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Erythrocyte Membrane Stabilization Method: An Empirical Evaluation of Anti-Inflammatory Activity of Methanol Extract of *Syzygium cumini* (Pulp)

Tanwy Chowdhury, Md. Abdul Motaleb Bhuiya*, Sumaiya Nahid, Antica Barua

Department of Pharmacy, University of Science and Technology Chittagong, Chittagong, Bangladesh

Research Article

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***For Correspondence:**
Md. Abdul Motaleb Bhuiya, Department of Pharmacy, University of Science and Technology Chittagong, Chittagong, Bangladesh
E-mail: motaleb@ustc.ac.bd

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ABSTRACT

The motive of this experiment was to evaluate the anti-inflammatory activity of methanol extract of Syzygium cumini pulp or fruit. Fresh fruits of Syzygium cumini were collected, dried, powdered and extracted with methanol. In search of phytochemical constituents accountable for anti-inflammatory activity, primary phytochemical screening of crude methanol extracts of the pulp was done by using placed methods. The extract was then subjected to membrane stabilization method. Results brought out the presence of phytochemicals like alkaloid, flavonoid, anthocyanin, tannins, steroids, and saponin. In membrane stabilizing assay, crude methanol extract of pulp of Syzygium cumini was found to be very effective for stabilizing erythrocyte membrane in hypotonic solution. The highest concentration of the pulp extract (1000 µg/ml) showed 62.381% inhibition with methanol extract compared with the standard drug Diclofenac sodium, which accounted for 87.143% of inhibition. The result of the present study demonstrates a favorable baseline in progression for the possible use of pulp or fruit of Syzygium cumini to treat inflammation.

INTRODUCTION

Medicinal plants have performed an essential role in the improvement of human culture. It is confirmed by WHO that traditional, complementary, and alternative medicine can be used as an input to “modern” pharmaceutical

research, health care systems, but also as a source of effective treatment in its right. About 14 of the 15 therapeutic categories of pharmaceutical preparations encircle herbal products that are recommended by practitioners and considered as a significant part of the health care system in the western world [1]. Elucidating the phytochemical profiles of herbal medicine that are made up of natural components, are readily metabolized in the body and much safer than existing drugs, has become a research focus for all scientific communities. Ethno botanists are frequently involved in evaluating the safety and efficacy of herbal medicine especially pharmacological and toxicological testing are growing tendency as not to be contended with the mere collected list of botanically recognized native plant names, and their uses.

The chemical composition of herbal medicine can provide intimations for designing, screening, and developing multi-target therapeutics. However, many herbal formulations are crude preparations which possess insoluble character leading to lower bioavailability because of hydrophobic phytoconstituents and increased systemic clearance. Several experimental activities have found out that herbal medicines exhibit good activity in assays *in vitro*, which are not reproducible in experiments *in vivo*. Introduction of nanotechnology to herbal medicine may initiate the development of nanoherbal products, which will bring out a new era of herbal drug discovery. Because this drug delivery system has many advantages including the enhancement of pharmacological activity and stability, improvement of solubility, protection from toxicity, and physical and chemical degradation.

Syzygium cumini (family; Myrtaceae) which is an evergreen tree commonly known as Jamun, Jambu, Mahajambu, Indian Blackberry, Black plum, Surabhipatra, Raj Jambu, Jambul, Kalajam, Jambolan, Palendera, Jam, Mahaphala, and Kalojum and native to the Indian sub-continent, Thailand, Philippines, Florida, Brazil, California, Algeria and Israel, and many others regions of South Asia such as India, Bangladesh, Pakistan, Nepal, Burma, Sri Lanka and Indonesia.The synonyms of *Syzygium cumini* are *Syzygium jambolana Lam*., *Eugenia cumini, Eugenia djouant Perr., Eugenia jambolana Lam.* and *Myrtuscumini Linn*. Traditionally, all parts (bark, leaf, fruit, seed) of the *Syzygium cumini* treehave medicinal value because of the existence of anthocyanins, tannins, flavonoids, ellagic acid, polyphenol derivative, malic acid, gallicacid, oxalic acid which give anti-inflammatory, neuropsychopharmacological, antioxidant, antimicrobial, anti-HIV, antifungal, antifertility, anorexigenic, antibacterial, gastroprotective and anti-ulcerogenic, antidiarrheal and redioprotective activities [2-4]. Fruits of *Syzygium cumini* have a high amount of anthocyanins and contain polymerized phenolic compounds like hydrolysable tannins such as ellagitannins and gallotannins, and condensed tannins called as Proanthocyanidins (PACs) which multiply its antioxidant activity and prevent the formation of free radicals and reduce oxidative stress and heal inflammation. Anthocyanins, a flavonoids subclass, are known to inhibit lipid peroxidation.

Following to the best of our learning, many studies have reported the membrane stabilizing activity of methanol extracts of various plants, and there is not any scientific detailed report on *in vitro* anti-inflammatory activity of pulp of *Syzygium cumini*. Thus, we have selected the methanol extract of pulp of *Syzygium cumini* to investigate anti-inflammatory activity [5].

MATERIALS AND METHODS

Collection and preparation of plant material

The fully advanced fruits of *Syzygium cumini* (about 12 kg) were picked up from local market during the study period July 2019 (Bangladesh) and they were washed with freshwater and removed impurities. Then the pulp of the fruit was separated from the seed. The pulps were dried away from direct sunlight, then powdered by a blender and stored at room temperature. The methanol extract of the pulp was the sample used for the study.

Preparation of extract: 1250 grams of dried powder of pulp was weighed and taken into an aspirator. The aspirator must be washed and dried properly. 1360 ml of solvent (Methanol) was added gradually in the container and sealed on. It should be kept for 15-20 days with episodic shaking to allow the extractable components to dissolve in the solvent [6]. Cotton wool was used to filtrate the mixture and let it to concentrate by evaporating in dry and clean air. The obtained extract was kept in refrigerator until use. The % of yield is 17.19%.

Chemicals: Methanol was bought from Kuri and Company private limited Bangladesh. Normal saline solution (0.9% NaCl) and Diclofenac sodium were purchased from a nearby pharmaceutical shop.

Phytochemical screening: The methods of phytochemical screening of pulp extract elicited the presence of phytochemical components, namely, anthocyanins, flavonoids, steroids, alkaloids, tannins, and saponin.

***In vitro* anti-inflammatory test (Membrane stabilization method):** The present study was developed by certain alteration of the method proposed in 1964, based on *in vitro* determination of the human RBC cell caused by the control group and comparing it with the positive and test group. Blood (5 mL) was collected using venipuncture from a healthy volunteer (HRBC).The sample blood was mixed with same amount of sterilized alsever solution [7]. Alsever solution is a mixture of 2% (w/v) dextrose solution, 0.05% (w/v) citric acid, 0.42% (w/v) sodium chloride and 0.8% (w/v) sodium citrate that are dissolved in distilled water. The sample was centrifuged at 3000 r/min for 5 minutes and the resultant packed cells were washed three times using isosaline and 10% (v/v) isosaline (suspension) was made. To carry out the experiment, 1 mL phosphate buffer (pH 6.8), 2 mL hyposaline, and 0.5 mL HRBC suspension were used to prepare various concentrations of pulp extract and the control sample contained hypotonic buffered saline mixed 0.5 ml of erythrocytes and standard drug is Diclofenac sodium [8]. The mixtures were incubated at 37°C for 30 minutes and centrifuged at 3,000 r/min for 5 minutes. The absorbance of supernant was measured using UV analysis at 560 nm. The percentage of inhibition produced was estimated by using the following equation.

$$\%Membrane\ Stabilization = \%Membrane\ Stabilization - \left\{100 - \left(\frac{\text{Absorbance of Sample}}{\text{Absorbance of Control}}\right) \times 100\right\}$$

RESULTS

Phytochemical screening

The methanol extract of pulp of *Syzygium cumini* in different chemical tests showed the presence of anthocyanins, flavonoids, steroids, alkaloids, tannins, and saponin (Table 1).

Table1: Qualitative phytochemical screening of Syzygium cumini pulp extract.

Chemical constituent	Results of the methanol extract of *Syzygium cumini* (Pulp)
Alkaloid	+
Flavonoid	+
Anthocyanins	+
Tannin	+
Saponin	+
Steroid	+
Note: (+) Positive result	

In-vitro anti-inflammatory test

Membrane stabilization is a way to stabilize the membrane in which anti-inflammatory drugs are involved in maintaining the integrity of both erythrocyte membrane and lysosomal membrane. At the concentration range of 250-1000 µg/ml, anti-inflammatory activity of pulp of *Syzygium cumini* increases with increasing concentration. The highest concentration of the pulp extract (1000 µg/ml) afforded 62.381% inhibition. At a concentration of 1000 µg/ml, 87.143% inhibition was shown by Diclofenac sodium.

DISCUSSION

The results of screening tests of pulp extract of *Syzygium cumini* represent the presence of anthocyanins, alkaloids, flavonoids, tannins, steroids, and saponin. Denaturation of proteins occurs due to the application of external compounds including strong acid or base, organic solvents or by heat or stress, which is a known cause of inflammation as proteins lose their distinct structure and biological function. Inflammation results due to the tissue injury caused by destruction of macromolecules when enzymes are released into the cytosol by lysosomal membrane anti-inflammatory drugs work to stabilize the erythrocyte and lysosomal membrane from inflammation by blocking the release of lysosomal enzymes [9].

Extensive studies are required to explore more anti-inflammatory agents from natural sources, as excessive use of anti-inflammatory drugs causes stomach problem, heart diseases, kidney problem, allergic reaction, etc. The RBC membrane and components of lysosomal membrane are analogous to each other. Sometimes cell lysis is promoted when in contact with hypotonic conditions due to the excessive oxidative stress in the cell wall. Fruit extract of *Syzygium cumini* protects the RBC membrane from destruction means that it may also stabilize the lysozomal membrane because of the similarities between the two membranes. Comparing with the anti-inflammatory activity of standard drug diclofenac sodium, a considerable similar activity was displayed for the pulp extract of *Syzygium cumini*. Stabilization of Human Red Blood Cell Membrane (HRBC) by hypo tonicity induced membrane lysis can be used as an *in vitro* measure of anti-inflammatory activity of the drugs or plant extracts [10].

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CONCLUSION

In the present work, phytochemical screening tests were done to prove the presence of antioxidant molecules such as flavonoids, anthocyanins, and polyphenols. Antioxidant molecules are responsible for preventing oxidative stress and inflammation. Anthocyanins present in the extract of *Syzygium cumini* pulp inhibit lipid peroxidation, enzymatic and non-enzymatic lipid peroxidation, protect DNA cleavage, enzyme inhibition, regulate immune responses, and provide anti-inflammatory activity. Therefore, the outcomes of the investigation of methanol extract of *Syzygium cumini* pulp indicate that it possesses anti-inflammatory properties. The inhibitory effect is founded to be dependent on concentration. The extract can also be used as a topical preparation

All in all, the outcomes of the present experiment provide evidence to use methanol extract of pulp of *Syzygium cumini* in inflammation. Though it gives significant anti-inflammatory activity, further study needs to establish its therapeutic dose, dosage form, route of administration as well as its mode of action to treat cancer, neurological disorders, ageing problems and other possible diseases.

AUTHORS CONTRIBUTIONS

This work was accomplished by the team work of all authors. Md. Abdul MotalebBhuiya acted as a corresponding author. Md. Abdul MotalebBhuiya, Tanwy Chowdhury, and AnticaBarua executed the overall study design, data analysis and interpretation, manuscript preparation, and statistical analysis. Final version of the manuscript was reviewed and approved by all authors.

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