International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304, ISSN(Online): 2455-9563
Vol.13, No.03, pp 295-304, 2020

Phytochemical Characterization of Natural Dye Extracted from *Senna siamea* Pods

S.H.Patil[1]*, D.D. Kurlapkar[2] and D.K. Gaikwad[1]

[1]Department of Botany, Shivaji University Kolhapur, Maharashtra – 416004, India
[2]S.B. Khade Mahavidyalaya, Koparde, Kolhapur, Maharashtra – 416005, India

Abstract : An increasing eco-consciousness among peoples has been shifting the use of natural dyes for textile dyeing and in other realms too as food, pharmaceuticals and cosmetics. The present study was focussed on aqueous extraction of natural dye from the pod husk of *Senna siamea* (Lam.) H.S. Irwin & Barneby, its characterization through spectroscopic (UV-VIS and FT-IR) and chromatographic (GC-MS/ LC-MS) technique. It was noticed that the percent recovery was 16%, while FTIR results indicates different functional groups present in the dye, total 16 constituents were identified in the GC-MS analysis of *Senna* dye such as D-Fructose,3-O-methyl-, Stigmast-5-en-3-ol,oleate, Benzaldehyde,2-hydroxy-4-methyl-, 3'-Methoxybenzo[1',2'-b]-1,4-, Tetrapentacontane, n-Hexadecanoic acid, 2,3-Dihydroxypropyl elaidate 3-Hydroxy-4-methoxybenzoic acid, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6, 4-Hydroxy-2-methylacetophenone, Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl), Maltol, Methyl 14-methyl-eicosanoate, Bis(2-ethylhexyl) phthalate, 9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydro, Benzeneacetaldehyde, and the LCMS analysis exhibits the presence of 20 major bioactive compounds among these N-Cyclohexane carbonyl pentadecylamine, Docosanedioic acid, Emmotin A, 3α,12α-Dihydroxy-5β-chol-7-en-24-oic Acid, 4-Hydroxyphenylglyoxylate, Hexadecyl Acetyl Glycerol, 2-oxo-nonadecanoic acid, 1-Monopalmitin, Spisulosine and N,N-dimethyl-Safingol showed highest retention time. Thus the Senna dye is a rich source of natural bioactive compounds.
Keywords : Natural dye, UV-VIS, FTIR, GC-MS, LCMS.

Introduction

Since the dawn of civilization, the art of making natural dye is one of the oldest methods known to humankind[1]. The discovery of synthetic dye was almost replaced the natural dyes. This could have happened because of its own drawbacks like divergence of colour, less availability and lack of fastness properties[2]. Due to toxic and allergic reactions associated with synthetic dyes, ban has been imposed on their uses by Germany and other European countries. Hence there is renaissance in the use of natural dyes in the textile dyeing, for food, pharmaceutical and in cosmetics[3].

DOI= http://dx.doi.org/10.20902/IJPTR.2019.130322

Natural dyes obtained from flora and fauna are non-toxic, non-carcinogenic and biodegradable in nature and almost non-pollutant so do not pose serious hazards to environment[4]. The colour range is correlated to chemical structure and chromogen–chromophore correlated with auxo chrome. In textile coloration chromophore and auxo chrome are considered as most important chemical constituents of dyes[5, 6]. The colour of dyed fabrics depends on the nature of dye constituents[7]. The constituent molecules contain aromatic ring structure coupled with azide chain are usually required for resonance and thus to impart colour. Due to lack of knowledge on the extraction and dyeing of natural dye, it has not been employed commercially like synthetic dyes[8]. But now a day's considerable research work being started throughout the world on the application of natural dyes[9]. Added to this natural dyes are gaining popularity as UV protective and antimicrobial clothing dyes[10].

Now a days there has been increasing interest in production of antibacterial textiles because clothing and other textile materials provide essential requirements in the form of moisture and nutrients to pathogenic and odour generating microbes. Natural dyes possess antimicrobial properties because of presence of considerable amount of antimicrobial compounds such as anthraquinones, flavonoids, tannins, naphthoquinones etc[11]. Natural dye extracted from *Punica granatum* reported as antibacterial agent as it possesses considerable amount of tannins. Some other sources of plant dyes such as lawsone from henna, juglone from walnut and lapachol from alkanet are rich in naphthoquinones also exhibit antibacterial properties[12, 13, 14]. There is need to reinvestigate the renewable resources as the alternative raw material as source of natural dyes[15, 16].

Senna siamea (Lam.) H.S.Irwin & Barneby Formerly, *Cassia siamea* belongs to family Fabaceae. It is commonly known as kassod tree, cassod tree and cassia tree. In traditional medicine, the fruit is used to prevent convulsion in children. The young fruits and leaves are also eaten as vegetables in Thailand[17]. The plant also possesses antimalarial, antidiabetic[18], antitumor or anticancer[19, 20], laxative[21] anti-inflammatory, analgesic, antipyretic, anxiolytic, antidepressant, and sedative[22] properties. The plants are rich in array of secondary metabolites such as polyphenols, flavonoids, isoflavonoids, phenolic acids, triterpenoids, chromones, anthraquinones, bianthra-quinones, sennosides, steroids, and carotenoids etc[23] which have been found to possess antimicrobial properties in vitro. In the present investigation an attempt has been made to extract the natural water soluble dye from pods of *S. siamea*, to characterize the prepared dye for its chemical constituents and to assess its antibacterial potential.

Figure 1: Picture off *Senna siamea* Pods

Material and Methods

1. Preparation of Raw Material

Pods of *S. siamea* were collected from campus of Shivaji University, Kolhapur. The plant material was identified using Flora of Maharashtra state [24] and authenticated by referring to type specimen. A voucher specimen (SHP-001) has been deposited at the Department of Botany, SUK herbarium. The material was washed thoroughly to remove adhering dirt and dried at room temperature. Subsequently dried material was grind into powder. The powder was passed through a sieve of 25µm mesh size to obtain uniform particle size of material. The sieved powder was used for dye extraction at different experimental conditions.

2. Extraction of Dye in Powder Form

The dye from the pod husk of *S. siamea* was extracted by heating the husk powder and distilled water (M: L-10:100) at 80^0c-90^0c for 2 hr. The dye solution was filtered and transferred in evaporating dish and dried in oven at 60^0c then it is cooled and weighed to determine the weight of extract from which the yield (16%) was calculated. The percentage of the dye powder was calculated from equation[25]

$$\% \text{ dye powder} = \frac{\text{g of purified dye}}{\text{g of plant material}} \times 100$$

3. Characterization of Dye

3.1 UV- VIS spectrum analysis

To detect the UV-VIS spectrum profile of *Senna* dye, aqueous extract was scanned in the wavelength ranging from 200-800 nm using UV-VIS spectrophotometer and the characteristic peak were detected.

3.2 FT-IR Spectral Analysis

The most characteristics auxo chromic functional group present in *S.siamea* dye were recorded using FT-IR spectrophotometer (BRUKER ALPHA 100508). The spectrum was located in the range from 500–4000 cm^{-1} with a resolution of 4cm^{-1} and each spectrum composed of 32 scans. Peaks in the spectrum were analysed in accordance with the literature.

3.3 GC-MS Analysis

GC-MS analysis of the sample was performed on a SHIMADZU GC-2010.GCMS QP-2010. Column used was Restec Rtx-5MS measuring 60 mm × 0.25 mm ID thickness of 0.25µm composed of 95% dimethyl polysiloxane. Helium gas was used as carrier gas at a flow rate of 1ml/min and injection volume of 1µl was utilized. During the process the oven temperature was programmed initially at 60^0c for 5 minutes then an increased to 240^0c for 5 minutes then programmed to increased up to 280^0C for 2 minutes at a rate of 10^0C per minutes ending with a 5 minutes. Interpretation of mass spectrum of GCMS was done using database of National Institute standard and Technology (NIST) library. Measurement of peak area and data processing were carried out by Real time analysis software ver.2.6.

3.4 LC-MS analysis

Aqueous extract of *Senna* dye was subjected to LCMS analysis. An Agilent 6540 UHD QTOF LCMS instrument was used to perform untargeted sample analysis. Zorbax SB-Aq, 3.0×100 mm; 3.5 µM column (Agilent 1,290 Infinity Binary pump, well plate auto sampler, thermostatted column compartment) with a flow rate of 0.6 ml/min and 20 µl injection volume was used. MS with both positive and negative modes was used (Agilent 6540 UHD QTOF LC/MS/MS). The operating parameters for the LCMS detection were as follows- nebulizing gas flow 30 psi, drying gas pressure 12 $Lmin^{-1}$ and gas temp. 325°c, Skimmer voltage 65v, octapole

RF 750v, capillary voltage 3.5kv and fragmented voltage 150 v. Data were collected using Mass Hunter Molecular Feature Extractor (MFE) tool.

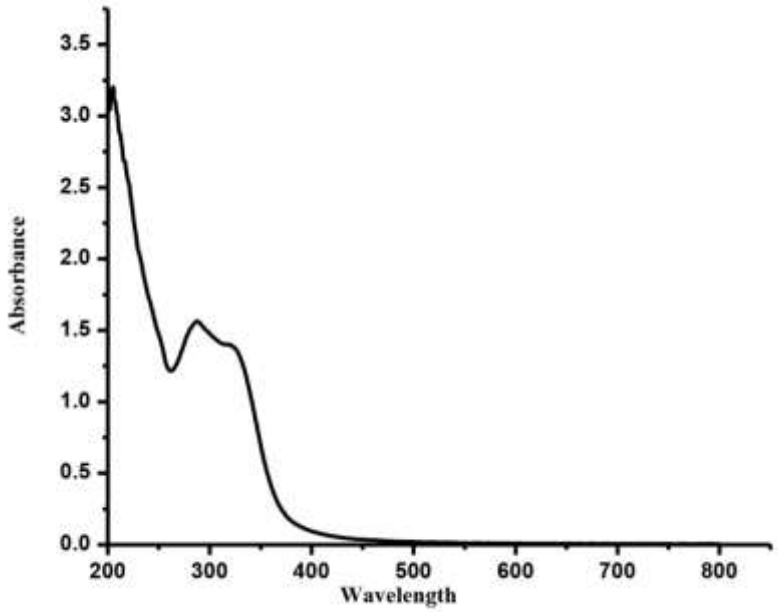

Figure 2: UV-VIS spectrum of *Senna* dye.

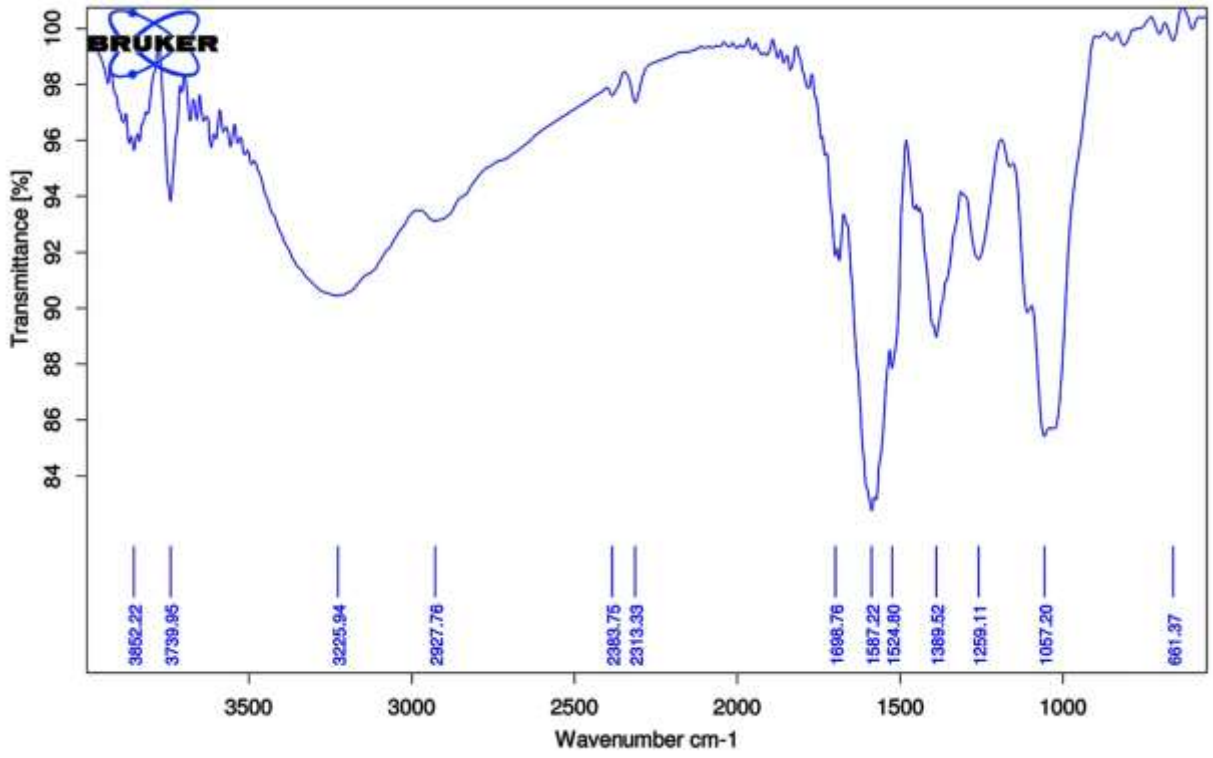

Figure 3: FTIR spectrum of *Senna* dye.

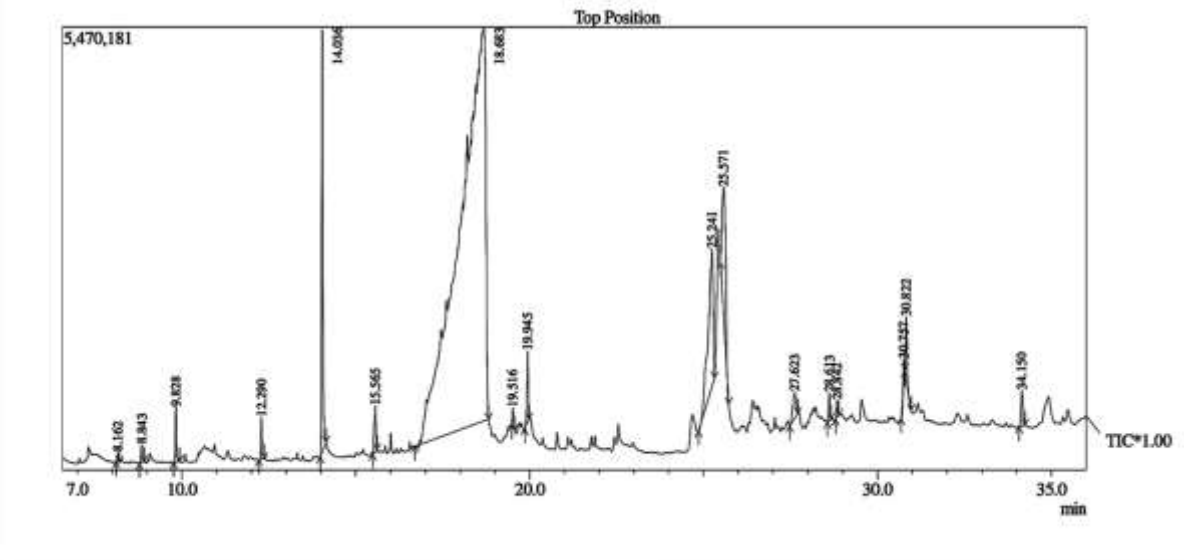

Figure 4: Chromatogram of *Senna* dye by GC-MS.

Table 1: Functional group analysis of *Senna* dye using FT-IR spectroscopy

Sr. No.	Wavenumbers cm^{-1}	Assignments
1	3852.22	Alcohol(OH)
2	3739.95	
3	3225.94	
4	2927.76	Alkane(C-H)
5	2383.75	Carboxylic acid(O-H)
6	2313.33	
7	1698.76	Carbonyl(C=O)
8	1587.22	Aromatic(C=C)
9	1524.80	Aromatic(C=C)
10	1389.52	Alkane (C-H)
11	1259.11	Akyl aryl ether(C-O)
12	1057.20	Fluroalkane(C-X)
13	661.37	Chloroalkane(C-X)

Table 2: Bioactive compounds detected in *Senna* dye extract by GC-MS

Peak No.	RT	Name of Compound	Molecular Formula	Molecular weight	Area%
1.	18.683	D-Fructose, 3-O-methyl-	C7H14O6	194	81.96
2.	25.241	Stigmast-5-en-3-ol, oleate	C47H82O2	678	5.41
3.	14.036	Benzaldehyde, 2-hydroxy-4-methyl-	C8H8O2	136	3.92
4.	25.571	3'-Methoxybenzo[1',2'-b]-1,4-	C11H14N2O	190	3.64
5.	27.623	Tetrapentacontane	C54H110	758	0.70
6.	19.945	n-Hexadecanoic acid	C16H32O2	256	0.69
7.	30.822	2,3-Dihydroxypropyl elaidate	C21H40O4	356	0.67
8.	15.565	3-Hydroxy-4-methoxybenzoic acid	C8H8O4	168	0.63
9.	9.828	4H-Pyran-4-one, 2,3-dihydro-3,5-	C6H8O4	144	0.51
10.	12.290	4-Hydroxy-2-methylacetophenone	C9H10O2	150	0.37
11.	28.613	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethy)	C19H38O4	330	0.27
12.	8.843	Maltol	C6H6O3	126	0.18
13.	19.516	Methyl 14-methyl-eicosanoate	C22H44O2	340	0.16
14.	28.842	Bis(2-ethylhexyl) phthalate	C24H38O4	390	0.15
15.	30.757	9,12-Octadecadienoic acid (Z,Z)-, 2,3-	C21H38O4	354	0.14
16.	8.162	Benzeneacetaldehyde	C8H8O	120	0.08

Table 3: Bioactive compounds detected in *Senna* dye extract by LC-MS

Sr. No.	Name of Compound	Molecular formula	Score	Mass	RT
1.	N-Cyclohexanecarbonylpentadecylamine	C22 H43 N O	98.47	337.3343	22.0719
2.	Docosanedioic acid	C22 H42 O4	96.87	370.3073	21.9896
3.	Emmotin A	C16 H22 O4	99.24	278.1522	21.9358
4.	3α,12α-Dihydroxy-5β-chol-7-en-24-oic Acid	C24 H38 O4	99.02	390.2769	21.935
5.	4-Hydroxyphenylglyoxylate	C8 H6 O4	98.72	166.0271	21.9336
6.	Hexadecyl Acetyl Glycerol	C21 H42 O4	99.11	358.3082	20.7433
7.	2-oxo-nonadecanoic acid	C19 H36 O3	95.76	312.2664	18.9349
8.	1-Monopalmitin	C19 H38 O4	98.28	330.2768	18.9327
9.	Spisulosine	C18 H39 N O	96.49	285.3029	12.9222
10.	N,N-dimethyl-Safingol	C20 H43 N O2	98.63	329.3292	12.832
11.	DL-3-Phenyllactic acid	C9 H10 O3	96.35	166.0634	11.7741
12.	Estra-1,3,5(10)-triene-3,6alpha,17beta-triol triacetate	C24 H30 O6	99.4	414.2041	11.6812
13.	C16 Sphinganine	C16 H35 N O2	99.77	273.2666	8.8219
14.	Carnosol	C20 H26 O4	99.47	330.1831	8.3337
15.	5,7,2',3'-Tetrahydroxyflavone	C15 H10 O6	99.03	286.0479	7.8794
16.	PGE3 1,15-lactone	C20 H28 O4	97.69	332.1991	7.8
17.	5,7-nonadienal	C9 H14 O	99.22	138.1045	7.792
18.	Longicaudatin	C26 H22 O7	99.4	446.1365	7.5569
19.	Nodifloretin	C16 H12 O7	99.4	316.0586	7.455
20.	Emodic Acid	C15 H8 O7	96.72	300.0276	7.1173

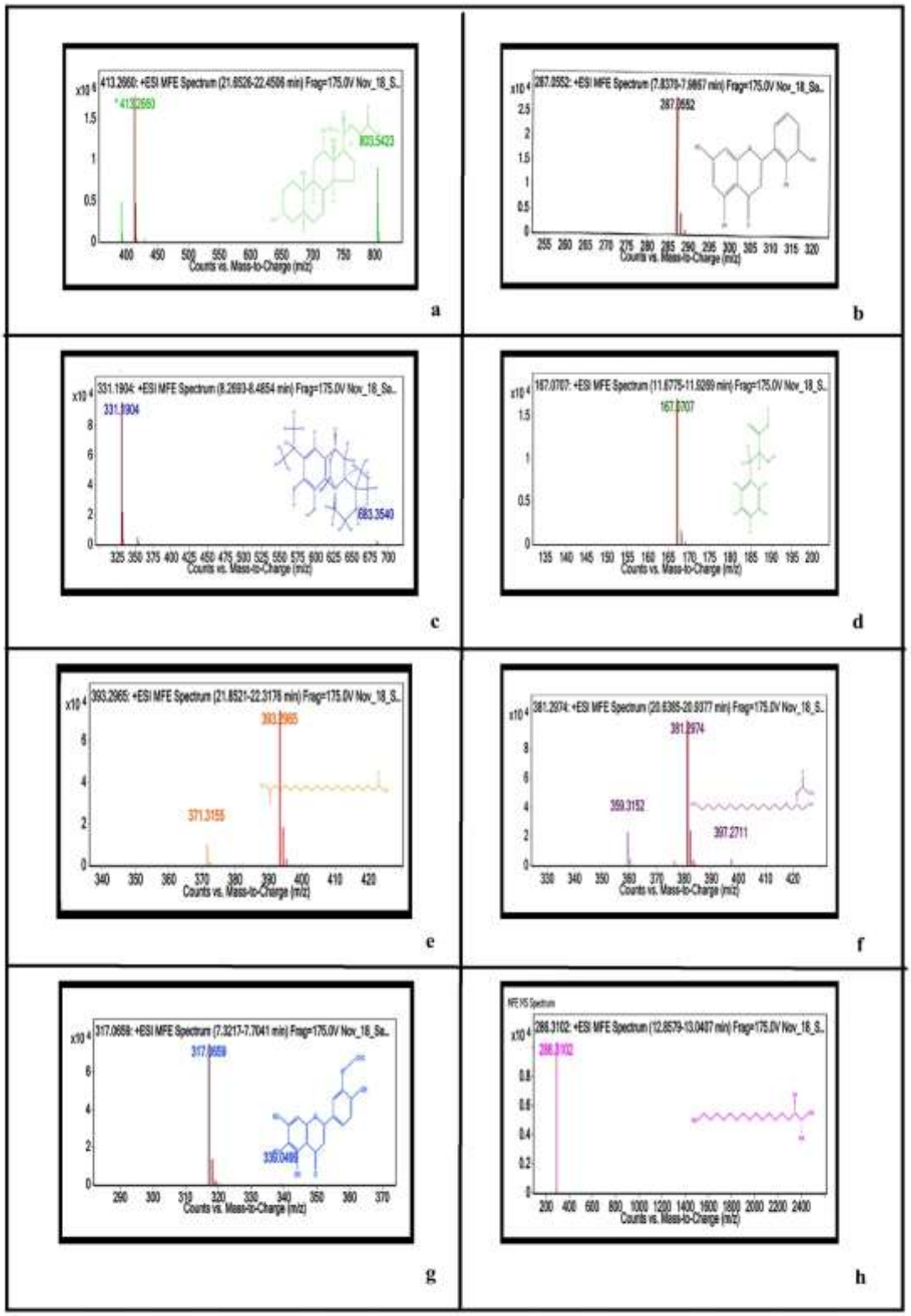

Figure 5: MS spectrum of LCMS compounds: a. Docosanedioic acid, b. 3α,12α-Dihydroxy-5β-chol-7-en-24-oic Acid, c. Hexadecyl Acetyl Glycerol, d.Spisulosine, e. DL-3-Phenyllactic acid, f. Carnosol, g. 5,7,2',3'-Tetrahydroxyflavone, h. Nodifloretin.

Results and Discussion

UV-VIS spectral analysis of natural dye was performed for qualitative analysis and for identification of certain classes of compound, main hue, and property of dye. The UV-VIS spectrum of *Senna* dye extract showed one peak at 287nm with the absorption of 1.563. Figure1 shows absorption spectrum of *Senna* dye powder.

The comparative data on the peak value with wave numbers and the possible functional groups during FTIR analysis of *Senna* dye powder are presented in table 1. FTIR spectrum (Figure 2) exhibit characteristic functional groups are present in *Senna* dye powder. Peak at 3852.22 cm-1, 3739.95 cm-1and 3225.94 cm-1 was indexed to the -OH group, at 2927.76 cm-1 assigned to C-H stretching in -CH_2 group[26]. Presence of C-H bond which usually considered as prominent band for flavonoid pigment[27]. Peak at 2383.75 cm-1 and 2313.33 cm-1 indicates O-H stretching vibration of carboxylic acid[28]. At 1587.22 cm-1 and 1524.80 cm-1 refers to the infrared absorption of C=C while 1259.11 cm-1 indicating C-O[29,30], Peak at 1389.52 cm-1 denotes the ether group, Other peaks at 1057.20 cm-1and 661.37 cm-1 indicates floroalkane and chloroalkane. The dye had its respective functional group like alcohol, alkane, carboxylic acid, carbonyl, ether, aromatic etc. Hence, the crude extract subjected to UV-VIS and FTIR analysis is used for the identification of chemical constituents present in dye. In addition, UV-VIS and FTIR spectroscopy is proved to be a reliable and sensitive method for detection of bio molecular composition.

The compounds present in the crude dye powder of *S.siamea* was identified by GC-MS and LCMS analysis. The GC-MS revealed the presence of 16 components are presented in table2.and Figure3 shows GCMS chromatogram spectrum of *Senna* dye powder. The identification of phytochemicals was based on the peak area, retention time and compound name. The active compounds along with their respective retention time, area percentage, molecular formula, molecular weight are presented in table 2. The principal constituents were identified in dye extract are D-Fructose, 3-O-methyl-, Stigmast-5-en-3-ol, oleate, Benzaldehyde, 2-hydroxy-4-methyl-, 3'-Methoxybenzo [1', 2'-b]-1, 4-, with percent area of 81.96, 5.41, 3.92 and 3.64 respectively.

The LCMS analysis exhibit 20 different major compounds listed in table 3., among these Docosanedioic acid used in antiseptics, top-grade coatings, painting materials, corrosion inhibitors, surfactants, and engineering plastics such as nylon 612[31], 3α, 12α-Dihydroxy-5β-chol-7-en-24-oic acid displays cholesterol elevating activity[32]. Hexadecyl Acetyl Glycerol inhibits the growth of HL-60 cells and induces differentiation to cells resembling mononuclear phagocytes[33]. Spisulosine have antiproliferative activity toward advanced malignant solid tumors[34, 35]. Whereas DL-3-Phenyllactic acid is used as natural antibiotic agent. Carnosol is known for its high antioxidative capacities, and have many industrial applications in the fields of foods and beverages, nutrition, and health[36].The compound like 5, 7, 2', 3'-Tetrahydroxyflavone and Nodifloretin displays the antiviral, insecticidal activities[37, 38]. The presence of these compounds (Figure4) in *Senna* dye shows a potential source of these bioactive compounds which might be utilized in the development of various pharmaceutical formulations as well as basis of anticancerous and antiviral drugs. Further study of these phytoconstituents may prove the medicinal importance.

Conclusion

The results of GC- MS and LCMS analysis highlighted that the *Senna* dye is good source of phytochemicals which have a variety of medicinal properties that can be useful for the cure of various diseases. It is clear from the study high bioactive compounds present in dye and also exhibit effective biomedical properties which might be helpful in the development of various drugs as well as pharmaceuticals and bio cosmetic ingredients. The data generated through this work may be used as a basis for studying the economic viability of producing the dyes on commercial scale.

Acknowledgement

The authors are grateful to the Department of Botany, Shivaji University,Kolhapur, India for providing necessary facilities to carry out the present research work.

References

1. Kannanmarikani, Kannan U. S. and Kanniappan R., Assessment of dyeing properties and quality parameters of natural dye extracted from *Lawsonia inermis*, Euro J Exp Bio, 2010, 7, 62-70.
2. Singh S. V. and Purohit M. C., Applications of eco-friendly natural dye on wool fibres using combination of natural and chemical mordants. Univers, J. environ. Res. technol, 2012, 2, 48-55.
3. Kumaresan M., Palanisamy P. N. and Kumar P. E., Application of eco-friendly natural dye on cotton using combination of mordants, Indian J Fibre Text, 2012, 37, 194-198.
4. El-Amoudy E. S. and El-Ebissy A. A., Characterization of a new natural dye applied on wool fabrics, MSAIJ, 2014, 12, 491-504.
5. Trotman E. R., Dyeing and Chemical Technology of Textile fibres. 6th Edition, 1993, 254-286.
6. Foulds J., Dyeing and Printing. A Handbook on Small Scale Textiles, Intermediate Technology Publications 103/105 Southampton Row, London, 1995, 1-27.
7. Padma S. V., Chemistry of Natural dyes. General Article. Facility for Ecological and Analytical Testing (FEAT) at the Indian Institute of Technology, Kanpur, India, Resonance, 2000, 73-80.
8. Siva R., Status of natural dyes and Dye-Yielding plants in India, Curr. Sci, 2007, 7, 916-925.
9. Acguah S. B. and Oduru K. A., Traditional cloth dyeing enterprise at Ntonso: challenges and opportunities, West Afr. J. Appl. Ecol, 2012, 1, 25-36.
10. Ali N. F., El-Khatib E. M. and El-Mohamedy R. S. S., Antimicrobial activity of wool fabric dyed with natural dyes, Res. J. Textile. Apparel, 2011, 15, 1-10.
11. Baliarsingh S., Panda A. K., Jena J., Das T. and Das N. B., Exploring sustainable technique on natural dye extraction from native plants for textile: Identification of colourants, colourimetric analysis of dyed yarns and their antimicrobial evaluation, J. Clean. Prod, 2012, 37, 257-264.
12. Gerson H., Fungi toxicity of 1, 4-napthoquinones to *Candida albicans* and *Trichophyton mentagrophytes*, Can J Microbiol, 1975, 21, 197-205.
13. Schuerch A. R. and Wehrli W., b-Lapachone an inhibitor of oncorna-virus reverse transcriptase and eukaryotic DNA polymerased inhibitory effect, thiol dependence and specificity, Eur J Biochem, 1978, 84, 197-205.
14. Wagner H., Kreher B. Lotter H., Hamburger M. O. and Cordell G. A., Structure determination of new isomeric naphthol [2, 3,-b] furan-4, 9-diones from *Tabebuia avellanedae* by the selective INEPT technique, Helv Chim Acta, 1989, 72, 659-67.
15. Gupta D., Khareb S. K. and Lahaa A., Antimicrobial properties of natural dyes against Gram-negative bacteria, Color. Technol, 2004, 120, 167-171.
16. Sarkar R. K., Purushottam D. and Chauhan P. D., Bacteria- resist finish on cotton fabrics using natural herbal extracts, Indian J Fibre Text, 2003, 28, 322-331.
17. Kiepe P.L., Effect of cassia sianea hedgerow barriers on soil physical properties, Geoderma. J. integrative Med, 2001, 68, 113-720.
18. Odason E.E. and Kolawole J., Anti-diabetic properties and brine shrimp toxicity of the aqueous extract of the root of *Cassia siamea* Lam. (Ceasalpiniaceae). Nigerian Journal of Pharmaceutical Research, 2007, 6, 66-69.
19. Kupradinun P., Tepsuwan A., Tantasi N., Meesiripun N., Rungsipipat A. and Kusamran W.R., Anticlastogenic and anticarcinogenic potential of Thai bitter: Gourd fruits. Asian Pacific Journal of Cancer Prevention, 2011, 5, 1299-1305.
20. Nsonde Ntandou G.F., Banzouzi J.T., Mbatchi B., Elion-Itoua R.G.D., EtouOssibi A.W., Ramos S., Benoit-Vicale B., Abena A.A., Ouamba J.M., Analgesic and antiinflammatory effects of *Cassia siamea* Lam. stem bark extracts. Journal of Ethnopharmacology, 2010, 127,108-111.
21. Morales M.A., Hernández D., Bustamante S., Bachiller I. and Rojas A., Is Senna laxative use associated to cathartic colon, genotoxicity, or carcinogenicity? Journal of Toxicology, 2009, 1–8.
22. Wongwitdecha N., Neuro-psychopharma-cological studies the effects of barakol, a traditional Thai medicine, Srinagarind Medical Journal, 2007, 22, 325-332.
23. Eddouks M., Bidi A., Bouhali B. el., Hajji L., and Zeggwagh N.A., "Antidiabetic plants improving insulin sensitiv-ity", The Journal of Pharmacy and Pharmacology, 2014, 9, 1197–1214.
24. Singh N.P., Lakshminarasimhan P., Karthikeyan S. and Prasanna P.V., Flora of Maharashtra State, Botanical Survey of India, Calcutta, 2001.
25. Muangthai P., Promrong N. and Wannawong C., Preparation of Purified Dye Powder from the Bark of *Livistona speciosa*, Sci. J. UBU, 2010, 2, 65-70.

26. Patel B. H., Desai K. U. and Jha P. K., *Azadirachta indica* Mediated Bioactive Lyocell Yarn: Chemical and Colour Characterization, Advances in Chemistry, 2014, 1-8.
27. Aksoz B. E. and Ertan R., Spectral properties of chalcone II, FABAD, J. Pharm. Sci, 2012, 4, 205-216.
28. Saravanakumar K., Adaikala R. and Umaiyambigai D., GC-MS and FT-IR Profiling of leaves methanol extract from the *Pleiospermium alatum* (Wall. ex Wt. & Arn) Swingle Rutaceae family, J Phytopharmacol, 2016, 5, 201-204.
29. Ramprasath R., Kavi G. G. and Rathi T. S., Isolation of Natural Dyes from *Hibiscus Rosa Sinensis* and Marigold Flower and Dyeing Properties of the Dyes on Cotton Cloth. IOSR J. Appl. Chem, 2017, 5, 74-79.
30. Ludin N. A., Mahmoud A. M., Al-Alwani, Mohamad A. B., Abd. Kadhum A. H., Norul Hamid N.H., Ibrahim M.A., Teridi M.A.M., Tarik M., Hakeem A., Mukhlus A. and Sopian, K., Utilization of Natural Dyes from *Zingiber officinale* Leaves and *Clitoria ternatea* Flowers to Prepare New Photosensitizers for Dye-Sensitized Solar Cells. Int. J. Electrochem, 2018, 13, 7451 – 7465.
31. https://en.wikipedia.org/wiki/Dodecanedioic_acid.
32. https://www.trc-canada.com/product-detail/?A727553 while.
33. http://www.bertin-20bioreagent.com/pr11529/hexadecyl-acetyl-glycerol.
34. Cuadros R., Montejo De., Garcini E., Wandosell F., Faircloth G., Fernández, Sousa J. M. and Avila J., The marine compound Spisulosine, and inhibitor of cell proliferation promotes the disassembly of actin stress fibers, Cancer Lett., 2000, 152, 23–29.
35. Faircloth G., Cuevas C.D. and Marchante M., *Molluscs: From Chemo-Ecological Study to Biotechnological Application.* Vol. 43. Berlin, Germany: Springer; Kahalalide F and ES285: potent anticancer agents from marine molluscs, 2000, 363–379.
36. Birtic S., Dussort P., Pierre F.X., Bily A.C. and Roller M. (2015) Carnosic acid. Phytochemistry, 2015, 115: 9–19.
37. Singh S., Jatav V.K. and Sharma S., Virtual screening & molecular docking of glycoprotein b inhibitor as a treatment of herpes simplex virus, International Research Journal of Humanities, Engineering & Pharmaceutical Sciences, 2015, 10, 20-24.
38. Zheng L., Application of *Lippia nodiflora* extract to preparing medicinal preparation for treating hepatitis, China patent application CN. 2007-10084260 20070224, 2008.

*****