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Research Article

PHYTOCHEMICAL SCREENING AND DETERMINATION OF ANTIOXIDANT ACTIVITY OF *HELICIA NILAGIRICA* BEDD., AN ETHNOMEDICINAL PLANT OF MIZORAM, INDIA

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ABSTRACT

Objectives: The present study was undertaken to screen the phytochemical constituents and determine the antioxidant activity of Helicia nilagirica Bedd., an ethnomedicinal plant of Mizoram, India.

Methods: Phytochemical screening was carried out according to standard procedures on the methanolic bark extract of the plant. The methanolic extract was subjected to in-vitro screening models such as DPPH radical scavenging assay, hydroxyl scavenging activity assay and reducing power assay using BHA and ascorbic acid respectively as standards. The total phenolic and total flavonoid content were determined using Gallic acid and Quercetin respectively as standards.

Results: The presence of flavonoids, glycosides, steroids and carbohydrates was indicated by the tests conducted. The methanol extract exhibited IC50 values of 3.92 µg/ml and 6.86 µg/ml respectively in DPPH and hydroxyl radical scavenging activity assay. The reducing power of the extract was lower than that of the standard ascorbic acid. The total phenolic content and flavonoid content was 62.75 mg/g and 56 mg/g of gallic acid and quercetin equivalent respectively.

Conclusion: The results of the present study concluded that the methanolic extract of the bark of Helicia nilagirica Bedd. possess antioxidant activity.

Keywords: Helicia nilagirica, Phytochemicals, Antioxidant activity, Diphenyl-1-picrylhydrazyl, Phenol and flavonoid.

INTRODUCTION

New drugs have been discovered from a variety of natural sources. They may be found quite by accident or as a result of many years of the tireless pursuit. Throughout medical history, plant materials have served as a reservoir of potential new drugs [1]. The studies of the traditional system of medicines have resulted in the development of valuable compounds. Antioxidant compounds such as phenolic acids, polyphenols, and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases [2]. Anti-inflammatory, anti-carcinogenic etc., Flavonoid has antioxidant activity in in vivo studies with rats, protecting their gastrointestinal mucosa against the reactive oxygen species (ROS) generated by acute and chronic stress. Protection against oxidative stress in the human gastrointestinal tract, direct antibacterial activity, synergistic activity with antibiotics [3].

Oxygen derived free radicals implication in the pathogenesis of a wide variety of clinical disorders and gastric damage, caused by physical, chemical, and psychological factors that lead to gastric ulceration in human and experimental animals [4].

Antioxidants may guard against ROS toxicities by the prevention of ROS construction, by the disruption of ROS attack, by scavenging reactive metabolites and converting them to less reactive molecules or by enhancing the resistance of sensitive biological target to ROS attack [5]. Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxyltoluene, tertiary butylated hydroxyquinone, and gallic acid esters have been suspected to be carcinogenic. Hence, strong limitations have been placed on their use, and there is a trend to replace them with naturally occurring antioxidants. Moreover, these synthetic antioxidants also show low solubility and moderate antioxidant activity [6].

Helicia nilagirica Bedd. is a medium-sized tree belonging to the family Proteaceae. It is commonly known as Pasaltakaza in Mizoram, Northeast India. Traditionally, a decoction prepared by boiling the leaves or bark is used for various stomach ailments including peptic ulcer and indigestion by the people of Mizoram. It is also used in scabies and other skin diseases [7]. There are no scientific reports regarding the activities of the bark of the plant, however, there are reports on the isolation of compounds from the leaves and seeds. Two compounds were isolated from the leaves of H. nilagirica, Compound 1 was elucidated as1-O-3-D-glucopyranosyl-(2S,3S,4R,8Z)-2-[(2'R)-2'-hydroxylignocenoyl-amino]-8-octadecene-1, 3,4-triol. Compound 2 was an analog of compound 1 [8]. Five compounds were isolated from the dichloromethane and n-butanol extracts of the seeds, identified as p-hydroxybenzaldehyde, p-hydroxybenzoic acid, gallic acid, helicide, 4-formylpymyl-O-beta-D-glucopyranoside [9]. The present investigation was carried out to investigate the phytochemical constituents and antioxidant activity on the methanolic bark extract of H. nilagirica.

MATERIALS AND METHODS

Plant material

The plant material of Helicia nilagirca Bedd. was collected from Sesawm village in the southern part of Mizoram, India. It was authenticated by Botanical Survey of India, Kolkata.

Preparation of plant extract

The bark of H. nilagirica was air-dried and coarsely powdered. It was then extracted successively with petroleum ether and methanol using the Soxhlet apparatus for 24 hrs. The methanolic extract was evaporated under vacuum in a rotary evaporator, kept in air tight container and used for the experiment.

Phytochemical screening

The methanolic extract was subjected to phytochemical screening and the presence of steroids, flavonoids, glycosides, and carbohydrates were detected [10]. Wide ranges of phytoconstituents are responsible for anti-inflammatory activity including phenolics, alkaloids, and

terpenoids [11]. Flavonoids exhibit varied biological activities that include analgesic, anti-inflammatory, antioxidant, hepatoprotective, and antiulcer activities [12]. The presence of a phytochemical of interest may lead to its further isolation, purification, and characterization. It can then be used as the basis for a new pharmaceutical product.

Determination of diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The antioxidant activity of H. nilagirica Bedd. and the standard were estimated on the basis of the radical scavenging effect of the stable DPPH free radical according to the method described by Blois (1958) with minor changes [13]. BHA was used as reference standard. 0.5 ml of DPPH solution in methanol (0.1 mM) was mixed with 3 ml of the extract and 3 ml of standard prepared in various concentrations (10, 20, 40, 60, 80, and 100 µg/ml), respectively. The extract and standards were incubated for 30 minutes at 37°C. Absorbance was measured at 517 nm using ultraviolet-visible (UV-Vis) spectrophotometer. Control reading was also taken. The scavenging effect of DPPH free radical was calculated using the following equation.

$$\% \text{ DPPH radical scavenging} = (Abs_{cont.} - Abs_{ext.}/Abs_{cont.}) \times 100$$

Where, $Abs_{cont.}$ is absorbance of control and Abs_{ext} is absorbance of the extract.

Determination hydroxyl radical scavenging activity

The scavenging capacity for hydroxyl radical was measured according to the modified method of Halliwell et al. (1987) [14]. Ascorbic acid was used as a standard. The assay was performed by adding 0.1 ml of ethylenediamine tetraacetic acid (EDTA) (1 mM), 0.01 ml of ferric chloride (10 mM), 0.1 ml of hydrogen peroxide (10 mM), 0.36 ml of deoxyribose (10 mM), 1 ml of different dilutions of the extract and standard solutions (10, 20, 40, 60, 80, and 100 µg/ml), dissolved in distilled water, 0.33 ml of phosphate buffer (pH 7.4), and 0.1 ml of ascorbic acid in sequence. The mixture was then incubated at 37°C for 1 hr. 1 ml of the incubated reaction mixture was mixed with 1 ml of 10% trichloro acetic acid and 1 ml of 0.5% thiobarbituric acid (in 0.025 M, NaOH and BHA) to develop the pink chromagen. Absorbance was measured at 532 nm using UV-Vis spectrophotometer. The hydroxyl radical scavenging activity of the extract is reported as % inhibition of deoxyribose degradation and is calculated as,

$$OH\text{-scavenged } (\%) = (Abs_{cont.} - Abs_{ext.}/Abs_{cont.}) \times 100$$

Where, $Abs_{cont.}$ is the absorbance of control and $Abs_{ext.}$ is the absorbance of the extract.

Determination of reducing power

The reducing power of the extract of H. nilagirica determined by the method of Oyaizu (1986) using ascorbic acid as standard [15]. 1 ml of the extract and 1 ml of the standard with various concentrations (10, 20, 40, 60, 80, and 100 µg/ml) were mixed with 2.5 ml of phosphate buffer (6.6 pH) and 2.5 ml of 1% potassium ferricyanide. The mixture was then incubated at 50°C for 30 minutes. The reaction was stopped by adding 2.5 ml of 10% trichloroacetic acid and the mixture was centrifuged at 3000 rpm for 10 minutes. 2.5 ml of the supernatant was mixed with 2.5 ml of distilled water, and 0.5 ml of 0.1% ferric chloride solution and the absorbance was taken at 700 nm using UV-Vis spectrophotometer. Higher absorbance of the reaction mixture indicated that the reducing power is increased.

Determination of total phenolic content

The total phenolic content of the leaf extract was determined separately using the method of Mcdonald et al. [16] with modifications. Calibration curve was prepared by mixing 1 ml of methanolic solution of gallic acid (10, 20, 40, 60, 80, and 100 µg/ml) with 5 ml Folin–Ciocalteu reagent (diluted tenfold). After 3 minutes, 4 ml of sodium carbonate solution (0.7 M) was added, and the mixture was allowed to stand for 1 hr at room temperature. Absorbance was measured at 765 nm using UV-Vis spectrophotometer. 1 ml extract (50 µg/ml) was also mixed with the reagents above and after 1 hr the absorbance was measured to determine total plant phenolic content. From the calibration curve, the amount of phenolic compounds was determined and expressed as milligrams of gallic acid equivalent (GAE)/g of the dried extract.

Determination of total flavonoids content

The total flavonoid content of the extract of H. nilagirica was determined by the aluminum chloride method [17]. 1 ml of the extract (50 µg/ml) was mixed with 2 ml of distilled water. After 5 minutes, 3 ml of 5% sodium nitrite ($NaNO_2$) and 0.3 ml of 10% aluminum chloride ($AlCl_3$) were added. After 6 minutes, 2 ml of NaOH (1 M) was added, and the volume was made up to 10 ml with distilled water. After 1 hr, absorbance reading was taken at 510 nm. A standard curve was prepared with quercetin at different concentrations (5, 10, 20, 40, 60, 80, and 100 µg/ml). From the calibration curve of the reference standard, the total flavonoid content was determined and expressed as milligrams of quercetin equivalent (QE/g) of dried extract.

RESULTS AND DISCUSSION

The methanol bark extract of the plant was found to have glycosides, flavonoids, steroids, and carbohydrate. In general, flavonoids have been known to be cyclo-oxygenase inhibitors and so, the plants containing them may be considered to have anti-inflammatory properties [11]. Some steroids are also known to have anti-inflammatory activity and so the plants containing them may also have anti-inflammatory property [12]. The present study shows that H. nilagirica Bedd. contains phytochemicals which may be a potential new drug for the future.

It is increasingly being realized that many of today's diseases are due to the "oxidative stress" that results from an imbalance between the formation and neutralization of pro-oxidants. These excess free radicals react with biological macromolecules such as proteins, lipids, and DNA in healthy human cells and this results in the induction of carcinogenesis, atherosclerosis, cardiovascular diseases, ageing, and inflammatory diseases. These harmful radicals have to be eliminated from biological systems by enzymes such as superoxide dismutase, catalase and peroxidase, or compounds such as ascorbic acid, polyphenols, tocopherol, and glutathione which possess antioxidant properties [18].

DPPH is a free radical which is stable at room temperature, and this method is often employed to determine the antioxidant activity of many plant extracts. DPPH is a stable nitrogen-centered free radical, the color of which changes from violet to yellow upon reduction by either the process of hydrogen-or electron-donation. Substances which are able to perform this reaction can be considered as antioxidants and, therefore, radical scavengers [19]. The concentration in µg/ml of the extract to scavenge 50% of the DPPH radical is called inhibitory concentration 50 (IC_{50}), and lower IC_{50} values indicate higher antiradical activity. From the scavenging activity, the IC_{50} was calculated by linear regression method using % DPPH scavenging activity in Y-axis and concentration in

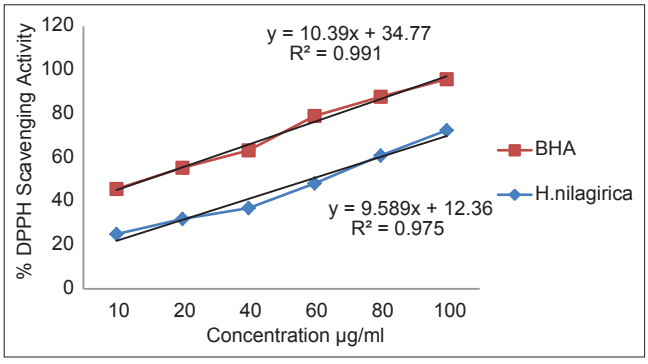

Fig. 1: Diphenyl-1-picrylhydrazyl radical scavenging assay

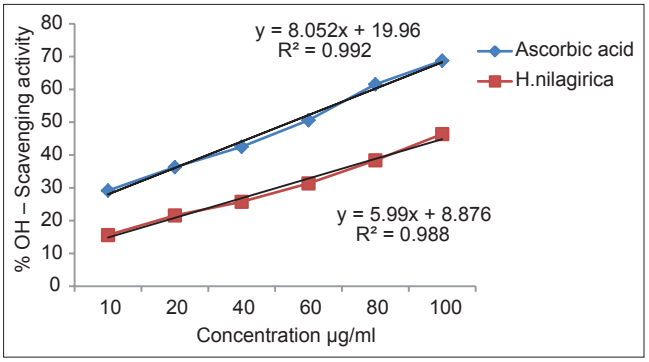

Fig. 2: Hydroxyl radical scavenging activity

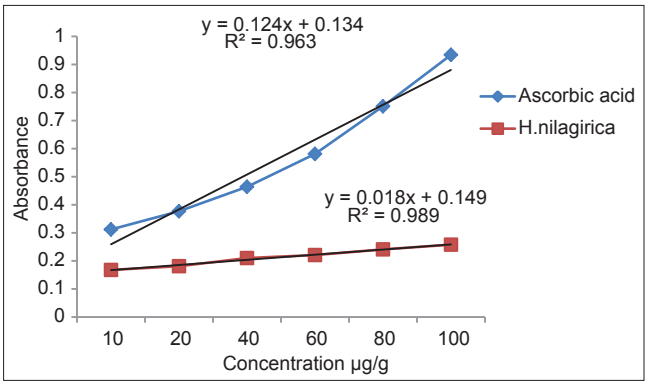

Fig. 3: Reducing power

X-axis. The IC_{50} of the extract of H. nilagirica Bedd. exhibited 3.92 µg/ml as compared to standard BHA, which exhibited 1.46 µg/ml (Fig. 1).

Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and test compound for hydroxyl radical generated from the Fe^{3+}/ascorbic acid/EDTA/H_2O_2 system. The hydroxyl radical attacked deoxyribose, which resulted in thiobarbituric acid reacting substance formation based on the inhibition rate of 2-oxyribose oxidation by hydroxyl radical. The IC_{50} of the sample was found to be 6.86 µg/ml, and the IC_{50} of the standard was found to be 3.73 µg/ml (Fig. 2).

The reducing power assay is often used to evaluate the ability of an antioxidant to donate an electron. Fe^{3+} reduction is often used as an indicator of electron-donating activity, which is an important mechanism of phenolic antioxidant action [20]. In this assay, the presence of antioxidant in the extract resulted into reduction of the ferric cyanide complex (Fe^{3+}) to the ferrous cyanide form (Fe^{2+}). Higher is the absorbance of the reaction mixture, higher would be the reducing power. The reducing power of the extract increases as the concentration increases suggesting that some compound in the extract may be able to terminate the radical chain reaction. The reducing power of the extract of H. nilagirica was lower when compared to the standard Ascorbic acid. However, the antioxidant present in the extract cause the reduction of Fe^{3+} to Fe^{2+} and thus proved its reducing power (Fig 3).

Phenolic substances have been shown to be responsible for the antioxidant activity of plant materials [21]. Phenolic compounds are very important plant constituents because they exhibit antioxidant activity by inactivating lipid free radicals or preventing decomposition of hydroperoxides into free radicals [22]. Therefore, the amount of total phenols in the extracts was investigated by the Folin–Ciocalteu method. The total phenolic compounds detected in the extract of H. nilagirica was 62.75 mg GAE/g dry weight of the sample (Fig 4).

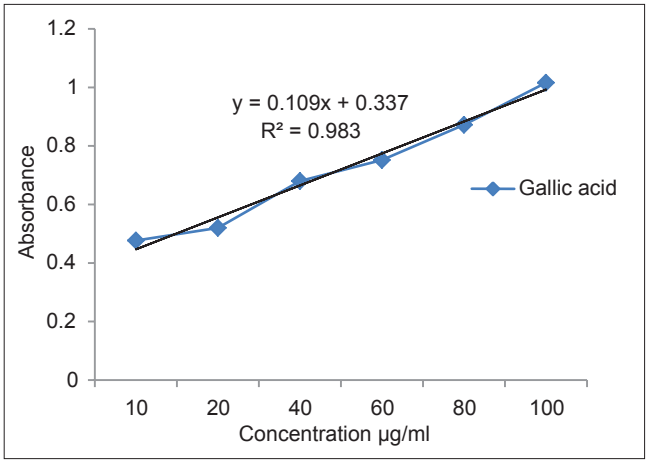

Fig. 4: Calibration plot for total phenols

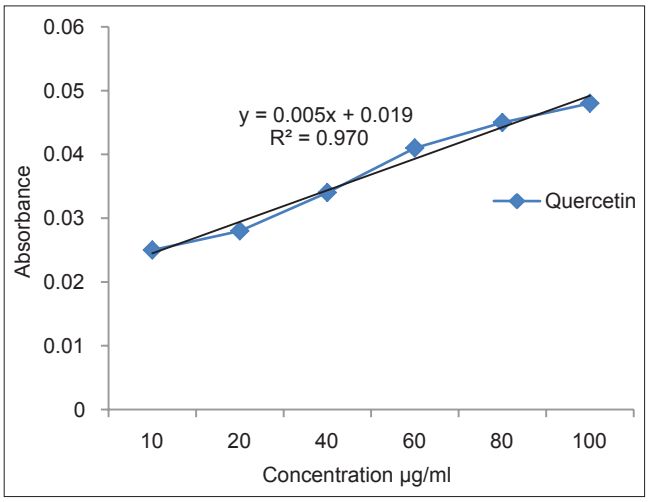

Fig. 5: Calibration plot for total flavonoids

The flavonoids act through scavenging or chelating process [23]. So far as plant phenolics constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators, it was reasonable to determine their total amount in the selected plant extracts. Flavonoids as one of the most diverse and widespread group of natural compounds are probably the most important natural phenolics [24]. These compounds possess a broad spectrum of chemical and biological activities including radical scavenging properties. Such properties are especially distinct for flavonols. The total flavonoid content of the extract was found to be 56 mg QE/g dry weight of the sample (Fig. 5).

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