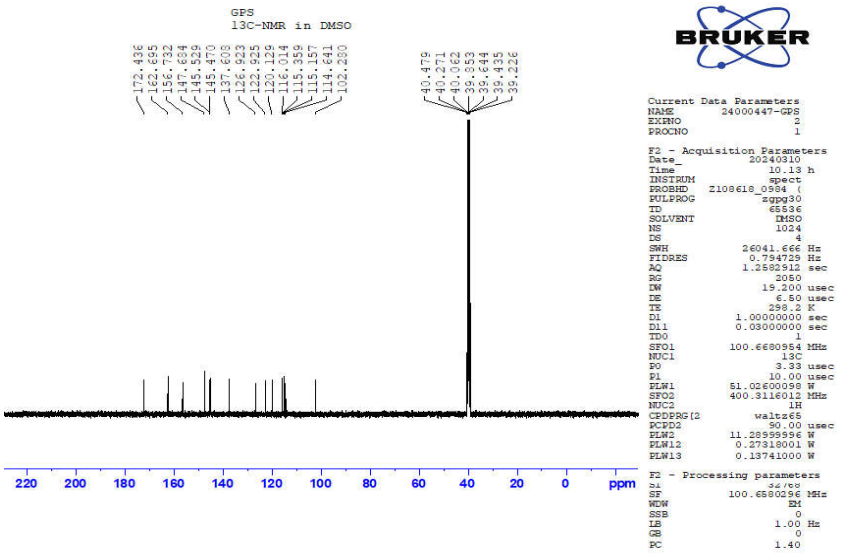

Figure 7. C-13 NMR spectrum of isolated compound from EtOH extract of *S.uniflora* seeds

FTIR spectra is shown in figure 9. Notable peaks were observed at 3519 cm^{-1} and 3348 cm^{-1} indicative of the -OH group, 2996cm^{-1} suggestive of the =CH stretching. The stretching band of the carbonyl group C=O was detected at 1606 cm^{-1}, while the aromatic double bond was identified at 1569 cm^{-1}. In the fingerprint region, the C-H bending vibration peaks were noticed at 1332 cm^{-1}. The molecular weight of the isolated compound was determined using high-resolution mass spectrometry, and the molecular ion peak was observed at m/z 287.07 (M+1) as shown in figure10. Combining the results of the spectral analysis of the isolated compound, it could be concluded that the isolated compound could have a molecular structure same as that of fisetin shown in figure 11.

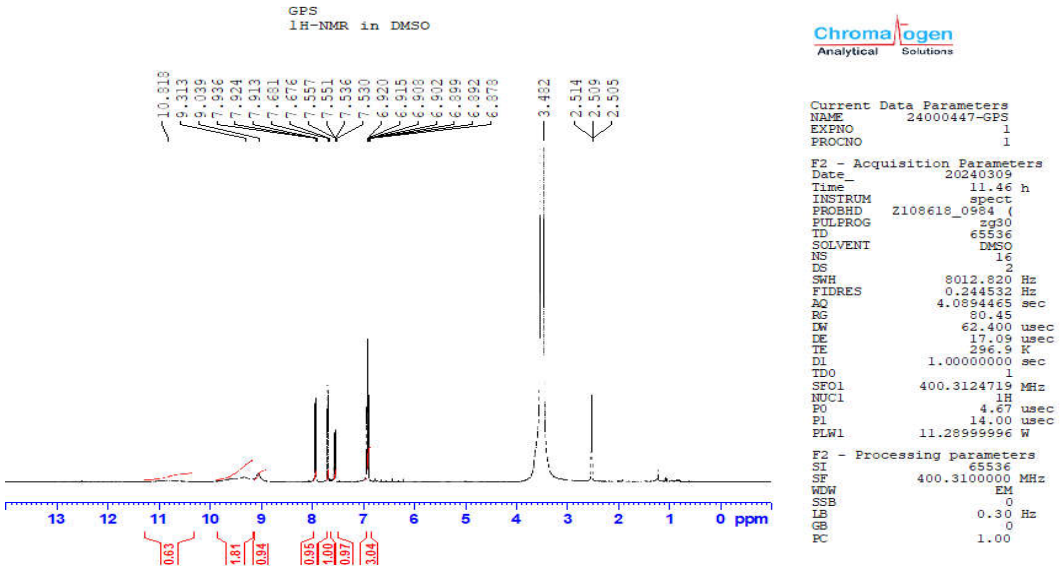

Figure 8. [1] H NMR spectrum of of isolated compound from EtOH extract of *S.uniflora* seeds

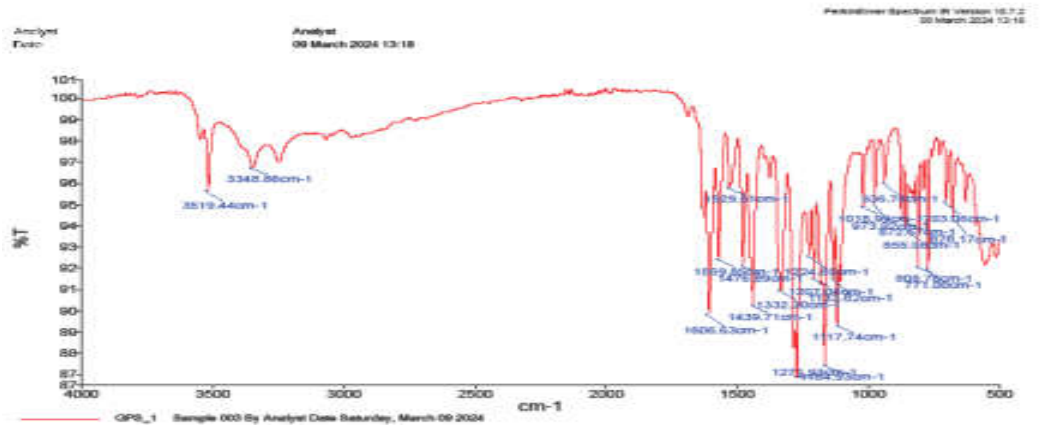

Figure 9. FTIR spectrum of isolated compound from EtOH extract of *S.uniflora* seeds

4. Discussion

Pharmacognostical evaluations like determination of ash values, extractive values, analysis of phytoconstituents, powder characterization help in setting standards for plant drugs. Similarly macroscopical evaluation and microscopical studies of all parts of the plants individually are also identifying parameters. The powder characters of different parts of the plant individually, and of the whole plant gives an idea about the identity and genuinity of the plant drugs. These studies help in the determining the time of collection, storage and processing of medicinal plants It also helps to avoid adulteration in medicinal plants. The study of morphology of *S. uniflora* has shown similar characters to that of *Senna tora* like: only four leaflets with glands between all pairs, presence of trichomes on leaves and stems which is varied or distinctive character from other *Senna spp*. The very least studies reported on *S. uniflora* may be due to similarity between *S. uniflora* and *S. tora* [18]

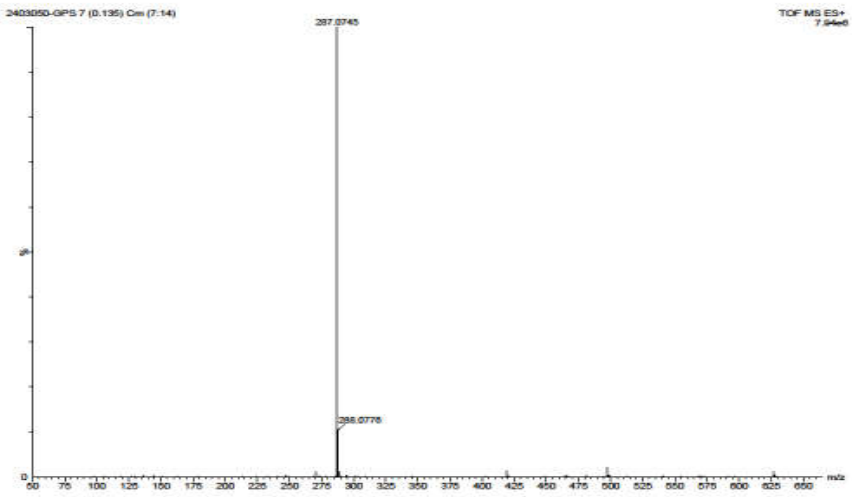

Figure 10. Mass spectrum of isolated compound from EtOH extract of *S.uniflora* seeds

The comprehensive analysis of the phamacognostic features of seeds of *Senna uniflora* has been presented here in this research paper for the first time. All types pharmacognostical and phytochemical

evaluation studies and their result is discussed here. It involves macroscopical evaluation that may help to identify the plant and its seeds by external morphology. The macroscopical evaluation might help significantly to explain the usefulness of these morphological characters established in the study as a parameter in the botanical identification of the plant and its seeds. Since this research paper involves the pharmacognostical evaluation of the seeds of *S. uniflora* seeds and its powder characterization was also performed and evaluated. The transverse section of the seeds and its cotyledons could be used for the evaluation and identification of the seeds of *Senna uniflora*. The powder characters of the seeds have been evaluated by powder microscopical studies. It reveals the presence of aleurone grain, starch granules, prismatic crystals, collapsed cells, palisade cells. This can also help in the identification and purity of the powdered drugs. All these microscopical parameters can also be used for the identification of the seed powder and help in the determination of adulteration in the seed powders. With regard to the fluorescent analysis the powdered drug was mixed with different solvents and viewed under daylight and UV light. The solvents used were NaOH, HNO_3, H_2SO_4, I_2 in NaOH, nitrocellulose in amyl acetate etc. The powder drug when viewed under day light and UV light, exhibited different colours. These characters can be used to evaluate the seed powder qualitatively. This fluorescence character is an important parameter in the pharmacognostical evaluation [19].

The phytochemical evaluation of the seed powder extracts revealed the presence of alkaloids, gums and mucilages, flavonoids, phenolic compounds, sterols, fats, proteins etc. The evaluation of physical parameters of the seeds and its powder namely ash values, extractive values, LOD, moisture content also can be used in the identification and determination of quality and to confirm the authenticity of *Senna uniflora* seed. The ethanol extracts of the seed powder exhibited the presence of large amounts of phytochemicals. So EtOH extract of the seed powder was subjected to separation into its components and also isolation of the major component. Polarity gradient method of separation of constituents in the EtOH extract into individual constituent was adopted for isolation. A two phase solvent system or mixture of solvents of different polarities was used. A hexane: ethyl acetate followed by Ethyl acetate: chloroform and then by chloroform: methanol solvent system was adopted in order to extract and separate all the polar and non-polar components. Among the 78 fractions collected, the fractions showing similar Rf value on TLC were combined and concentrated. Similar Rf value indicates that the compound present the selected fractions is a single component and present in larger amount.

The chemical structure of the isolated plant constituents can be identified which will help in future studies, like pharmacological activity, SAR studies, and structural modification to increase the bioactivity or decrease the side effects, synthesis of active phytoconstituents to meet the needs worldwide. Since very little amount of phytoconstituents are isolated from the plant extract, the structural elucidation of phytoconstituents is not possible completely by chemical reactions. So identification of phytoconstituents can be performed using spectral analytical methods which will use very little amount of the sample.

The isolated compound was characterized by spectral analysis namely FT-IR, NMR both ^{13}C & ^{1}H and Mass spectrometric analysis. The molecular ion peak was observed at m/z 287.07 (M+1) for the isolated compound. All these spectral analysis confirmed that the compound isolated could be fisetin [20] characterized by by Mass FT-IR, NMR-^{13}C, ^{1}H NMR. The structure of fisetin is shown in the figure 11.

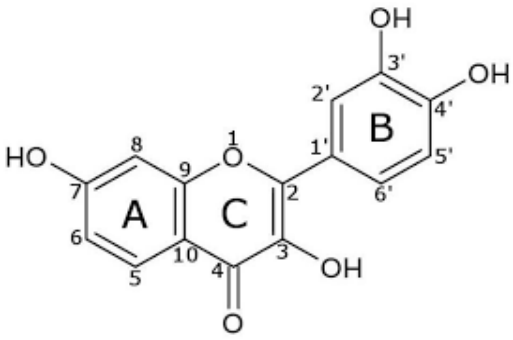

Figure 11. Structure of Fisetin present in the isolated EtOH extract of *S.uniflora* seeds

5. Conclusion

The seeds of *S. uniflora* have been used for numerous therapeutical properties. So it was necessary to standardize the seeds by morphological parameters, Phytochemical and pharmacognostical parameters as a means of identification and also to avoid adulteration of the seeds and its powder. The phytochemical study of ethanol extract of the seed of *Senna uniflora* revealed the presence of alkaloids, flavonoids, phenolic compounds, gum and mucilage. This implies that the seed part of the plant could be used as the potential source for identifying novel phytocompounds which can have a good antioxidant activity, anti diabetic activity and anticancer activity. The isolated compounds were recognized through FT-IR, NMR (both C-13 & proton) and mass spectroscopy. A simple easy, rapid and highly efficient extraction method and standardization procedure has been devised and designed for the seeds of *Senna uniflora* plant.

6.Acknowledgement

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