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To perform phytochemical screening and study the antioxidant potential of isolated compound from *Hemidesmus indicus*

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ABSTRACT

"Anantmul" is an important and widely used medicinal plant. The study aimed to determine the physicochemical composition, bioactive compounds and antioxidant activity of *Hemidesmus indicus* (Asclepiadaceae). The roots of the plant were collected and sequentially extracted using petroleum ether, ethyl acetate, and methanol. The preliminary phytochemical screening of extracts was carried out and found to be a good source of bioactive compounds. Biological activities of flavonoid and phenolic compounds have been discovered in several latest studies. Further phytochemical isolation was carried out, and Lupeol was isolated. Phenols are shown to be multifunctional antioxidants which will perform as singlet oxygen quenchers. Lupeol was evaluated for in-vitro antioxidant activity. It showed a correlation with antioxidant activity by DPPH (IC_{50} = 0.52, P 6 0.05) and H_2O_2 (IC_{50} = 0.43, P 6 0.05). The results show promising perspectives for the exploitation and use of anantmul rhizome as a constituent of anti-aging as well as anticancer diet.

Keywords: Anantmul, Lupeol, Antioxidant, DPPH

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INTRODUCTION

Plants drugs are traditionally utilized in customary therapeutic frameworks; ethnomedicine, folk remedy, and herbalism give a rational and obvious source of contenders for focused identification of lead substances with unique structures, combinations, and mechanisms of action.

They moreover have the additionally preferred standpoint that, as a medicament, their safety and efficacy profiles are appropriately settled through old use or long time span human experience[1].

Hemidesmus indicus universally accepted as Indian sarsaparilla (figure 1, 2 and 3), belonging to family Asclepiadaceae. Its vernacular name "Anantmul" may be an Indic word which implies 'endless root' [2].

Figure 1 *Hemedesmus indicus* plant

Figure 2 *Hemedesmus indicus* flower

Figure 3 *Hemedesmus indicus* root

H. indicus may be a slender laticiferous, twig, typically prostrate or semi-erect woody plant, occurring in greater part of Indian Subcontinent. Anantamul is often distinguished by its slender, twisted, rigid, cylindrical and aromatic root and rhizome. Its bark is rust-colored and corky.

Furthermore, it has wrinkled with annulated cracks. Its stems are varied, slender, terete, thickened at the nodes. Leaves are opposite, variable, elliptic-oblong to linear-lanceolate, usually variegated with white above and pubescent beneath. Flowers are light-green outside and deep purple within huddled in subsessile axillary cymes. Follicles are slender, four inches long, cylindrical, typically arciform and divaricate. Its seeds are numerous, black planate. Phytoconstituents of *H. indicus* ranges from hydrocarbons, glycosides, oligoglycosides, and terpenoids to steroids[3].

In folk medicine, the root of *Hemidesmus indicus* R. Br. is reported as aphrodisiac, antipyretic, anti-diarrheal, alleviates leprosy, leucoderma, skin diseases, and useful in piles. Further, it is also used as diuretic; in the joints- pain, syphilis, and leucoderma. The leaves are good for vomiting, cold, wounds, leucoderma- the stem has a bitter bad taste; diaphoretic, diuretic, laxative; lessens inflammation; good for diseases of the brain, the liver, the kidney; useful in syphilis, gleet and urinary discharges, uterine complaints, leucoderma, paralysis, cough, asthma; gargle good for toothache.[2]

H. indicus roots have been reported for many pharmacological actions, most notably antimicrobial activity[4], antioxidant[5], wound healing activity[6], antihyperglycemic, antidyslipidemic [7], anti-arthritic activity[8], Cytotoxic activity[9] to cite a few.

This study aimed to perform the phytochemical screening and characterization of the isolated compound from *H. indicus* root, followed by the evaluation of its antioxidant activity.

MATERIALS AND METHODS

Collection and Authentication of Plant Material

Sample collection: Roots and rhizome powder of *Hemidesmus indicus* R.Br. (locally called Anantmul) were obtained and authenticated from NISCAIR-PUSA (Ref. no. NISCAIR/ RHMD/ consult/2013/2224/05).

Preparation of Anantmul Extracts

Dried powder of *H indicus* (100 gm) was exhaustively extracted with 500 ml petroleum ether and then with methanol in Soxhlet apparatus for 24 hours and dark brown residue (3.7 gm) was obtained after evaporation of the solvent. The dried extract (HIME) was stored in amber colored airtight container at 2.0°C temperature[10].

Preliminary Phytochemical Study

For the identification of various phytochemical constituents, the different extracts were subjected to qualitative tests as per the standard procedure[11].

Isolation and purification of the compound.

A small quantity of HIME was dissolved in chloroform, and the solution was spotted on TLC plates. The plates were developed using several solvent systems; notably, Hexane / Chloroform (9:1) and Chloroform / Ethylacetate (5:1) gave better separation of the components and were used in the TLC monitoring of the Column Chromatography. 10g of the chloroform fraction (CF) was subjected to column chromatography on a silica gel (60 – 120 mesh) with gradient elution using Hexane and Chloroform[12,13]

Spectroscopic Characterization

Different spectroscopic methods were used to elucidate the structure of HM_1, including IR, ^{1}H NMR, and ^{13}C NMR techniques. The IR spectrum was recorded on a Jasco FTIR V 460 plus spectrometer using Diffuse Reflectance Attachment, the ^{1}H NMR spectra were recorded on Varian Mercury YH 300 (300 MHz FT NMR), and ^{13}C NMR spectra were recorded on a JEOL GSX 400 NB, 400 MHz FT NMR spectrometer in deuterated chloroform with TMS as an internal standard.

Antioxidant Activity Assessment

In-Vitro Antioxidant Activity conducted on H indicus extracts was DPPH (2, 2-diphenyl-picryl-hydrazine) test as per Silva[14] and H_2O_2 assay as per Yang[15] using ascorbic acid as standard. All the studies were carried out in triplicate.

RESULTS

Preliminary Phytochemical Analysis

The study results showed a spectrum of secondary metabolites (Table 1). It was also determined that extracts of *H indicus* contained a high concentration of secondary metabolites like Terpenoids, Saponins Flavonoids, Glycosides, Phytosterols, Tannins, all of which were reported to have antioxidant as well as cytotoxic properties.[16]

Table 1: Phytochemical Analysis of *Hemidesmus indicus*

S. No	Compounds	Analysis
1	Alkaloids	+ +
2	Carbohydrate	-
3	Fats & Oils	-
4	Flavonoids	++
5	Glycosides	++
6	Protein & amino acid	+
7	Phenols	+
8	Phytosterol	++
9	Resins	+
10	Saponin	+++
11	Tannins	++
12	Terpenoids	+++

Isolation and purification of the compound

A total of seventy collections, based on their TLC profiles, were made and pooled into half - dozen major fractions. Fraction four suggested a greater proportion of the interest compound and was further purified by preparatory TLC using the hexane/ethyl acetate solvent system (9:1). A uniform spot with two different Hexane / Ethylacetate solvent systems (9:1) and (5:1) was obtained on TLC. This compound, coded (HM_1), appeared as white needles and was subjected to spectral analysis.

Chemical Characterization result by various spectroscopy:

Different spectroscopic methods were used to elucidate the structure of HM_1.The Spectral data is presented in Table 2.

Table 2: Various Spectroscopy techniques result data

S. N.	Spectroscopy technique	Data
1	FTIR ($CDCl_3$)	3139.59cm^{-1}(br, OH), 2945.31 cm^{-1}, 2872.60 cm^{-1}(C-H Str. In CH_3 and CH_2), 1637.98 cm^{-1}(C=C Str.) 1453.15 cm^{-1}(C-H deformation in CH_2/CH_3), 1400.31 cm^{-1}(C-H deformation in gem dimethyl), 1043.20cm^{-1}(C-O Str. Of secondary alcohol), 880.06cm^{-1}(exocyclin CH_2)
2	^{1}HNMR ($CDCl_3$)	δ 4.665(s,1H, H-29), δ 4.583(s,1H,H-27), δ 3.202(d,1H, H-3), δ 2.370(m,1H,H-19), δ 1.938(m,1H, H-21), δ 1.704(s,3H,H-30), δ 1.679(t,1H,H-13), δ 1.626(s,3H,H-2A), δ 1.542(s,3H,H-2B), δ1.519(d,1H,H-11), δ1.418(d,1H,H-14), δ 1.389(q,1H,H-6), δ 1.335(s,1H,H-21),δ1.287(s,1H,H-9), δ1.253(s,1H,H-9), δ 1.055(s1H,H-23), δ 1.018(d,1H,H-15), δ0.989(s,3H,H-23), δ0.966(s,3H,H-27), δ 0.905(t,1H,H-18), δ0.879(s,3H,H-25), δ 0.787(s,3H,H-28), δ0.691(d,1H,H-5)
3	^{13}C NMR (CDCL3)	δ 151.178, δ109.481,δ 79.135,δ 55.477, δ48.493, δ43.014, δ41.031, δ37.354, δ34.463, δ30.019, δ27.626, δ25.334, δ21.108, δ19.490, δ14.760

These assignments are in good agreement for the structure of lupeol as per Jain[17].

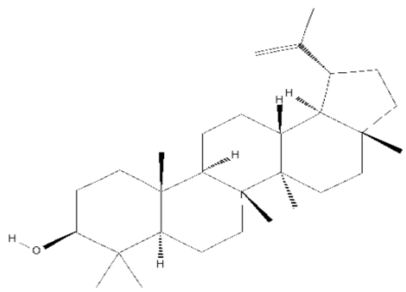

Figure 4: Lupeol : pentacylic tri-terpenoid

Antioxidant Activity Assessment

Mostly because of the complex nature of phytochemicals, the antioxidant effects of plant products must be measured by incorporating two or more different in vitro assays to acquire satisfactory data. Each of these tests is based on one feature of the antioxidant activity, such as the ability to scavenge free radicals, or the metal ion chelation. The results are presented in Table 3 and 4. Overall, our study indicates that the high antioxidant properties of lupeol obtained from *H. indicus* root extract and may inhibit cellular lipid peroxidation and ameliorate other oxidative damage caused by free radicals[18].

Table 3: Antioxidant activity of Lupeol by H_2O_2 Free Redical Scavenging Activity

S.No.	Dose µg/ml.	% Inhibitor	IC_{50}
1	Lupeol 10	35.76±0.963	30 µg/ml
2	Lupeol 25	45.62±1.043	30µg/ml
3	Lupeol 50	74.76±1.873	30µg/ml

Table 4: Antioxidant activity of Lupeol by DPPH Radical Scavenging Activity.

S.No.	Dose µg/ml.	% Inhibitor	IC_{50}
1	Lupeol 10	33.12±0.934	30 µg/ml
2	Lupeol 25	41.53±1.768	30 µg/ml
3	Lupeol 50	78.76±1.532	30 µg/ml

CONCLUSION

Phytochemical screening of the HIME showed the presence of triterpenoids, tannins, glycosides, flavonoids, polyphenols etc. Our findings strongly suggest that the Anantmul roots are promising sources of natural antioxidants, as indicated by their high contents of polyphenols, flavonoids, tannins, etc. All these classes of compounds have good antioxidant potential and their effects on human nutrition and health are significant. Also the considerable DPPH free radical-scavenging activities and H_2O_2 values of Lupeol further support these finding. Further studies m,ay be carried out to evaluate its in-vivo anti cancer potential.

Conflict of Interest: None

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