

THE IDENTIFICATION OF PHYTOCHEMICALS OF MEDICINAL IMPORTANT IN SENNA OCCIDENTALIS (L.) LINK

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Abstract

Plants or plant based traditional medicines are used to take care of issues related to human health using since long time. The present study was aimed to investigate the phytochemicals of aqueous, ethanol and hexane extracts of whole plant, leaves and seeds of *Senna occidentalis*. Phytochemicals like alkaloids, flavonoids, betaxanthin, coumarins, resin, steroids, terpenoids and tannins *etc.* have been reported in these extracts. GC-MS chromatogram of ethanol and hexane extracts of seeds has publicised eighteen and six compounds respectively. Some of the major compounds include eicosane 7-hexyl-, tricaproin, benzene, (ethoxymethyl)-, dasycarpidan-1-methanol, acetate (ester), astaxanthin. FTIR spectroscopic analysis revealed the presence alcohols, alkynes, alkenes, aldehydes amines and esters in different parts. The reported compounds have curative potential for different human health problems. Significance of utilizing *Senna occidentalis* in traditional medicines gets supported through these findings.

Key words : Senna occidentalis, Whole plant, Leaves, Seeds, Phytochemicals, GC-MS analysis, FTIR analysis.

Introduction

Plants are important for sustainable life on the earth including humans. Since large number of plants is used as a source of therapeutics, plants and their products have attracted the researchers in the field of ethnomedicine. Various studies have mentioned the use of plant materials in medicines because of their curative potential and minimum side effects. Medicinal plants are believed to be important source of new chemical substances with potential therapeutic effects (Sofowora, 1993). The medicinal value of plants pertains to presence of various metabolites (alkaloids, flavonoids, terpenoids, phenolics etc). It is pertinent to record that the chemical constituents of the plants depends upon the species, variety, growth conditions, geographical area, time of collection, climatic conditions and part of the plant used. The above mentioned phytochemicals, elements and some time their derivatives are utilized in the preparation of medicines (Ebomoyi et al., 2004, Kabila et al., 2017).

Different species of genus *Senna* L. are being used in various health care practices. This genus is a member of family fabaceae (subfamily caesalpinoideae). It grows in wild as weed and is distributed throughout the world. Most commonly found growing in unused lands along the roadside. Its high density has been reported in India, particularly in Western U. P. Uttarakhand, Punjab and Haryana, where it grows on the wasteland (Singh et al., 2013, Choudhary and Nagori, 2014, Rekha et al., 2016). There is an infinite literature on the medicinal importance of S. occidentalis but search for more, better and an effective compound is still to be found. Different plant parts such as roots, leaves, flowers and pods of the species contain useful phytoconstituents. Senna occidentalis is a major ingredient of a drug Liv-52 for the treatment of liver disorders (Wagh and Vidhale, 2010; Kaur et al., 2014; Firdous et al., 2015). It is useful in curing skin ailments and diseases such as ringworm, eczema, scabies, asthma, antibacterial, antidiabetic, antioxidant, antimalarial and possesses significant hepatoprotective and antiinflammatory activities (Chukwujekwu et al., 2006, Yadav et al., 2009).

Senna occidentalis is an erect, branched herb and usually 2 to 3 m tall. The members of this species are annual and perennial. Leaves of the plant are alternate, paripinnately compound and petiole possesses the dark purple brown ovoid gland near the stem junction. Leaflets

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are 3-5 (usually 5) in pairs, larger ones are lanceolate or oblong- ovate and smaller leaflets are ovate with short stalk. Leaflets are acuminate at apex and possess foetid smell. Flowers are yellow coloured, short axillary racemes or terminal leaf axils. Pods are flat, thin, straight and slightly curved. Each pod contains around 30-40 seeds. Seeds are pale brown with dull surface (Vashishtha, *et al.*, 2009; Nassar, *et al.*, 2011).

In this study metabolomic analysis has been undertaken to characterize the useful metabolites of *Senna occidentalis*.

Materials and Methods

Collection of Plant Material

Plant material is collected from the natural habitat in district Mohali and Rupnagar, Punjab. The collected specimens were identified using the available literature, floras, manuals and by consulting the Department of Botany, Herbarium, Panjab University, Chandigarh.

Processing of Material

Plant material was washed first with tap water and

then distilled water. The plant material was 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 analysis.

Preparation of Extracts

Aqueous extract- 20 g of powdered was soaked in 100 ml of distilled water taken in a conical flask. Kept it on a rotary shaker for about 24 hrs. Filtered the mixture through muslin cloth and then through Whattman filter paper No. 1. The filtrate (aqueous extract) was collected in vials and stored in the refrigerator till further use.

Ethanol and Hexane Extracts - 10 gm plant powder was extracted in 130 ml of solvent (Ethanol and Hexane). The extraction was carried out in a Soxhlet apparatus at a temperature ranging between 50°C to 60°C in Ethanol and 40°C to 50°C in Hexane. After complete extraction, the extract was allowed to evaporate at room temperature till the volume reduced to 1/3rd of the original and stored at 4°C.



Fig. 1: (a-f): Senna occidentalis (a, b) Whole plant, (c) Plant with pods, (d, e) Flower, (f) Seeds.

Phytochemical Analysis

The whole plant and selected plant parts of *Senna occidentalis* have been screened for phytochemicals such as alkaloids, flavonoids, saponins, coumarins, anthraquinones, terpenoids, steroids *etc*. The analysis was carried out using standard procedures (Trease and Evans, 1989; Sofowora, 1993; Kokate, 1994; Harborne, 1998; Kokate *et al.*, 2005; Roopshree *et al.*, 2008; Evans, 2009; Njoku *et al.*, 2009; Basumatary, 2016; Sidhu and Thakur, 2016; Sidhu and Sharma, 2016).

GC-MS Analysis

The analysis is carried out by 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 Laborartory, Panjab University, Chandigarh. Column TG 5MS composed of 5% Diphenyl; 95% dimethyl polysiloxane operating in electron impact mode and helium 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) injected temperature 250°C. The oven temperature is programmed for 60°C with hold time of 1 min and increased of 10°C/min at 220°C with hold time of 4 min. Mass spectra transfer line temperature is 250°C. Ion source temperature is 230°C and Mass range from 50-700. The compound identification was done by comparison of retention time and mass spectra of GC-MS.

In Gas chromatography, heat separates the compound mixture into different individual substances and the compound was identifed using the databse of National Institute of Standards and Technology (NIST) Library 2.0. The unknown component mass spectrum has been compared with spectrum of known component of NIST library. This analysis has provided the name, molecular weight, molecular formula and area under the peak of the components.

FT-IR Spectroscopy Analysis

Fourier Transform Infrared (FTIR) Spectroscopy is helpful in the identification and classification of chemical bonds/functional groups pertaining to phytochemicals. The chemical bonds present in the spectrum has absorbed a light of particular wavelength. Thus, the chemical bonds present in the reported 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 scan range from 400 to 4000 cm⁻¹. Plant samples powder was used for analysis and different functional groups were reported.

Results and Discussion

Phytochemical Analysis

The bioactivity of a plant species is likely due to the presence of different chemical compounds and elements. Various types of phytochemicals (alkaloids, phenolics, tannins, terpenoids, saponin etc.) have been reported during the present study. They are produced by the plant as per their need and some of them possess antimicrobial activity. Most of the studied phytochemicals get extracted in aqueous and ethanol extracts. Phytochemical like betaxanthin, cardiac active glycosides, glycosides and terpenoids are present in all the extracts except aqueous extract of leaves, whole plant and seeds and ethanol extract of seeds. Saponins are only present in aqueous extract of whole plant and seeds. Similarly, gum and mucilage are abundant in all the aqueous extracts of samples. Steroids have been recorded in all the extracts of whole plant but in case of leaves and seeds it is present only in aqueous and ethanol respectively. Amino acids, reducing sugar and anthocyanin are absent in all the extracts except traces in aqueous extract of whole plant and seeds. Similarly, anthraquinones, diterpenoids, lignins, phenolics, phlobataninns, quinones, and saponins are absent in all hexane extracts. This might be due to the less polarity of solvent to extract the metabolites. The presence and absence of the particular phytochemical is also dependent on the particular qualitative test e.g. KOH test of tannins and NaOH test of flavonoids gives promising result as compared to FeCl, and H₂SO₄ tests respectively. The aqueous extract of leaves contains maximum number of phytochemicals followed by ethanol and aqueous whole plant extract and the least in hexane extracts of leaves and seeds.

The qualitative phytochemical characterization of different extracts prepared from the whole plant, leaves and seeds have revealed the presence of many metabolites such as alkaloids, anthraquinones, carbohydrates, coumarins, flavonoids, phenolics, steroid, terpenoids etc that have supported the pharmacological importance of this plant species. It has also been reported that the extraction of phytochemicals of the plant through polar solvent is the most efficient. The hexane extract has also shown some important phytochemicals. Odega et al., (2014) studied the phytochemicals of Senna occidentalis leaves in hexane, ethanol and methanol extracts. Alkaloids were present in all the extracts whereas reducing sugar and glycosides were recorded only in hexane extract. Flavonoids, phlobatannins, steroids and cardiac glycosides were absent in all the extracts. Phytochemical profiling of leaves of C. occidentalis had

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S.N.	Phytochemicals	Whole plant			Leaves			Seeds		
		Aqs	Eth	Hex	Aqs	Eth	Hex	Aqs	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) $H_2 SO_4 Test$	+	+	-	+	-	-	+	-	±
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, Test	+++	++	-	-	++	-	±	+++	-

Table 1: Phytochemical analysis of *Senna occidentalis* whole plant, leaves and seeds.

+++ = Abundant, ++ = Moderate, + = Less and $\pm =$ Traces.



occidentalis leaves in six different solvents. Carbohydrates, glycosides, saponins, cardiac glycosides, phenolic compounds and tannins were reported in the ethanol and aqueous extracts. Alkaloids were absent in all the six extracts. Phytochemical analysis of the whole plant (*C. occidentalis*) had revealed the presence of fourteen metabolites including carbohydrates, saponins, terpenes, cardiac glycosides, anthraquinones etc. in the extracts whereas tannins, phenols, volatile

Fig. 2: GC-MS chromatogram of ethañol extract of Senna occidentalis seeds.

shown the presence of tannins, cardiac glycosides and anthraquinones in ethanol, methanol and aqueous extracts whereas alkaloids and flavonoids were absent (Sadiq *et al.*, 2012). Similarly, Shittu *et al.* (2014) reported tannins and flavonoids in aqueous and ethanol extracts but anthraquinones, saponins and phenols were present only in aqueous extract of *S. occidentalis*. Mohammed *et al.* (2012) carried out phytochemical screening of *C.* oil and phlobatannins were absent in methanol extract (Egharevba *et al.*, 2010).

Tannins, phenols, flavonoids, alkaloids, glycosides, steroids and saponins were reported in leaves of *Senna occidentalis* (Aja *et al.*, 2017). Similarly, Sani (2016) reported alkaloids, tannins, saponins and phenols in aqueous and ethanol extracts of the plant leaves. The roasted seeds of *S. occidentalis* contain saponins,

19.51

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17.22 18.40

glycosides, oxalate, alkaloids, phenolics etc (Olapade and Ajayia, 2016). Similarly, Veerchari *et al.*, (2012) studied the leaf phytochemicals of *C. occidentalis* using ethanol, methanol and ethyl acetate as solvents. It had

RT: 4 00 - 20 77 SM: 7G

100-

90 80

70

carbohydrates, steroids, terpenoids, cardiac glycosides, phlobatannins and alkaloids in all the extracts whereas anthraquinones and anthocyanosides were present in methanol, flavonoids in methanol and ethanol extracts.

> Some of the phytochemicals observed in the present study, were not reported from the earlier work such as flavonoids, phlobatannins, cardiac active glycosides and betaxanthinin leaves ethanol extract. Coumarins are present in all extracts except ethanol and hexane seeds extracts, diterpenoids in aqueous and ethanol extracts of whole plant and leaves whereas, lignins and quinones are present in ethanol whole plant and leaves (traces in aqueous leaves) and all ethanol or aqueous extract of leaves respectively.



Time (min)

S.N.	RT Value	Compound Name	Molecular Formula	Molecular Weight
1.	12.65	Astaxanthin	$C_{40}H_{52}O_{4}$	596.852 g/mol
2.	12.65	Pregnan-20-one, 3,17,21-tris[(trimethylsilyl)oxy]-, O-(phenylmethyl)oxime, (3á,5á)-	C ₃₇ H ₅₆ NO ₄ Si ₃	672.185 g/mol
3.	12.65	Di-tungsten, tris(cyclooctatetraene)	C ₂₄ H ₂₄ W ₂	680.136 g/mol
4.	17.22	(5á)Pregnane-3,20á-diol, 14à,18à-[4-methyl-3-oxo- (1-oxa-4-azabutane-1,4-d iyl)]-, diacetate	C ₂₈ H ₄₃ NO ₆	489.644 g/mol
5.	17.22	.psi.,.psiCarotene, 3,3',4,4'-tetradehydro-1',2'-dihydro- 1-hydroxy-1'methoxy (Hydroxyspirilloxanthin)	C ₄₁ H ₅₈ O ₂	582.913 g/mol

 Table 2: GC-MS analysis of Senna occidentalis hexane extracts of seeds.

Table 3: GC-MS analysis of ethanol extracts of Senna occidentalis seeds.

S.N.	RT Value	Compound Name	Molecular Formula	Molecular Weight
1.	4.54	Benzene, (ethoxymethyl)-	C ₉ H ₁₂ O	136.194 g/mol
2.	4.54	2-Butanol, 3-benzyloxy-	$C_{11}H_{16}O_{2}$	180.247 g/mol
3.	4.54	Benzene, 1,1'-[oxybis(methylene)]bis-(Dibenzyl Ether)	C ₁₄ H ₁₄ O	198.265 g/mol
4.	5.48	Camphor	C ₁₀ H ₁₆ O	152.237 g/mol
5.	6.00, 7.89	Heptadecane, 9-hexyl-	$C_{23}H_{48}$	324.637 g/mol
6.	6.00, 7.89	Dodecane, 5,8-diethyl-	C ₁₆ H ₃₄	226.448 g/mol
7.	6.00	Dodecane	C ₁₂ H ₂₆	170.34 g/mol
8.	7.89	Heptacosane, 1-chloro-	$C_{27}H_{55}Cl$	415.187 g/mol
9.	17.99	Tricaproin	C ₂₁ H ₃₈ O ₆	386.529 g/mol
10.	17.99	Spiro(1,3-dioxolane)-2,3'-[5'-androsten-16'-trimethylsilyloxy)-	$C_{24}H_{40}O_3Si$	404.666 g/mol
11.	17.99	Hexanethioic acid, S-decyl ester	C ₁₆ H ₃₂ OS	272.491 g/mol
12.	19.69	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	C ₂₆ H ₅₄	366.718 g/mol
13.	19.69	Heptadecane, 9-hexyl-	C ₂₃ H ₄₈	324.637 g/mol
14.	19.69	Eicosane, 7-hexyl-	C ₂₆ H ₅₄	366.718 g/mol
15.	19.80	Dasycarpidan-1-methanol, acetate (ester)	$C_{20}H_{26}N_{2}O_{2}$	326.44 g/mol
16.	19.80	9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-	C ₂₇ H ₅₂ O ₄ Si ₂	496.879 g/mol
		1-[[(trimethylsilyl)oxy]methyl]ethyl ester, (Z,Z,Z) (2-Monolinolenin, 2TMS derivative)		
17.	19.80	9-Hexadecenoic acid, 9-octadecenyl ester, (Z,Z)- (Oleyl palmi	toleate) $C_{34}H_{64}O_2$	504.884 g/mol
18.	20.72	Diisooctyl phthalate	C ₂₄ H ₃₈ O ₄	390.564 g/mol

IR Frequencies	(Wave num	ber cm ⁻¹)	IR frequencies range for the respective functionality (Wave number cm ⁻¹)	Groups (Coates, 2000; Pavi <i>et al</i> ., 2006)			
Whole plant	Leaves	Seeds					
<u> </u>	3627.87		3650-3300	O-H Stretch			
	3550.74						
	3523.95						
	3469.59						
	3269.59	3275.06	3400-3200	Normal "polymeric" OH stretch, O-H stretching in alcohols, phenols and carboxylic acids, Sp, C- H Stretch			
3186.83	<u>-</u>		3300-3030	Ammonium Ion			
2915.64	2921.69	2966.17	3000-2800	O-CH ₃ C-H Stretch, Methyl Ester, Alkanes, Sp ³ C-H Stretch			
2851.76	2844.70	2923.52					
	_	2843.59					
	2187.88		2200-2100	Alkyne			
	2163.59						
	2130.52						
	2039.69		2100-1800	Transition metal Carbonyls			
	1962.83						
1728.04			1740-1705	C=O stretch in aldehydes, ketones			
		1752.55	1760-1740	Alkyl Carbonate			
		1635.44	1650-1550	N-H Bend, Secondary amine			
1593.72	1597.04	1535.98	1650-1550	N-H Bend, Secondary amine			
		1	600-1500	C=C Stretch			
1421.57	1412.89	1462.43	1475-1350	CH ₂ /CH ₃ Bending Vibration			
		1405.22					
1372.53			1440-1200	C-O-H Bending Vibrations			
1319.41		1237.68					
1229.51							
1053.69	1053.12	1056.11	1350-1000	C-O Alcohols, Ethers, Esters, Carboxylic acid, Anydrides			
1005.71	1032.97	1000.68					
	1011.52						
882.18			900-670	C-H, Aromatic compounds			
514.59		518.50	600-500	C-I Stretch, Aliphatic Iodine Compounds			
	499.16		500-470	S-S Stretch, Polysulphide			
	445.08	444.95	500-430	S-S Stretch, Aryl Sulphide			

Table 4: FTIR peak values and functional groups of Senna occidentalis.

These metabolites are the less studied phytohemicals in *S. occidentalis* as per the available literature.

GC-MS Analysis

GC-MS has provided the detailed account of phytoconstituents present in ethanol and hexane extracts of seeds of *Senna occidentalis*. The mass spectrophotometery identifies the different compound in the samples. The GC-MS disclose various compounds with relative concentration getting eluted as per the retention time. The chromatogram of ethanol and hexane extracts of *S. occidentalis* seeds showed eight and two peaks respectively, corresponding to the presence of major chemical compounds at different peaks (Tables 2 and 3).

In previous GC-MS studies revealed the presence of 31 and 6 compounds in methanol leaf extracts (Ibrahim *et al.*, 2015, Javaid *et al.*, 2017). According to Panigrahi *et al.* (2015) GC MS analysis of *S. occidentalis* seeds



Fig. 4: FTIR Spectrum of Senna occidentalis whole plant.



Fig. 5: FTIR Spectrum of Senna occidentalis Leaf.



Fig. 6: FTIR Spectrum of Senna occidentalis Seeds.

has resulted into sixty one compounds at different peaks. The hexane and chloroform extracts of aerial parts of this species showed seven and ten phytocompounds respectively. Some of the reported compounds included dodecanoic acid, n-hexadecanoic acid, phytol and tetradecanoic acid etc at different retention time (Manikandaselvi *et al.*, 2016). Recently, Essien *et al.*, (2019) reported forty one and thirty eight compounds in volatile oil of fresh fruits of *S. occidentalis* and *Senna hirsuta* respectively. The phytoconstituents that are not reported in earlier available literature included benzene,

(ethoxymethyl)-, Tricaproin, 9-Hexadecenoic acid, 9-octadecenyl ester, (Z,Z)- (Oleyl palmitoleate), Dasycarpidan-1-methanol, acetate (ester). Astaxanthin and Hydroxyspirilloxanthin. Some of the reported compounds from this study have importance like Camphor (antidote, tooth ache, antihelminthic, antiseptic, stimulant etc.), 9-Hexadecenoic acid, 9-octadecenyl ester, (Z,Z)- (Oleyl palmitoleate) (cholesterol metabolism, improves secretory function, helps in insulin activity), Astaxanthin (antioxidant, anti-inflammatory, anticancer and immuno modulating properties), and Tricaproin (anticancer activity) (Jose et al., 2018; Kim, et al., 2019; Dr. Duke and Ethnobotanical Databses). Presence of these chemical constituents in the plant extracts provides the evidence of medicinal properties of the plant and its part.

FT-IR Analysis

The results pertaining to FTIR spectrum of *Senna occidentalis* whole plant, leaves and seeds are presented in (Figs. 4 to 6) and (Table 4). The peak values of the spectrum are important for identification of probable functional groups of different compounds in the plant samples.

Khyade et al. (2015) studied the FTIR spectrum of leaf extract of Cassia occidentalis. Cassia tora and Cassia uniflora and reported aldehydes, alcohols, carboxylic acid, alkenes, aromatic compounds, ester and nitro compounds. Different functional groups such as alkyl groups, methyl groups, alcohols, ethers were reported in the root, stem, flower and leaf tissues of Cassia occidentalis by FTIR analysis (Arora, 2015). Alcohol, aliphatic compounds, aldehydes, alkynes, alkenes, amines, alkyl carbonate and esters have been reported in the whole plant, leaf and seeds of S. occidentalis during the present study. The observations of the present study are compliance with the earlier findings except aryl sulphide, anhydrides, transition metal carbonyls and polysulphide. The variations in FTIR data is likely be due to use of different plant part or geographical variations.

Conclusion

Senna occidentalis is of immense medicinal importance and used in the treatment of various diseases. Several phytoconstituents in the whole plant, leaves and seeds of aqueous, ethanol and hexane extracts of this plant have been reported during the present study. The aqueous and ethanol extracts of leaves and whole plant have yielded more promising results. The GC-MS analysis has shown eighteen and five phytocompounds in ethanol and hexane extracts of seeds respectively including Benzene, (ethoxymethyl)-, Camphor, Tricaproin, Spiro (1, 3-dioxolane) - 2,3'-[5'-androsten-16'trimethylsilyloxy)- etc. The FTIR analysis of plant samples has shown major functional groups such as aldehydes, alcohols, aliphatic compounds, alkynes, alkenes and amine etc. Therefore, it is clear that the plant contains phytochemicals of medicinal interest and further studies are required to isolate and investigate the bioactivity of these compounds individually or in combination. Depending upon the activity, specific plant or plant part can be recommended to the pharmaceutical sector for the preparation of new or alternate medicine.

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