PRELIMINARY PHYTOCHEMICAL SCREENING AND ANTIOXIDANT POTENTIAL OF *Blepharis maderaspatensis* (L.) B.Heyne ex Roth LEAF

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ABSTRACT

**Objectives:** To evaluate the phytochemical screening and enzymatic and non-enzymatic antioxidants activity of the leaf extract of *Blepharis maderaspatensis*. **Methods:** Phytochemical screening of the leaf extract was done to determine the phytochemical constituents in the various solvents. The enzymatic antioxidants (Superoxide dismutase, Catalase, Glutathione-s-transferase and Glutathione peroxidase) and non-enzymatic antioxidants (Reduced glutathione, Vitamin C and Vitamin E) were estimated. **Results:** The phytochemical analysis comprehensively validates that the leaf extract of *B.maderaspatensis* possessed therapeutically important secondary metabolites such as Alkaloids, Phenols, Flavanoids, Steroids, Saponins, and Tannins. The levels of enzymatic and non-enzymatic antioxidants in the leaf extract of *B.maderaspatensis* shown significant scavenging activity which emphasis its antioxidant status. **Conclusion:** The results of the present study revealed that the leaf extract of *Blepharis maderaspatensis* has exhibited a broad spectrum of antioxidant activities and could be serve as an excellent source of natural antioxidants.

**Keywords:** *Blepharis maderaspatensis* (L.) B.Heyne ex Roth, Phytochemical, Enzymatic antioxidants, Non-enzymatic antioxidants

INTRODUCTION

Plants have been used as healers and health-rejuvenators since time immemorial. The use of plant products in the form of local medicines dates back to 4000-5000 B.C. Even today, plants play an important role in the health care of about 80% of the world population and is estimated that more than half of the drugs under clinical use at present owe their origin to plants [1]. The traditional medicine all over the world nowadays revealed by an extensive...
activity of researches on different plant species and their therapeutic principles. Reactive oxygen species (ROS) including superoxide anion radical, hydroxyl radical, and hydrogen peroxide, are formed and degraded by all aerobic organism, can cause oxidative damage of all major groups of biomolecules (DNA, protein, lipids and small cellular molecules), which in turn leads to cardiovascular and neurodegenerative diseases [2]. Therefore, it is very important to find out new sources of safe and inexpensive antioxidants of natural origin. Recently, many researchers have shown interest in edible and medicinal plants for their phenolic contents and related total antioxidant activities [3, 4]. Phenolic and flavonoids, as natural antioxidants and free radical scavengers, have involved substantial interest due to their importance in food and pharmacological industry [5]. The antioxidant defense systems including enzymatic (superoxide dismutase, catalase and glutathione peroxidase) and non-enzymatic defense (gluthatione, vitamins C and E) play an important role in scavenging oxidants and preventing cell injury [6,7]. Plants contain phytochemicals with various bioactivities including antioxidant, anti inflammatory and anticancer activities. Currently, about 25% of the active component was identified from plants that are used as prescribed medicines [8]. *Blepharis maderaspatensis* (L.) B.Heyne ex Roth., Acanthaceae, is known as “Nethirapoondu”, in Tamil, is a prostrate, creeping, wiry plant, rooting at the nodes. It is seen commonly on slopes among rocks, poor gravelly soil on the hills up to 1400 m. It is used to treat the disorders such as boils, bone fracture, diarrhoea and lactation [9]. Paste of leaves is mixed with lime juice and applied on cuts [10]. Therefore, the present study was aimed to screen the phytochemicals and assess the antioxidant activities of the leaf extract of *B. maderaspatensis*.

**MATERIALS AND METHODS**

**Sample collection and preparation**

The leaf of *Blepharis maderaspatensis* were collected from Kolli Hills in Namakkal District, Tamil Nadu, India. The plant was authenticated by Plant Anatomy Research Centre, Institute of Herbal Botany, Chennai, Tamil Nadu, India. The samples were washed thoroughly in distilled water, shade dried at room temperature and then ground to a fine powder in a mechanic grinder. The powdered material was then extracted using various solvents (Aqueous, Methanol, Ethanol Hexane, Petroleum ether and Chloroform) in the ratio 1:10 using Soxhlet apparatus. After extracting all coloring material, the solvent was removed by evaporating in a water bath, which gave rise to a solid mass of the extract.
Phytochemical screening of the extracts
Chemical tests were carried out for all the solvent extracts of *B. maderaspatensis* as per the standard methods described [11,12] for the screening of phytochemical constituents like Alkaloids, Flavonoids, Phenols, Tannins, Saponins, Glycosides and Steroids. Each test was repeated at least three times to confirm the presence or absence of the phytochemicals.

Preparations of enzymes extracts
For determination of antioxidant enzymatic and non-enzymatic activities, extracts are prepared according to Nayyar and Gupta (2006) [13] with some modifications. Each leaf (0.5 g) was ground with 8 ml solution containing 50 mM potassium phosphate buffer (pH 7.0) and 1% polyvinylpolypyrrolidone. The homogenate was centrifuged at 15000 rpm for 30 min and supernatant was collected for enzymes assays. (Mansor et al., 2009) [14].

Enzymatic Antioxidant
Assay of Superoxide Dismutase
The activity of superoxide dismutase (SOD) was assayed according to the procedure of Kakkar et al., (1984) [15]. 0.5 ml of homogenate was diluted to 1 ml with water. Then 2.5 ml of ethanol and 1.5 ml chloroform (all reagents chilled) was added. This mixture was shaken for 1 minute at 4°C and then centrifuged. The enzyme activity in the supernatant was determined. The assay mixture contained 1.2 ml of sodium pyrophosphate buffer (0.025 M, pH 8.3), 0.1 ml of 186 μM phenazine methosulphate, 0.3 ml of 30 μM nitroblue tetrazolium, 0.2 ml of 780 μM nicotinamide adenine dinucleotide (reduced), appropriately diluted enzyme preparation and distilled water in a total volume of 3.0 ml. The reaction was started by the addition of nicotinamide adenine dinucleotide (reduced). After incubation at 30°C for 90 seconds, the reaction was arrested by the addition of 1.0 ml of glacial acetic acid. The reaction mixture was stirred vigorously and shaken with 4.0 ml of n-butanol. The intensity of the chromogen in the butanol layer was measured at 560 nm against a blank containing n-butanol. The activity of SOD was expressed as units/mg protein. One unit of the enzyme activity is defined as the enzyme concentration required inhibiting the OD at 560 nm of chromogen production by 50% in one minute.

Assay of Catalase
The activity of Catalase was determined by the method of Sinha (1972) [16]. To 0.9 ml of phosphate buffer, 0.1 ml of homogenate and 0.4 ml of hydrogen peroxide were added. After
60 sec. 2.0 ml of dichromate acetic acid mixture was added. The tubes were kept in boiling water bath for 10 minutes and the colour developed was read at 620 nm. Standards in the range of 2-10 µmol were taken and preceded as test with blank containing reagent alone. The activities were expressed as µmol of H₂O₂ consumed / min / mg protein.

**Assay of Glutathione Peroxidase**

Glutathione peroxidase was estimated by the method of Rotruck et al., (1973) [17]. To 0.2 ml of trisbuffer, 0.2 ml of EDTA, 0.1 ml of sodium azide and 0.5 ml of homogenate were added. To the mixture, 0.2 ml of glutathione followed by 0.1 ml of hydrogen peroxide was added. The contents were mixed well and incubated at 37°C for 10 minutes along with a tube containing all the reagents except sample. After 10 minutes the reaction was arrested by the addition of 0.5 ml of 10% TCA, centrifuged and the supernatant was assayed. The activities were expressed as µg of GSH consumed/ min/mg protein.

**Assay of Glutathione - S - Transferase**

Glutathione-S-transferase activity was assayed by the method of Jackoby and Habig (1980) [18]. The reaction mixture contained 1.0 ml of phosphate buffer, 0.1 ml of CDNB, 0.1 ml of homogenate and 0.7 ml of distilled water. The reaction mixture was incubated at 37°C for 5 minute then the reaction was started by the addition of 0.1 ml of 30mM glutathione. The absorbance change was read at 340nm for 5 minutes. Reaction mixture without the enzyme was used as the blank. The activities were expressed as µmoles of CDNB-GSH conjugate formed/min/mg protein

**Non enzymatic antioxidants**

**Estimation of Reduced Glutathione**

Reduced glutathione was estimated by the method of Ellman (1959) [19]. A known weight of plant tissue was homogenized in phosphate buffer. From this 0.5 ml was pipetted out and precipitated with 2.0 ml of 5% TCA. 1.0 ml of the supernatant was taken after centrifugation and added to it 0.5 ml of Ellman’s reagent and 3.0 ml of phosphate buffer. The yellow colour developed was read at 412 nm. A series of standards were treated in a similar manner along with a blank containing 3.5 ml of buffer. The amount of glutathione was expressed as µg/mg protein.
Estimation of Vitamin C
Ascorbic acid was estimated by the method of Omaye et al., (1979) [20]. 0.5 ml of tissue homogenate was mixed thoroughly with 1.5 ml of 6% TCA and centrifuged for 20 minutes at 3500 g. To 0.5 ml of the supernatant, 0.5 ml of DNPH reagent was added and mixed well. The tubes were allowed to stand at room temperature for an additional 3 hours. Removed, placed in ice-cold water and added 2.5 ml of 85% sulphuric acid and allowed to stand for 30 minutes. A set of standards containing 10-50 g of ascorbic acid were taken and processed similarly along with a blank, containing 0.5 ml 4% TCA. The colour developed was read at 530 nm. Ascorbic acid values were expressed as µg/mg protein.

Estimation of Vitamin E
The Vitamin E was estimated by the method of Baker et al., (1980) [21]. To 0.5 ml of tissue homogenate, 1.5 ml of ethanol and 2.0 ml of petroleum ether were added, mixed and centrifuged. The supernatant was evaporated to dryness at 80°C. To this, 0.2 ml of 2, 2'-dipyridyl solution and 0.2 ml of ferric chloride were added, mixed well and kept in dark for 5 minutes and 2.0 ml of butanol was added. The red colour developed was read at 520 nm. Vitamin-E values are expressed as µg/mg protein.

RESULTS AND DISCUSSION
Phytochemical screening
Medicinal plants contain several active principles with specific therapeutic effects. They represent a source of chemical compounds such as tannins, flavonoids, saponins, resins and alkaloids with curative properties, often not provided by synthetic chemical compounds [22]. Phytochemical evaluation of the various solvent leaf extracts of B. maderaspatensis were screened for the presence of Alkaloids, Flavonoids, Phenols, Tannins, Saponins, Glycosides and Steroids and the results are presented in Table 1. Many medicinal plants contain large amount of antioxidants such as polyphenols, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. Many of these phytochemicals possess significant antioxidant capacities that are associated with lower occurrence and lower mortality rates of several human diseases [22].
Table 1: Preliminary Phytochemical Screening of *B. maderaspatensis*

<table>
<thead>
<tr>
<th>Phytochemical Constituents</th>
<th>Aqueous</th>
<th>Methanol</th>
<th>Ethanol</th>
<th>Hexane</th>
<th>Petroleum ether</th>
<th>Chloroform</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Phenols</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+: presence; -: Absence;

**Enzymatic antioxidant status of *B. maderaspatensis***

Free radicals are produced by exogenous and endogenous factors in the human body. The most common reactive oxygen species (ROS) includes superoxide anion ($O_2^-$), hydrogen peroxide ($H_2O_2$), peroxyl radicals (ROO) and nitric oxide (NO). ROS play an important role in cell metabolism including energy production, phagocytosis and intercellular signaling. These ROS produced by sunlight, ultraviolet light, ionizing radiation, chemical reactions and metabolic processes have a role in wide variety of metabolic diseases such as DNA damage, carcinogenesis and various degenerative disorders such as cardiovascular diseases, aging and neuro-degenerative diseases, atherosclerosis and rheumatoid arthritis [24]. Epidemiological studies have revealed that the consumption of antioxidants is positively associated with the reduced risk of developing chronic and ageing related diseases. On the other hand, synthetic antioxidants have been shown to be potentially toxic. Therefore, there is a growing interest in searching for antioxidants naturally present in plants [25]. Table 2 shown the level of Enzymatic antioxidants such as SOD, CAT, GPx and GST of the fresh leaf extract of *B. maderaspatensis*. The activity of SOD, CAT, Glutathione peroxidase and Glutathione - S Transferase level was found to be 36.41 ± 1.2 units/mg protein, 42.72 ±0.51 μmole of $H_2O_2$ consumed/min/mg proteins, 255.68 ± 0.41 μg of glutathione oxidized/min/mg protein and 271.43 ± 0.62 μmoles of CDNB-GSH conjugate formed/min/mg protein respectively. Superoxide dismutase (SOD), catalase, and glutathione peroxidase as well as smaller molecules such as Vitamin E, are mainly responsible for the primary defense against oxidative damage [26]. Although synthetic antioxidants have often been used to protect against free radicals by scavenging reactive oxygen or ending radical chain reactions, recent
health concerns draw much attention to the use of natural antioxidative compounds [27],[28]. The glutathione-S-transferase are a ubiquitous, multifunctional family of enzymes that catalyses the conjugation of glutathione to a wide variety of electrophilic, lipophilic substrates. A number of GSTs also have a role in counteracting oxidative stress by way of an additional glutathione peroxidase activity with a substrate preference for organic hydroperoxides [29].

**Table 2: Level of enzymatic antioxidants in fresh Leaf extract of *B. maderaspatensis***

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superoxide dismutase</td>
<td>36.41 ± 1.2</td>
</tr>
<tr>
<td>Catalase</td>
<td>42.72 ±0.51</td>
</tr>
<tr>
<td>Glutathione peroxidase</td>
<td>255.68 ± 0.41</td>
</tr>
<tr>
<td>Glutathione S transferase</td>
<td>271.43 ± 0.62</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD
Units: SOD: Units/mg protein, Catalase: μmole of H2O2 consumed/min/mg protein, GPx: μg of glutathione oxidized/min/mg protein; GST: μmoles of CDNB-GSH conjugate formed/min/mg protein.

**Non - Enzymatic status of *B. maderaspatensis***

The level of Non – Enzymatic antioxidants such as Reduced Glutathione, Vitamin – C, Vitamin E of the fresh leaf extract of *B. maderaspatensis* were depicted in Table – 3. Among the Non - Enzymatic antioxidants estimated *B.maderaspatensis* possess highest amount of Vitamin E 246.21 ± 0.16 μg/mg plant tissues followed by Vitamin – C 178.45 ± 0.45 μg/mg plant tissues and Reduced Glutathione 53.63 ± 0.11 μg/mg plant tissues respectively. Glutathione, a major non protein thiol in living organisms, plays a central role in coordinating the body’s antioxidant defense processes. Excessive peroxidation causes increased glutathione consumption. Reduced thiols have long been reported to be essential for recycling of antioxidants like vitamin E and vitamin C [34]. Vitamin E scavenges peroxyl radical intermediates in lipid peroxidation and is responsible for protecting poly unsaturated fatty acid present in cell membrane and low density lipoprotein (LDL) against lipid peroxidation [27]. Vitamin C is a water soluble antioxidant. It acts as a free radical scavenger. It scavenges peroxy radicals [36].
Table 3: Level of Non-enzymatic antioxidants in fresh Leaf extract of B. maderaspatensis:

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reduced glutathione</td>
<td>53.63 ± 0.11</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>178.45 ± 0.45</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>246.21 ± 0.16</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD

Units: Total reduced glutathione - µg/mg plant tissues; Vitamin C - µg/mg plant tissues; Vitamin E - µg/mg plant tissues.

CONCLUSION
The present study demonstrated that the leaf extract of B. maderaspatensis posses potential phytochemicals and antioxidant activities. Thus, the leaf extracts can be considered as a new source of effective natural antioxidant and could serve as a valid tool in the prevention of human diseases related to oxidative damage. Therefore, it is suggested that further work can be performed on the isolation and identification of the bioactive compounds in Blepharis maderaspatensis.

CONFLICT OF INTEREST STATEMENTS
We declare that we have no conflict of interest.

ACKNOWLEDGEMENT
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