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ESTIMATION OF PHYTOCHEMICAL CONSTITUENTS, DPPH FREE RADICAL SCAVENGING ACTIVITY AND FT-IR ANALYSIS OF FLOWERS EXTRACT OF *SENNA SURATTENSIS (BURM. F.)*

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ABSTRACT

Objective: To study phytochemicals, determination of total phenol and flavonoid content, antioxidant activity (DPPH Scavenging) and FT-IR spectral analysis in floral parts of *Senna surattensis*. **Methods:** The solvents methanol and aqueous are used for extraction of *Senna surattensis* plant parts. Phytochemical screening of plant parts was carried out in both the solvents. Determination of total phenol content was carried out using Folin - Ciocalteau method and total flavonoid content using Aluminium chloride spectrophotometric method. Antioxidant activity of methanolic extract of plant samples were evaluated with DPPH standard method. The FT-IR is a very useful technique for identifying the functional groups present in the mixture. **Results:** The result revealed the presence of flavonoids, saponins, Tannins, Triterpenoids, Anthraquinone, Reducing Sugar, Phenolic compound, steroids in methanol and aqueous extract but tannins. Alkaloids were absent in aqueous extract. Total phenol content was expressed in mg of Gallic Acid Equivalent (GAE) per g of dry weight. In results it was found that methanol extract shows highest phenol content 475.94± 0.27mg/g in flowers. The content of flavonoids was expressed in mg of Quercetin Equivalent (QE) per g of dry weight. It was evaluated that total flavonoid content found highest in flowers 465.22± 0.18mg/g in methanol extract. IC_{50} for standard ascorbic acid was found to be 51.36µg/ml and for methanol and aqueous extract flower was found to be 68.78µg/ml and 92.73µg/ml respectively. The DPPH radical scavenging activity of *Senna surattensis* was evaluated and compared with ascorbic acid. The presence inhibition of flowers extract was calculated at various concentration (50, 100, 150, 200, 250 etc.) as well as standard ascorbic acid. The highest scavenging activity of methanolic and aqueous extract were 186.4±0.58% and 138.2±0.58% at concentration of 250µg/ml. The FT-IR spectrum *Senna surattensis* showed the presence of alkane (C-H), methylene (C-H), (O-H) stretch, (C-N) stretch, (C-O) group, (N-H) stretch, p-directing benzene ring, alkyl halide (C-Cl) and aromatic amine compounds. FT-IR analysis of methanol flower and aqueous extracts of *Senna surattensis* confirmed the presence of phenols, alcohols, carboxylic acid, amide, aldehydes, ketones, alkanes, alkenes, aromatics, amines and alkyl halides which show major peaks. **Conclusion:** The results obtained from the preliminary standardization of *Senna surattensis* are very helpful in the determination of the quality and purity of the crude drug. The refurbished findings of *Senna surattensis* are promising and further research is important to identify the bioactive compounds, thereby developing nutritional supplements and medications through therapeutic compound isolation.

KEYWORDS

Senna surattensis, Phytochemical Screening, Total phenolic, Total flavonoids, DPPH Scavenging, Antioxidant activity FT-IR spectral, Pharmacological activity, Flavonoids and Steroids.

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INTRODUCTON

Since ancient times, several societies have resorted to nature, mainly to plants as medical and healthy sources. Today, a great percentage of the world population, particularly in developing countries, use plants for facing the primary needs of medical

assistance[1]. Medicinal plants have been used for thousands of years in folk medicines in developing and developed countries. According to the WHO, some nations still reply of plant-based treatments as their main source of medicine and developing nations are utilizing the benefit of naturally sourced compounds for therapeutic purpose[2]. Flowering plants have played a significant role in society, focusing on their aesthetic value rather than their food potential. This study's goal was to look into flowering plants for everything from health benefits to other possible applications. This review presents detailed information on 119 species of flowers with agri-food and health relevance. Data were collected on their family, species, common name, commonly used plant part, bioremediation applications, main chemical compounds, medicinal and gastronomic uses and concentration of bioactive compounds such as carotenoids and phenolic compounds[3].

Medicinal plants are useful in the treatment of many ailments and diseases among rural dwellers, indigenous users, traditional medicine practitioners and livestock owners in many African countries. The traditional knowledge of medicinal plants if harnessed, can give insights into the vital role that medicinal plants play in drug development[4,5]. Often, a single medicinal plant can have multiple uses, and sometimes different parts of the same plant may be used for the treatment of more than one disease condition. Other times, the same plant could be used as an ingredient in herbal preparations for a synergistic effect[1,4,5]. This is made possible due to the range of phytochemicals that are present in medicinal plants along with their diversities of bioactivities. Significantly, more than 50% of the species were only analysed for total concentrations of carotenoids and phenolic compounds, indicating a significant gap in identifying specific molecules of these bioactive compounds[6,7]. These potential sources of bioactive compounds could transform the health and nutraceutical industries, offering innovative approaches to combat oxidative stress and promote optimal well-being[8,9].

Senna surattensis Burm.f./Cassia surattensis Burm.f. syn.C.glauca Lam. (Caesalpinaceae) is commonly known as Glaucos cassia. It is a small tree or large shrub, distributed throughout India. Bark and leaves are useful in diabetes and gonorrhoea[10] aerial parts is used to treat diabetes[11]. The plant is known for its use in diabetes, gonorrhoea and blennorrhoea[12]. The beads made from wood are hanged in neck to cure jaundice[13]. The plant found to contain anthraquinone, flavonol glycosides, chrysophanol, physcion, kaemferide and quercetin[14-16]. As there is no scientific proof and data available about the antioxidant properties of this plant, we explored the antioxidant potency of the extract using various models in vitro. *Senna surattensis* is spread across tropical and subtropical countries, it is used as a food and as an ingredient in herbal mixtures. The roots are used for the treatment of gonorrhoea and snake bites, the leaves are used to treat dysentery and the flowers as a pugative[17,18]. The crude extracts and phytochemical constituents from *Senna surattensis* have shown antimicrobial[19], antioxidant[20], antidiabetic[21], antidiarrheal[22], hepatoprotective[23,24], antihyperlipidemic and antihyperglycemic activities[25].

MATERIAL AND METHODS

Collection of plant material

The flower of plants *Senna surattensis* was authentified by Prof. Rijwan Ulla, Department of Botany, Rajeev Gandhi Govt. Autonomous Post Graduate College Ambikapur, Surguja, Chhattisgarh, India. They were collected from different areas of Mainpat forest districts of Surguja, India. Collected materials was washed in running tap water, rinsed properly in distilled water and then subjected to drying at room temperature for about 5 days in open air. This air-dried material was grind into powdered and stored under refrigeration until their further utilization.

Preparation of flower extracts

Dry powder of plant parts was percolated in a soxhlet apparatus with solvents such as methanol and aqueous. The filtrates were evaporated to get concentrated residue. This residue treated as experimental drug for the present study. The extract was stored at 4^{o}C until assay was completed[26,27].

Qualitative phytochemical analysis

Phytochemical screening of flower extracts was carried out qualitatively for the presence of alkaloids, flavonoids, saponins, tannins, steroids, glycosides, terpenoids, amino acids and protein and using the standard methods[28,30].

Alkaloid screening

10mg of extract was dissolved with 2mL of 5% HCl and filtered. The filtrate was divided into 4 tubes. Drops of Mayer, Dragendorff, and Bouchardat reagents were added to each tube. The fourth filtrate serves as a positive control. The formation of a yellowish-white precipitate (Mayer), red–orange precipitate (Dragendorff) and brown precipitate (Bouchardat) indicates the presence of alkaloids[31].

Flavonoid screening

A few drops of concentrated HCl were added to a small amount of the extracts of the plant material. Immediate development of a red color was taken as an indication of the presence of flavonoids[32].

Tannin screening

0.5g of extract was boiled in 20mL of water in a test tube and then filtered. A few drops of 0.1% $FeCl_3$ was added and observed for brownish green or a blue-black coloration[33].

Phenolic screening

0.2g of thick extract was added with 2mL of 5% $FeCl_3$ solution. A positive result is indicated by the formation of a bluish color[34].

Saponin screening

0.5g of extract was shaken with 10mL of hot water. If foam produced persists for 5 min, it indicates the presence of saponins[35].

Terpenoid screening

Terpenoids screening was determined by Salkowski test 2mL of sample was mixed in 2mL of $CHCl_3$ and 2mL concentrated H_2SO_4 was carefully added to form a layer. A reddish-brown color produced in the lower chloroform layer indicates the presence of terpenoid[36].

Test for glycosides

A small amount of alcoholic extract was taken in 1mL of water in a test tube and a few drops of aqueous NaOH were added. A yellow coloration indicates the presence glycosides.

Test for steroids

A few drops of acetic acid and a drop of conc. H_2SO_4 were added in 1g plant extract. The presence of steroids was indicated by the appearance of green color.

Test for reducing sugar

1 mL of water and 5-8 drops of Fehling's solution were added to 0.5mL of plant extract and was heated. The appearance of brick red precipitation indicated the presence of reducing sugar.

Test for protein and amino acids

Biuret test

To 0.5mg of extract equal volume of 40% NaOH solution and two drops of 1% copper sulphate solution was added. The appearance of violet colour indicates the presence of protein.

Ninhydrin test

About 0.5mg of extract was taken and 2 drops of freshly prepared 0.2% ninhydrin reagent were added and heated. The appearance of pink or purple colour indicates the presence of proteins, peptides or amino acids.

Test for anthraquinone

Borntragers test

About 0.5gm of the bark extract was taken into a dry test tube and 5ml of chloroform was added and shaken for 5 min. The extract was filtered and the filtrate was shaken with an equal volume of 10% ammonia solution. A pink violet or red colour in the lower layer indicates the presence of anthraquinone.

Quantitative phytochemical analysis

Determination of total flavonoids content

Total Flavonoid Content (TFC) was assessed by spectrophotometric method with minor modification[37]. For each sample, 500µL of the samples (1000µg/mL) were mixed with 2.2 mL of aquadest and 150µL of 5% $NaNO_2$. After 5 min, 150µL of 10% $AlCl_3$ was added. Then, 6 min later, 2mL of 1M NaOH was added. The absorbance was read at 510nm. Quercetin was taken as standard for the calibration curve. The total flavonoid content was calibrated using the calibration curve based linear equation. The total flavonoid content was expressed as mg quercetin equivalent/g dry extract.

Determination of total phenolics content

The total phenol content was determined by using the Folin-Ciocalteu method[37] with few modifications. For each sample, 500µL of the samples (1000µg/mL) was added to 3.5mL distilled water and 250µL of 2N FC reagent. The mixture was incubated at room temperature for 8 min and then 250µL of 20% Na_2CO_3 was added to the mixture, and incubated again for 2 h. The absorbance was read at 765nm. Gallic acid was taken as standard for the calibration curve. The total phenol content was expressed as mg gallic acid equivalent/g dry extract.

Determination of antioxidant activity by DPPH

DPPH Free Radical Scavenging Assay

DPPH (1, 1-diphenyl-2- picrylhydrazyl (a, a-diphenyl-bpicrylhydrazyl) radical scavenging analysis was performed according to the reported method with slight modifications. Briefly, 1 mg/ml solutions of compounds and ascorbic acid were prepared by dissolving them into DMSO (Dimethyl sulfoxide). 50, 100, 150, 200 and 250µL of each was added separately to 10.0mL amber color volumetric flasks containing 2.0ml of 0.01mM DPPH (prepared in ethanol). The final volume was made up to 3.0ml and allowed to stand for 30 minutes in the dark and after 30 min absorbance was checked at 517nm by using UV-visible spectrophotometer[38-41]. Pure DPPH solution (0.01mM) was used as a control and ethanol was as a blank. The decrease of in absorbance equates the DPPH radical scavenging capacity. The above process was repeated three times for ascorbic acid and compounds/ samples. The radical scavenging ability was calculated

according to the formula:

Radical scavenging activity = $(A_0 - A_T / A_0) \times 100$

Where, A_0 is the absorbance of pure DPPH solution (0.01mM) and A_T is the absorbance of (DPPH) and compounds / samples.

Fourier Transform Infrared Spectrophotometer (FT-IR)

Fourier Transform Infrared Spectrophotometer (FT-IR) is perhaps the most powerful tool for identifying the types of chemical bonds/functional groups present in the phytochemicals. The wavelength of light absorbed is salient feature of the chemical bond as can be seen in the annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a compound can be determined. Dried powder of the plant extracts of *Senna surattensis* was used for FT-IR analysis. 10mg of the dried extract powder was encapsulated in 100mg of KBr pellet, in order to prepare translucent sample discs. The powdered sample of each extract was loaded in FT-IR spectroscope (Perkin Elmer Spectrum Two FT-IR Version 10.7.2), with a range from 400 to 4000cm^{-1} with a resolution of 4cm^{-1} [142,43].

RESULTS AND DISCUSSION

Preliminary phytochemical screening

The phytochemical screening including qualitative as well as quantitative estimation revealed that the three flowers used were rich in phenols, terpenoids and little flavonoids, with antioxidant, hydroxyl radical scavenging and nitric oxide radical scavenging activity. Thus from these biochemical investigations, it is quite evident that flowers *Senna surattensis* are very rich source of secondary metabolites.

The study of the chemical constituents and the active principles of the medicinal plants have acquired a lot of importance all over the world. The present study includes the phytochemical screening of the plants *Senna surattensis*. The qualitative chemical tests for the ethanolic extracts were performed. The investigation showed that *Senna surattensis* contains, flavonoids, saponins, tannins, Steroidal glycosides, Triterpenoids, Anthraquinone, Reducing Sugar, Phenolic compound, steroids present in methanol and aqueous extract but tannins, alkaloids were absent in aqueous extract.

The results given in Table No.3 show that the total phenol content and total flavonoid of *Senna surattensis* flowers are methanolic 475.94±0.27, 345.20±0.45 and aqueous extract 465.22±0.18, 315.75± 0.20 respectively.

Phytochemical is a natural bioactive compound found in plants, such as vegetables, fruits, medicinal plants, flowers and leaves, to protect against

diseases. Some of the most important bioactive phytochemical constituents such as alkaloids, essential oils, flavonoids, tannins, terpenoids possess antioxidant[44-46], antiulcer[47], protective effects[48], inhibitory effects[49], in addition to hydroxyl radical scavenging activity. Phytochemical analysis of medicinal plants revealed the presence of major phytocompounds like Flavonoids, terpenoids, alkaloids, Steroids glycosides, phenolic and tannins as reported[50].

DPPH scavenging activity

DPPH scavenging activity of *Senna surattensis* flower extracts against DPPH radical were determined and the results are shown in table (3.3, 3.4, 3.5). DPPH scavenging activity has been used by various researchers as a rapid, easy and reliable parameter for screening the in vitro antioxidant activity of plant extracts. DPPH is a stable free radical and accepts an electron to become a stable diamagnetic molecule. The absorption maximum of a stable DPPH radical in methanol was at 517nm. IC_{50} for standard ascorbic acid was found to be 51.36µg/ml and for methanol and aqueous extract flower was found to be 68.78µg/ml and 92.73µg/ml, respectively. In order to study the effects of these compounds on biological system more studies are needed as these compounds might be responsible for use of this plant in different diseases[51]. The DPPH radical scavenging activity of *Senna surattensis* was evaluated and compared with ascorbic acid. The presence inhibition of flowers extract was calculated at various concentration (50, 100, 150, 200, 250 etc.) as well as standard ascorbic acid. The highest scavenging activity of methanolic and aqueous extract were 186.4±0.58% and 138.2±0.58 % at concentration of 250µg/ml.

Phenolic compounds are very important plant constituents because of their scavenging ability due to their hydroxyl groups[52]. The polyphenolic compounds may contribute directly to the antioxidative action[53]. In addition, it was reported to play an important role in stabilizing lipid peroxidation[54]. The extract contain polyphenol is used for the prevention and cure of various diseases which is mainly associated with free radicals[55]. DPPH is a stable free radical in aqueous or ethanol solution and accepts an electron or hydrogen radical becomes a stable diamagnetic molecule. DPPH is usually used as a substrate to evaluate antioxidative activity of antioxidants[56]. In the presence of an electron-donating anti-oxidant, the purple colour typical of the free DPPH radical diminishes in intensity, a change that can be followed spectrophotometrically at 517nm. The radical scavenging activities of the extract measured as decolorizing activity following the trapping of the unpaired electron of DPPH. The inhibitory effect of these extracts may be attributed to the presence of phenolic compound. It is well known that antioxidants can seize the free radical chain of oxidation and form stable free radicals. This would not initiate or propagate further oxidation DPPH has been used extensively as a fee radical to evaluate reducing substance[57,58].

FTIR spectral data interpretation

FT-IR study **Table No.7** shows the functional groups of the organic and inorganic compounds of the plant extract. The FTIR methods was performed on a spectrophotometer system, which was used to detect the characteristics peak values and their functional group. Infra spectrum shows beak area 3269.84cm^{-1} (O–H stretch, free hydroxyl, phenols and 3011.05cm^{-1} is presence of OH groups. The peak area at 2923.40, 2853.33cm^{-1} vibration C-H stretching alkane and C-H stretching aldehyde. The beak area at 2179.92 and 2026.57cm^{-1} shows C≡C stretching alkyne and aromatic compound. A strong stretching vibration at 1743.63cm^{-1} and 1621.02cm-1 shows the presence of carbonyl(C=O) and alkene(C=C) groups. The beak at 1529.51 cm^{-1} N-O stretch (in–ring) Nitro compound and 1462.76cm^{-1} show C–H bend alkanes. The beak area a 1336.78cm^{-1} shows O-H bending phenol. The beak at 1229.40cm^{-1} shows Alkyl amine group. 857.05cm^{-1} beak shows C-H out plane bending. The beak areas 715.02cm^{-1}, shows halogen compounds like C-Cl, compounds. FT-IR analysis of methanol flower and aqueous extracts of *Senna surattensis* confirmed the presence of phenols, alcohols, carboxylic acid, amide, aldehydes, ketones, alkanes, alkenes, aromatics, amines and alkyl halides which show major peaks.

Spectral differences are the objective reflection of componential differences. By using FT-IR spectrum, we can confirm the functional constituent's presence in the given parts and extract, identify the medicinal materials from the adulterate and even evaluate the qualities of medicinal materials. The results of the present study coincided with the previous observations observed by various plant biologist and taxonomist many researchers applied the FT-IR spectrum as a tool for distinguishing closely associated plants.

Table No.1: FT-IR bond and functional group

S.No	Frequency, cm^{-1}	Bond and functional group
1	3853-3985	O–H stretch, free hydroxyl, alcohols, phenols
2	3340-3200	O–H stretch, H–bonded alcohols, phenols
3	2904-3000	C-H stretching Alkane
4	2347-2260	C≡N stretch nitriles
5	1697-1745	C=O stretch, Carbonyl compounds ketones(C=O)
6	1600-1564	C–C stretch (in–ring) aromatics
7	1445-1412	C–H bend alkanes
8	1335-1250	C–N stretch aromatic amines
9	1231-1020	Alkyl amine
10	835-805	C–H -Aromatic compound
11	800-753	C–Cl stretch, alkyl halides

Table No.2: Phytochemical Screening of *Senna Surattensis* flower Methanolic and Aqueous extract

S.No	Phytochemicals	Methanolic Extract	Aqueous Extract
1	Flavonoid	+	+
2	Saponins	+	+
3	Tannins	+	_
4	Steroidal Glycoside	-	+
5	Triterpenoids	+	+
6	Anthraquinones	+	+
7	Reducing Sugar	+	+
8	Alkaloid	+	-
9	Steroids	+	+
10	Phenolic compound	+	+
11	Protein and amino acid	-	+

(+): Presence, (-): Absent

Table No.3: Result of quantification studies *Senna surattensis* flower

S.No	Extract	Test Parameter	Results(mg/g) (±SEM)
1	Methanol	Total phenolic Total Flavonoids	475.94± 0.27 465.22± 0.18
2	Aqueous	Total phenolic Total Flavonoids	345.20± 0.45 315.75± 0.20

Table No.4: Free radical scavenging capacity of ascorbic acid

S.No	Concentration (µg/ml)	DPPH Scavenging %
		Methanol Extract
1	50	51.2±0.36
2	100	86.7±0.27
3	150	129.8±0.98
4	200	167.1±0.24
5	250	212.3±0.76
6	IC_{50}	51.36

Table No.5: Free radical scavenging capacity of Methanol extract of *Senna Surattensis*

S.No	Concentration (µg/ml)	DPPH Scavenging %
		Methanol Extract
1	50	40.1±0.58
2	100	69.5±0.58
3	150	108.4±0.58
4	200	142.7±0.58
5	250	186.4±0.58
6	IC_{50}	68.78

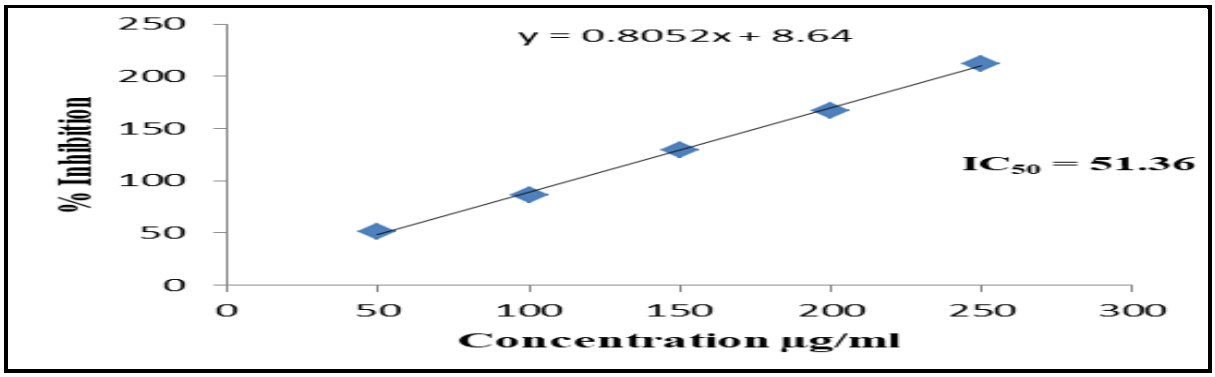
Values are mean ± SEM of three determinations

Table No.6: Free radical scavenging capacity of aqueous extract of *Senna Surattensis*

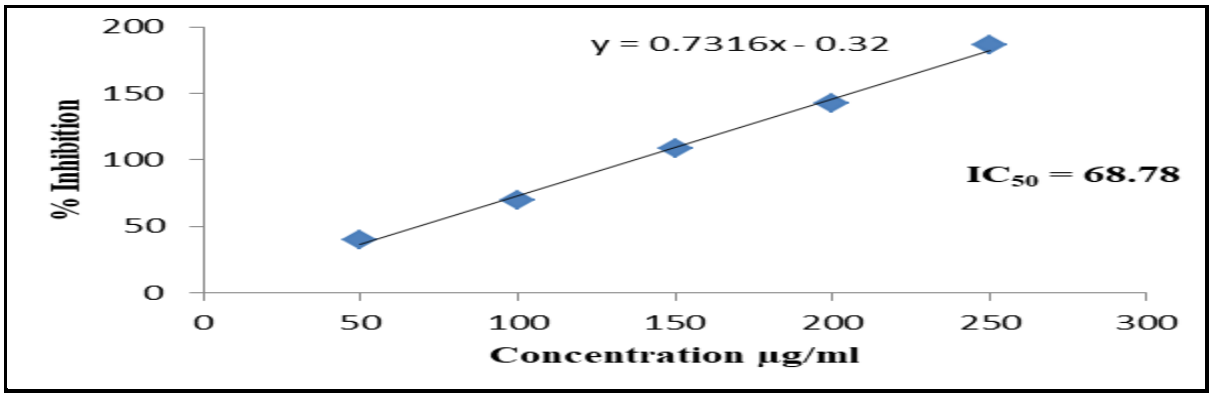
S.No	Concentration (µg/ml)	DPPH Scavenging %
		Aqueous Extract
1	50	31.7±0.47
2	100	52.1±0.36
3	150	76.8±0.92
4	200	101.4±0.37
5	250	138.2±0.58
6	IC_{50}	92.73

Table No.7: FT-IR spectrum of Methanolic and Aqueous extract of *Senna surattensis*

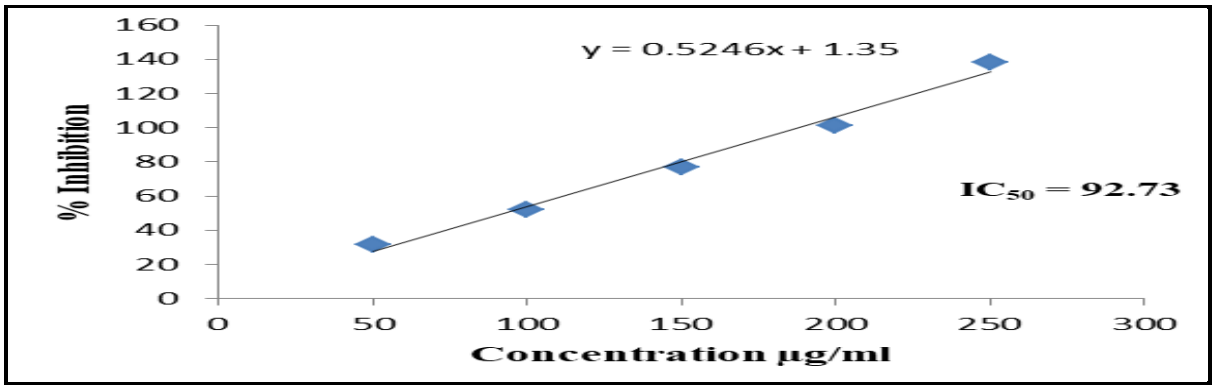
S.No	Extract	Peak Value (cm^{-1})	Group	Class	Peak Detail
1	Methanolic Extract	665-730 857.05 1068.13 1163.75 1229.40 1336.78 1416.59 1462.76 1529.51 1621.02 1743.63 1870.25 2026.57 2179.92 2350.36 2853.33 2923.40 3011.05 3269.84	C=C bending C=C bending C-O stretching C-O stretching C-N stretching O-H Bending O-H Bending C-H Bending N-O Stretching C=C Stretching C=C Stretching C=O Stretching C-H Bending C≡C stretching O=C=O stretching C-H Stretching C-H Stretching O-H Stretching O-H Stretching	Alkene alkene Primary Alcohol Ether Amine Phenol Aldehyde Alkane Nitro Compound Alkene Carboxylic acid Conjugated Anhyd. Aromatic Compound Alkyne Carbon dioxide Aldehyde Alkane Alcohol Alcohol	Strong Medium Strong Strong Medium Medium Medium Strong Medium Weak Strong Strong Weak Weak Strong Medium Medium Weak, Broad Strong, Broad
2	Aqueous Extract	665.73 893.37 1067.73 1098.17 1232.16 1262.75 1392.03 1591.18 1986.97 2126.72 2929.68 3224.85	C=C bending C=C bending C-O Stretching C-O Stretching C-O Stretching C-N Stretching C-H Bending N-H Bending C=C=C Bending C≡C stretching C-H Stretching O-H Stretching	Alkene Alkene Primary alcohol Ether Aryl ether Aromatic Amine Aldehyde Amine Allele Alkyne Alkene Alcohol	Strong Strong Strong Strong Strong Strong Medium Medium Medium Weak Medium Strong, Broad



Values are mean ± SEM of three determinations



Values are mean ± SEM of three determinations



Values are mean ± SEM of three determinations

Figure No.1: Flowering plant of *Senna surattensis*

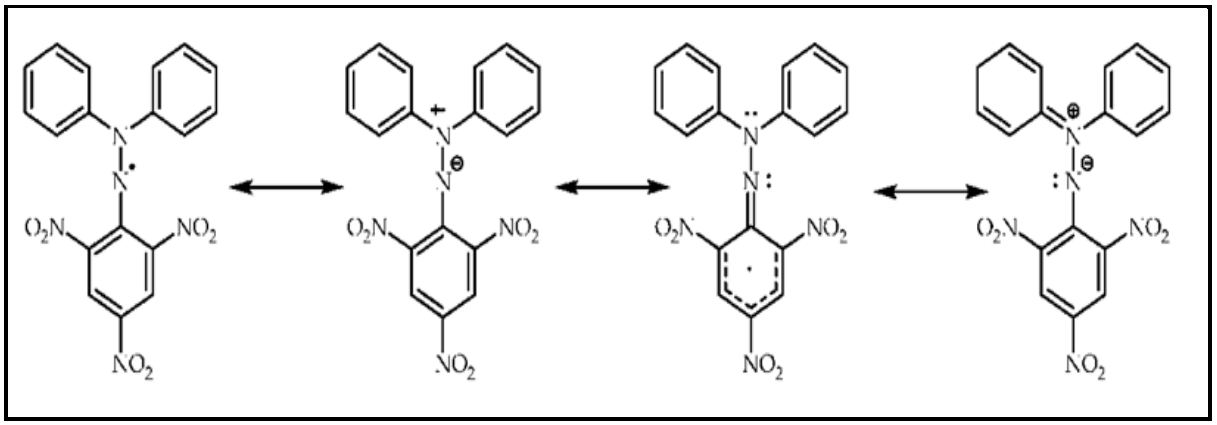


Figure No.2: The chemical structures of a 1, 1-diphenyl-2-picrylhydrazil radical (DPPH)

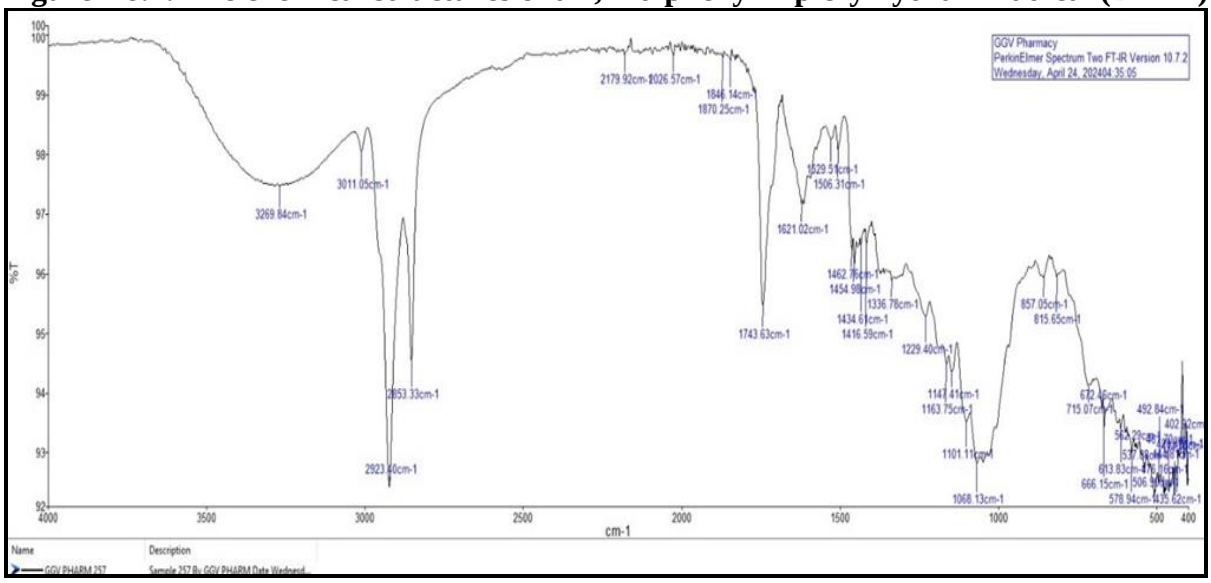


Figure No.3: Shows the FT-IR frequency range of methanolic Senna surattensis flower

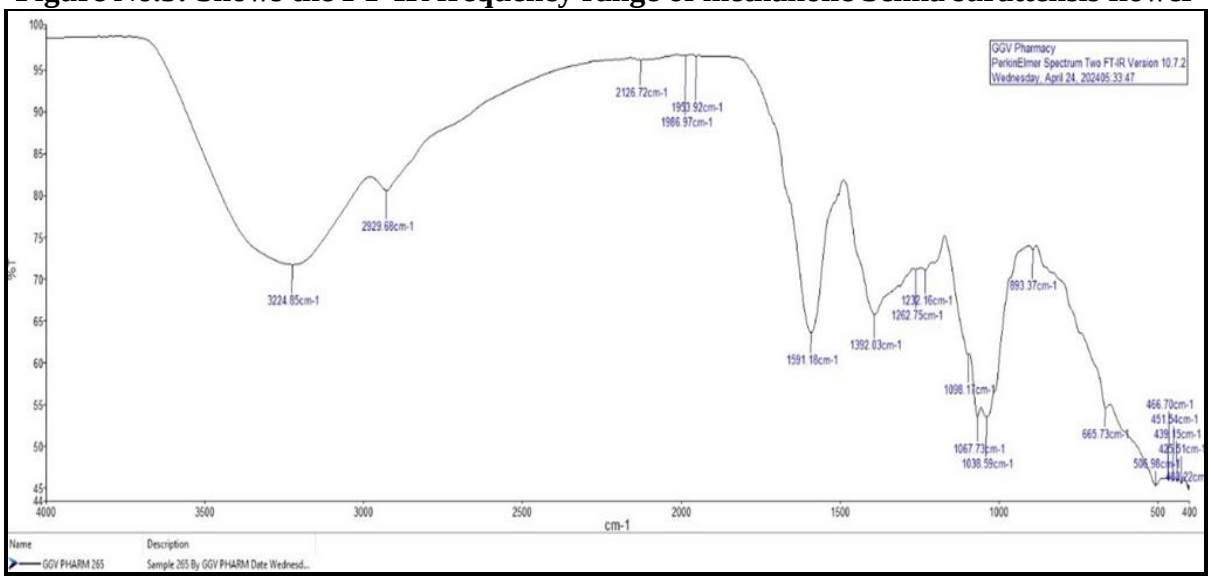


Figure No.4: Shows the FT-IR frequency range of aqueous *Senna surattensis* flower

CONCLUSION

The present study indicates that *Senna surattensis* flowers is a rich source of secondary metabolites. The presence of active constituents shows moderate to significant anti-oxidant activity. Our study also revealed the importance of screening already identified and useful medicinal plants, as an aid in the discovery of new bioactivities which may be useful in the future development of new alternative therapeutic drugs.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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