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Research Article

Antihyperglycemic, Antioxidant and Hepatoprotective Properties of *Smilax wightii* A.DC. : an Endemic Plant of Western Ghats

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ABSTRACT

Phytotherapy is an inevitable companion of human civilization. *Smilax wightii* is an ethnomedicinal plant in Smilacaceae, with unexplored scientifically therapeutic potential. The antihyperglycemic, antioxidant and hepatoprotective capabilities of the methanolic extract of leaf, stem, rhizome and root of *S. wightii* were inspected in the present study. Hyperglycemia is a manifestation of the prevalent metabolic disorder, type 2 diabetes mellitus (T2DM). Inhibitors of α –glucosidase and α – amylase could be efficiently employed in diabetes mellitus therapy as hypoglycemic agents. In the α –glucosidase and α – amylase inhibitory assays, root and rhizome extracts recorded better antihyperglycemic activity. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and total antioxidant capacity were the parameters employed to determine the antioxidant activity. Hepatoprotectivity determines the capability of samples to safeguard the hepatocytes from damage. Novel hepatoprotective agents are in demand since the incidence of liver impairment is high among the global population. The rhizome extract showed comparatively superior hepatoprotectivity followed by the leaf, stem, and root extracts. Rhizome, at 100µg/ml guaranteed a cell viability percentage of 77.43 in the Chang liver cell line treated with Carbon tetrachloride. So the root and rhizome of *S. wightii* are the therapeutically significant plant parts with hypoglycemic, free radical scavenging and hepatoprotective potentialities.

Introduction

Plants and plant-based compounds play a significant role in alleviating the contemporary ailments. *S. wightii* is a prickled woody climber in the family Smilacaceae, endemic to the Western Ghats and particularly seen at high altitudes. It is reported to be sporadically located in the open areas amid the evergreen and montane forests. Leaves of the plant are simple with cordate base and obtuse, cuspidate apex and reticulate venation. The umbel Inflorescence contains greenish-yellow perianth. *S. wightii* is used in ethnomedicine to combat various conditions of the nervous system, skin, urinary system as well as venereal diseases.[1] Even though the plant's therapeutic potential is least explored, few studies have reported the anti-inflammatory property of its fruit and the antioxidant and anti-diabetic activity of the whole plant extracts.[2-4]

As per the World Health Organization (WHO), India has the highest number of Diabetic patients globally, and by 2030, it would be around 80 million.[5] Chronic hyperglycemia in diabetes mellitus (DM) cases arises due to the lack of or insufficient insulin secretion from the pancreas, with or without the deterioration of simultaneous insulin action.[6] The regulation of the glucose in blood plasma is the prominent aspect of diabetes therapy, and α–glucosidase and α – amylase are two key enzymes involved in the carbohydrate metabolism that leads to the formation of glucose. Of late, the application of plants in the treatment of diabetes has been endorsed by the WHO.[7] Saponins were observed in appreciable quantity in the phytochemical screening of *S. wightii.* This is a potent class of bioactive molecules with promising anti-diabetic properties and has the

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potential to be developed into effective anti-diabetic medicine.[8,9]

Antioxidant activity determines the ability to counteract the potentially damaging free radicals inside the living system. Free radicals are related to several diseases, *viz* cancer, arthritis, liver damage, etc.[10] Liver toxicity is an important health hazard of the era, which may also occur through alcohol abuse, exposure to poisonous chemicals, drug overdose etc. Despite the advancements in medicine, liver diseases remain a critical health issue due to limited alternatives for its prevention and treatment.[11] Other than synthetic drugs, there are many effective hepatoprotective agents available, *viz* L-carnitine, vitamin C, N-acetylcysteine, silymarin etc. The hepatotoxic haloalkane, Carbon tetrachloride is reported to induce centrilobular hepatic necrosis and hepatocellular fatty degeneration.[12] So, CCl_4, which is the hepatotoxin in 80% of the hepatoprotective research globally, was used to induce damage to the hepatocytes in the present study.

Numerous plant-based formulations are employed in curing hyperglycemia and liver toxicity.[13,14] Studies have revealed increased use of phytomedicine in Europe and the United States in recent years, and nearly 65% of patients with liver damage take herbal remedies.[15] Screening plants for their bioactivities, thereby leading to the discovery of novel bioactive molecules, is important in modern medicine. The present analysis aimed to investigate the influence of methanolic extract of various parts of *S. wightii* on free radical scavenging, hyperglycemia and hepatic toxicity.

Materials and Methods

Plant Material

Smilax wightii was collected from Idinjar forest range of Thiruvananthapuram, Kerala, India at 8.751855°N and 77.070835°S. Leaf, stem, rhizome, and root were separately washed in running tap water and shade dried. The dried plant materials were powdered using an electrical blender and stored in an airtight container for further analysis.

Solvent Extraction

The plant powders were separately subjected to hot continuous extraction using a Soxhlet apparatus for 6 hours. The extracts were then evaporated to dryness with the help of a rotary vacuum evaporator. Solvents of increasing polarity like Petroleum ether, Chloroform, Methanol, and Distilled water were used. Extract yield was calculated using the equation:

$$\text{Extract yield} = \frac{\text{Weight of dried extract}}{\text{Weight of plant sample}} \times 100$$

Antihyperglycemic Activity

α-glucosidase inhibitory assay and α–amylase inhibitory assay was conducted on methanolic extracts of various parts of *S. wightii* to determine their antihyperglycemic activity.

(a) α -glucosidase Inhibitory Assay

The inhibition of α-glucosidase by the samples was determined according to the procedure by Kim *et al.*[8] A volume of 200 µL α-glucosidase (0.067 U/mL) from *Saccharomyces cerevisiae* was preincubated with various concentrations of the sample for 10 minutes. Then 200 µL of 3.0 mM p-nitrophenyl glucopyranoside (pNPG) dissolved in a 0.1M sodium phosphate buffer (pH 6.9) was added to initiate the reaction mixture was incubated at 37°C for 20 minutes. The reaction was stopped by the addition of 2 mL of 0.1 M Na_2CO_3. α-glucosidase activity was determined by analyzing the yellow-colored para nitrophenol released from pNPG. Absorbance was measured at 400 nm using a spectrophotometer, and results were expressed as a percentage of inhibition of the enzyme.

(b) α–amylase Inhibitory Assay

The inhibitory activity was determined through a modification of the protocol described in the Worthington Enzyme Manual.[16,17] A 0.5 mg/mL α- amylase enzyme and different concentrations (25, 50, 100, and 200 µg) of plant extract were added to 500 µL of 0.02 M sodium phosphate buffer of pH 6.9 with 0.006 M NaCl. The mixture was pre-incubated at 25°C for 10 minutes. Then 500 µL of 1% starch solution in 0.02 M sodium phosphate buffer of pH 6.9 with 0.006 M NaCl was added to each tube. Dinitrosalicylic acid (1.0 mL) was used to stop the reaction. The test solutions were incubated in a boiling water bath for 5 minutes and cooled to room temperature. They were made up to 10 mL with distilled water, and the absorbance was measured at 540 nm using UV-visible light spectrophotometer. Then the percentage of inhibition was calculated.

Antioxidant Activity

DPPH radical scavenging assay and total antioxidant activity assay were conducted on the methanolic extracts of the plant parts of *S. wightii* to determine the antioxidant activity.

(a) 2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay

The DPPH radical scavenging assay described by Brand–Williams *et al*. was used with slight modification.[18] One mL of 0.1 mM DPPH in the methanol solution was mixed with the various plant extract concentrations (0.2 to 1.0 mg/mL) in methanol. The mixture was shaken vigorously and left to stand for 30 minutes under dark room temperature. The control solution contained methanol and DPPH solution. Absorbance was read at 517 nm, and the activity was recorded as the percentage of inhibition. Ascorbate was used as a standard.

(b) Total Antioxidant Capacity Assay (TAC)

The total antioxidant capacity of the extract was evaluated by the phospho-molybdenum method, described by Prieto *et*

al.,[19] 100 µL of the sample was mixed with 3 mL of reagent solution (0.6M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95°C for 90 minutes. Then, the absorbance of the solution was measured at 695 nm against blank after cooling to room temperature. The total antioxidant activity is expressed as the number of gram equivalent of ascorbic acid. The calibration curve was prepared by mixing ascorbic acid with methanol.

Hepatoprotectivity Assay

Confluent Chang liver cells were cultured in the growth media, Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum (FBS) in a sterile 96-well *microtiter plates*. The cells with a density of 5 × 104 cells/well were incubated overnight. Post incubation, it was treated with varying concentrations of plant samples *viz* 25, 50, and 100 µg/mL and incubated for 24 hours. Then 73µM of H_2O_2 (IC50 of H_2O_2) was added as hepatotoxicant and incubated for another 24 hours. The treated cells were washed with PBS (Phosphate-buffered saline) and incubated with growth media containing MTT [3-(4, 5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide]. Later medium was removed, and the formazan crystals were dissolved using dimethyl sulfoxide. Absorbance was measured at 570 nm. With the untreated cells as a control, the percentage of cell viability in treated cells was calculated.

RESULTS

Solvent extraction

The extracts collected from Soxhlet extraction were employed in the analysis. Among the four solvents used, the maximum extraction of phytochemical was observed in the hot continuous extraction using the more polar solvent, methanol in all the plant parts. The highest extract yield was obtained in methanolic extraction of the leaf of *S. wightii* (13.23%), followed by the rhizome (10.5%), stem (9.029), and root (8.858%), respectively. The results are recorded in Table 1.

Table 1: Extract Yield in the Soxhlet Extraction using Various Solvents

Plant part	*Solvent*	*Extract yield (%)*
Leaf	Petroleum ether	1.18 ± 0.017
	Chloroform	0.676 ± 0.13
	Methanol	12.36 ± 0.63
	Distilled water	8.11 ± 0.49
Stem	Petroleum ether	0.743 ± 0.047
	Chloroform	1.155 ± 0.023
	Methanol	9.033 ± 0.158
	Distilled water	6.45 ± 0.477
Rhizome	Petroleum ether	0.466 ± 0.031
	Chloroform	1.357 ± 0.22
	Methanol	10.77 ± 0.15
	Distilled water	6.811 ± 0.23
Root	Petroleum ether	0.924 ± 0.042
	Chloroform	5.93 ± 0.347
	Methanol	8.376 ± 0.276
	Distilled water	7.055 ± 0.154

Antihyperglycemic Activity

Alpha-amylase and alpha-glucosidase are pivotal proteins related to type II diabetes (T2D). Inhibitory assays for α -glucosidase and α- amylase were conducted to evaluate the antihyperglycemic property of *S. wightii.* In the α -glucosidase inhibitory assay, all the extracts showed an increase in inhibitory activity in a concentration-dependent manner (Fig. 1). At the concentrations ranging from 3.0 µg/mL to 25 µg/mL the leaf extract inhibited α –glucosidase enzyme in the range from 58.47 to 77.62 %, the rhizome extract from 63.01 to 79.90%, and the root methanol extract from 42.46 to 76.25%. The stem extract exhibited the lowest inhibitory activity ranging from 15.5 to 41.09%. The IC50 (concentration where 50% inhibition occurs) values were also compared. The leaf's calculated IC50 values, stem, rhizome, and root were 13.854, 31.79, 23.816, and 4.903 µg/mL, respectively.

In α-amylase inhibitory assay all the plant parts showed inhibitory potential different from α -glucosidase inhibitory potential (Fig. 2). In the concentration range of 3.0 to 25µg/mL, the range of inhibition percentage by the leaf, stem, rhizome, and root were 37.37 to 83.69%,

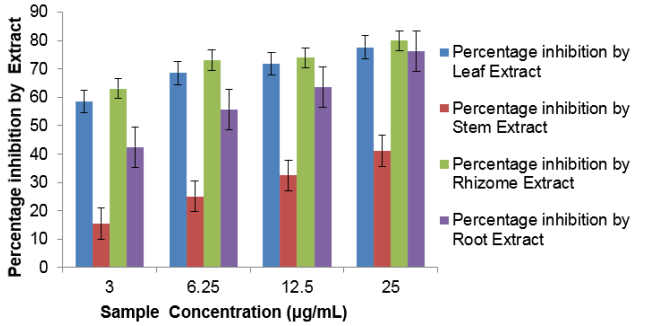

Fig. 1: α -glucosidase inhibitory activity of the methanolic extract of the leaf, stem, rhizome and root of *S. wightii*

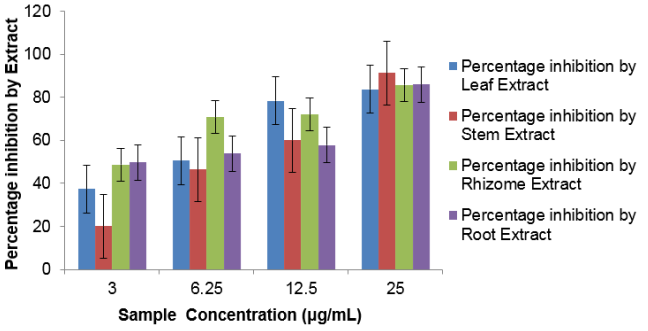

Fig 2: α- amylase inhibitory activity of the methanolic extract of the leaf, stem, rhizome and root of *S. wightii*

20.07 to 91.25%, 48.70 to 85.68 % and 49.70 to 85.88% respectively. The IC50 values of the leaf, stem, rhizome, and root methanolic extract were calculated to be 5.589, 10.17, 2.254, 4.50 µg/mL, respectively. All the plant samples showed higher inhibition to α- amylase than α –glucosidase enzyme.

Antioxidant Activity

The antioxidant property is the potential of plant sample to protect the body from free radicals damage, thereby inhibiting associated diseases.[20] DPPH is a stable free radical which is pink in color and turns yellow once scavenged. The DPPH free radical scavenging assay uses this property of DPPH to determine the free radical scavenging activity of samples. All the extracts showed a concentration-dependent improvement in antioxidant activity (Fig. 3). Rhizome methanolic extract exhibited the highest DPPH free radical scavenging activity, and the IC50 value was observed to be 93 µg/mL. It was followed by leaf (114 µg/mL), root (537 µg/mL), and stem (646 µg/mL) extract with moderate free radical scavenging activity. Ascorbic acid was used as the standard, which had an IC50 value of 0.517 µg/mL.

Total antioxidant capacity (TAC) assay is an effective method to predict crude plant extracts' overall antioxidant capacity. In the TAC analysis, also the root, rhizome, and leaf showed considerable antioxidant properties (Fig. 4). Among the four plant parts root extract showed highest antioxidant content, i.e., 215.78µg/g followed by the rhizome (171.57 µg/g), leaf (159.47 µg/g) and stem (86.3 µg/g), respectively. The values were compared with the standard ascorbic acid.

Hepatoprotectivity Assay

The effect of the different extracts of *S. wightii* on liver toxicity was analyzed through 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Chang's liver cell line protectivity against H_2O_2 induced toxicity by the samples, was measured, and it was found that all the extracts exhibited considerable hepatoprotective activity. Plant extracts were treated in three concentrations viz 25, 50, and 100 µg/mL, and the cell lines sowed viability in the range of 50 to 77% in all the treatments (Figs. 5 and 6). Rhizome extract showed comparatively better activity, followed by the leaf, stem, and root extracts, respectively. At 100 µg/mL concentration of the rhizome extract, the cell viability percentage was 77.43, whereas the cell viability in the leaf methanolic extract was 75.84. Stem and the root extract showed lesser activity.

Discussion

Hyperglycemia-linked T2DM is a significant metabolic disorder that can result in many related health issues as well. α-glucosidase and α-amylase are prominent proteins that function in carbohydrate breakdown leading to glucose synthesis, which results in hyperglycemia. α-amylase functions in the lysis of the long-chain sugars whereas, α-glucosidase converts starch and other disaccharides into glucose.[21] Control over postprandial hyperglycemia is a matter of concern in diabetic patients and this is possible through the inhibitors of α-amylase and glucosidase enzymes. They are considered as potential lead molecules in drug development for diabetes therapy.[22] Maheswari *et al*. in 2014 investigated the anti-diabetic activity of the methanolic extract of the whole plant of S. wightii by analyzing the biochemical parameters like serum-protein, albumin, globulin, serum glutamic pyruvic transaminase (SGPT), serum glutamic-oxaloacetic transaminase (SGOT), and systematic assessment of licensee performance (SALP) levels in streptozotocin-induced diabetic rats. The results proved that the whole plant extract of S. wightii could improve protein metabolism and can be used to prevent diabetic complications.[4] In the present analysis, the root

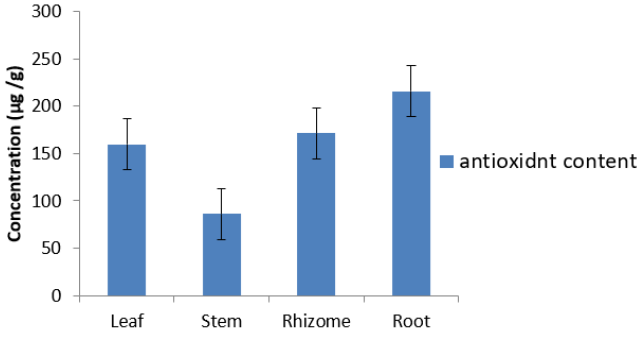

Fig. 4: Total antioxidant content of various parts of *S. wightii*

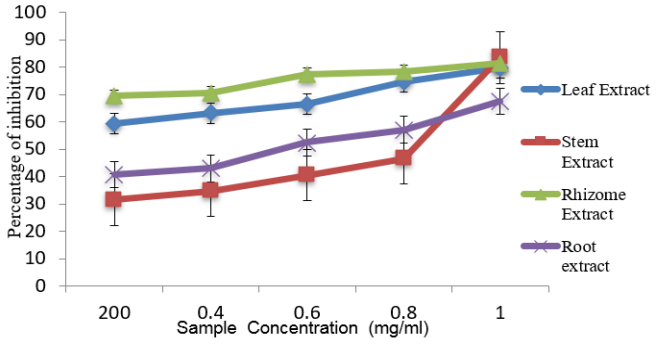

Fig. 3: Comparative antioxidant activity of various parts of *S. wightii* through DPPH radical scavenging assay

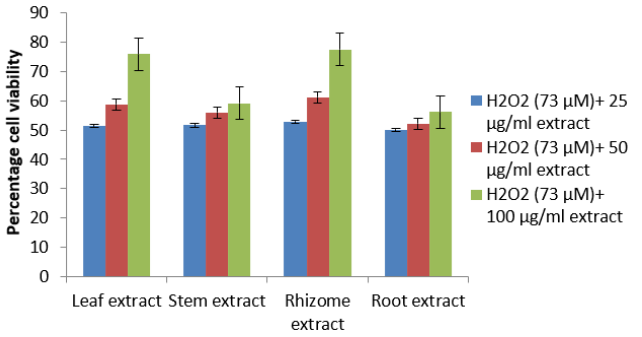

Fig. 5: Comparative hepatoprotectivity of different parts of *S. wightii*

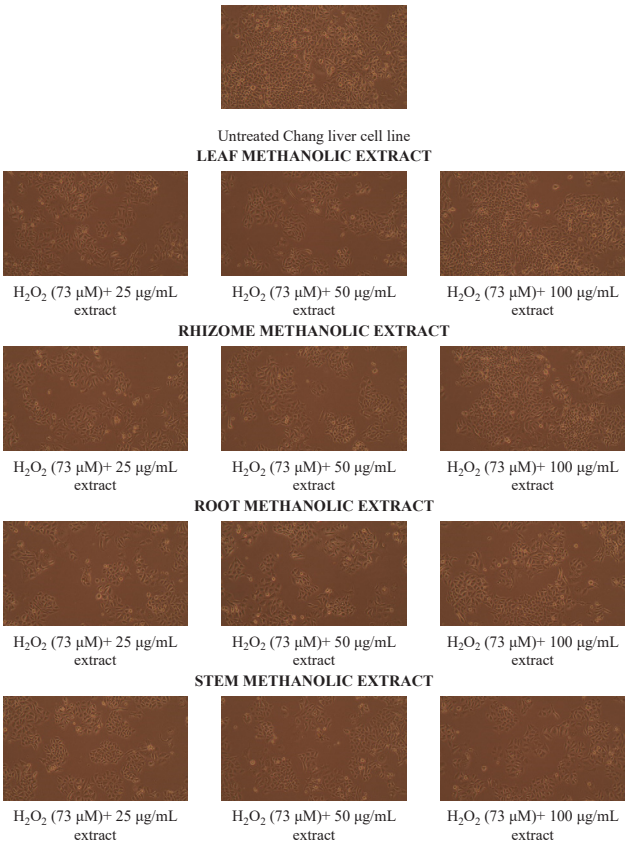

Fig. 6: Hepatoprotectivity of the various plant parts on Chang liver cell line

and rhizome's methanolic extract showed considerable inhibition to α -glucosidase and α–amylase *in vitro*. The lowest IC50 values were recorded in the α–amylase inhibition assay than the α–glucosidase inhibition. So S. wightii could inhibit α–amylase better than α–glucosidase.

In the α -glucosidase inhibitory assay to determine the antihyperglycemic activity, rhizome extract showed high inhibitory activity, rhizome, leaf, and the root methanolic extract showed considerable hypoglycemic potential, with the root extract recording the lowest IC50 value of 4.903 µg/mL. Whereas in α- amylase inhibitory assay, rhizome, leaf, and the root extract showed comparable inhibitory property, with the rhizome and the root extract recording the lowest IC50 values 2.254 and 4.50 µg/mL, respectively. In both the assays, the stem extract showed the lowest activity.

The analysis proved that the root and the rhizome of *S. wightii* contain some hypoglycemic bioactive principle. The phytochemicals like polyphenols, alkaloids, flavonoids, terpenoids, tannins, and steroids present in the plant may be responsible for the property since there are reports regarding these metabolites resulting in the anti-diabetic property of plant extract.[23] Doss and Vijayasanthi (2016) examined the α -glucosidase and α –amylase inhibitory property of flavonoids, phenolics, proanthocyanidin, and tannins from *Coccinia grandis* and found that flavonoids exhibit maximum inhibition to α –amylase.[5]

Oxidative stress, which leads to reactive oxygen species build up in the system, is a major health hazard and has a role in neurodegenerative diseases like Alzheimer's and Parkinson's diseases, cancers, heart diseases, aging, damage to biomolecules, tissue damage etc. Antioxidant compounds are the path to combat oxidative stress. Considerable activity was recorded by the rhizome and root extract in DPPH radical scavenging and Total antioxidant activity assays, which are widely accepted protocols to determine the antioxidant potential. Maheswari *et al*. (2014) studied the free radical scavenging activity of methanol extract of *S. wightii* whole plant through DPPH, Nitric oxide, and ABTS radical scavenging assays and showed that the extract caused an inhibition percentage of 72.16 at 50 µg/mL.[2] This potential of the plant may be due to the presence of secondary metabolites like phenols and flavonoids. The present analysis could contribute to the quest for natural antioxidant molecules in the world.

The liver has many functions in the body like detoxification and excretion of compounds from the body, including chemicals and biotransformation. Common reasons for liver failure include consumption of alcohol, exposure to toxic chemicals, and intake of some drugs like paracetamol, acetaminophen, aflatoxin, chemotherapeutic agents etc. Plant-derived hepatoprotective agents can cure drug-induced liver damage. Numerous plants in India have been screened for anti-diabetic as well as hepatoprotective activity and effective drug formulations could be developed using indigenous medicinal plants after further experiments and clinical trials.[24] Rhizome extract of *S. wightii* showed high hepatoprotectivity on H_2O_2 induced toxicity in the Chang liver cell line followed by the leaf extract. The rhizome methanolic extract contains some phytochemicals that can protect the hepatocytes from the toxicity induced by H_2O_2. Metabolites like flavonoids, alkaloids, saponins, glycosides etc., from plants have been reported as effective hepatoprotective compounds.[25] Andrographolide (*Andrographis paniculata*), Curcumin (*Curcuma longa*), Catechin (*Annacardium occidentale*), Fumaric acid (*Sida cordifolia*) etc., are some plant-derived compounds with hepatoprotective ability.[26] The present analysis pointed out the presence of bioactive phytochemicals in the plant extract that can be developed into a prospective drug lead.

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