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Research Article

EVALUATION OF PHYTOCHEMICAL COMPOUNDS AND ANTIMICROBIAL ACTIVITIES OF *HEMIDESMUS INDICUS*

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ABSTRACT

Objective: The present study was aimed to investigate the phytochemical screening, antibacterial activities, and identification of bioactive compounds by gas chromatography-mass spectrometry (GC-MS) and high-performance liquid chromatography (HPLC) in *Hemidesmus indicus* root extracts.

Methods: The qualitative and quantitative phytochemical analysis was carried out using standard methods. Bioactive compounds were identified by GC-MS and HPLC analysis. Antibacterial activity of different solvent extracts was carried out using the agar well diffusion method.

Results: Preliminary phytochemical analysis showed the presence of phenols, tannins, alkaloids, flavonoids, proteins, reducing sugar, glycosides, amino acids, steroids, terpenoids, resins, volatile oil, emodols, and coumarins. Total phenolic and flavonoid contents were higher in the methanolic extracts compared to other solvent extracts. 2-hydroxy 4-methoxy benzaldehyde was identified as the major compound. The maximum zone of inhibition was observed in the methanolic extracts of root against *Klebsiella pneumoniae*, *Staphylococcus aureus,* and *Escherichia coli*.

Conclusion: Our results suggest that *H. indicus* plant contains many chemical compounds and its root exhibits the wide range of antimicrobial activity. Further studies have to be carried out regarding the *in vitro* multiplication of plant and to enhance the root flavoring compounds for medicinal, food, and beverage industries.

Keywords: *Hemidesmus indicus,* methanolic extracts, phytochemicals, antimicrobial activity.

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INTRODUCTION

Plants are the "back bone" of traditional medicine, where about 80% of the world population rely exclusively on traditional medicine for their primary health care needs [1]. Nowadays, the term "Alternative Medicine" became very common in western culture; it focuses on the idea of using the plants for medicinal purposes. In developing countries, low-income people such as farmers, people of small isolated villages and native communities use folk medicine for the treatment of common infectious diseases [2]. Medicinal plants frequently used as raw material for extraction of active ingredients which are used in the synthesis of different drugs, such as laxatives, blood thinners, antibiotics, and antimalarial medications, contain ingredients from plants [3]. The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including plants. Increasing dependence on the use of medicinal plants in the industrialized societies for extraction and development of several drugs. Medicinal plants have a promising future, and most of them have not yet investigated the medicinal activities and could be decisive in the treatment of present or future problems [4].

Hemidesmus indicus is one of the important medicinal plants, belongs to the family Asclepiadaceae**,** which is derived from the word "Asklepios" – means "God of Medicine." It is commonly known as Indian Sarsaparilla, and in Sanskrit, it is termed as "Anantmool," which means "endless root." It is a slender, laticiferous semi-erect shrub, distributed in upper Gangetic plains and from Central to South India, growing under mesophytic to semi-dry conditions in the plains and an altitude of 600 m [5,6]. Roots of this plant contain steroids, terpenoids, flavonoids, saponins, phenolic compounds, tannins and lignins, cardiac glycosides, proteins, and carbohydrates. Roots and stem act as a laxative, diaphoretic, and diuretic and are useful in the treatment of syphilis, cough, asthma, leukoderma, etc. [7].

H. indicus is used to treat nutritional disorders, fever and foul odor from the body, bronchitis, piles, rat bite poisoning, epileptic fits in children, and "tridosha" disease of the blood, leukorrhea, and kapha and vata. The root extract was found to protect microsomal membranes as evident from the reduction in lipid peroxidation values [8]. Leaves of *H. indicus* possess a promising role in the treatment of wounds, especially chronic wounds of diabetic and cancer patients. Lupeol acetate and methoxy benzoic acid isolated from *H. indicus* root extract significantly neutralize lethality, hemorrhage induced by *Daboia russellii* venom, particularly root has antivenom potential [1]. 2-hydroxy 4-methoxy benzaldehyde (2H4MB) is an isomer of vanillin; it is one of the major compounds in the volatile oils of *Decalepis hamiltonii* and *H. indicus* [9]. Based on the above medicinal importance, the present work is carried out on the phytochemical and antibacterial screening of the different extracts of the *H. indicus* root.

MATERIALS AND METHODS

Collection of plant material

The plants of *H. indicus* were collected from the Southern part of Karnataka and authenticated in the Department of Botany, Bangalore University, Bengaluru. A voucher specimen is deposited in the herbarium (BUB, No. 2288). Fresh roots were separated from the plants and rinsed thoroughly in running tap water. The shade dried roots were finely powdered with the help of a blender and stored for further analysis (Fig. 1).

Preparation of extracts

The 20 g of dried root powder was weighed and packed with Whatman No. 1 filter paper. The extraction was done by Soxhlet extraction method, extracted with methanol, petroleum ether, and aqueous solvents about 10–12 h. Each extract was collected and concentrated by evaporation, stored at 4°C for further analysis [10].

Fig. 1: (a) Natural habitat (b) fresh root and (c) powdered form of *Hemidesmus indicus* root

Preliminary phytochemical analysis

The preliminary phytochemical analysis was carried out by standard methods [11]. The lyophilized extracts were dissolved in respective solvents and screened for the qualitative analysis for the presence of alkaloids, flavonoids, proteins, amino acids, phenols, tannins, steroids, terpenoids, glycosides, coumarins, reducing sugar, resins, volatile oils, and emodols [12,13].

Quantitative analysis

Total phenol content

The total phenol content was determined by the method of Singleton *et al.* [14]. 0.5 ml of root extract was mixed with 0.5 ml of FC reagent and allowed to stand for room temperature (RT) for 2–3 min. Followed by, 1 ml of 7% sodium carbonate was added and the final volume was made up to 5 ml with distilled water (d. w). After 90 min of incubation at RT in the dark, the absorbance was read at 725 nm using ultraviolet-visible spectrophotometer in triplicates. Gallic acid was used for calibration of the standard curve. The results were expressed as mg of gallic acid equivalent (mg GAE/g) of the dry weight of the material.

Total flavonoid content

The total flavonoid content was determined by the aluminum chloride method with slight modifications. 0.5 ml of sample extract was mixed with 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate, 1.5 ml of 90% ethanol, and 1.8 ml of d. w. After incubation at RT for 30 min, the absorbance of the reaction mixture was measured at 415 nm with a spectrophotometer. The final absorbance of each sample was compared with a standard curve plotted from quercetin. The total flavonoid content was expressed in micrograms of quercetin equivalents per mg extract [15].

Total tannin content

The total tannin content was determined using catechin hydrate as a standard. 0.5 ml of sample add 3 ml of 4% vanillin solution in methanol and 1.0 ml of concentrated hydrochloric acid were added. The mixture was then shaken and incubated at room temperature for 15 min the absorbance was measured at 500 nm against a blank. The tannin content was expressed as mg of catechin equivalent per g of dry weight (mg CE/g DW) [16].

Extraction of volatile oil

Fresh and healthy roots were collected, washed under running tap water to remove the dust and adherent rotten material and the tuberous roots were shade dried under RT. The dried roots (100 g) were powdered with the help of a mechanical blender, subjected to hydrodistillation using a Clevenger-type apparatus for 5 h. The essential oil was collected through a funnel containing anhydrous sodium sulfate to remove the water content [17].

Gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS analysis of methanolic root extract and root oil of *H. indicus* were analyzed with the help of GC-MS analyzer [18]. The Clarus 680 GC was used in the analysis employed a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250 μm df) and the components were separated using helium as carrier gas at a constant flow of 1 ml/min. The injector temperature was set at 260°C during the chromatographic run. The 1 μl of extract sample injected into the instrument the oven temperature was as follows: 60°C (2 min); followed by 300°C at the rate of 10°C/min; and 300°C, where it was held for 6 min. The mass detector conditions were transferred line temperature 240°C; ion source temperature 240°C; and ionization mode electron impact at 70 eV, a scan time 0.2 s, and scan interval of 0.1 s. The fragments were elevated from 40 to 600 Da. The spectrums of the components were compared with the database of the spectrum of known components stored in the GC-MS NIST (2008) library.

High-performance liquid chromatography (HPLC) analysis

HPLC analysis was done according to the procedure of Zishan *et al*. [19]. The methanolic extract was redissolved in methanol, filtered through a 0.45 μm membrane filter and used for HPLC analysis. About 10 μl of the volume was injected for quantitative detection of C_{18} analytical column. The mobile phase comprising of methanol/water (80:20, v/v) for 2H4MB at pH 5 with the flow rate of 1 ml/min was used. The chromatograms were monitored at 280 nm. The peak identification of extract was based on the comparison of retention time with those of standard in the same condition with a total running time of 6 min for a standard calibration plot of the compound, three distinct calibration levels were used to set the calibration plot. The calibration plot was drawn by plotting the peak area against the concentration of the compound.

Antibacterial activity

Preparation of inoculum

The solvent extracts were assayed against the following organisms *Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas fluorescence,* and *Staphylococcus aureus*. All the stocks were obtained from the Department of Microbiology and Biotechnology, Bangalore University, Bengaluru. Loopful of overnight grown bacterial culture was inoculated in a known volume of nutrient broth (NB) and incubated at 37°C for 6–8 h. The actively growing culture suspension was adjusted with NB to obtain a turbidity that could be visually comparable with a 0.5 McFarland standard. The turbidity is approximately equal to 1×10^8 CFU/ml.

In vitro antibacterial activity was performed by agar well diffusion method according to the protocol of Johnson *et al*. [20]. To determine the inhibitory activity of different solvent extracts in various concentrations was prepared in DMSO. Wells were bored into nutrient agar using a sterile 8 mm diameter cork borer. Streptomycin was used as a standard. Different concentration of the solvent extracts was added into the wells using sterilized pipettes and allowed to diffuse at RT for 2 h. The plates were incubated at 37 °C for 24 h. After the incubation, the diameter of the zone of inhibition was recorded in millimeter and compared with the standard antibiotics. The experiments were repeated thrice in triplicates.

Statistical analysis

The results were expressed as mean ± standard deviation, and data were analyzed statistically by one-way analysis of variance followed by Duncan's multiple range tests using SPSS software. $p<0.05$ was considered as significant.

RESULTS AND DISCUSSION

Preliminary phytochemical analysis

A primary metabolite is directly involved in normal growth, development, reproduction, etc. Secondary metabolites are end products of the primary metabolites, which play an important role in the production of bioactive compounds in plants. Preliminary phytochemical screening of methanol, petroleum ether and aqueous extracts of *H. indicus* roots were subjected to various tests. All the extracts showed the presence of phenols, tannins, alkaloids, and

Table 1: Qualitative analysis of different extracts of *Hemidesmus indicus* roots

Phytochemicals	Method	Methanol	Petroleum ether	Aqueous
Phenols	Ferric chloride	+	-	+
Tannins	Gelatine	+	-	+
Alkaloids	Dragendroff's	+	+	+
Flavonoids	Alkaline reagent	+	-	+
Proteins	Biuret	+	+	+
Amino acid	Ninhydrin	+	-	+
Reducing sugar	Fehling's reagents	+	+	+
Glycoside's	Keller–Kiliani	+	+	+
Steroids	Acetic anhydride	+	+	+
Terpenoids	Chloroform	+	+	+
Resins	Turbidity	+	-	+
Volatile oil	Ethanol and $FeCl_3$	+	-	+
Emodols	Ammonia	-	-	-
Coumarins	Alcoholic KOH	+	-	+

+: Present, -: Absent

Table 2a: Compounds identified in the methanolic root extract of *Hemidesmus indicus* by gas chromatography-mass spectrometry

Serial number	Name of the compounds	MF	MW	RT	Peak area (%)
1	URS 12 – En – 28 Oic acid, 3 hydroxy, methyl ester (3β)	$C_{31}H_{50}O_3$	470	29.95	09.08
2	Lupeol	$C_{30}H_{50}O$	426	30.36	39.76
3	3 - O Acetyl-6 - methoxy - Cycloartenol	$C_{33}H_{54}O_3$	498	30.60	17.15
4	3 - O Acetyl-6 - methoxy - Cycloartenol	$C_{33}H_{54}O_3$	498	30.66	28.58
5	1, 3 – Bis – T-Butyl per oxy - Phthalan	$C_{16}H_{24}O_5$	296	30.885	03.66
6	N - Acetyl - 2 - ethoxy amphetamine	$C_{13}H_{19}O_2$ N	221	31.14	01.75

MF: Molecular formula, MW: Molecular weight, RT: Retention time

Table 2b: Compounds identified in root oil of *Hemidesmus indicus* by gas chromatography-mass spectrometry

Serial number	Name of the compounds	MF	MW	RT	Peak area (%)
1	Pyrolo (3, 2 - D) Pyrimidin – 2, 4 (1H, 3H) - Dione	$C_6H_5O_2N_3$	151	15.96	30.76
2	Vanillin	$C_8H_8O_3$	152	16.21	14.94
3	2 - Hydroxy 4 - methoxy Benzaldehyde, Acetate	$C_{10}H_{10}O_4$	194	16.34	14.69
4	Benzaldehyde 3 - hydroxy 4 - methoxy	$C_8H_8O_3$	152	16.59	06.66
5	Benzaldehyde 2 - hydroxy 4 - methoxy	$C_8H_8O_3$	152	16.74	04.07
6	Benzaldehyde 2, 4 Di hydroxy 6 - methyl	$C_8H_8O_3$	152	16.81	15.19
7	2 - Hydroxy 4 - methoxy Benzaldehyde, Acetate	$C_{10}H_{10}O_4$	194	17.24	03.89
8	2 (1H) – Pyridinone, 1 - Cyclohexyl - 3, 4, 5, 6 – Tetra methyl	$C_{15}H_{23}ON$	233	17.40	04.69
9	4 – Methoxy formanilide	$C_8H_9O_2N$	151	17.60	05.07

MF: Molecular formula, MW: Molecular weight, RT: Retention time

flavonoids, proteins, reducing sugar, glycosides, amino acids, steroids, terpenoids, resins, volatile oil, emodols, and coumarins. Among the three extracts, methanolic extract shows the presence of all these phytochemicals [Table 1]. The results are concordance with the Devi *et al*. [21], where alkaloids, flavonoids, phenols, and saponins are identified in different parts of *H. indicus*.

Quantitative analysis

Total phenol content

Phenolics play an important role in plant development, particularly in lignin and pigment biosynthesis. They also provide structural integrity and scaffolding support to plants. In this study, the higher amount of total phenolic content was recorded in the methanolic root extract of *H. indicus* (11.975 ± 2.001 mg/g GAE), followed by aqueous and petroleum ether extract (16.672 ± 0.74 mg/g and 7.512 ± 0.318 mg/g), respectively. Devi *et al*. [21] also reported higher phenolic content in the aqueous extract of *H. indicus* root (Fig. 2).

Total flavonoid content

Flavonoids play an important role in the protection of plants against plant-feeding insects and herbivores. Their presence can alter the palatability of the plants and reduce their nutritive value, decrease digestibility or even act as toxins in few cases. The present work shows

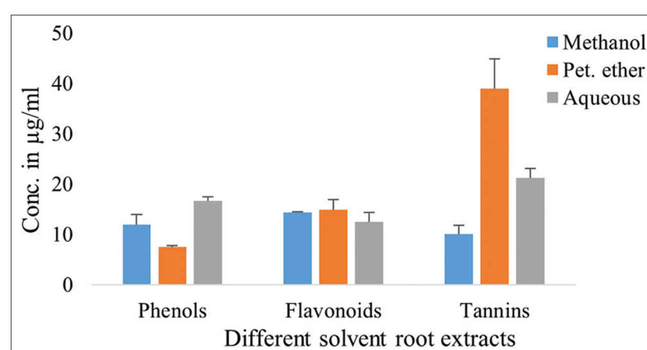

Fig. 2: Quantitative analysis of phenols, flavonoids, and tannins of *Hemidesmus indicus* root.

maximum total flavonoid content in the methanolic extract of *H. indicus* (14.876 ± 2.087 mg/g) followed by petroleum ether and aqueous extract (14.361 ± 0.195 mg/g and 12.495 ± 1.873 mg/g), respectively (Fig. 2). Samydurai and Thangapandian [22] reported the highest phenolic and flavonoid contents in the methanolic root extracts when compared to other extracts in *D. hamiltonii*. Banothu *et al.* [23] also reported that the polar solvents showed higher content of flavonoids in leaf and stem extracts of *Physalis minima.*

Total tannin content

Tannins are the phenolic substances present in wood, bark, and other plant materials. Tannins are extracted with water or organic chemicals. The total tannin content was higher in the methanolic extract of *H. indicus* root which is around 38.922 ± 5.98 mg/g (CE), followed by aqueous and petroleum ether extract (21.163 ± 1.918 mg/g and 10.01 ± 1.77 mg/g), respectively (Fig. 2).

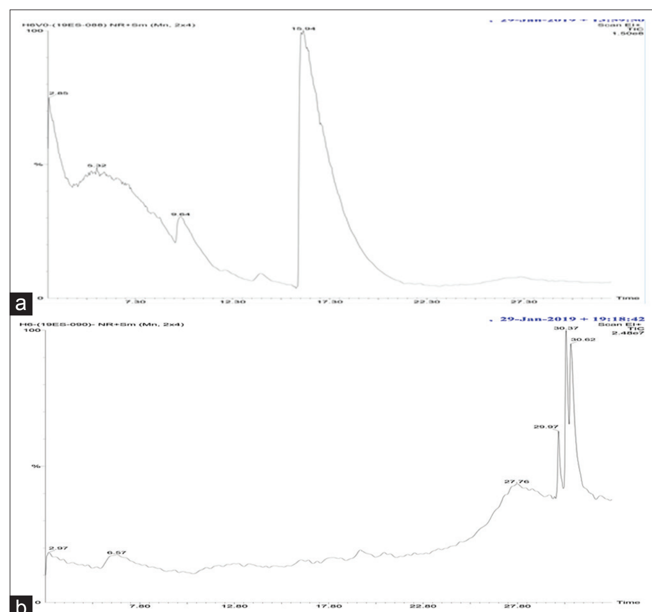

Fig. 3: Gas chromatography-mass spectrometry chromatogram of (a) methanolic extract of root and (b) Root oil of *Hemidesmus indicus*

Hydrodistillation is one of the conventional methods to isolate essential oil. The yield of volatile oil extracted from the tuberous root of *H. indicus* was 0.07% (v/w) obtained by hydrodistillation. The chemical composition of the oil was analyzed by GC-MS and HPLC.

GC-MS

GC-MS is one of the premium techniques to the identification of compound occurs on the basis of molecular mass, molecular structure, and fragments. The mass spectrum of *H. indicus* showed six and nine prominent peaks in the methanolic extract of root and root oil (Table 2a and b). The relative amount of each compound in percentage was determined by comparing its average peak area to the total area. Analysis of *H. indicus* root oil showed the presence of many compounds mainly they are Pyrolo (3, 2-D) pyrimidin-2, 4 (1H,3H)– Dione, Vanillin, 2-hydroxy 4-methoxy benzaldehyde acetate, and benzaldehyde 2, 4 di hydroxy 6-methyl with the retention time 15.96, 16.21, 16.34, and 16.74, respectively (Fig. 3a). Methanolic root extracts showed the presence of compounds such as lupeol, 3-O acetyl-6-methoxy-cycloartenol, and 3-O Acetyl-6-methoxy-cycloartenol with the retention time 30.36, 30.60, and 30.66, respectively (Fig. 3b). The results were concordance with the reports of Arunodaya *et al.* [24] where stem bark oil of *Litsea glutinosa* showed the presence of these compounds.

HPLC

HPLC is a useful technique for the characterization and quantification of secondary metabolites in plant extracts. The chromatogram of the 2H4MB standard compound and the peak was recorded at the retention time 3.936 min. The peaks of the methanolic root extract show the 3.891 and root oil exhibits 3.861 retention time, respectively (Fig. 4). Nagarajan and Rao isolated 2-hydroxy-4-methoxybenzaldehyde from

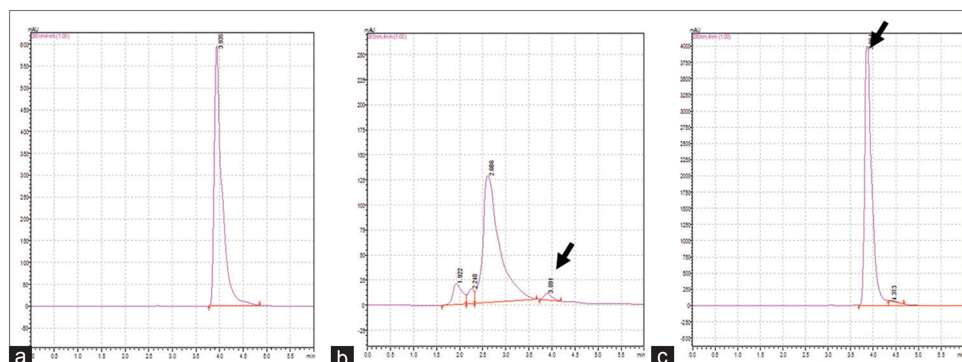

Fig. 4: High-performance liquid chromatography analysis report of (a) standard 2-hydroxy 4-methoxy benzaldehyde and (b) methanolic root extract and (c) root oil of *Hemidesmus indicus*

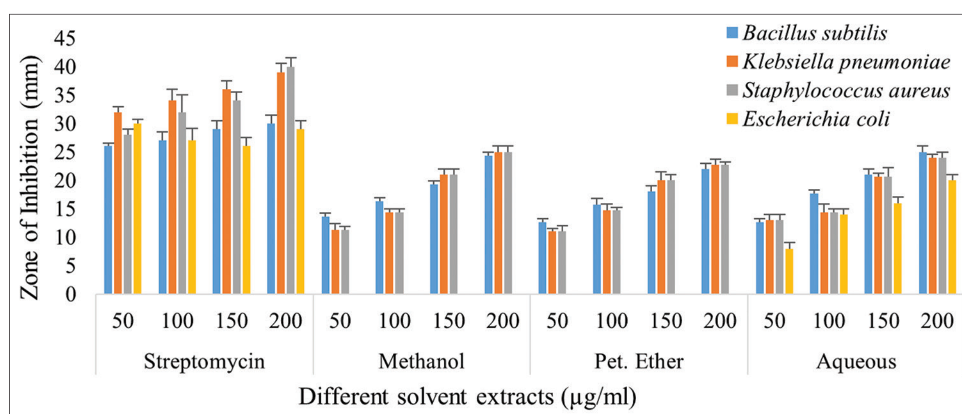

Fig. 5: Antibacterial activity of *Hemidesmus indicus* against different bacterial strains.

the roots of *D. hamiltonii* and *H. indicus,* which is responsible for its aromatic nature. The results are concordance with reports of Kundu and Mitra [25], where aqueous:methanol extracts of *H. indicus* root showed a high amount of methoxybenzaldehydes, which shows promising inhibitory potential against acetyl cholinesterase enzyme.

Antibacterial activity

The antibacterial activity of different extracts and their effectiveness was quantitatively assessed by the presence or absence of a zone of inhibition. The maximum zone of inhibition was recorded in methanolic extract of root against *S. aureus* (25.00 ± 1.00 mm), petroleum ether extract against *K. pneumoniae* (24.00 ± 1.00 mm), and aqueous extract against *B. subtilis* (25.00 ± 1.00 mm), respectively (Fig. 5). The results of the work carried out by Kavitha *et al*. [8] reported that the roots of *H. indicus* showed effective growth of the zone of inhibition for *B. subtilis* in the methanolic extract and no zone of inhibition for other extracts. Similar results were reported by Mohan *et al.* [6] where the potent growth of zone of inhibition was observed in the methanolic root extract of *H. indicus.*

CONCLUSION

The present study indicates that *H. indicus* have many bioactive components such as alkaloids, phenols, flavonoids, tannins, and volatile oils. 2-hydroxy 4-methoxy benzaldehyde was observed in both root and root oil through GC-MS and HPLC method. The maximum content of 2H4MB was recorded in root oil, which might be helpful in the pharmaceutical and food industries. Further investigations are needed for *in vitro* multiplication and to enhance the phytoconstituents of roots using different promoters.

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AUTHOR'S CONTRIBUTIONS

Ashalatha has done a biochemical and antibacterial activity. Raja Shantha Reddy collected the plant material and prepared the extract. Raveesha coordinated the work and writing of the manuscript.

COMPETING INTEREST

Authors declared that they have no conflicts of interests.

REFERENCES

1. Chatterjee S, Banerjee A, Chandra I. *Hemidesmus indicus*: A rich source of herbal medicine. Med Aromat Plants 2014;3:1-3.
2. Hassan BA. Medicinal plants (importance and uses). Pharm Anal Acta 2012;3:10.
3. Singh R. Medicinal plants: A review. J Plant Sci 2015;3:50-5.
4. Ahvazi M, Sigaroodi FK, Charkhchiyan MM, Mojab F, Mozaffarian VA, Zakeri H. Introduction of medicinal plants species with the most traditional usage in Alamut region. Iran J Pharm Res 2012;11:185-94.
5. Balaji VK, Muthuswamy S, Easow JM. Qualitative and quantitative analysis of phytochemicals in *Hemidesmus indicus* (L.) R.Br. World J Pharm Pharm Sci 2017;6:1083-92.
6. Mohan CH, Sridhar S, Mohan B, Krishnaveni SB, Reddy SK. Antibacterial activity and phytochemical screening of *Hemidesmus indicus* L. BR. Int J Pure Appl BioSci 2015;3:221-5.
7. Joshi A, Lad H, Sharma H, Bhatnagar D. Evaluation of phytochemical composition and antioxidative, hypoglycaemic and hypolipodaemic properties of methanolic extract of *Hemidesmus indicus* roots in streptozotocin-induced diabetic mice. Clin PhytoSci 2018;4:7.
8. Kavitha BT, Ramachandra YL, Rai SP, Sudeep HV, Krishana S. Antibacterial activity of the root extracts of *Hemidesmus indicus*. Pharmacologyonline 2010;2:711-6.
9. Nagarajan S, Rao LJ. Determination of 2-hydroxy-4-methoxybenzaldehyde in roots of *Decalepis hamiltonii* (Wight and arn.) and *Hemidesmus indicus* R.Br. J AOAC Int 2003;86:564-7.
10. Samydurai P, Saradha M. Effects of various solvent on the extraction of antimicrobial, antioxidant, phenolic from the stem bark of *Decalepis hamiltonii* Wight and Arn. Asian J Res Pharm Sci 2016;6:129-33.
11. Harbone JB. Phytochemical Methods. London: Chapman and Hall Ltd.; 1973. p. 33-80.
12. Mohan B, Nayak JB, Kumar RS, Kumar LP, Mohan C, Rajani B. Phytochemical screening, GC-MS analysis of *D. hamiltonii* Wight and Arn. An endangered medicinal plant. J Pharm Phytochem 2016;5:10-6.
13. Kota S, Govada VR, Anantha RK, Verma MK. An investigation into phytochemical constituents, antioxidant, antibacterial and anti-cataract activity of *Alternanthera sessilis,* a predominant wild leafy vegetable of south India. Biocatal Agric Biotechnol 2017;10:197-203.
14. Singleton VL, Orthofer R, Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Meth Enzymol 1999;299:152-78.
15. Hossain MA, Shah MD. A study on the total phenols content and antioxidant activity of essential oil and different solvent extracts of endemic plant *Merremia borneensis*. Arab J Chem 2015;8:66-71.
16. Medini F, Fellah H, Ksouri R, Abdelly C. Total phenolic, flavonoid and tannin contents and antioxidant and antimicrobial activities of organic extracts of shoots of the plant *Limonium delicatulum.* J Taibah Univ Sci 2014;8:216-24.
17. Rassem HA, Nour NA, Yunus RM. Techniques for extraction of essential oils from plants: A review. Aust J Basic Appl Sci 2016;10:117-27.
18. Mickymaray S, Al Aboody MS, Rath PK, Annamalai P, Nooruddin T. Screening and antibacterial efficacy of selected Indian medicinal plants. Asian Pac J Trop Biomed 2016;6:185-91.
19. Zishan A, Anwar S, Shiwali S. Evaluation of *in vitro* antioxidant activity, HPLC and GC–MS analysis along with chemoprofling of *Decalepis arayalpathra*: A critically endangered plant of Western Ghats, India. Rend. Fis. Acc. Lincei 2017;28:711-20.
20. Johnson M, Wesely EG, Hussain MI, Selvan N. *In vivo* and *in vitro* phytochemical and antibacterial efficacy of *Baliospermum montanum* (Willd.) Muell. Arg. Asian Pac J Trop Med 2010;3:894-7.
21. Devi BR, Mohan CH, Manjula P, Kumar BK, Naresh B, Devi BP. Phytochemical and micropropagation studies in *Hemidesmus indicus* (L.) R. Br. J Indian Bot Soc 2014;93:76-81.
22. Samydurai P, Thangapandian V. Nutritional assessment, polyphenols evaluation and antioxidant activity of food resource plant *Decalepis hamiltonii* Wight and Arn. J Appl Pharm Sci 2012;2:106-10.
23. Banothu V, Adepally U, Lingam J. *In vitro* total phenolics, flavonoids contents, antioxidant and antimicrobial activities of various solvent extracts from the medicinal plant *Physalis minima* Linn. Int J Pharm Pharm Sci 2017;9:192-8.
24. Arunodaya HS, Krishna V, Shashikumar R, Kumar KG. Antibacterial and antioxidant activities of stem bark essential oil constituents of *Litsea glutinosa* C.B. Rob. Int J Pharm Pharm Sci 2016;8:258-64.
25. Kundu A, Mitra A. Flavoring extracts of *Hemidesmus indicus* roots and *Vanilla planifolia* pods exhibit *in vitro* acetylcholinesterase inhibitory activities. Plant Foods Hum Nutr 2013;68:247-53.