Pharmacogn. Res., 2025; 17(2):679-688.
https://www.phcogres.com

Original Article

FTIR Analysis, Total Phenolic Content, Antioxidant and Antidiabetic Activities of *Hildegardia populifolia* (Roxb.) Schott and Endl. (Malvaceae)

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ABSTRACT

Background: Diabetes has become a major public health issue in developing countries, with rising prevalence in urban populations. Type II diabetes, the most common form, can be managed with medication, diet and exercise. In India, Ayurvedic medicine utilizes botanicals for treating various conditions. *Hildegardia populifolia*, an endangered plant, has shown promise due to its therapeutic properties. **Objectives:** The aim and objectives of this study were to identify the active constituents by preliminary phytochemical screening, characterise the functional groups using Fourier Transform Infrared Spectrosocpy, analyse the total amount of phenols and to study the antioxidant and antidiabetic activities of aqueous leaf extract of *Hildegardia populifolia*. **Materials and Methods:** Total phenolic content was measured with the Folin-Ciocalteu reagent and antioxidant activity was determined using DPPH free radical scavenging assay. Anti-diabetic potential was assessed by 3,5-dinitrosalicylic acid method using α-amylase inhibition assay. **Results:** Phytochemical study of the extract revealed the presence of phenols, flavonoids, glycosides, tannins and terpenoids. The total phenolic content was 27.05 mg Gallic Acid Equivalent (GAE) per gram of extract. FTIR analysis showed the presence of 16 peaks representing functional groups like OH, C=C, C=O, C-Br, etc. The plant extract demonstrated good antioxidant and anti-diabetic activities, likely to the standard, ascorbic acid and acarbose respectively. **Conclusion:** These findings suggest that *Hildegardia populifolia* has strong potential for future research in developing therapeutic agents with both antioxidant and anti-diabetic properties, contributing to the search for innovative treatments for diabetes.

Keywords: 3,5-dinitrosalicylic acid, α-amylase, Acarabose, Folin-Ciocalteu.

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Received: 14-11-2024;
Revised: 28-01-2025;
Accepted: 07-03-2025.

INTRODUCTION

Diabetes mellitus is an important threat to global human health and is considered as one of the leading noncommunicable diseases. Globally, an estimated 537 million adults aged 20 to 79 are currently living with diabetes (10.5% of all adults in this age range). By 2020, this number was expected to rise to 643 million people and by 2045, it will reach 783 million. In South-East Asian (SEA) countries, the occurrence of diabetes has been rising steadily for over 20 years and current projections have exceeded all previous forecasts, according to the Indian Development Foundation (IDF) 10th edition.[1] Of these, 90% of people have type 2 diabetes, which is rising consistently with the overall growth of diabetic population.[2] It is linked with several risk factors, including age, obesity, high systolic blood pressure, low levels of High-Density Lipoproteins (HDL), elevated triglycerides, marriage, low education, genetics and modern lifestyles.[3] One promising strategy for the prevention and treatment of type 2 diabetes is delaying the breakdown of carbohydrates in digestive system. The digestive enzyme, α-amylase breaks down polysaccharide glycosidic linkages into monosaccharides. Thus, blocking α-amylase can aid in delaying the breakdown of carbohydrates in small intestine and lowering blood sugar levels.[4] Interestingly, α-amylase inhibitors are naturally present in plants.

Few synthetic inhibitors of these enzymes, such as acarbose and voglibose, have been developed, however, these inhibitors do have some side effects, such as flatulence and digestive and liver function disorders.[5] Thus, it is preferable to use natural sources

DOI: 10.5530/pres.20252113

of inhibitors with no adverse effects are preferred. Numerous research studies have examined these phytochemicals potential as antidiabetic agents both *in vitro* and in animal models.[6] Efforts have been made to generate a physiologically functional food or lead chemicals for use in antidiabetic drugs by effective inhibition of α-amylase from natural sources.

The genus Hildegardia Scchott and Endl. comprises deciduous trees and is a member of the tribe Sterculieae and sub-family Sterculioideae of the larger Malvaceae family.[7] The genus comprises of 12 species with a pantropical distribution that includes West Africa, East Africa, Madagascar, southern India, Philippines, Indonesia, northern Australia and Cuba.[8] *Hildegardia populifolia* is the single species found in India distributed primarily in Andhra Pradesh, Tamil Nadu and a small area in Karnataka.[9] Previously this plant was named as *Sterculia populifolia*.[10] According to Nayar and Sastry (1990) information given in the Red data Book of Indian plants, this limited endemic species faces significant threats from both extrinsic and intrinsic sources.[11] It is an enigmatic species whose conservation status has been variously classified as critically endangered by World Conservation Monitoring Centre in 1998. Previously, it was known to be represented by a single surviving population of roughly 20 trees in Tamil Nadu's Kalarayan Hills and was classified as endangered. Rao *et al.*, in 1998, identified five subpopulations of this endangered species in the Rayalaseema district of Andhra Pradesh.[12] Later, in 2011, Rao and colleagues classified the species as Vulnerable.[13]

Stem bark from *Hildegardia populifolia* is valuable economically because the fibres are arranged in uniaxial manner, loosely connected and slight interlacing. In packaging, it serves as an alternative to glass, carbon and synthetic fibres. Because it degrades naturally, it can also be utilised as reinforcement in green composites.[14] The bark of this plant is used in traditional medicine to cure malaria and dog bites.[15,16] The plant exhibits strong antioxidant, antibacterial, anti-diabetic, antimalarial, anticancer and antiinflammatory properties. This plant has been shown to have alkaloids, tannins, saponins, terpenoids, flavonoids, glycosides and polyphenols as the main phytoconstituents.[17] Despite the plant's high medicinal potential, limited research has been done on it. In this article, we have provided a literature review of the pharmacological and phytochemical studies available, summarized in Tables 1 and 2. Based on the literature survey, no studies have yet addressed for Fourier Transform Infrared Spectroscopy (FTIR) analysis, antidiabetic activity, total phenol estimation and antioxidant activity for the aqueous leaf extract of the plant. We have therefore selected these studies to explore the medicinal potential of this endangered plant further.

MATERIALS AND METHODS

All chemicals used were of analytical reagent grade. 3,5 Dinitrosalicylic Acid (DNSA), gallic acid, Folin-Ciocalteu's reagent, 2,2-Diphenyl-1-Picrylhydrazyl (DPPH), α-amylase and Dimethylsulfoxide (DMSO) were purchased from SRL chemicals. UV-visible spectrophotometer (Shimadzu), FTIR (Bruker) and centrifuge (Remi) were used for the analysis.

Collection and authentication of plant material

Fresh leaves of *Hildegardia populifolia* were collected from Dr. BRR Government Degree College, Zadcherla, Telangana and their authenticity was confirmed by botanist Mr. Sadasivaiah, Associate Professor, Department of Botany, Dr. BRR Government Degree College, Zadcherla, Telangana, India.

Extract preparation

Aqueous leaf extract was prepared by the maceration process for 3 days followed by reflux extraction for 3 hr. The extract was dried in a vacuum oven at 60°C. This was used further for studies.[29]

Phytochemical Screening

In order to identify the presence of secondary metabolites that underline a variety of biological activities and the therapeutic qualities of plants and herbs, phytochemical screening is an initial and crucial step. To find out if secondary metabolites were present, the aqueous leaf extract was screened. Using the alkaline and Shinoda tests, flavonoids were found. Tannins, terpenoids and saponins were found using the phenazone, Salkowski and foam tests, in that order. The ferric chloride test and Mayer's test were used to identify phenolic chemicals and alkaloids, respectively. To find lipids and carbohydrates, Liebermann Burchard and Fehling's test were used. The biuret test was used to test proteins.[30]

FTIR analysis

FTIR spectrum of the aqueous leaf extract of *Hildegardia populifolia* was obtained using a CE Bruker FTIR spectrophotometer, model Alpha (2 01054), with Opus software (version 7.5 Build). The spectrum was recorded over a wavelength range of 400-4000 cm^{-1}.[31]

Total phenolic content

Folin-Ciocalteu's method was used to detect total phenolic content of *Hildegardia populifolia* spectrophotometrically. After combining 100 µL of the extract (1 mg/mL) with 100 µL of Folin-Ciocalteu's phenol reagent, the mixture was left for 5 min. Next, 1.3 mL of deionised water and 1 mL of 7% Na_2CO_3 solution were added to the reaction mixture. The mixture was stored in the dark at room temperature for 90 min. At 750 nm, the absorbance was measured. Equivalents of Gallic Acid (EGA) were used to quantify the number of phenolic compounds found in one

Table 1: Literature review of *Hildegardia populifolia* on pharmacological activities.

Name of Activity	Solvent	Part of the Plant	Model	Method	Standard	References
Anti-inflammatory	Methanol	leaves	Carrageenan, formalin and histamine induced paw edema.	*In vivo* Wistar rats	indomethacin	[17]
	Methanol	Stem bark	Carrageenan, formalin and histamine induced paw edema.	*In vivo* Wistar rats	---	[18]
Analgesic	Methanol	leaves	Acetic acid induced writhing response hot plate method.	*In vivo* Swiss albino mice	Aspirin and Pentazocine	[17]
Anti-nociceptive	Methanol	Stem bark	Acetic acid induced writhing response hot plate method.	Swiss albino mice	---	[18]
Antioxidant	Petroleum ether, methanol and choloroform	Leaves, node, internode and petiole explants	DPPH radical scavenging activity ABTS assay.	*In vitro*	Rutin and Quercetin Trolox	[19]
	Methanol	Leaves and stem bark	(i)DPPH radical scavenging activity (ii) Hydroxyl radical scavenging activity (iii) Reducing power activity (iv) ABTS+assay (v) Metal chelating activity.	*In vitro*	BHT BHT Ascorbic acid Trolox EDTA	[20]
	Ethanol	Leaves	Hydroxyl radical scavenging activity Hydrogen peroxide radical scavenging activity Reducing power assay.	*In vitro*	Ascorbic acid	[21]
	Methanol	Leaves and stem bark	Nitric oxide scavenging activity Inhibition of β - carotene bleaching activity.	*In vitro*	Quercetin and BHT Quercetin and BHT	[22]
	Ethanol	Stem bark	DPPH radical scavenging activity.	*In vitro*	Ascorbic acid	[23]
Anti-hemolytic	Methanol	Leaves and stem bark	H_2O_2 mediated haemolysis on erythrocyte membrane ghost.	*In vitro*	Quercetin and BHT	[22]
Sun protection	cream	Stem bark	UV method	*In vitro*	---	[23]
Antimicrobial	Ethanol	Leaves	Staphylococcus aureus, Pseudomonas aeruginosa and Aspergillus niger.	*In vitro*	---	[24]
	Ethanol	Leaves	Staphylococcus aureus, Pseudomonas aeruginosa and Aspergillus niger.	*In vitro*	---	[25]

Name of Activity	Solvent	Part of the Plant	Model	Method	Standard	References
Antifungal	Methanol	Leaves and stem bark	Aspergillus fumigatus, Aspergillus niger, Candida albicans, Paecilomyces lilacinus, Trichoderma viride, Verticillium lecanii, Fusarium sp., Mucor sp. and Penicillium sp.	*In vitro*	Ampicillin	[26]
Antibacterial	Hexane, chloroform, methanol and aqueous extracts.	leaves	*Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus* and *Pseudomonas fluorescens.*	*In vitro*	Streptomycin	[27]

gram of *H. populifolia* extract. Gallic acid was used as standard reference at 10, 20, 30, 40 and 50 µg/mL concentrations to create a calibration curve.[32]

Antioxidant activity by DPPH Radical Scavenging

Antioxidant activity was tested using DPPH scavenging assay given by Chu *et al.*, with some modifications. In brief, 1 mL of 0.1 mM DPPH radical solution was gently mixed with different concentrations (50, 100, 250,500 and 1000 µg/mL) of plant extract. For 30 min, the reaction mixture was left to settle at room temperature in the absence of light. Ascorbic acid was used as a positive control. Absorbance was measured at 517 nm.[33]

Antidiabetic activity by α-amylase inhibitory study

The DNSA technique was used to perform the α-amylase inhibitory study. The aqueous leaf extract of *Hildegardia populifolia* was prepared in the concentration ranges from 10 to 1000 µg/mL dissolved in a small amount of 10% DMSO and phosphate buffer 0.02 M containing 0.0006 M NaCl at pH 6.9. 200 µL of the extract was mixed with 200 µL of α-amylase solution (2 units/mL) and the mixture was incubated for 10 min at 30°C. Following that, 200 µL of the 1% w/v starch solution was added to each tube and incubated for 3 min. To stop of the reaction, 200 µL of DNSA reagent was added to the mixture, which was then boiled in a water bath at 85-90°C for 10 min. After heating, the mixture was allowed to cool to room temperature before being diluted with 5 mL of distilled water. The absorbance was measured at 540 nm using a UV-visible spectrophotometer.

For the blank with 100% enzyme activity, the plant extract was replaced with 200 µL of buffer. Similarly, a blank reaction was prepared at each concentration using the plant extract without adding the enzyme solution. Acarabose was used as a positive control at the same concentrations as the plant extract and the reaction was carried out identically to the reaction with the plant extract as described above.[2]

Statistical Analysis

Data were expressed as mean±standard error. The statistical analysis was performed using GraphPad Prism 6 software. Two-way ANOVA *Posthoc* Wilcoxon Tests were used to determine the significant differences between the means of multiple groups. The differences between the means were considered significant at the probability level ($p<0.05$).

RESULTS

Phytochemical Screening

The phytochemical investigation of plant extract has shown the presence of phenols, favonoids, glycosides, tannins and terpenoids.

FTIR analysis

The extract showed a total of 16 peaks in the spectrum. The firstfour peaks were closely present at 3742.85 cm^{-1}, 3699.67 cm^{-1}, 3648.09 cm^{-1} and 3565.61 cm^{-1} representing O-H stretch in water molecule, alcohol and carboxylic acid and N-H stretch in amines. A broad band was observed between 2980.79 cm^{-1} and 2359.92 cm^{-1} with four distinct sharp peaks. These include two small peaks at 2867.18 cm^{-1} and 2843.05 cm^{-1}, a medium sharp peak at 2980.79 cm^{-1} and a high sharp peak at 2359.92 cm^{-1} corresponding to C-H stretch in alkanes and aldehydes, O-H stretch in carboxylic acids and C≡N stretch in nitriles respectively. Three additional sharp peaks were observed at 1669.51 cm^{-1}, 1507.86 cm^{-1} and 1455.72 cm^{-1} assigned to C-C stretch in alkenes, N-O stretch in nitro compounds and C-O stretch in alcohol. In the fingerprint region, there was one high sharp peak at 1054.98 cm^{-1}, two moderate sharp peaks at 1032.83 cm^{-1} and1012.57 cm^{-1} and two small sharp peaks at 675.19 cm^{-1} and 668.47 cm^{-1}. These peaks represent C-O stretch in alcohol, C-O stretch in carbonyl, C-F and C-Br stretch in alkyl and aryl halides and C-Cl stretch in alkyl and aryl halides respectively. The results were expressed in Table 3 and Figure 1.

Total phenolic content

The Total Phenolic Content (TPC) of the plant extract was determined using the Folin-Ciocalteu assay. A standard curve was constructed with gallic acid by correlating absorbance with concentration. The resulting calibration curve for gallic acid showed linearity, with an equation of $y=0.41x$ and a correlation coefficient (R^2) of 0.9902. The TPC is given in milligrams per gram of extract as equivalents of gallic acid. From the calibration curve, the extract showed a phenolic content of 27.05 mgGAE/g. The results were presented in Figure 2A.

Antioxidant activity

The plant extract's ability to scavenge DPPH radical was used to measure its antioxidant capability. The concentration of antioxidant needed to reduce the initial DPPH radical concentration by 50% is referred to as the Inhibitory Concentration (IC_{50}) value. Higher antioxidant power is correlated with a lower IC_{50} value. A good amount of antioxidant activity (IC_{50}=10.35 µg/mL) was observed by the plant extract. In contrast, the IC_{50} value of 12.14 µg/mL was observed for the standard ascorbic acid. Concentration dependent reduction of DPPH was observed in this study. The results were presented in Figure 2B.

Antidiabetic activity

Figure 2C shows the effects of the plant extract and the standard (Acarbose) on pancreatic α-amylase activity using an *in vitro* assay. By analyzing the plot of % α-amylase inhibition against varying extract concentrations, IC_{50} values were determined. The aqueous extract demonstrated a lower IC_{50} value of 9 µg/mL, compared to the IC_{50} of 14 µg/mL for Acarbose. The extract showed significant inhibitory effectiveness compared to standard.

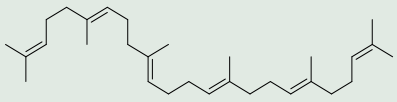
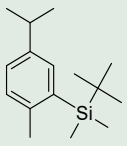
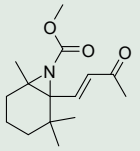
DISCUSSION

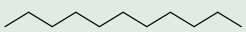
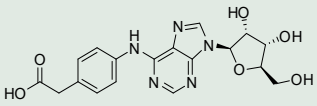
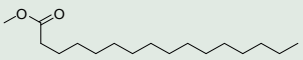
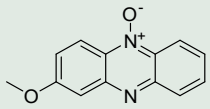
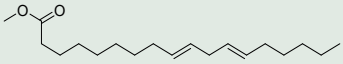
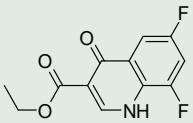
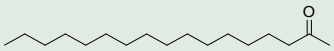
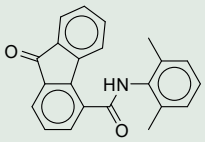
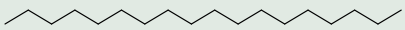
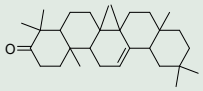
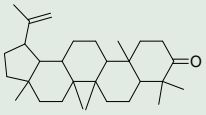
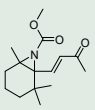
The practice of testing medicinal plants for antidiabetic effectiveness has become more common because it's critical to find new, potent treatments for the condition. Compared to conventional medications, traditional medicine based on plant extracts has been found to be less expensive, more clinically effective and have fewer side effects.[34]

Phytochemical screening is a preliminary and an important step in the study of plant drugs since it reveals the presence of constituents known for their various biological activities and medicinal properties.[35] The plant extract has shown the presence of phenols, favonoids, glycosides, tannins and terpenoids.

Fourier Transform Infrared (FTIR) spectroscopy is one of the main non-destructive analytical techniques used to identify the functional groups of active chemical constituents. This

Table 2: Literature review on phytochemistry of *Hildegardia populifolia*.

Compound No.	Name of the Part and Extract	Compound name and its structure	References
1	Methanolic leaf extract	 2,6,6-Trimethyl-bicyclo [3.1.1] heptane	[28]
2		 Squalene	[28]
3		 tert-Butyl (5-isopropyl-2-methyl phenyl) dimethylsilane	[28]
4		 1-Benzazirene-1-carboxylic acid, 2,2,5a-trimethyl-1a-[3-oxo-1-butenyl] perhydro-methyl ester	[28]

Compound No.	Name of the Part and Extract	Compound name and its structure	References
5	Methanolic stem bark extract	 Undecane	[28]
6		 Adenosine, N6-phenylacetic acid	[28]
7		 Hexadecanoic acid, methyl ester	[28]
8		 Phenazine 2-methoxy 5-oxide	[28]
9		 9,12-Octadecadienoic acid, methyl ester	[28]
10		 3-Quinolinecarboxylic acid, 6,8-difluoro-4-hydroxy-, ethyl ester	[28]
11		 2-Heptadecanone	[28]
12		 9H-Fluorene-4-carboxylic acid, 9-oxo-, (2,6-dimethyl phenyl)amide	[28]
13		 Octadecane	[28]
14		 4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2H-picen-3-one	[28]
15		 Lup-20(29)-en-3-one	[28]
16		 1-Benzazirene-1-carboxylic acid, 2,2,5a-trimethyl-1a-[3-oxo-1-butenyl] perhydro-, methyl ester	[28]

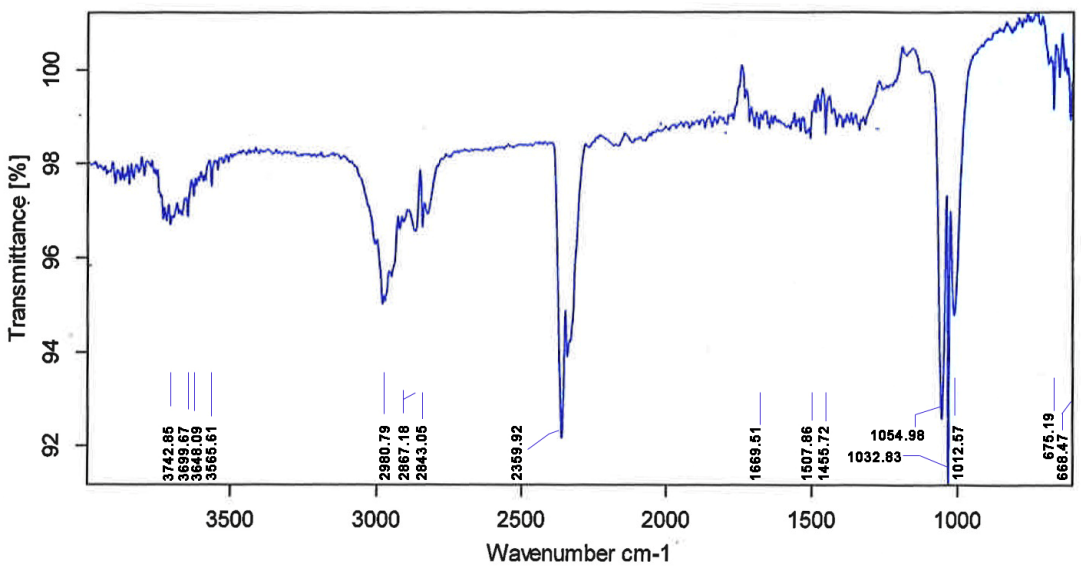


Figure 1: FTIR analysis of aqueous leaf extract of *Hildegardia populifolia.*

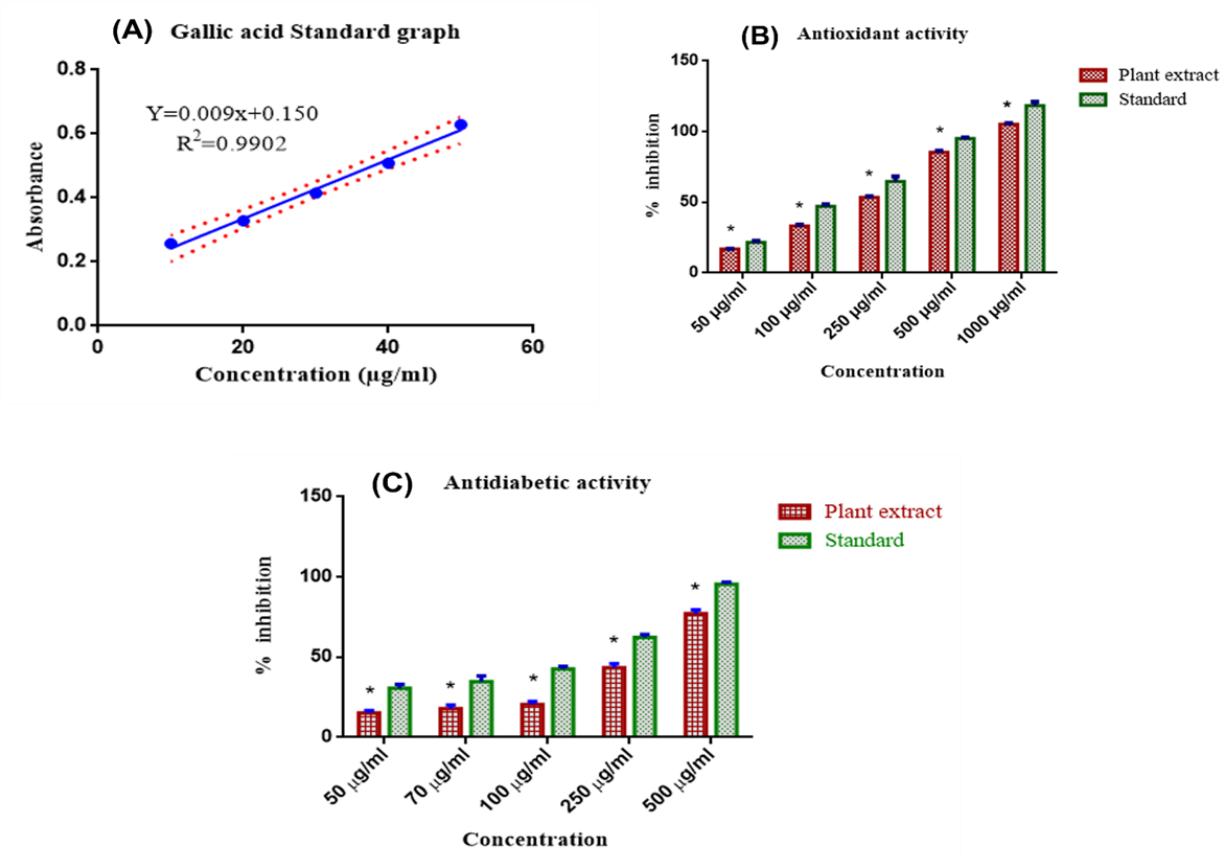


Figure 2: (A) Standard graph of Gallic acid (B) *In vitro* antioxidant activity of aqueous leaf extract of *Hildegardia populifolia* (C) Antidiabetic activity of aqueous leaf extract of *Hildegardia populifolia* [Two way ANOVA with *Posthoc* Wilcoxon Test were performed to determine the statistical significance. The results are mean±S.E. of three parallel measurements. $p<0.05$. The arterisk (*) denotes that the data are significantly differ from standard].

Table 3: FTIR analysis of aqueous leaf extract of *Hildegardia populifolia*.

Peak No.	Wave numbers (cm^{-1})	Class of compounds	Group
1	668.47	Alkyl and Aryl Halides	C-Cl stretch
2	675.19	Aromatic compounds Alkyl and Aryl Halides	C-H bend C-Br stretch
3	1012.57	Alkyl and Aryl Halides	C-F stretch
4	1032.83	Carbonyl	C-O stretch
5	1054.98	Alcohol	C-O stretch
6	1455.72	Aromatic compounds	C=C stretch
7	1507.86	Nitro Compounds	N-O stretch
8	1669.51	Amides Ketones, conj. Alkenes	C=O stretch C=O stretch C=C stretch
9	2359.92	Nitriles	C=N stretch
10	2843.05	Carboxylic acids	O-H stretch
11	2867.18	Alkanes Aldehydes	C-H stretch C-H stretch
12	2980.79	Carboxylic acids	O-H stretch
13	3565.61	Amine	N-H Stretch
14	3648.09	Carboxylic acid	O-H stretch
15	3699.67	Alcohol	O-H stretch
16	3742.85	Water	O-H stretch

technique is widely used for quality control in the herbal analysis, pharmaceutical, food and beverage industries. Recently, FTIR spectroscopy has advanced rapidly due to its low noise, quick speed, high repeatability, easy process, low expense and so on.[36] FTIR has become increasingly valuable in the field of assessing the quality of herbal drugs. FTIR analysis revealed the presence of 16 peaks representing functional groups like OH, C=C, C=O, C-Br.

Free radicals are believed to play a major role in the onset of chronic illnesses.[37] The ability of plant polyphenols to mitigate oxidative stress-induced tissue damage, which is linked to several chronic diseases, is crucial components of the human diet due to their documented antioxidant properties. These polyphenolic compounds form a diverse group of active constituents in plants, displaying variation in both distribution and concentration across different plant species.[38]

The phenolic content values in this study differ from those reported in the literature. Ch. Subbalakshmi *et al.*, have conducted phytochemical analysis of this plant using four extracts i.e. hexane, ethylacetate, ethanol and aqueous. They found polyphenols were present in all the four extracts.[24] M. Sarada *et al.*, developed a protocol for efficient callogenesis from leaf, node, internode and petiole explants. They found phenolic content was higher in 60 days old leaf callus cultures and it was found to be 18.20±0.87 mg/GAE/g extract.[19] S. Paulsamy *et al.*, reported total pheonolics of 2254.26±9.30 mg GAE/g in methanolic leaf extract.[20] In our study, we performed total phenol estimation in aqueous leaf extract and found 27.05 mgGAE/g. The variation in phenolic compound concentrations may be due to the presence of different secondary metabolites that are present at different amounts as well as other variables such as genotypes, plant age, plant part used, extraction techniques, solvent polarity, phytogeographical region and the time of year the plants were collected.[39]

DPPH is a stable free radical that shows a deep purple color with an absorption maximum at 517 nm. This purple color fades when an antioxidant quenches DPPH free radicals by electron donation in redox reactions.[40] Research indicates that the antioxidant activity in medicinal plants may be largely due to phenolic compounds, including flavonoids, which possess hydroxyl groups with redox capabilities.[38] A review of the literature shows different authors have performed antioxidant activity using DPPH assay and other scavenging assays. M. Sarada *et al.*, reported an IC_{50} value of 407.43±13.09 µg/mL for callus produced from petiole explants after 20 days of study.[19] S. Paulsamy *et al.*, found an IC_{50} of 92.86 µg/mL for methanolic leaf extract.[20] In our study, the IC_{50} of the extract was found to be 10.35 µg/mL and 14 µg/mL for the standard, ascorbic acid. These differences in antioxidant activity can be attributed to variations in secondary metabolite concentration in different parts of the plant and the solvent used

for extraction which significantly influence the pharmacological activities of the medicinal plants.

The well-known enzyme alpha-amylase present in saliva and pancreatic juice converts big, insoluble starch molecules into smaller absorbable ones.[41] α-amylase inhibitors reduce the postprandial blood glucose surge and postpone the breakdown of carbohydrates in the small intestine. Since they impede or delay the body's absorption of starch. These α-amylase inhibitors also known as starch blockers prevent the hydrolysis of 1,4-glycosidic bonds, thereby blocking the conversion of starch and other oligosaccharides into maltose, maltriose and other simple sugars.[42] Many α-amylase inhibitors have been identified from therapeutic plants, offering a more potent alternative with fewer side effects than current synthetic medications. Review of the literature shows that anti diabetic activity was not studied previously for this plant and hence we have done this diabetic activity by α-amylase inhibitory assay. We found comparable IC_{50} of 9 µg/mL for the extract and 14 µg/mL for the standard, acarabose.

CONCLUSION

Hildegardia populifolia is an endangered and vulnerable plant with limited distributed in India. The study findings suggest that the aqueous leaf extract exhibited significant antioxidant and antidiabetic activity, possibly attributed to the presence of phenolic compounds. Therefore, further research to isolate and characterise these bioactive constituents is warranted. Additionally, comprehensive studies on pharmacokinetics, safety and efficacy of these compounds are necessary to fully understand their therapeutic potential.

ACKNOWLEDGEMENT

The authors would like to thank the management of our college for providing facilities and their constant support and encouragement.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

FTIR: Fourier Transform Infrared Spectroscopy; **GAE:** Gallic acid equivalents; **DPPH:** 2,2-Diphenyl-1-picrylhydrazyl; **SEA:** South-East Asian; **IDF:** Indian Development Foundation; **HDL:** High Density Lipoproteins; **DMSO:** Dimethylsulfoxide; **DNSA:** 3,5-dinitrosalicylic acid; **TPC:** Total phenolic content; **IC_{50}:** Inhibitory concentration.

ETHICAL APPROVAL

This study did not involved animal subjects. Therefore, ethical approval is not required.

SUMMARY

Hildegardia populifolia, an endangered plant used in Ayurvedic medicine, was studied for its antioxidant and anti-diabetic properties. The aqueous leaf extract was analyzed for its active constituents through preliminary phytochemical screening, functional group analysis by FTIR spectroscopy, total phenolic content estimation and biological assays. Phytochemical analysis confirmed the presence of phenols, flavonoids, glycosides, tannins and terpenoids. The total phenolic content was 27.05 mg GAE/g of extract. FTIR analysis identified 16 functional groups, including OH, C=C and C=O. The extract exhibited strong antioxidant activity and significant α-amylase inhibition, comparable to ascorbic acid and acarbose. These findings highlight the potential of *H. populifolia* for developing novel therapeutic agents for diabetes management.

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Cite this article: Mahalakshmi SVVNS, Penmetsa DB, Yennam KR, Byalla S. FTIR Analysis, Total Phenolic Content, Antioxidant and Antidiabetic Activities of *Hildegardia populifolia* (Roxb.) Schott and Endl. (Malvaceae). Pharmacog Res. 2025;17(2):679-88.