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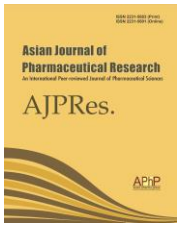

RESEARCH ARTICLE

Evaluation of Antioxidant and Anti-inflamatory Activity of Ethanolic Extract of *Glochidion acuminatum* Leaves

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ABSTRACT:
The present study was undertaken to investigate the antioxidant and anti inflammatory activity of ethanolic extract of *Glochidion acuminatum* leaves. Ethanolic extract of the leaves of the plant was subjected to various in-vitro assays. Antioxidant potential was tested through total phenol content, total flavonoid content, DPPH free radical scavenging capacity and total anti-oxidant capacity assay. Anti-inflammatory activity was tested against the denaturation of protein. *Glochidion acuminatum* exhibited good antioxidant potential on total phenol content assay, TPC as GAE was 507.24±19.48mg/gm. It showed remarkable antioxidant potential on total flavonoid content assay, TFC as QE was 486.28±16.44mg/gm. In DPPH free radical scavenging assay IC_{50} value was 14.97µg/ml while for standard ascorbic acid it was found to be 8.87µg/ml which express potent antioxidant activity. It also showed excellent antioxidant potential on total antioxidant capacity assay, TAC as AAE was 712.5±5.89µg/ml. Anti-inflamatory assay showed that the plant leaves extract possess very good anti-inflamatory potential. These effects may be due to the presence of different phytoconstituents present in the extract. Further studies are required to evaluate these effects and due to the findings which warrants further more sophisticated research.

KEYWORDS: *Glochidion acuminatum*, Ethanolic Extract, DPPH, Antioxidant and Anti-inflammatory Activity.

INTRODUCTION:
For the treatment of new diseases and the existence diseases, different drugs are required. We have noticed that human body have become resistant to drugs which are available in market. Also because of using synthetic drugs, various toxic and side effects occur. So recently, curiosity has grown in world for herbal medicines which are obtained from medicinal plant. Although the toxicity profile of most medicinal plants have not been thoroughly evaluated, it is generally accepted that medicines derived from plant products are safer than their synthetic counterparts[1-2].

The role of free radical reactions in disease pathology is well established and is known to be involved in many acute and chronic disorders in human being such as diabetes, atherosclerosis, aging, immunosuppression and neurodegenerator[3]. Recent interest in naturally occurring antioxidants has considerably increased for use in food, cosmetic and pharmaceutical products because they possess multiphase in their multitude and magnitude of activity and provide enormous scope in correcting imbalance[4-5]. Recent reports indicated that there is an inverse relationship between the dietary intake of anti-oxidant rich food and incidence of human diseases. Denaturation of proteins is a well-documented cause of inflammation and rheumatoid arthritis. Phenylbutazone, salicylic acid, flufenamic acid (anti-inflammatory drugs) etc, have shown dose dependent ability to inhibit thermally induced protein denaturation. As a part of the

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investigation on the mechanism of the anti-inflammatory activity, ability of extract to inhibit protein denaturation was studied.

Glochidion acuminatum is a shrub or a tree of Phyllanthaceae family it is an evergreen tree growing up to 6 meters tall. The tree is harvested from the wild for local use as a food and wood. The plant has been traditionally known for its medicinal properties. Traditionally it is used in stomach ailments. This species is found in Bangladesh, China, Northern India, Nepal, Myanmar, and Thailand where it lives in the open forests up to hilly altitudes. In Bangladesh the local name of this plat is Anguti.

MATERIALS AND METHODS:

Collection and Identification of Plant:

Leaves of *Glochidion acuminatum* were collected from Lawachara National Park, Srimongol, Sylhet, Bangladesh. Later it was recognized by Bangladesh National Herbarium, Bangladesh (DACB accession number 48255) and verification was done by the corresponding authority.

Preparation of Plant samples:

The experimented plant leaves were separated from undesirable plant parts. Then air dried in shed for 5 days. After completion of drying, dried leaves were pulverized into coarse powder by suitable grinding machine. Powders were kept in clean airtight glass containers with desiccator for further use. Here the grinder was properly cleaned so that contamination with previous ground material on other foreign matter can be avoided. The weight of leaf powder was 665gm. Finally it is placed in dry and cool area until experiment begins.

Extraction of powdered samples:

After drying and grinding 300gm dried leaves powder was soaked with 500ml ethanol in an amber glass jar for 3 days with periodically stirring. Then the extract was filtered by using sterilized cotton plug followed by Whatman filter paper. After the extraction process the solvent was evaporated by rotary vacuum evaporator (Bibby RE-200, Sterilin Ltd., UK) at 160rpm, 40^{o}C temperature, 2 Pa pressure. Then the oily crude extract was collected on a sterile petri dish after completion of evaporation. Then the crude extract was preserved at +4^{o}C.

Chemicals: All the chemicals and reagents used throughout the investigation were of reagent grade.

In-vitro Antioxidant Potential Evaluation:

The selected oily crude extract of *Glochidion acuminatum* was subjected to various in-vitro models of antioxidant activity described below.

Determination of Total Phenolic content:

The content of total phenolic compounds was determined by using the Folin-Ciocalteu Reagent (FCR).[6] Extract (200µg/ml) was mixed with 400µl of the Folin-Ciocalteu reagent and 1.5ml of 20% sodium carbonate. The mixture was shaken thoroughly and made up to 10ml using distilled water. The mixture was allowed to stand for 2 hrs. Then the absorbance at 765 nm was determined. The concentration of total phenol content in the extract was then determined as mg of gallic acid equivalent by using an equation that was obtained from standard gallic acid graph.

Determination of Total Flavonoid Content:

Flavonoid content of the plant extract was determined according to the method described by Kumaran and Karunakaran[7]. 1.0ml of plant extract (200µg/ml) was taken in test tube and 3ml of ethanol was added into the test tube. Then 200µl of 10% aluminium chloride solution and 200µ1 of 1M potassium acetate solution were added to the mixtures in the test tube. Then 5.6ml of distilled water was added into the test tube and was incubated for 30 minutes at room temperature to complete reaction. Then the Standard (Quercetin) solutions of different concentration were prepared at the same manner. The absorbance of the solutions was measured at 415nm using a UV-VIS spectrophotometer against blank. Then the concentration of the Flavonoid content was determined as mg of Quercetin equivalent using an equation that was obtained from standard Quercetin graph.

DPPH Radical Scavenging Assay:

DPPH free radical scavenging activity of the plant extract was determined following the method described by Braca et al.[8]. Free radical scavenging activity of the plant extract was determined using stable free radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH). Plant extract (0.1 ml) was added to 3ml of a 0.004% ethanol solution of DPPH. Absorbance at 517nm was determined after 30 min using a UV-VIS spectrophotometer and the percentage inhibition activity was calculated from [(A0–A1)/A0] x100, where A0 is the absorbance of the control, and A1 is the absorbance of the extract/ standard. The inhibition curves were prepared and IC50 values were obtained.

Determination of Total Antioxidant Capacity:

Total antioxidant capacity of the plant extract was determined using the method described by Prieto et al.[9] The assay is based on the reduction of Mo (vi) to Mo(v) by the extract and formation of a green Phosphate/ Mo(v) complex at acid pH. 300µl of extract solution was taken in a test tube and 3ml of reagent solution (0.6 M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate) was added into the test tube. The test tube was incubated at 95°C for 90 minutes to

complete reaction. The Standard (Ascorbic Acid) solutions of different concentration were prepared at the same manner. Then the absorbance of the solutions was measured at 695nm using a UV-VIS spectrophotometer against blank after cooling to room temperature. For the preparation of blank solution 300µl ethanol was used instead of sample. The antioxidant capacity was expressed as the number of gram equavalents of Ascorbic acid.

Evaluation of in vitro anti-inflammatory activity:

The extract at different concentrations was incubated with egg albumin in controlled experimental conditions and subjected to determination of absorbance to assess the anti-inflammatory property. Diclofenac sodium was used as the reference drug.[10] 1ml of plant extract and standard drug of different concentrations (500, 400, 300, 200 and 100µg/ml) was mixed with 1ml of 5% egg albumin. The pH (5.6±0.2) of the reaction mixtures was adjusted by 1 N HCl solution, and incubated at 27^{c} for 15 minutes.The mixture was kept at 70^{c} in a water bath for 10 minutes to induce denaturation.After Cooling the turbidity was measured spectrophotometrically at 660nm. Percentage of inhibition of denaturation was calculated from control where no drug was added.

RESULTS:

In-vitro Antioxidant Potential Evaluation:

Total Phenolic Content Determination: The Phenol content of the *G. Acuminatum* was determined by using the calibration curve of Gallic acid (Fig-1) and the value was expressed as Gallic acid equivalent (GE) which was 507.25±2.57.

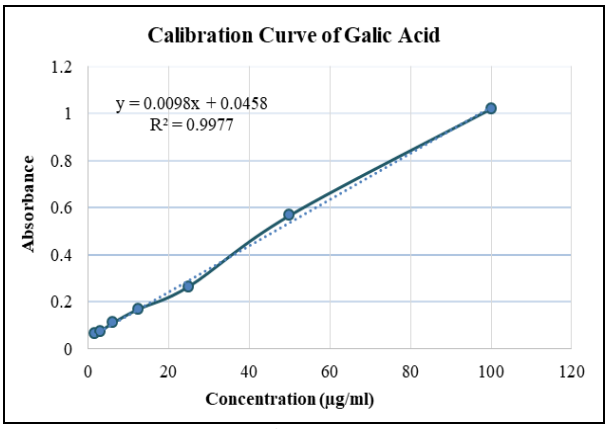

Figure 1: Calibration Curve of Gallic Acid

Total Flavonoid Content:

Flavonoids are a large clsss of benzo-pyrone derivatives, ubiquitous in plants and exhibit antioxidant activity. Flavonoids content of the *G. Acuminatum* was expressed as Quercetin equivalent (QE) and the value was 497.91±1.25

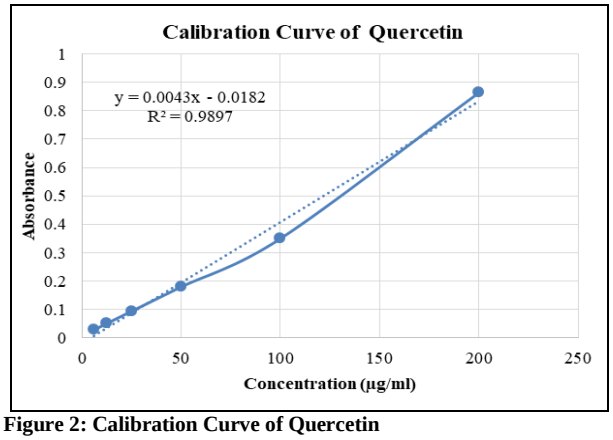

Figure 2: Calibration Curve of Quercetin

DPPH Free Radical Scavenging Activity:

The plant extract showed dose dependent free radical scavenging activity. The percentage inhibition is shown in fig-3. The extract possessed DPPH free radical scavenging activity, IC50= 14.97 compared with standard Ascorbic acid (IC50= 8.87)

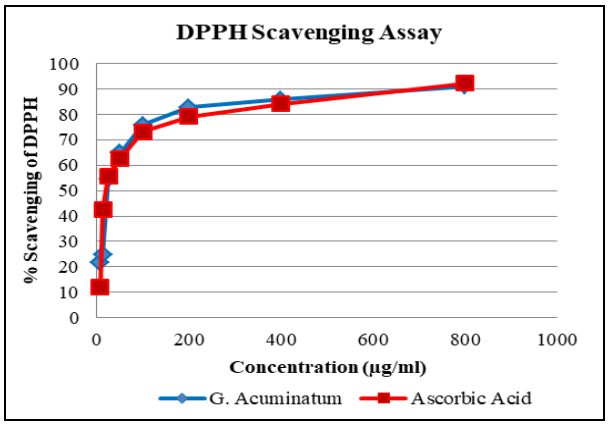

Figure 3: DPPH Scavenging Assay

Determination of total antioxidant capacity:

Total antioxidant capacity of the *G. Acuminatum* was calculated using the standard curve of ascorbic acid (Fig-4) which was expressed as ascorbic acid equivalents (AAE) per gram of plant extract. The value was 712.5±2.89mg/gm

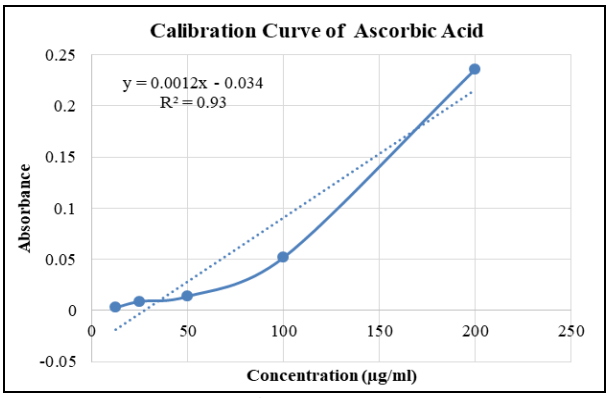

Figure 4: Calibration curve for ascorbic acid.

In vitro anti-inflammatory activity:

Ability of the extract of *Glochidion acuminatum* to inhibit thermal denaturation of protein is possibly a contributing factor for its anti-inflammatory activity. The extract exhibited dose dependent inhibition of protein denaturation in comparison to standard Diclofenac Sodium which reflect that the extract possess prominent anti-inflamatory activity.

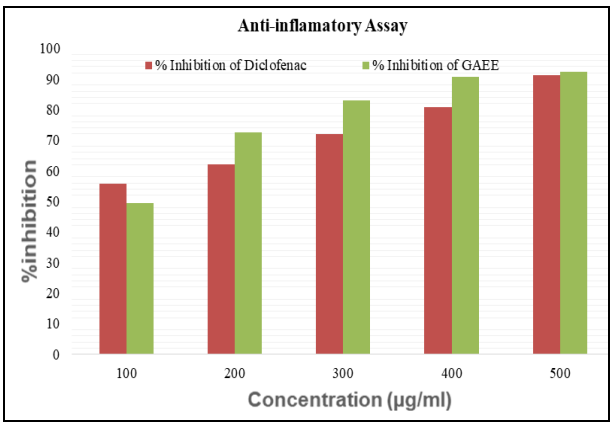

Figure 5: Anti-inflamatory Activity of Diclofenac Sodium and GAEE (*Glochidion acuminatum* Ethanolic Extract)

DISCUSSION:

This study revealed that the ehanolic extrac of the leaves of *Glochidion acuminatum* possess remarkable antioxidant activity which was evaluated through the determination of total phenolic compounds and flavonoids, DPPH scavenging assay and total antioxidant capacity.

The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching single and triplet oxygen, or decomposing peroxides.[11] The anti-oxidative effect of the extract may be due to presence of phenolic components. Aluminium chloride colorimetric method was used to determine the total flavonoid contents of the extract. Flavonoids, a subclass of polyphenols, are the most common polyphenolic compounds found in nature and are further divided into several subclasses including flavones, flavonols, isoflavones, anthocyanins, flavanols, and proanthocyanidins. Flavonoids exerts their antioxidative properties of by several different mechanisms, such as scavenging of free radicals, chelation of metal ions, such as iron and copper, and inhibition of enzymes responsible for free radical generation.[12] When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance. Antioxidant properties, especially radical scavenging activities, are very important due to the deleterious role of free radicals in foods and in biological systems. Excessive formation of free radicals accelerates the oxidation of lipids in foods and decreases food quality and consumer acceptance. The DPPH assay is often used to evaluate the ability of antioxidants to scavenge free radicals which are known to be a major factor in biological damage caused by oxidative stress. Total antioxidant capacity is expressed as the number of equivalents of ascorbic acid. The phosphomolybdenum method is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate/Mo (V) complex. Phenolic compounds and flavonoids have been reported to be associated with antioxidative action in biological systems, acting as scavengers of singlet oxygen and free radicals[13]. Among the numerous naturally occurring antioxidants; ascorbic acid, carotenoids and phenolic compounds are more effective. They are known to inhibit lipid peroxidation, to scavenge free radicals and active oxygen species by propagating a reaction cycle and to chelate heavy metal ions. Denaturation of proteins is a well-documented cause of inflammation. Several anti-inflammatory drugs have shown dose dependent ability to inhibit thermally induced protein denaturation.

CONCLUSION:

It was observed from the present study that the ethanolic extract of the leaves of *Glochidion acuminatum* has the remarkable total phenol content and total flavonoids. The extrac also possesses prominant DPPH scavenging activity and total antioxidant capacity. Again The extract exhibited dose dependent inhibition of protein denaturation. All the conducted experiments in the present study are based on crude extract and are considered to be preliminary and more sophisticate research is necessary to rich a concrete conclusion about the findings of the present study.

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