

Potential Investigation of Anti-Inflammatory Activity and Phytochemical Investigations of Ethanolic Extract of *Glycosmis Pentaphylla* Leaves

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Abstract The present study is carried out with the ethanolic extract of leaves of *Glycosmis pentaphylla*. Traditional use of it inspired us to investigate the blood corpuscular protective power of this plant as it used as blood tonic in Chinese traditional medicine. By the phytochemical screening we have both flavonoids and steroids. There's also alkaloids are found which have extensive physiologic action embodying analgesic activity. This investigation is made following the most simple, reliable and less time consuming method. As the human red blood corpuscular membrane is similar to lysosomal membranes that influence inflammatory process. Significant result obtained using ethanolic extract of *G. pentaphylla* have better acceptance as it shows good response in inhibiting hemolysis (55.16%) at highest concentration and these investigation surely stimulate further screening and isolation process.

Keywords: *G. pentaphylla*, lysosome, membrane stability, phytochemicals investigation

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1. Introduction

Inflammation is one common and major cause of sufferings now and every time past. Those drugs that are available are known as NSAID, i.e. non-steroidal anti-inflammatory drugs, act by inhibiting the function of prostaglandin. Prostaglandin is an autocoid that release extracellularly and initiate pain. Anti-inflammatory agents either block this autocoid synthesis by inhibiting COX enzyme or protecting lysosomal membrane from break down.

Plant is a source of wide variety of chemicals. Most of them need to be synthesized. One plant may consist of several compounds that have several effects on physiology. The main source of medicine from the beginning of mankind till modern time is plant. The synthesis of plant is both dangerous and harmful as they may be toxic. But in our daily life deliberately or undeliberately we take plants as food and they shows their regular biochemical action which is unnoticed to us, like tea shows stimulatory action for caffeine, beetle nut cause aphrodisiac action for some alkaloidal content etc. *Glycosmis pentaphylla* is a species of small shrub and flowering plant in the citrus family, Rutaceae, known commonly as orange berry (English). [1] *G. pentaphylla* has a long history of usage in

traditional medicine against various ailments around the world. In Ayurvedic and other traditional medicinal practices the plant has been used against diseases like bilious complaints, cough, worms, jaundice, fever, inflammation, rheumatism, anaemia and vermifuge. Phytochemicals like alkaloids, flavonoids, terpenes and sterols have been isolated. [2] The literature review says *G. pentaphylla* has hypoglycemic, anti-inflammatory, [3] anti-oxidant [4] and more recent search on this, suggest that it is more apoptotic than others [5].

2. Methods and Materials

2.1. Plant Material Preparation

The plant material was collected from wild and hilly part of Bangladesh (Bandarban, Chittagong) and was identified by Forest Research Institute of Bangladesh. The collected plant was dried for a few days in natural way and then at hot air oven ($37\pm 2^\circ\text{C}$) for 3 hours. It macerated to powder form and about 250 gm powder was dissolved in 500 ml methanol (95%) following cold extraction [6,7]. It takes couple of days for proper dissolution then filtered through Buchner funnel and again dried at water bath (40°C) for evaporation of methanol and extract preserved at $<4^\circ\text{C}$ for next use [8].

2.2. Primary Phytochemical Screening

Phytochemicals of the selected plants were carried out by using aqueous and powdered form of the plant following methods described by Md. Reyad-ul-ferdous [9].

2.3. Test for Tannin

About 0.5 gm powdered sample was boiled in water (20 ml), filtered it, and a few drops of 0.1% ferric chloride solution was added to see the brownish green or blue black coloration.

2.4. Test for Saponin

About 2 gm of dried sample was taken with 20 ml of distilled water for boiling in water and filtered. 10 ml filtrate was added with 5 ml distilled water and vigorous shaking; 3-4 ml olive oil was added for formation of emulsion.

2.5. Test for Flavonoid

5 ml of ammonium solution was added to a portion of aqueous extract following the addition of concentrated H₂SO₄. Yellow coloration confirms the presence of flavonoids. On standing for few moments it disappears. 1% aluminum solution, 2-3 drops, was added to a portion of aqueous extract filtrate. Yellow coloration concludes the presence of flavonoid [10].

2.6. Test for Steroid

5 ml of aqueous extract was added to 2 ml chloroform successively 3ml concentrated H₂SO₄ added, cautiously for reddish brown intermittent layer, which confirm positive result.

2.7. Test for Alkaloid

Alkaloids are basic nitrogenous compounds with definite physiological and pharmacological activity. The alkaloid solution produces white-yellowish precipitation, when a few drops of Meyer's reagent were added [11]. Most alkaloids are precipitated from neutral or slightly acidic solution by Meyer's reagent [12]. The ethanolic extract was evaporated to dryness and the residue was heated on boiling in water bath with 2% HCl. After cooling, the mixture was filtered and treated a few drops of Meyer's reagent. The samples were then holding to observe turbidity or yellowish white precipitation.

2.8. Anti-inflammatory Activity by Membrane Stability Assay

Anti-inflammatory activity of ethanolic extract of *Glycosmis pentaphylla* was evaluated by using *in vitro* human red blood cell stability method. Blood sample was collected from a fresh volunteer, who doesn't have anti-inflammatory or contraceptive drugs at least since a week. The collected blood was mixed with sterilized Alsever solution. Alsever solution was prepared by 2% dextrose, 0.8% sodium citrate, 0.05% citric acid and 0.42% sodium chloride dissolved in distilled water. Blood sample was centrifuged at 3000 rpm and packed cell was washed with isosaline and a 10% (V/V) suspension of isosaline was made [13,14]. Three different solution of *G. pentaphylla*

were mixed with 1 ml phosphate buffer, 2 ml hyposaline and 0.5 ml HRBC suspension. Diclofenace was used as contrastable drug and instead of hyposaline 2 ml water was used as control. The hemoglobin content in supernatant was calculated using spectrophotometer at 560 nm spectrum.

The result was estimated by following equations [15].

$$\text{Percentage of hemolysis} = \frac{\text{OD of test}}{\text{OD of control}} \times 100$$

The percent of membrane protection was calculated by the following equation

$$\text{Percent of protection} = 100 - \frac{\text{OD of test}}{\text{OD of control}} \times 100$$

2.9. Statistical Analysis

Statistical analysis was conducted using one way analysis of variance (ANOVA) followed by Dennett's multiple tests. Results are expressed as mean±SD. statistical analysis shows significant value as p<0.005.

3. Results and Discussion

The extractive preliminary phytochemical analysis that performed earlier results the presence of alkaloid, flavonoid, steroid, saponin etc. It was qualitative analysis only, performed to find out and predict why the plant has anti-inflammatory effect. Alkaloid, a nitrogenous group of phytochemical that has wide diversity in classification and distribution, has good evidence of pain killing activity [16]. Recent observations from animal and human studies have demonstrated anti-inflammatory effects of phytochemicals. For example, several animal and human studies report reductions in the levels of pro-inflammatory cytokines, including C-reactive protein, after consumption of dietary plant sterols [17]. Flavonoids have the hepatoprotective reputation as anti-oxidant phytoagent. So anyone in the present phytomaterials may cause the pain reduction that needs further concern. Present study exhibit several phytoconstituents mention in the (Table 1).

Table 1. Preliminary phytochemical screening of ethanolic extract of *G. pentaphylla* leaves

Phytochemicals	Status
Alkaloid	+
Glycoside	+
Terpenoid	-
Flavonoid	+
steroid	+
Tannis	+
Reducing sugar	-
Resin	+
Antraquinone	+
Saponin	+
phllobotanin	-

(+) = present, (-) = absent

The main function of anti-inflammatory agent is to inhibit the function of cyclooxygenase (COX) enzyme that is responsible for conversion of arachidonic acid to prostaglandin (PG). When the nociceptor or pain receptor activated it influence the release of that enzyme and which then function then extracellularly by converting arachidonic acid to prostaglandin. Non-steroidal anti-

inflammatory drugs either stop or inhibit the conversion or protect the lysosomal membrane to inhibit inflammation.

The Table 2 shows the anti-inflammatory action of *G. pentaphylla* ethanolic extract. This was only a preliminary testing where we have such kind of concentration dependent percent of membrane protection. As the HRBC membrane is likely to the membrane of lysosome

therefore the stabilizing ability of HRBC will be implied as its ability to protect the lysosomal membrane as well. In this test Diclofenac, as standard, has 75.34% of protection at 1000 µg/ml where extract has 55.16% at the same concentration. It seems the extract has significant activity on anti-inflammatory functioning.

Table 2. Anti-inflammatory activity of ethanolic extract of *G. pentaphylla* leaves by using HRBC membrane protection testing

No. of sample	Concentration				
	1000 µg/ml	500 µg/ml	250 µg/ml	125 µg/ml	62.5 µg/ml
Diclofenac	75.34±1.08%	69.70±1.78%	54.82±0.67%	34.43±0.65%	16.55±0.77%
<i>G. pentaphylla</i>	55.16±0.97%	47.65±0.83%	41.05±1.22%	25.32±1.06%	7.54±1.11%

n=3, mean ± SEM, this percentage represents the percent of protection provided by both groups, the statistical analysis shows significant value as p<0.005.

4. Conclusion

In the conclusion it can be said that the experiment was helpful for further isolation of natural product as in pain reduction purposes. Most of drugs are not safe when they came from synthetic source but if they are from nature, it becomes better than synthetic. No agent can be allowed for clinical trial or for animal model induction before it go through in vitro study or primary tests, because it is quite harmful and offensive as well so if the agent have in vitro good result then these steps can be considered. Now from this study we have both phytochemical knowledge and membrane stabilizing data for *G. pentaphylla* and it provide us good result to precede further steps. As the study shows that *G. pentaphylla* has property of pain or inflammation healing and from phytochemical analysis we found the presence of alkaloid and steroid, so it could also have anti-coagulation property as well. The presence of flavonoid enhances the scope to find out its anti-oxidant property, it seems this would be an ideal agent as anti-inflammatory agent if properly modified.

4.1. Ethical Consent

Blood samples were withdrawn from those healthy persons who were volunteers themselves and also after getting clearance from our institutional ethical committee.

Conflict of Interest

All authors declare that there is no conflict of interest.

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