© 2018 IJSRST | Volume 5 | Issue 2 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X
Themed Section:Scienceand Technology

Exploring Anti-Inflammatory Potential in Leaves of Jamun (Syzygium Cumini)

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ABSTRACT

Inflammation is initiated as healing process by the tissue in response to injury by pathogens, irritants or cell damage. Anti-inflammatory drugs used today to cure the disease have serious side-effects. The focus of research is to study the bioactive compounds from medicinal plants with anti-inflammatory property. The work is carried out with Syzygium cumini leaves (family-Myrtaceae). Leaves are reported to posses anti-inflammatory activity in crude extract of leaves and bark. In our study, ethanolic extract of leaves was screened for the anti-inflamatory activity and bioactive compound was partially purified by Thin Layer Chromatography (TLC) technique. Partially purified bioactive compounds were further analysed using various in vitro models such as inhibition of albumin denaturation assay and HRBC membrane stabilization assay. Aspirin and diclofenac sodium were used as standard drug. It is cleared from the result that the bioactive compound tannins at concentration of 100µg/ml posses 99.50% inhibition of heat induced protein denaturation as compared with the standard drug Aspirin i.e. 89.26%. In HRBC membrane stabilization activity tannins at concentration of 1mg/ml showed 82.94% protection of HRBC membrane while the standard diclofenac sodium showed 70.41% protection. Overall study claims that ethanol extract of S. cumini leaves posses potential anti-inflamatory activity due. Further study is required to purify and identify the specific bioactive compound.

Keywords: Herbal preparation, Bioactive compounds, S. Cumini, TLC, Aspirin, Diclofenac sodium

I. INTRODUCTION

Inflammation is the healthy and an essential immune response shown by the body that may enable body to survive during infection or injury which is also termed as host defense mechanism. Inflammation process also maintains tissue homeostasis in noxious conditions [1]. Its key features are being characterized by redness, warmth, swelling, pain in joints with loss of joint function [2]. Inflammation is mainly classified into Acute and Chronic inflammation. Acute inflammation is an initial response given by the body to harmful stimuli and is achieved by increase in the movement of plasma and leukocytes (especially granulocytes) from the blood into the injured tissues. A various biochemical pathways such as the local vascular system, the immune system and various cells within the injured tissue show the inflammatory response [3]. Chronic inflammation is chiefly marked by new tissue formation and is prolonged. Currently inflammation is treated with most commonly worldwide used drug NSAIDs [4]. In inflammation, injured tissue cells release kinins, prostroglandins and histamine that are collectively involves increase in vasodilation (widening of blood capillaries) and permeability of the capillaries which lead to increase blood flow to the injured site. Management of inflammation related disease is the real issue in the

rural community; the population in this area uses many alternative drugs such as various different substances produced from medicinal plants, [5] Most of the anti-inflammatory drugs available in market have problems with efficacy and side effects and the need of safe novel effective anti-inflammatory compounds can be fulfilled by herbal medicine as they have no side effects [6]. Syzygium cumini (Family Myrtaceae), also called as Eugenia cimini and Syzygium jambolanum. Other common names used for this are jambul, Black Plum, java Plum, jamblang, jamun, Indian Blackberry etc. Tree is found in Asian subcontinent, Eastern Africa, South America etc. The tree fruit is annually and is sour to taste. Different parts of jamun are reported to have medicinal properties including anti-diabetic, antioxidant, anti-inflammatory, macological, anti-bacterial, anti-fungal, anti-HIV, anti-diarrheal, anti-ulcerogenic, antifertility, anti-leshmanial, nitric oxide scavenging, free radical scavenging and radioprotective [7, 8].Ethanol S. cumini seeds is reported to have potential antidiabetic activity[9] . Insufficient data is present which can show the effective anti-inflammatory activity on s.cumini .Very few literatures are available for the activity of seed, leaf and bark. We did not find any literature on isolated compounds on S.cumini leaf and bark extract.

II. METHODS AND MATERIAL

Collection of Plant material and Extract preparation

Syzygium cumini leaves were collected from Nakshatra Udyan, Vidya pratishthan, Baramati, Dist:Pune, (MS), India. The leaves were shade dried for 5-7 days and powdered with morter pestle. The Powder was then filtered by using 85mm mesh and extracted by soaking 30g of powder in 50ml of ethanol for 72 hrs at 37ºc in dark. The extract was filtered and concentrated by evaporation [10].

Determination of mineral content

The 5gm crude leaf powder was converted into ash by keeping in muffle furnace for 2 hours, mixed with 100ml nitric acid and filtered through filter paper . Standard working solution was prepared to determine mineral content in atomic absorption spectrophotometer [11].

Qualitative analysis of phytochemicals

The leaf extract of S.cumini were analysed for the presence of phytochemicals according to standard methods reported [11, 12].

Test for alkaloids: 2ml of extract was added to 2N HCL. and treated with few drops of Mayer's reagent were added. Inferenced by Cream colour precipitate.

Test for flavonoids: 3-5 drops of 1N NaOH were added to 2ml extract. Inferenced by formation of yellow orange color.

Test for phenolic compounds: 3-5 drops of 5% Fecl3 solution were added to 2ml extract. Inferenced by deep blue color formation.

Test for saponins: 2ml of extract was mixed with 6 ml of water. Persistent foam was observed.

Test for tannins: 2ml of the aqueous extract was mixed with 2ml of D/W and few drops of fecl3 solution were added. Formation of green precipitate confirmed the presence of tannis.

Test for phlobatanins: 2ml of extract was mixed with 2ml of 1%HCL and the mixture was boiled. Inferenced by deposition of red precipitate.

Test for coumarins: 3ml of 10% NaOH was added to 2ml of aqueous extract. Formation of yellow color was observed

Test for anthocyanins: 2ml of aqueous extract was mixed with 2ml of 2N HCL and ammonia. Turning of Pink-red to blue violet was observed

Test for leucoanthocyanin: 5ml of aqueous extract was added to 5ml of isoamyl alcohol. Inferenced by formation of upper layer red in colour

Test for terpenoids: 2ml of extract was mixed with 2ml of acetic anhydride and con of H2So4. Formation of blue green ring confirmed the presence of terpenoids.

Test for steroids: 1ml of extract was taken and mixed with 10ml of chloroform & equal volume of concentrated sulphuric acid was added slowly from the

side. Upper layer turned red & sulphuric acid layer showed yellow with green fluorescence.

Test for fatty acid: 0.5 ml of extract was mixed with 5ml of ether, extract was allowed to evaporate on filter paper & filter paper was dried. The transparency on paper was observed.

Thin layer chromatography

The leaf extracts were analysed with the help of Thin Layer Chromatography (TLC) to separate specific bioactive compound from the extracts. TLC was done using different solvent ratio described in [9,13]. The slide were then dried and kept in iodine saturation tank for spot visualization. The movement of the analyte was expressed by its retention factor (Rf). Values were calculated for different sample by formula [14].

Rf (Retention factor) = Distance travel by solute / Distance travel by solvent

The active compounds were retrieved in 1ml of ethanol and filtrate was concentrated by evaporation. Further the concentrated filtrates of different bioactive compounds were stored for further analysis [15].

In-vitro

Inhibition of albumin denaturation assay

The assay was determined by methods described by [5] and [16] with slight modification. The 2ml reaction mixture contained test extract at different concentrations (100µg/ml-1000 µg/ml) and aqueous solution of bovine serum albumin fraction (1%). pH of reaction mixture was adjusted to 6.3 by 1N HCl. The samples were incubated at 37 ℃ for 20 min and then heated at 57 ℃ for 30 min. After cooling the samples, 1ml of Phosphate buffer saline was added to each sample tubes. The turbidity was measured spectrophotometrically at 660 nm against blank. The experiment was performed in triplicate. Standard drug used was aspirin at concentration of 1mg/ml [17]. Percent inhibition of protein denaturation was calculated as follows

Percentage inhibition (%) = (O.D control – O.D sample) X 100/ O.D control

HRBC membrane stabilization assay

HRBC membrane stabilization method has been used to study the cyto-protective activity. The assay was performed according to simple modification of [18,19]. Healthy human blood was collected and mixed with equal volume of sterilized alsever solution (2% dextrose, 0.8% sodium citrate, 0.5 citric acid, 0.42% sodium chloride in distilled water).The blood was centrifuged at 3000rpm & packed cells were washed with iso-saline (0.85%, pH 7.2) and suspension was made with iso-saline. Assay mixture contained 1ml phosphate buffer (0.15M pH 7.2), 2ml of hypo saline (0.36%), 0.5ml of RBC suspension & 1ml of various concentration of test samples i.e. (200ug/ml, 400ug/ml, 600ugml, 1000uglml). Diclofenac sodium was used as standard drug. In control 2ml of Distilled water was used instead of hypo saline. All reaction mixtures were incubated at 37º c for 30min and centrifuged at 3000rpm for 20min.Absorbance was taken at 560nm. The percent haemolysis was calculated by comparing with the control produced in 100% Distilled water. The % HRBC membrane stabilization was calculated by using the standard formula.

% Protection = 100 – (Absorbance of control - Absorbance of sample/Absorbance of control) × 100

III. RESULT AND DISCUSSION

Mineral analysis

Mineralization of S.cumini leaf and bark extract by atomic absorption spectrophotometry analysis has revealed the presence 6 important elements including 2 macro nutrients like Ca++ & Mg++ and 4 micro nutrients like Fe++, Zn++, Mn++ and Cu++. Major elements are found higher as compared to trace element. Each nutrient yielded as Ca++ 48.80ppm, Mg++-5.37ppm, Fe++-3.8ppm and Zn++-1.7ppm in leaf extract and Ca++ 64.44ppm, Mg++- 5.3ppm, Fe++-2.5ppm and Zn++-1.3ppm in bark extract which were far lesser then the reported values Ca++-156ppm, Mg++-11.2ppm, Fe++-12,7ppm and Zn++-2.38ppm[20].

The presence of macro and micro elements reflect their function as essential nutrient elements and often as cofactor activators in metal- ligand complexes [21].

Table 1- Mineralization of leaf and bark extract by atomic absorption spectrophotometry

	Mineral content (mg/L)					
Elements / P Part	Ca++	Cu++	Fe++	Mg++	Zn++	Mn++
Leaves extract	48.80	1.43	3.88	5.31	1.70	2.54
Bark extract	64.4	1.53	2.52	5.37	1.30	1.61

In plant leaf and bark extract the minerals found to be dominant were calcium followed by magnesium, iron and manganese, whereas zinc and copper were found to be in trace amounts. Ca++ showed highest concentration in both plant extract than the other elements.

Table 2 Phytochemical analysis of aqueous leaf and bark extract. (+): Positive (-): Negative

Secondary metabolites	Bark extract	Leaf extract
Alkaloids	+	+
Phenolic compounds	+	+
Flavonoids	+	+
Saponins	+	+
Tannins	+	+
Phlobatannins	+	+
Coumarins	_	_
Anthocyanins	_	_
eucoanthocyanins	_	_
Emodins	_	_
Steroids	+	+
Fatty acids	+	- --

As per the evaluation Out of 12 phytochemicals 8 bioactive compounds were present in bark extract, while 7 were present in leaf extract. One of which might be the responsible factor for the anti-inflammatory activity for the plant extracts.

Thin layer chromatography.

Profiling of plant leaf extract in different solvent system confirmed the presence of diverse group of phytochemicals like alkaloids, flavonoids, tannins and phenols. The separation was confirmed by comparing RF values with reported values in literature as shown in Table3. TLC is simple cost-effective technique which has been used routinely from several decades to separate chemical and biochemical compounds. Study revealed the application of qualitative estimation of bioactive compounds from medicinal plants. The presence of bioactive compounds like alkaloids, tannins, phenol and flavonoids was determined and confirmed by comparing obtained Rf values 0.71, 0.74, 0.97 and 0.41 with reported Rf values 0.72, 0.74, 0.97 and 0.42 in literature [13, 14, 22, 23]. The separation of alkaloids was done by using benzene: methanol (80:20) which gave the best results. Mobile phase used for flavonoids - ethyl acetate: methanol: water (100:20:12), phenols- chloroform: methanol (27:0.3) and tannins- methanol: water (6:4) were used as reported in the reference [14] which resulted into the proper separation. Rf values of all the compounds was cross checked with the reported values in the references, results are tabulated in Table 3. The total yield of retrieved compound as for flavonoids- 3.12g/ml, alkaloid- 980mg/ml, phenols- 90mg/ml, tannins- 100mg/ml which were used as stock concentrations for further analysis. That were separated by using specific solvent ratio and Rf value obtained was confirmed by crosschecking with the reported values in the literature [13,22,23]

Sr. No	Purified bioactive compound	RF value	Reported value	Solvent used	Total yield

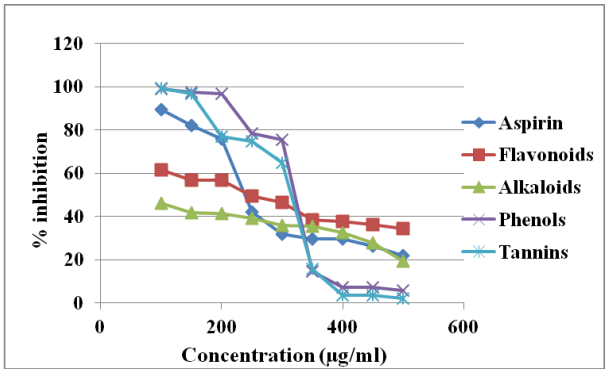
1	lkaloid s	0.41, 0.90, 0.97, 0.72, 0.18	0.41, 18, 0.55, 0.64, 82, ,0.92 [14]	Benzene: Methanol (80:20)	980mg/ ml
2	lavonoi ds	0.89, 0.74, 0.62, 0.33	85, 0.73, 0.62, .57,0.33[14]	thylacetate: Methanol: Water (100:20:12)	.12g/ml
3	Tannins	0.96, 0.42, 0.74, .93 ,0.3 9	0.83, 94, 0.46, 0.74 [22]	lethanol :W ater (6:4)	100mg/ ml.
4	Phenols	.98,0.52 ,0.81, .46,0.76 ,0.70	.52,0.65, .87,0.83. 0.46 [23]	Chloroform: Methnol (27:0.3)	0mg/ml

Inhibition albumin denaturation assay

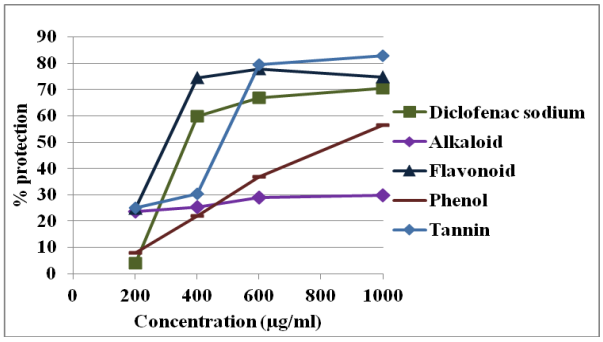
Inflammation is nothing but the normal protective response to tissue injury caused by physical, chemical or microbial agents. Denaturation of protein is well documented cause of inflammation. Protein denaturation is the process in which protein lose their secondary and tertiary structure. Most of the biological protein loses their biological function when denatured [5]. Our study revealed the mechanism of anti-inflammatory activity and the ability of bioactive compounds to inhibit the protein denaturation. BSA denatured by heat treatment. The denatured BSA expresses antigens associated to type 2 hypersensitive reaction that are related to various diseases. Heat denatured proteins are as effective as native protein in provoking delayed hypersensitivity [24]. Among the four bioactive compounds tannins showed maximum 99.50 % inhibition at the concentration of 100µg/ml as compared to standard anti-inflammation drug aspirin 89.26% as shown in Graph 1.

HRBC Membrane stabilization assay

The lysosomal enzyme released during inflammation produces various disorders. The extracellular activity of these enzymes is related to acute or chronic inflammation [25]. Since, HRBC membrane is similar to lysosomal membrane. Exposure of RBC's to injurious substances such as hypotonic medium shows haemolytic effect that result in lysis of the membrane and oxidation of haemoglobin. Membrane stabilization brings about prevention of leakage of serum proteins and fluids into the tissue during the period of increased permeability caused by inflammatory mediators [18]. Thus the phytochemicals from the leaf extract possess the potent anti-inflammatory property. The anti-inflammatory activities are probably due to their inhibitory effect on enzymes that are involved in production of chemical mediators of inflammation and metabolism of arachidonic acid [26]. A bioactive component of leaf extract at concentration 200-1000µg/ml protects the human RBC membrane against lysis induced by hypotonic solution. At concentration of 1000µg/ml the active component tannins shows maximum 82.94% protection as compared with standard diclofinac sodium 70.41% at same concentration as shown in Graph 2.



Graph 1- Effect of bioactive compounds on heat induced percent denaturation



Graph 2- Effect of bioactive compounds on hypotonicity induced haemolysis of HRBCs

IV. CONCLUSIONS

Overall study concluded that S.cumini leaves and bark contains total 7 phytochemicals out of which tannins showed highest activity, hence proved to be potent anti-inflammatory agent. Ca is present in higher concentration in both the extracts.

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