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Research Article

In vitro Cyclooxygenase inhibitory activity and GC-MS profiling of bioactive compounds in *Bauhinia racemosa* Lam.

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Abstract

Non-steroidal anti-inflammatory drugs (NSAIDs) are a common treatment for chronic pain and inflammation. While NSAIDs have been shown to reduce inflammation and pain associated with them, they have an array of side effects. Researchers investigated the COX inhibitory action of NSAIDs. They revealed that there are two distinct COX enzymes: COX-1 and COX2. Developing COX inhibitors has remained critical for producing innovative and safe anti-inflammatory drugs. *Bauhinia racemosa* Lam belonging to the family Fabaceae has a wide range of medicinal properties. The plant is used to treat various diseases in traditional medicine, but there is less knowledge based on clinical efficacy as an anti-inflammatory agent. In the present study *Bauhinia racemosa* Lam hydroalcoholic extract were screened for COX inhibition and GC-MS analysis was done to screen phytochemicals. The results obtained were found to inhibit 89% of COX activity significantly as compared to standard drug (Diclofenac sodium). GC-MS analysis revealed a total of ten potent bioactive compounds. The samples were identified by comparing retention time and peak area to literature and interpreting mass spectra. Results observed from the study suggest that the hydroalcoholic extract of *Bauhinia racemosa* Lam possess anti-inflammatory activity and can be utilized as an alternate source for NSAIDs.

Keywords: COX, *Bauhinia racemosa* Lam, Anti-inflammatory, NSAIDs.

Introduction

Inflammation is a complex biological response to stimuli, including irritants, injured cells, and pathogens. Injured cells release arachidonic acid[1], which is processed via the cyclooxygenase (COX)[2] and lipoxygenase (LOX) pathways[3]. These pathways are involved in various inflammatory diseases like arthritis[4], chronic pain, fever[5], burns, sepsis[6,7], carcinogenic progressions in colorectal cancer[8], and inflammatory bowel disease[9]. The intricate processes linked to inflammatory responses often involve reactive oxygen species (ROS)[10]. Protection against ROS through antioxidant compounds, such as phenolics, can help protect against inflammation.

Cyclooxygenase enzymes (COXs) catalyze two reactions: adding molecular oxygen to arachidonic acid (AA) to form prostaglandin G2 (PGG) and converting PGG2 to PGH2 through peroxidase function. This enzyme initiates the AA metabolic cascade, leading to the formation of pro-inflammatory prostaglandins, thromboxanes, and prostacyclins[10]. Prostaglandins regulate smooth muscle contractility, blood pressure, platelet aggregation, pain, and fever. Inhibiting cyclooxygenase activity allows NSAIDs to exert their analgesic, antipyretic, anti-inflammatory, and antithrombotic effects[11].

COX-1 maintains physiological prostanoid biosynthesis, while COX-2 is an inducible isoform linked to inflammation[12]. Prolonged use of NSAIDs can cause severe side effects like gastro-intestinal hemorrhage[11]. New COX-2 selective drugs are not risk-free, as some COX-2 inhibitors cause cardiovascular problems[13]. Steroids are essential for treating inflammatory diseases but are toxicity-prone and only suitable for serious cases. Therefore, there is a need for natural products with minimal side effects, as steroids are only effective in severe cases.

Plant secondary metabolites, also known as phytochemicals, are naturally occurring compounds with potential disease-inhibiting properties[14]. They contain bioactive compounds like volatile oils, steroids,

alkaloids, and natural antioxidants like flavonoids and phenolic compounds. Screening for active compounds and determining antioxidant activity from plants has led to the discovery of novel drugs for various diseases[15]. These plants are easily available, less expensive, safe, and efficient, with few side effects[16]. Modern methods for identifying and quantifying active constituents in plant materials may help standardize herbal drug formulations. Gas chromatography (GC-MS) is a technique used for analyzing components in traditional medicines and medicinal plants. It is increasingly used for analyzing non-polar components and volatile essential oils, fatty acids, lipids, and alkaloids in medicinal plants, as it has proven to be a valuable method in recent years[17].

Bauhinia racemosa Lam., commonly known as the bidi leaf tree, is a small deciduous tree with dark scabrous bark, found in tropical regions of South, Southeast, and East Asia, characterized by harsh climatic conditions[18].The tree is important for its nutritional and economic value, providing fodder for livestock and fuel from its hard, heavy wood[18]. *B. racemosa* is widely used in traditional medicine due to its medicinal properties, particularly in its flower buds, which have anti-ulcerogenic properties[19], the seeds can be utilized for their potential antibacterial properties[20], the compounds isolated from the roots show significant antibacterial and antifungal properties[21]. The plant has antihyperglycemic and anthelmintic properties in its leaf extracts, and its stem bark is medicinally important for treating various ailments like headache, fever, skin diseases, and diarrhea[22]. The study aims to evaluate the anti-inflammatory viz., cyclooxygenase (COX) inhibitory activity of *B. racemosa* Lam hydroalcoholic extract and identification of bioactive compounds by GC-MS analysis.

MATERIALS AND METHODS

Fresh leaves of *B. racemosa* Lam. were collected from Veermata Jijabai Udyan (Byculla), Mumbai, India and authenticated at the Blatter Herbarium, St. Xaviers College, Mumbai (Accession no. PD-431).

Preparation of extract

Leaves were cleaned and dried for a week to prevent loss of volatile phytoconstituents. The leaf powder was extracted by Soxhlet extraction using a hydroalcoholic solvent (40:60), resulting in a dark green solvent. The extract was collected, and the solvent was removed through evaporation. The concentrated extract was used for further investigation.

In vitro anti-inflammatory activity

Cyclooxygenase (COX-2) inhibition test

Cyclooxygenase-2 Inhibitor Screening Kit (COX-2) was obtained from Sigma-Aldrich. The assay is based on fluorometric detection. Diclofenac sodium (MW=296.14) was used as a positive control for inhibiting COX-2. *B. racemosa Lam.* hydroalcoholic extract was used of varying concentration (10-50µl/ml). The assay was performed according to the manufacturer's instructions.

GC-MS profiling

The compounds of the hydroalcoholic extract isolated from *B. racemosa* were identified using GC-MS. The Clarus 600C system was used for the analysis, which included a Gas Chromatograph, a Mass Spectrometer, and a GSBP-5 MS column made of 5% diphenyl/95%dimethyl polysiloxane. The column was 30m long and had an internal diameter of 0.25mm and a film thickness of 0.25µm.

The GC-MS technique uses an electron ionization device in electron impact mode with an ionization energy of 70 eV. The procedure used helium (99.999%) as the carrier gas, with a constant flow rate of 14 mL/min and an injection volume of 1 µL, with an 80:1 split ratio. Temperatures for the injector and ion source were kept at 250 °C and 220 °C, respectively, to comply with requirements. The oven temperature was set at 70 °C for 1 minute in an isothermal environment. Later, the temperature was increased from 190 °C/min to 260 °C under 10 minisothermal conditions. The entire analytical procedure lasted 35 minutes. At 70 eV, mass spectra were found ranging from 50 to 650 m/z. Several *B. racemosa* components were identified by comparing their mass spectra to those from Wiley and NIST libraries, including those mentioned by Adams, and comparing retention indices.

RESULTS AND DISCUSSION

In vitro anti-inflammatory activity

Cyclooxygenase inhibition activity

COX-2 inhibitors function by directly inhibiting the production of prostaglandins, which are fatty acid derivatives present throughout the body and have inflammatory and immune response effects[23]. COX-2-specific inhibitors offer various advantages over traditional NSAIDs. Plants are recognized to have crucial roles in discovering and developing new drugs. Flavanones isolated from *Sorghum bicolor* selectively inhibit COX-2 activity[24]. *Berberis* species are used in traditional medicine to treat a wide range of inflammatory diseases. Numerous studies have revealed that the anti-inflammatory function is linked to the presence of berberine, an alkaloid contained inside the *Berberis* sp.[25] *Amaranthus tricolour* inhibits the COX-1 enzyme by 78, 63, and 93%, and the COX-2 enzyme by 87, 74, and 95%, respectively[26]. At various concentration viz., 10,20,30,40 and 50 µg/mL the % inhibition, exhibited by standard diclofenac sodium (Standard) were found to be 18%, 30%, 40% and 50% whereas, for *B. racemosa* Lam. Hydroalcoholic extract was found to be 23%, 48%, 67%, 73% & 89% respectively. Dose dependent inhibition of COX-2 was exhibited by both standard and plant extract (Figure 1). The study found that *B. racemosa* Lam. has an 89 % inhibition level, making it a viable alternative to synthetic NSAIDs that may produce adverse effects.

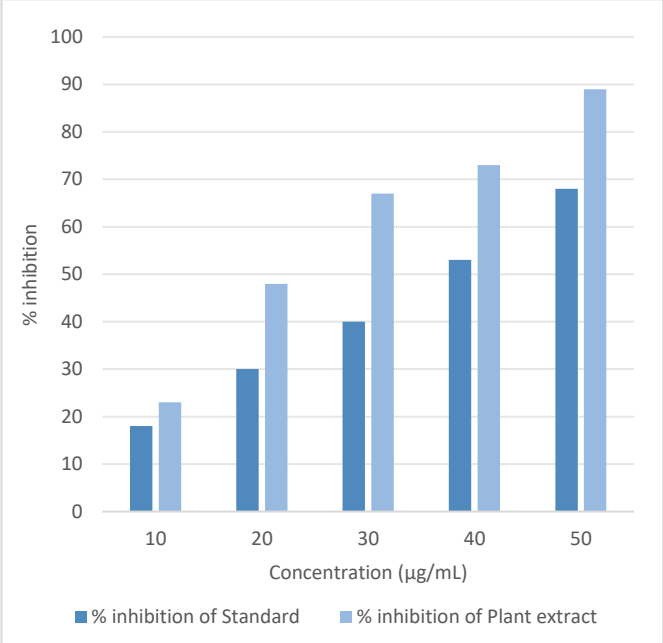

Figure 1: Effect of *B. racemosa* Lam. hydroalcoholic extract on COX-2 activity

Determination of Bioactive compounds by GC-MS analysis

Gas Chromatography-Mass Spectroscopy (GC–MS) analysis of hydroalcoholic extract of *B. racemosa* revealed diverse range of chemical compounds. The analysis resulted in the identification of 10 compounds, constituting the total bioactive component, as depicted in Table 1 & Figure 2. The GC-MS chromatogram reveled peak of various component, the most abundant compound was found to be n-Hexadecanoic acid (100%), Bromoform (73.62%) and Phenol, 4,4'-(1-methylethylidene) bis (50.48%) each of which with a different retention time. n-Hexadecanoic acid has antioxidant, 5-alpha reductase inhibitor, anti-fibrinolytic, haemolytic, antimicrobial, hypocho-lesterolemic nematicide, pesticide, antiandrogenic flavour, and haemolytic properties[27]. 5-Aminosalicylic acid, N,O,O'-tris(trimethylsilyl) is antioxidant and antiinflammatory in nature[28,29]. 2-Hexadecanol exhibits antimicrobial and antibacterial activity[30], Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13, 15,15-hexadecamethyl also exhibit antimicrobial activity[31]. Earlier studies on bark identified compounds which are antitumor, anti-tubercular and anti-fungal properties[32].

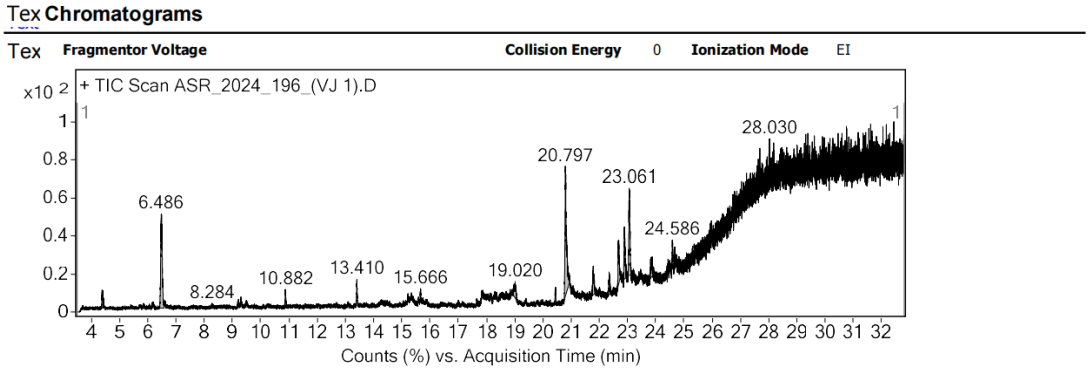

Figure 2: GC-MS chromatogram of hydroalcoholic extract of *B. racemosa* Lam.

Table 1: GC-MS spectral analysis of hydroalcoholic extract of *B. racemosa* Lam.

No.	RT	Name of Compound	Mol. formula	Mol. weight	Peak area %
1.	6.486	Bromoform	$C_2HBr_3O_2$	294	73.62
2.	8.284	6-Methoxy-1-(3-methoxybenzyl)-3,4-dihydroisoquinoline	$C_{18}H_{19}NO_2$	281	1.87
3.	10.882	5-Aminosalicylic acid, N, O, O'-tris(trimethylsilyl)	$C_{16}H_{31}NO_3Si_3$	369	6.99
4.	13.410	Dodecamethylcyclohexasiloxane	$C_{12}H_{36}O_6Si_6$	444	9.01
5.	15.666	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	$C_{18}H_{52}O_7Si_7$	576	3.38
6.	19.020	2-Hexadecanol	$C_{16}H_{34}O$	242	14.01
7.	20.797	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	100
8.	23.061	Phenol, 4,4'-(1-methylethylidene) bis	$C_{15}H_{16}O_2$	228	50.48
9.	24.586	4-Acetyloxyimino-6,6-dimethyl-3-methylsulfanyl-4,5,6,7-tetrahydro-benzo[c]thiophene-1-carboxylic acid methyl ester	$C_{15}H_{19}NO_4S_2$	341	8.44
10.	28.030	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl	$C_{16}H_{50}O_7Si_8$	578	2.1

CONCLUSION

This study aimed to validate the use of *B. racemosa* in traditional medicine as an anti-inflammatory agent. Hydroalcoholic extract of *B. racemosa* was tested for anti-inflammatory efficacy and GC-MS analysis. The anti-inflammatory activity results demonstrated a considerable suppression of cyclooxygenase-2 (COX-2). GC-MS study revealed 10 bioactive compounds. n-Hexadecanoic acid, Bromoform and Phenol, 4,4'-(1-methylethylidene) bis were the predominant potential bioactive compounds. Further research should focus on the isolation, purification, and characterization of active compounds from hydroalcoholic extracts. This approach has the potential to identify novel COX-2 inhibitors, which could offer effective anti-inflammatory properties while reducing the adverse effects associated with current treatments.

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Conflicts of Interest

Authors have declared that no conflicts of interests exist.

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