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Phytochemical, physicochemical, TLC, minerals analysis and in-vitro antioxidant activity of ethanolic extract of leaves of *Heldigardia populifolia*

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ABSTRACT

The aim of present study was to investigate the preliminary phytochemical, physicochemical, TLC, minerals analysis and In-vitro antioxidant activity of leaves of ethanolic extract of Heldigardia populifolia. The preliminary phytochemical screening of ethanolic extract showed the presence of triterpenoids, flavonoids, glycosides, sterols, steroids, phenols, carbohydrates and saponins. The composition of minerals found in the leaf powder was within the permissible limits. TLC analysis of ethanol extract showed the five spots which indicate the presence of five phytoconstituents. The extractive value of ethanol was high than acetone. Ash values were within the limits. The in-vitro antioxidant activity of ethanolic extract increased with increasing the concentration. The ethanolic extract in all the concentration showed the significant antioxidant activity.

Keywords: Heldigardia populifolia; ethanolic extract; phytochemical; physicochemical; minerals; antioxidant activity.

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INTRODUCTION

Management of diabetes mellitus is a global problem and successful treatment is very essential for preventing or at least delaying the onset of long-term complications of the disorder. Remedies to treat such chronic states are available in nature in the form of herbal medicines or drugs with very minimum adverse effects when compared to the available synthetic drugs[1]. Such herbal drugs as therapeutic agents are a born when compared to the severe adverse effects of the allopathic medical practice for diabetes, through the quest for a complete and permanent cure for the disease is being pursued relentlessly by eluding physicians and researchers[2]. These herbal remedies which exemplify the process of symbolism still remain unexplored amidst the modern technical advances, which has created a tremendous scope for folklore medicines. It is believed that the traditional medicines used for the treatment of diabetes mellitus alternate the progression of complications of the disease. Scientific validation of such plants is necessary, because though their medicinal use dates back to 1000 years, a scientific literature that supports it's therapeutic and pharmacological properties become necessary. The search for the effective herbal drugs for the treatment of diabetes based on ethnomedical clues still continues and in the long run, has yielded us invaluable herbal remedies[3].

Hildegardia populifolia is belonging to the family of Sterculiaceae. It is commonly called as 'Urumikol' in Malayalam. It is a medium sized evergreen shrub, widely distributed in the Peninsular India and Srilanka. Leaves are lanceolate, rounded at the acute

apex round at the base, nerves from the base and form midrib. Flowers are 3-5 together, pedicle 5mm long, and whitish. Stout calyx tube 2 mm long, campanulate, glabrous lobes 6mm long, narrow, corolla tube 2-2.5 cam long, lobes 1.5-1.7 cm long, oblong , acute berry 8×7 mm, ovoid, glabrous. *Heldigardia populifolia* is used in the traditional systems of medicine in India for the treatment of diabetes mellitus [4]. Therefore, the aim of present study was to investigate the preliminary phytochemical, physicochemical, TLC, minerals analysis and *In-vitro* antioxidant activity of leaves of ethanol extract of *Heldigardia populifolia.*

MATERIALS AND METHODS

Collection and authentication of plant material

The leaves of the '*Hildegardia populifolia' used* in this study were collected from talakona forest near Tirupathi (Chittoor district, Andhra Pradesh.) The plant was authenticated by Dr. P.Jayaraman, Professor, Department of Botany, Presidency College, Chennai.

Preparation of plant extract

The leaves of *Hildegardia populifolia* were dried at room temperature for 15 days and powder using the mechanical grinder. The powder was sieved through sieve no.22 to get uniform particle size. About 500g of coarse powder was cold macerated with ethanol (1lt) for 72 hours with occasional shaking. After completion of extraction, the ethanolic extract was filtered and concentrated at 55°C on a water bath. The residue was stored in air tight container.

Preliminary phytochemical analysis

The preliminary phytochemical analysis of an ethanolic extract of leaves of *Hildegardia populifolia* was carried out according to methods of Harborn and Evans [5-6].

Physicochemical constants

Physicochemical constants such as ash values and extractive values were carried out according to standard method and procedure[7].

Thin layer chromatography analysis

The ethanol extract of leaves of *Heldigardia populifolia* was subjected to TLC analysis using Ethylacetate:methanol: water (10:3:1) as a mobile phase[8].

Elemental analysis

Specified amount of leaves powder were placed in 100 ml volumetric flask and 30 ml of nitric acid was added. The flask was heated at 250 °C on a hot plate for 4 hrs and cooled. Then 15 ml of perchloric acid was added and heated the solution until the solution became the colorless. The solution was filtered and subjected to trace elements analysis by Atomic Absorption Spectrometer[9].

In-vitro Antioxidant activity

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity of the ethanolic extract was determined according to the method of Asharani et al[10]. The reaction mixture consist of 1 ml of various concentrations of the extract (100 to 600 µg/ml), 0.01 ml of 1 mM $FeCl_3$, 0.1 ml of 1 mM EDTA, 0.1 ml of 20 mM H_2O_2, 0.1 ml of 1 mM L-ascorbic acid and 0.36 ml of 30 mM deoxyribose in potassium phosphate buffer (pH 7.4). The reaction mixture was incubated for 1 h at 37 °C, and further heated in a boiling water bath for15 min after addition of 1 mL of 2.8% (w/v) trichloroacetic acid and 1 mL of 1% (w/w) 2-thiobarbituric acid. The absorbance of resulting solution was measured at 560 nm against a blank[10].

Hydrogen peroxide radical scavenging activity

Hydrogen peroxide radical scavenging activity of ethanol extract was carried out according to the method of Ruch et al with slight modification. The reaction mixture consists of 1 ml of various concentrations of the extract (100 to 600 µg/ml) and 0.6 ml of hydrogen peroxide solution. The absorbance of resulting solution was measured at 230 nm against a blank after 10 min[11].

Reducing power

The reducing power was determined according to the method of Oyaizu (1986). Ethanol extract (100–600 µg/ml) in methanol (2.5 ml) was mixed with 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide and the mixture was incubated at 50 °C for 20 min. After 2.5 ml of 10% trichloroacetic acid (w/v, Wako) were added, the mixture was centrifuged at 200g for 10 min. The upper layer (5 ml) was mixed with 5 ml of deionized water and 1 ml of 0.1% ferric chloride (Wako), and the absorbance was measured at 700 nm against blank in a spectrophotometer[12].

RESULTS AND DISCUSSION

Preliminary phytochemical studies

Preliminary phytochemical screening of ethanol extract mainly revealed the presence of triterpenoids, flavonoids, glycosides, sterols, steroids, phenols,

Table 1: Phytochemical screening of ethanolic extract of leaf of *Hildegardia populifolia*

S. No.	Constituents	Ethanolic Extract
1	Alkaloids	Absent
2	Carbohydrates	Present
3.	Protein	Absent
4.	Steroids	Present
5.	Phenols	Present
6.	Tannins	Absent
7.	Flavanoids	Present
8.	Gums and Mucilage	Absent
9.	Glycosides	Present
10.	Sterols	Present
11.	Saponins	Present
12.	Terpenoids	Present

Table 2: Physicochemical parameters of powdered leaf of *Hildegardia populifolia*

S. No	Parameters	Values in %
1	Total ash	9.11±0.020
2	Water soluble ash	10.15±0.058
3	Acid insoluble ash	3.08±0.029
4	Sulphated ash	11.13±0.036
5	Petroleum ether soluble extractive	3.11±0.038
6	Chloroform soluble extractive	3.82±0.032
7	Acetone soluble extractive	11.76±0.026
8	Ethanol soluble extractive	18.13±0.067
9	Ethylacetate soluble extractive	10.12±0.027

Table 3: TLC profile of ethanolic extract of leaves of *Hildegardia populifolia*

Parts used	Extracts	Solvents system	Distance travelled by solute	Distance travelled by solvent	Detecting agent	Rf value
Leaves	Ethanol	Ethylacetate: Methanol:water (10:3:1)	0.9	4.3	Iodine chamber	0.2
			1.8			0.4
			2.9			0.6
			3.5			0.8
			1.6			0.35

Table 4: Minerals content in leaves, soil, and water of *Hildegardia populifolia*

S. No	Name of Minerals	Minerals content (mg/kg)		
		Leaves	Soil	Water
1	Sodium	22	21	2.5
2	Potassium	17	26	1
3	Calcium	27	62.5	0.5

Table 5: Percentage of hydroxyl radical scavenging activity of ethanol extract of leaves of *Hildegardia populifolia*

S. No	Concentration (µg/mL)	% of activity (±SEM)	
		Ethanol extract	Ascorbic acid
1	100	13.8 ±0.79	49.37±0.001
2	200	36.8 ±0.80	57.22±0.001
3	300	45.13±0.79	59.11±0.001
4	400	53.7 ±0.61	69.80±0.002
5	500	60.87±0.61	70.43±0.002
6	600	71.75±0.61	73.26±0.002

carbohydrates, and saponins. The results are shown in Table 1.

Physicochemical constants

Physicochemical constants of leaf powder like ash values and the extractive value were presented in Table 2. The total ash, water soluble ash, acid insoluble ash, and sulfated ash values were found to be 9.11%w/w, 10.15%w/w, 3.08%w/w, and 11.13%w/w respectively on the basis of dry weight, whereas extractive values such as petroleum soluble, chloroform soluble, acetone soluble, ethanol soluble, and ethyl acetate soluble were found to be 3.11%w/w, 3.82%w/w, 11.76%w/w, 18.13%w/w, and 10.12%w/w respectively.

TLC analysis

The different R_f values of varies phytoconstituents were as recorded in Table 3. TLC analysis is used for analysis of phytoconstituents present in the medicinal plant extract.

Minerals analysis

A total of three minerals like sodium, potassium, and calcium were determined in the powdered leaves, soil, and water using an atomic absorption spectrometer (AAS). The result was shown in Table 4.

The concentrations of elements were found within in the permissible limit. The concentration of these minerals may be mainly due to an environmental condition where the plant grown such as soil and water. Minerals act as a cofactor for enzymes that involved in energy production in our body.

***In-vitro* antioxidant activity**

Assay of hydroxyl radical scavenging activity

The scavenging activity of hydroxyl radicals can be evaluated by Fenton's reaction. The hydroxyl radical is highly reactive oxygen species formed by the reaction of various hydroperoxides with transition metal ions. A hydroxyl radical is attacked the polyunsaturated fatty acid in the membrane and is abstracting the hydrogen atoms from polyunsaturated fatty acids and brings about the peroxidic reaction of membrane lipids. The hydroxyl radical scavenging effect of extract and ascorbic acid has been shown in Table 5. The percentage scavenging effects of extract as well as ascorbic acid were increased with the increase in concentration from 100 to 600 µg/mL. The extract, in all the concentrations, showed the significant hydroxyl radical scavenging activity. The ethanol extract showed the highest percentage of hydroxyl radical inhibition at the concentration of 600 µg/mL compared to ascorbic acid. The experimental data showed that percentage inhibition values of standard ascorbic acid were high when compared to ethanol extract.

Assay of hydrogen peroxide scavenging activity

Hydrogen peroxide scavenging activity is based on the loss or decomposition of hydrogen peroxide, when the hydrogen peroxide incubated with the scavenger. The hydrogen peroxide scavenging effect of extract and ascorbic acid has been shown in Table 6. The percentage scavenging effects of extract as well as ascorbic acid were increased with the increase in concentration from 100 to 600 µg/mL. The extract, in all the concentrations, showed the significant hydroxyl radical scavenging activity. The ethanol extract showed the highest percentage inhibition of hydrogen peroxide at the concentration of 600 µg/mL compared to ascorbic acid. The experimental data showed that percentage inhibition values of standard ascorbic acid were high when compared to ethanol extract.

Table 6: Percentage of hydrogen peroxide scavenging activity of ethanol extract of leaves of Hildegardia populifolia

S. No	Concentration (µg/mL)	% of activity (±SEM)	
		Ethanol extract	Ascorbic acid
1	100	13.8 ±0.79	49.37±0.001
2	200	36.8 ±0.80	57.22±0.001
3	300	45.13±0.79	59.11±0.001
4	400	53.7 ±0.61	69.80±0.002
5	500	60.87±0.61	70.43±0.002
6	600	71.75±0.61	73.26±0.002

*All values are expressed as mean ± SEM for three determinations

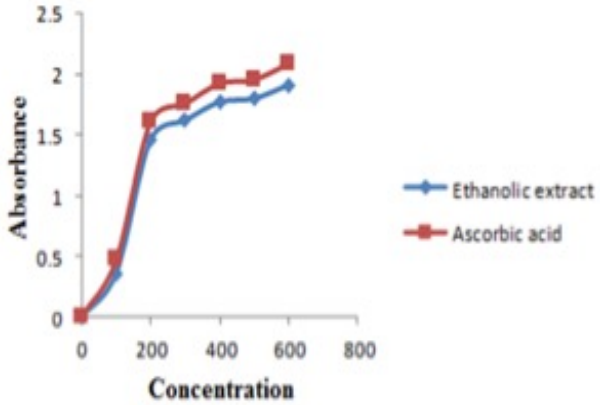

Figure 1: Reducing the power of ethanol extract of leaves of Hildegardia populifolia

Reducing power assay

Reducing the power of ethanol extract was shown in Figure 1: Reducing the power of ethanol extract of leaves of Hildegardia populifolia. Reducing power capabilities of extract and ascorbic acid increased with increases in the concentration. The extract with reducing power indicates that they are electron donors and can reduce the oxidized intermediate of lipid peroxidation processes.

CONCLUSION

This study will play an important role in the prevention of adulteration and quality control of the plants. The ethanolic extract of leaves of *Hildegardia populifolia* showed the good anti-oxidant activity.

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