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RARE MEDICINAL PLANT HUGONIA BELLI SEDGW. PHYTOCHEMICAL PROFILING AND IN VITRO ANTIMICROBIAL PROPERTIES OF THE METHANOLIC LEAF EXTRACT

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ABSTRACT

Hugonia belli, popularly known as Modira kanni gida, is a rare plant species in India. The main objective of this study was to examine the phytochemical constituents and in vitro antimicrobial activity of this plant. Qualitative screening revealed the presence of phenols, alkaloids, flavonoids, tannins, terpenoids, proteins and carbohydrates. Liquid chromatography-mass spectroscopy screening of the leaf methanol extract revealed the presence of 39 bioactive phyto-constituents. Hermalin, Caffeic acid Mytragyine, Apigenin Di methyl ether, Colforsin, Ganoderal A, Wilfortide A, Spirostane, Fructose base, Alpha – L -Mannopyranoside derivative and adernosterone were the major identified compounds. The methanolic leaf extract sample showed moderate antidiabetic potential, with IC50 values of 153.80 and 94.15 µg/ml for α amylase and α-glucosidase inhibition, respectively. It exhibited dose-dependent antibacterial activity against Staphylococcus aureus and Escherichia coli, with no effect at 1 mg and increasing inhibition zones up to 20.66 mm and 20.33 mm at 4 mg, respectively. Kanamycin showed strong antibacterial activity at all tested concentrations, with inhibition zones increasing slightly with dose, peaking at 22.33 mm and 21.66 mm at 100 µg and the sample showed moderate antidiabetic potential with IC50 values of 153.80 µg/ml for α-amylase and 94.15 µg/ml for α-glucosidase inhibition.

KEYWORDS: Hugonia belli, Rare medicinal plant, Chemical profiling.

INTRODUCTION

Hugonia belli Sedgwick is a lesser-known, endemic liana species native to the Southern Western Ghats of India, primarily found in the evergreen and semi-evergreen forests of Karnataka, Tamil Nad,u and Kerala. It is listed as a "rare" species in the Red Data Book of Indian Plants because of its restricted distribution and limited population, making it ecologically significant and in need of conservation.[1]

As a member of the Linaceae family, H. belli grows as a woody climber and relies on other plants for support. It is particularly adapted to humid and shaded conditions in tropical forest understories. The leaves are alternate, elongated (7–20 cm long and 2–7 cm wide), and covered in rusty-brown, velvety hairs (ferruginous-tomentose), with clearly visible veins on both surfaces of the leaf. The petioles are hairy and measure 6–11 mm in length. Its stipules are slender, divided, and approximately 15 mm long.[2]

Flowering typically occurs between November and January in the southern hemisphere. The plant bears attractive yellow flowers, followed by oblong capsules with thin, membranous textures. Typically, only one seed matures per fruit due to natural seed abortion during development.[2]

Despite limited documentation, Hugonia belli is of considerable interest because of its potential medicinal value. Plants from the Western Ghats are known to be rich sources of bioactive compounds, and H. belli is no exception. This study explores the methanolic leaf extract of H. belli.

To investigate its phytochemical composition and potential biological activity. Advanced techniques, such as LC-MS profiling, will help identify key secondary metabolites. In parallel, bioassays, such as antibacterial and enzyme inhibition tests (e.g., α-amylase and α-glucosidase), are conducted to evaluate therapeutic potential. These insights aim to support the future development of plant-based treatments for microbial infections and metabolic disorders, such as diabetes.[3,4] Given the increasing interest in plant-derived pharmaceuticals, the documentation and study of rare and endemic species, such as H. belli, could lead to the discovery of novel compounds with pharmacological significance. Thus, this study contributes to ethnopharmacology and aids biodiversity conservation through scientific validation.

MATERIALS AND METHODS

Collection of Plant Material

The leaves of Hugonia belli were collected in September from the evergreen forests of the Southern Western Ghats. The plant material was thoroughly washed with distilled water to remove dirt and dust. The leaves were shade-dried for 20 d to ensure complete moisture removal. After drying, the leaves were finely powdered to obtain a homogeneous sample.[5]

Preparation of Methanolic Extract

The powdered leaf material (25 g) was subjected to Soxhlet extraction using methanol as the solvent. A total of 250 mL of methanol was used for the extraction process, which was performed for 13 cycles. After extraction, the obtained extract was concentrated by evaporating the solvent in a water bath until a dry residue was obtained.[6, 7]

Preliminary phytochemical profiling

Initially, the sample underwent a qualitative phytochemical examination through standard chemical tests to identify the different classes of compounds it contained. Phenols were tested by adding lead acetate solution, which indicated their presence by precipitate formation.[8] Alkaloids were detected using Wagner's and Dragendorff's reagents, which produce characteristic precipitates.[8, 9] Flavonoids were identified using the alkaline reagent and lead acetate tests, based on color changes and precipitation.[9] Tannins were tested using a ferric chloride solution that formed a color change.[8] Terpenoids were detected using the Salkowski test, which involves chloroform and concentrated sulfuric acid.[10] Saponins were identified using the foam test after vigorous shaking with distilled water.[9] Glycosides were detected using the Keller-Killiani test, which produces a brown ring in the presence of glacial acetic acid, ferric chloride, and sulfuric acid.[8] Steroids were identified using the Liebermann-Burchard test, based on color change in the presence of chloroform and sulfuric acid.[10] Proteins were tested using the Biuret test with sodium hydroxide and copper sulfate.[9] Carbohydrates were detected by heating the sample with Benedict's reagent, which produces a color change upon reaction.[8]

Liquid chromatography Mass spectroscopic profiling

Chemical profiling of the methanolic leaf extract of Hugonia belli was performed using Liquid Chromatography-Mass Spectrometry (LC-MS) to identify its bioactive constituents. The analysis was conducted using a Waters Acquity UPLC system coupled with a Waters Synapt G2 Mass Spectrometer.[11] The mobile phases used were 10 mM ammonium acetate in

water (Mobile Phase A) and acetonitrile (Mobile Phase B).[12] For sample preparation, 10 mg of the methanolic extract was dissolved in methanol and diluted to 10 mL, followed by filtration, and a 10 µL aliquot was injected into the LC system.[13] A gradient elution program was employed at a constant flow rate of 0.40 mL/min. The run started with 98% A and 2% B, held for 2 min, gradually changed to 50% A and 50% B at 6 min, and then to 2% A and 98% B by 10 min. This was maintained for 15 min, after which the initial conditions were restored at 17 min and maintained until 20 min.[11,13]

Antibacterial Activity

The antimicrobial activity of the methanolic extract was evaluated using the agar well diffusion method, as described by Ranjitha et al. (2023), with slight modifications.[14] The bacterial strains used for screening included Gram-positive Staphylococcus aureus (MTCC-7443) and Gram-negative Escherichia coli (MTCC-7410). The bacterial inoculum was adjusted to approximately 5×10^5 CFU/mL using a sterile saline solution. The extract was prepared as a stock solution of 20 mg/mL in dimethyl sulfoxide (DMSO), and varying concentrations (100–400 µg) were tested in separate wells. Mueller-Hinton agar was used as the culture medium, and the plates were incubated at 37°C for 24 h. Following incubation, the diameter of the inhibition zones (in mm) was measured to assess the antibacterial activity.[14,15]

Antidiabetic activity

The inhibitory activity of the methanolic leaf extract against carbohydrate-digesting enzymes was assessed using in vitro assays. α-Glucosidase inhibition was performed according to the protocol described by Patil et al. with minor modifications.[16] The enzyme solution (1 U/mL), prepared in 50 mM phosphate buffer (pH 6.9), was pre-incubated with different concentrations of the extract for 10 min at 37°C.[17] The reaction was initiated by adding p-nitrophenyl-α-D- glucopyranoside and allowed to proceed for 30 min before termination with sodium carbonate.[18] Absorbance was measured at 405 nm to determine the enzyme activity. For α-amylase inhibition, the procedure was performed according to Gharge et al.[17], using porcine pancreatic α-amylase (1 U/mL) in a phosphate buffer. After pre-incubation with various concentrations of the extract, the reaction was initiated by adding starch solution as the substrate.[19] The reaction was stopped using dinitrosalicylic acid (DNS) reagent, followed by boiling and the addition of potassium sodium tartrate.[20] Absorbance was then recorded at 540 nm. In both assays, a standard inhibitor (acarbose) was used as a positive contro.[16,20]

RESULTS

Preliminary qualitative phytochemical analysis

Preliminary qualitative phytochemical analysis of the extract samples revealed the presence of several bioactive compounds. As shown in **Table 1**, phenols, alkaloids, flavonoids, tannins, terpenoids, proteins, and carbohydrates were detected, whereas saponins, glycosides, and steroids were absent.

LC - MS Analysis of methonolic leaf extract of H.belli

Liquid chromatography-mass spectrometry (LC-MS) analysis of the extract sample revealed the presence of a wide variety of bioactive compounds. These compounds were detected at different retention times and exhibited distinct molecular structures. The analysis identified a complex mix of chemical classes, including medium- and long-chain fatty acids, alkaloids, flavonoids, triterpenoids, steroidal saponins, diterpenoids, and other specialized metabolites. This chemical diversity suggests that the extract may have multiple pharmacological properties and Potential therapeutic value. A detailed summary of the major identified compounds, along with their retention times, molecular formulas, and chemical classes, is presented in **Table 2**.

Antibacterial of leaf Methonolic extract of H.belli

The antibacterial activity of the HL sample was tested against Staphylococcus aureus and Escherichia coli at different concentrations (1–4 mg). The results showed no activity at lower concentrations but increased inhibition at higher concentrations. Kanamycin was used as the standard and exhibited consistent antibacterial activity across all tested concentrations. The zone of inhibition results are summarized in Table 3.

Antidiabetic Properties of leaf Methonolic extract of H.belli

The HL sample demonstrated inhibitory effects on enzymes involved in carbohydrate digestion, indicating its potential antidiabetic activity. Specifically, HL showed an IC50 value of 153.80 µg/ml for α-amylase inhibition and 94.15 µg/ml for α-glucosidase inhibition, as summarized in **Table 4**.

DISSCUSION

Qualitative phytochemical analysis of the HL extract revealed the presence of several important bioactive compounds, including phenols, alkaloids, flavonoids, tannins, terpenoids, proteins, and carbohydrates (Table 1). These phytoconstituents are known for their diverse

pharmacological properties, such as antioxidant, antimicrobial, anti-inflammatory, and anticancer activities, which may contribute to the therapeutic potential of the extract (21). The absence of saponins, glycosides, and steroids indicates that these compounds are either absent or present in negligible amounts in the HL extract. Phytochemical profiling is essential as it provides a basis for understanding the biological activities and medicinal uses of plant extracts.

Table 1: Qualitative Phytochemicals Estimation of Extract Sample.

Sl No	Phytochemical Tests	Result
1	Phenols	+
2	Alkaloids	+
3	Flavonoids	+
4	Tannins	+
5	Terpenoids	+
6	Saponins	–
7	Glycosides	–
8	Steroids	–
9	Proteins	+
10	Carbohydrates	+

Note: "+" indicates presence of the phytochemical; "–" indicates the absence of the phytochemical.

LCMS analysis provided an in-depth profile of the phytochemical constituents in the sample, revealing a complex mixture of bioactive compounds spanning diverse chemical classes, such as medium- and long-chain fatty acids, alkaloids, flavonoids, triterpenoids, and steroidal saponins. This chemical complexity underscores the multifaceted therapeutic potential of this extract.

Medium-chain fatty acids, such as nonic acid, and long-chain fatty acids, such as octadecanedioic acid, are well documented for their anti-inflammatory and antimicrobial properties. These fatty acids are integral components of many natural products used to treat microbial infections and inflammation-related disorders.[22, 23] Their role in modulating inflammatory pathways contributes to wound healing and immune system support in the body.

Alkaloids such as Harmaline and Mitragynine, which were detected in the sample, are bioactive compounds with diverse pharmacological activities. Harmaline, a β-carboline alkaloid, possesses neuroprotective effects, antioxidant properties, and potential anticancer

activity by inducing apoptosis in cancer cells.[24] Mitragynine, a major alkaloid found in Mitragyna speciosa, is recognized for its analgesic and anti-inflammatory effects, and ongoing studies are exploring its potential in pain management and opioid substitution therapy.[25]

Flavonoids, including caffeic acid and apigenin derivatives, are well-known for their potent antioxidant activities, free radical scavenging, and reduction of oxidative stress, which play a crucial role in chronic diseases such as cardiovascular disorders, cancer, and neurodegeneration.[26] These compounds also exhibit anti-inflammatory and cardioprotective effects, enhancing the overall therapeutic value of the extract.

Triterpenoids and steroidal saponins, such as Ganoderal A and Wilforlide A significantly contribute to the immunomodulatory and anticancer activities of medicinal plants. Triterpenoids have been shown to regulate immune responses and inhibit tumor growth by modulating multiple molecular targets.[27] Steroidal saponins exhibit cytotoxic activity against various cancer cell lines and possess anti-inflammatory properties.

Moreover, the presence of macrolides and diterpene lactones in the extract indicates additional antimicrobial and cytotoxic potential, supporting their traditional use in treating infections and tumors. These compounds disrupt microbial cell walls and interfere with cellular signaling pathways in cancer cells.[28]

Taken together, the diverse phytochemical profile identified by LC-MS suggests that the sample possesses a broad spectrum of biological activities. The synergistic interactions among these compounds may enhance the therapeutic efficacy of the extract, justifying the need for further detailed pharmacological and clinical studies to explore their medicinal applications.

Table 2: LCMS Identified Compounds with Retention Times and Molecular Formulas.

Sl No	Proposed Compounds	Retention Time (min)	Precur sor Ion	Adduct	Formula	Ontology
1	Nonic Acid	0.372	227.0023	[M+H]+	C9H16O4	Medium-chain fatty acids
2	1-tert-butyl-3-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine	1.049	268.1613	[M+H]+	C15H17N5	Phenylpyraz oles
3	Prolintane	4.398	218.1994	[M+H]+	C15H23N	Amphetamines and derivatives
4	(1R,3r,8S,9r)-1,8-dimethyl-3,6-diazatricyclo [4.3.1.1(3,8)] undecan-9-ol	5.142	197.1619	[M+H]+	C11H20N2O	1,4-diazepanes
5	N-(9-oxodecyl)ac etamide	5.447	214.1701	[M+H]+	C12H23NO2	Acetamides
6	Harmaline	5.514	215.1176	[M+H]+	C13H14N2O	Harmala alkaloids
7	Caffeic acid	6.461	181.1655	[M+H]+	C9H8O4	Hydroxycin na mic acids
8	(2S,3S)- 3,5,7- trihydroxy-2-(4- hydroxyphe nyl)-2,3-dihydrochro men-4-one	6.698	289.1935	[M+H]+	C15H12O6	Flavanonols
9	Cadin-4-En-10-Ol	6.901	261.1627	[M+K]+	C15H26O	Sesquiterpen oids
10	Mitragynine	6.935	399.2446	[M+H]+	C23H30N2O4	Corynanthea n-type alkaloids
11	1,4-dioxacycloh exadecane-5,16-dione	6.969	257.1792	[M+H]+	C14H24O4	Macrolides and analogues
12	Canavanine	7.003	177.0978	[M+H]+	C5H12N4O3	L-alpha-amino acids
13	(9Z,12E)-15,16-Dihydroxyoc tadeca-9,12-dienoic acid	7.172	313.2325	[M+H]+	C18H32O4	Linoleicacid s and derivatives
14	Indene-3-carboxylic acid	7.273	209.1618	[M+H]+	C13H20O2	Carboxylic acids
15	2,4- dihydroxy- 5-methoxy-1",6",8"- trimethyldis piro[bis(oxo la ne)-3,2':5',7"- [3]oxatricycl o[6.3.1.0?,??] dodecane]-2"-one	7.815	397.2229	[M+H]+	C21H32O7	O-glycosyl compounds
16	COLFORSIN	8.221	411.2418	[M+H]+	C22H34O7	Triterpenoids
17	Spirostane	8.288	883.4632	[M+H]+	C45H70O17	Steroidal saponins
18	Oxyphenoni um	8.356	349.254	[M+H]+	C21H34N O3	Benzeneand substituted derivatives
19	Apigenin Dimethyl Ether	8.458	299.2156	[M+H]+	C17H14O5	7-O-methylated flavonoids
20	Furostane base -2H + O-Hex	8.542	577.3824	[M-H2O+H]+	C33H54O9	Steroidal saponins
21	9,13- Epoxy-3,15,16,18-	8.559	357.2628	[M+H]+	C20H36O5	Diterpenoids

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22	Adrenostero ne	8.796	301.1942	[M+H]+	C19H24O3	Androgens and derivatives
23	Ganoderal A	8.83	459.3166	[M+Na]+	C30H44O2	Triterpenoids
24	Wilforlide A	8.897	455.3425	[M+H]+	C30H46O3	Triterpenoids
25	Octadecane dioic acid	9.033	315.2461	[M+H]+	C18H34O4	Long-chain fatty acids
26	(2R,3R,4R, 5R,6S)-2-[[(2R,3S,4S ,5R,6S)-6-[(2R,3R,4R, 5S,6S)-2-[(2E,6E,10 E)-14-[(2S,3R,4S, 5S,6R)-4,5-dihydroxy-6- (hydroxyme thyl)-3-[(2S,3R,4S, 5S,6R)- 3,4,5- trihydroxy-6- (hydroxyme thyl)oxan-2- yl]oxyoxan-2-yl]oxy-2,6,10,14- tetramethyl hexadeca-2,6,10,15-tetraenoxy]- 3,5-dihydroxy-6- methyloxan -4-yl]oxy- 3,4,5-trihydroxyo xan-2-yl]methoxy] -6-methyloxan e-3,4,5-triol	9.1	1085.532	[M+H]+	C50H84O2 5	Diterpene glycosides
27	Tetrahydrog ambogic Acid	9.168	671.3373	[M+H]+	C38H48O8	Pyranoxanthones
28	alpha-L-Mannopyra noside, (1beta,3alph a,8xi,9xi,14 xi ,25S)-3-hydroxyspir ost-5-en-1-yl6-deoxy-2-O-(6- deoxy-alpha-L-mannopyran osyl)-	9.27	723.4364	[M+H]+	C39H62O1 2	Steroidal saponins
29	(1R,4aS,5R, 8aS)-5-(5-hydroxy-3-methylpenty l)-1,4a- dimethyl-6-methylidene - 3,4,5,7,8,8a-hexahydro-2H-naphthalene -1- carboxylic acid	9.337	323.2582	[M+H]+	C20H34O3	Diterpenoids
30	2alpha-acetoxy-3beta,22alp ha-stictandiol	9.439	520.4313	[M+NH4]+	C32H54O4	Sesquiterpe noi ds
31	Scytophycin A	10.792	804.5237	[M+H-H2O]-	C45H75NO12	Diterpene lactones
32	Ferri triacetylfusa rinin C	11.807	906.3366	[M+H]+	C39H57N6O15	Macrolacta ms
33	Harmaline	5.517	213.1119	[M-H]-	C13H14N2O	Harmala alkaloids
34	(2R,7S,13R, 14R,16S,19 R, 20S)-19- (furan-3-yl)-9,9,13,20- tetramethyl-5,12,17- trioxo-4,8,15,18-	5.923	527.0614	[M-H]-	C28H32O1 0	Limonoids

	tetraoxahex acyclodocos an -11-yl acetate					
35	(2E,4E)-12- hydroxy-13-(hydroxyme thyl)-3,5,7-trimethyltetr adeca-2,4-dienedioic acid	8.969	341.2538	[M-H]-	C18H30O6	Long-chain fatty acids
36	(2R,3S,4S,5 R,6R)-2-(hydroxyme thyl)-6-[(E)-3-(4- hydroxyphe nyl)prop-2-enoxy]oxan e-3,4,5-triol	9.679	311.2438	[M-H]-	C15H20O7	Fattyacyl glycosidesof mono- and disaccharide s
37	[(4E)-7- acetyloxy-6-hydroxy-2-methyl-10-oxo-2,3,6,7,8,9- hexahydroo xecin-3-yl] (E)-but-2-enoate	10.018	325.2613	[M-H]-	C16H22O7	Tricarboxyli c acidsand derivatives
38	4-methoxy-6-[2-[4-(3-methylbut-2-enoxy)phenyl]ethyl]-1,3-benzodioxole	10.526	339.2743	[M-H]-	C21H24O4	Stilbenes
39	Sphingomy elin	12.253	763.6074	[M+CH3C OO]-	C39H81N2 O6P	Phosphosphingolipids

The antibacterial activity of the HL extract was evaluated against Staphylococcus aureus and Escherichia coli at different concentrations ranging from 1 to 4 mg. The results clearly show a concentration-dependent antibacterial effect.

For Staphylococcus aureus, the HL extract showed no activity at 1 mg, weak inhibition at 2 mg (9.66 mm), and significantly stronger activity at 3 mg (16.33 mm) and 4 mg (20.66 mm). This suggests the presence of bioactive compounds in the HL extract that effectively inhibit the growth of gram-positive bacteria, with increased potency at higher doses.

In the case of Escherichia coli, no antibacterial activity was observed at 1 and 2 mg. However, the extract demonstrated moderate (3 mg, 15.66 mm) and strong (4 mg, 20.33 mm) inhibition. The ability of the extract to inhibit E. coli, a gram-negative bacterium known for its resistance due to its outer membrane, indicates the presence of potent antimicrobial agents capable of breaching or disrupting bacterial defenses.

These findings align with previous research showing that phytochemicals, such as alkaloids, flavonoids, and terpenoids, possess antimicrobial properties (29, 30, 31). The dose-dependent nature of the antibacterial activity is consistent with the concentration and diversity of these compounds in the extract, as previously identified via LC-MS analysis. Thus, the HL extract

shows promising potential for development as an antibacterial agent, particularly for infections involving S. aureus and E. coli.

Table 3: Antibacterial Activity of Hugonia belli Extract Against Staphylococcus aureus and Escherichia coli.

Sl No	Concentration	Staphylococcusaureu s (Zoneof Inhibition in mm)	Escherichiacoli(Zon eof Inhibition in mm)
1	1 mg	---	---
2	2 mg	9.66	---
3	3 mg	16.33	15.66
4	4 mg	20.66	20.33

Note: '---' indicates no antibacterial activity at the tested concentration.

Hugonia belli leaf extract exhibited significant inhibitory effects against α-amylase and α-glucosidase, with IC50 values of 153.802 and 94.149 µg/ml, respectively. These enzymes are pivotal in carbohydrate digestion, breaking down complex polysaccharides into absorbable monosaccharides, thereby playing a crucial role in regulating postprandial blood glucose levels.[32, 33] The relatively stronger inhibition of α-glucosidase suggests that HL extract could effectively delays glucose absorption in the intestines, contributing to the reduction of post-meal hyperglycemia, a major therapeutic target in diabetes management.[34, 37]

The presence of various bioactive phytochemicals, such as flavonoids, phenols, and terpenoids, identified in Hugonia leaf extract through qualitative and LCMS analyses, is likely responsible for this enzyme inhibitory activity.[21, 35] Numerous studies have shown that these phytochemicals exhibit antidiabetic properties by modulating enzyme activity and enhancing insulin sensitivity.[38, 39]

Furthermore, plant-derived α-amylase and α-glucosidase inhibitors are gaining attention because of their lower side effects than synthetic drugs, highlighting the potential of HL extract as a natural alternative or complementary treatment for diabetes.[40, 41]

Table 4: Antidiabetic Activity of Hugonia belli leaf Extract Based on Enzyme Inhibition Assays.

Sl No	Enzyme	IC50 (µg/ml)	Interpretation
1	α-Amylase Inhibition	153.802	Moderate inhibitory activity
2	α-Glucosidase Inhibition	94.149	Stronger inhibitory activity

CONCLUSION

The Hugonia belli leaf extract showed a rich presence of useful plant compounds like phenols, flavonoids, alkaloids, and terpenoids. These are known for their health benefits, particularly their antioxidant and medicinal effects.

LC-MS analysis confirmed that the extract contained various bioactive compounds, including fatty acids, flavonoids, alkaloids, saponins, and triterpenoids. These compounds are likely responsible for the biological activities observed in this study.

In the antibacterial tests, the HL extract was effective against Staphylococcus aureus and Escherichia coli, with stronger effects at higher doses. This suggests that it may function well as a natural antibacterial agent. The extract also showed a good ability to block the enzymes α-amylase and α-glucosidase, which are important in controlling blood sugar levels, indicating possible antidiabetic benefits.

Overall, the study shows that HL extract has the potential to be used for both antibacterial and antidiabetic applications. More research, including animal studies and clinical trials, is needed to better understand its full medical value.

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