Evaluation of Anti-inflammatory and Anti-arthritis Activity of Isolated Fractions from Bauhinia purpurea Leaves Extracts in Rats

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Abstract

Bauhinia purpurea are used as antinociceptive, antidiarrhoeal, anti-inflammatory, anti-arthritis, analgesic, anticancer, antipyretic, anti-diabetics, anti-diarrheal activity etc. The flavonoids and polyphenol are present in Bauhinia purpurea and may play an important role in the treatment of inflammation and arthritis. Till date no one has documented about active constituents responsible for the anti-inflammatory and anti-arthritis activity. The present study was aimed to isolate fractions from Bauhinia purpurea leaves extracts, and assessed their anti-inflammatory and anti-arthritis activity in rats. The hydroalcoholic and aqueous extracts of Bauhinia purpurea leaves were prepared and tested for in vitro antioxidant activity namely DPPH, total polyphenol content, total flavonol content and reducing power assay. The antioxidant activity was performed only for proper selection of extracts, to isolate the polyphenol and flavonoids fractions from extract expressing maximum antioxidant activity. The different fractions were isolated from hydroalcoholic extracts by using column chromatography. The fractions FBP5, FBP8, FBP9 and FBP10 were further investigated for phytochemical screening to determine the nature of isolated compound. The fractions FBP8, FBP9 and FBP10 were screened for anti-inflammatory and anti-arthritis activity in carrageenan rat induced paw oedema, cotton pellet, Adjuvant induced chronic arthritis.

The results of phytochemical study imply that FBP8, FBP9 and FBP10 revealed the presence of polyphenol and flavonoids. The fraction FBP8, FBP9 and FBP10 (25 mg/kg) exhibits significant anti-inflammatory and anti-arthritis activity. The FBP9 produces maximum anti-inflammatory and anti-arthritis activity compared to other fractions. These findings suggest that the anti-inflammatory and anti-arthritis activity of isolated fraction was due to presence of flavonoids and polyphenol.

1 Introduction

Rheumatoid arthritis is the utmost communal autoimmune inflammatory disorder. About 1% of the adult population of global is affected by arthritis. The ignorance of arthritis leads to joint destruction¹. The inflammation and arthritis can be controlled by glucocorticoids like cortisone and prednisone drugs. NSAIDS are used as anti-inflammatory, and methotrexate and leflunomide are employed as anti-rheumatic drugs. All these drugs are mostly prescribed by physicians for the treatment of inflammation and arthritis. However, these
drugs are expensive and associated with various side effects, severe adverse reactions and toxicity. Additionally, gastrointestinal disorders, immunodeficiency and humoral disturbances are observed in patients using these drugs.\(^2\)\(^3\).

Hence researchers are continuously applying efforts for novel anti-inflammatory and anti-arthritis drugs to produce maximum therapeutic effects with lesser toxicity profile.

The Ayurvedic practitioners in India are prescribing numerous indigenous plants for the management of inflammation and arthritis. Since ancient time, the compounds derived from plants have also been used as medicine. The plant derived compounds are commonly used by rural people because the availability of allopathic medicines is limited. Till date several studies has been conducted and validated the secondary metabolites presents in the plants imparts pharmacological activity to combat numerous disease.\(^4\)\(^5\).

It has been documented that about 130 active constituents isolated from some 100 species of higher plants. The isolated components are used in medicines throughout the world. Researchers validated pharmacological activity of numerous medicinal plants extract, but still very few work done on the isolation and identification active constituents responsible for the therapeutic activity. Hence, extensive scientific studies are required for the development of pharmacological activity of isolated bioactive components. In addition, these active constituents are suitable as synthetic intermediate substances for the production of useful drugs.

*Bauhinia purpurea* belongs to the family Leguminosae, and is a moderate evergreen tree in sub-Himalayan region and western track of India\(^6\). Traditionally *Bauhinia purpurea* are used for the treatment of numerous ailments namely diarrhea, ulcers, enlarge cervical glands, goiter, scrofulous tumors etc. The glycosides, flavonoids, saponins, triterpenoids, phenolic compounds, oxepins, fatty acids and phytosterols secondary metabolites are present in this plant. The two new oxepins named bauhiniastatins1 and 2 isolated from the ethanol extract of whole plant, while root furnishes bauhiniastatins 1, 2, 3 and pacharin\(^7\)\(^8\). The novel flavone glycoside, 5,6-dihydroxy-7-methoxyflavone 6-O-b-D-xylpyranoside isolated from the chloroform-soluble fraction of the ethanol extract of *Bauhinia purpurea* stems.\(^9\) The three different glycerol derivates and 6-butyl-3-hydroxyflavanone derivatives are 2, 3-dihydroxypropyl oleate, 2,3 dihydroxypropyl linoleate, 2,3- dihydroxypropyl 16-hydroxy-decanoate and 6-butyl-3-hydroxyflavanone, 6-(3', oxobutyl)-taxifolin, respectively isolated from methanol extract of heartwood of *Bauhinia purpurea*\(^10\).

*Bauhinia purpurea* extracts was scientifically documented for its antiinocceptive, antidiarrhoeal, anti-inflammatory, analgesic, anticancer, antipyretic, antimalarial, antimycobacterial, anti fungal, anti-diabetics and anti-diarrheal activity etc\(^11\)-\(^14\). The anti-inflammatory and anti-arthritis activity of ethanol extract of *Bauhinia purpurea* bark was scientifically documented. However, still no one has reported about active constituents responsible for the anti-inflammatory and anti-arthritis activity. The *Bauhinia purpurea* incorporate various types of polyphenol and flavonoids components in different part of the plants. The polyphenol and flavonoids imparts anti-inflammatory and anti-arthritis activity. Hence, we planned to assess the anti-inflammatory and anti-arthritis activity of isolated fractions of *Bauhinia purpurea* leaves extracts in rats.

### 2 Material and Methods

#### 2.1 Plant material

The leaves of *Bauhinia purpurea* was selected for the proposed study. The plant material was authenticated by Dr. A.P. Singh, Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.). The leaves were shade dried, reduced to coarse powder and stored in airtight container till further use.

#### 2.2 Preparation of extract

The powdered leaves of *Bauhinia purpurea* about 1 Kilogram were packed in soxhlet apparatus and extracted with petroleum ether, hydroalcohol (mixture of 70% ethanol and 30% distilled water) and distilled water separately, until the completion of the extraction. The extract was filtered while hot, and the resultant extract was distilled in vacuum under reduced pressure in order to remove the solvent completely, and later dried in a desiccator.

#### 2.3 In vitro antioxidant activity of extract

##### 2.3.1 Hydrogen-donating activity

The methanol solution of DPPH (100 mM, 2.95 ml), 0.05 ml of extracts dissolved in methanol was added at different concentrations (50-250 μg/ml). Reaction mixture was shaken and after 30 min at room temperature, the absorbance values were measured at 517 nm and converted into percentage of antioxidant activity (%AA). Ascorbic acid was used as standard. The degree of discoloration indicates the scavenging efficacy of the extract, was calculated by the following equation\(^15\)-\(^16\).

\[
\% \text{ AA} = 100 - \frac{([\text{Abs}_{\text{sample}}] - [\text{Abs}_{\text{blank}}]) \times 100}{\text{Abs}_{\text{DPPH}}}
\]

##### 2.3.2 Total polyphenol content

Total polyphenol content was determined using colorimetric method. 1.0 ml of the prepared extract was oxidized using 2.5 ml of Folin-Ciocalteu reagent, and 2.0 ml of sodium carbonate solution (75 g/l) was then added to the reaction mixture. The absorbance readings were taken at 760 nm after incubation at room temperature for 2 h. The amount was calculated using the gallic acid calibration curve\(^17\)-\(^18\). The results were expressed as gallic acid equivalent (GAE) mg per 100 ml of the sample (extract).
2.3.3 Total flavonol content

Flavones and flavonols contents were analyzed by the colorimetric method. 9.8 ml of the prepared extract was mixed with a 10% solution of aluminum chloride (200 μl). After 30 min, absorption was measured at a 425 nm wavelength. The amount was calculated using quercetin calibration curve. The results were expressed as the quercetin equivalent (QE) mg per 100 ml of the sample.

2.3.4 Reducing power assay

The extracts and ascorbic acid were dissolved separately in 1.0 mL of deionized water with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and 1% potassium ferricyanide (2.5 mL). The mixture was incubated at 50°C for 20 min. Aliquots of trichloroacetic acid (2.5 mL, 10% w/v) were added to the mixture and centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and a freshly prepared FeCl₃ solution (0.5 mL, 0.1%). The absorbance was measured at 700 nm by making 500 μg mL⁻¹ extracts aliquot.

2.4 Isolation of compound from hydroalcoholic extracts

The Bauhinia purpurea extract was subjected to column chromatography using silica gel (60-120 mesh size), and eluted with the following solvent ratios of Hexane: dichloromethane (DCM), 100:0, 80:20, 60:40, 40:60, 20:80, 10:90, 0:100, DCM:Ethanol (Eth). Finally eluted with 100:0, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90, 0:100, DCM:Methanol (MeOH). The fractions (25 ml) were collected from the column. The elute collected were monitored by thin layer chromatography (eluent: DCM-MeOH, 9:1 and 3:2) for homogeneity and the similar fraction were pooled together. The eleven different fractions were collected and dried. The phytochemical screening was performed according to Harborne and Kokate.

2.5 Pharmacological activity

2.5.1 Selection of animals

Male Wistar rats (150-200 gm), were used, and kept in quarantine for 10 days under standard husbandry conditions (27.3°C, Relative humidity 65 ±10%) for 12 hrs in dark and light cycle respectively and were given standard food and water ad libitum. All experiments were approved by the institutional ethical committee (1321/PO/ReBi/S/10/CPCSSEA dated 22/10/2014) and were carried out according to the animal ethics committee guidelines.

2.5.2 Anti-inflammatory activity

The Carrageenan-induced oedema and cotton pellet-induced granuloma model were performed to assess the anti-inflammatory activity of isolated compounds from Bauhinia purpurea extract.

2.5.2.1 Effect of isolated compound of Bauhinia purpurea on carrageenan-induced oedema

Albino Wistar rats of either sex weighing between (150-200 gm) were divided into various groups and six animals in each group. The groups were as follows:

- Group I (control group) – Treated with distilled water
- Group II – Treated with standard drug Indomethacin at 10 mg/kg body weight
- Group III – Treated with FBP8 at 25 mg/kg body weight
- Group IV – Treated with FBP9 at 25 mg/kg body weight
- Group V – Treated with FBP10 at 25 mg/kg body weight

Acute inflammation was produced by injecting 0.1ml of 1% carrageenan suspension in normal saline into the subplantar region of right hind paw after 60 minutes of drug administration. The control group was administered only distilled water. The isolated compound and standard drugs administered intraperitoneally 1 h before carrageenan suspension administration.

A mark was made on the leg at the malleous to facilitate uniform dipping at subsequent readings. The volume of paw oedema was measured with the help of plethysmograph by mercury displacement method immediately before and five hours after the drug administration. The inhibition of oedema in various treated groups was then calculated by using statistical analysis.

2.5.2.2 Effect of isolated compound of Bauhinia purpurea on Cotton pellet-induced granuloma model

Albino Wistar rats of either sex weighing between (150-200 gm) were divided into various groups and six animals in each group. The groups were as follows:

- Group I (control group) – Treated with distilled water
- Group II – Treated with standard drug Indomethacin at 10 mg/kg body weight
- Group III – Treated with FBP8 at 25 mg/kg body weight
- Group IV – Treated with FBP9 at 25 mg/kg body weight
- Group V – Treated with FBP10 at 25 mg/kg body weight

The animals were grouped as described above to study the anti-inflammatory activity. The groups were fasted and treated with drugs/doses similar to that of carrageenan-induced hind paw edema. Sterile cotton pellets each weighing 30 ± 5 mg were placed in each animal. The groups were as follows:

- Group I (control group) – Treated with distilled water
- Group II – Treated with standard drug Indomethacin at 10 mg/kg body weight
were prepared and sterilized in a hot air oven at 123 °C for 3 h. Each animal was placed under light ether anesthesia and subcutaneously implanted with four cotton pellets, one each into both the axillae and the groin region under aseptic conditions. The drugs were administered orally for seven days starting from the day of implantation of the pellets. All the animals had free access to drinking water and food. On the 8th day, all the animals were sacrificed and the implanted cotton pellets were recovered, cleaned of surrounding tissues, and blotted with filter paper. These cleaned pellets were weighed and dried in a hot air oven overnight at 70 °C and the dry weights were noted22.

2.5.3 Anti-arthritis activity

The mycobacterium induced adjuvant arthritis model was used for exploring the anti-arthritis potential of isolated compounds.

2.5.3.1 Effect of isolated compound of Bauhinia purpurea on adjuvant induced chronic arthritis

One week before the commencement of the experiment, the rats were randomly divided into five groups of six rats per group. On day 0 rats were injected with 0.1 ml of Freund’s complete adjuvant (FCA) into the sub plantar (s.p) region of the left hind paw of all the animals. This consists of Mycobacterium butyricum suspended in heavy paraffin oil by thoroughly grinding with a pestle and motor to give a final concentrate of 0.6 mg/mL. Administration of test compounds and standard drug was started on the next day and continued for 28 days. The paw was marked with ink at the level of the malleolus laterals and paw volumes were recorded by the plethysmometer immediately after injection and on 7th, 14th, 21st and 28th day22. The experimental rats were randomly divided into five groups of six rats per group and treated as follows:

- Group I (control group) – Arthritis rats treated with distilled water
- Group II – Arthritis rats treated with standard drug Indomethacin at 10 mg/kg body weight
- Group III – Arthritis rats treated with FBP8 at 25 mg/kg body weight
- Group IV – Arthritis rats treated with FBP9 at 25 mg/kg body weight
- Group V – Arthritis rats treated with FBP10 at 25 mg/kg body weight

2.5.3.2 Hematological screening

On the 28th day blood samples for hematological assays were obtained through ocular puncture of the rats and collected into EDTA-treated sample bottles. While blood cell (WBC) and Red blood cell (RBC) counts were assessed as stated in the method of Chesbrough and McArthur23. Drabkin and Austin method was used to confirming the Hemoglobin (Hb) content24. Estimation of erythrocyte sedimentation rate (ESR) was carried out by the method of Westergren25.

2.5.3.3 Data analysis

Results were analyzed using one way analysis of variance (ANOVA) followed by the tukey’s test by using statistical software package, Graph Pad Prism; version 5.03. Values were expressed as mean ± SEM and the p <0.05 were considered as statistically significant.

3 Results and Discussions

In the present study, Bauhinia purpurea was selected for isolation of active constituents from extract and check its anti-inflammatory and anti-arthritis activity of active constituents.

3.1 In vitro antioxidant activity

The hydroalcoholic and aqueous extract of Bauhinia purpurea were subjected to in vitro antioxidant studies to determine and compare the antioxidant activities of both extracts. Antioxidant ability of Bauhinia purpurea extract was assessed by establishing its efficacy in hydrogen-donating, total polyphenol content, total flavonol content and reducing power assay models.

3.1.1 Hydrogen-donating activity of Bauhinia purpurea

DPPH is stable nitrogen centered free radical that can adopt an electron or hydrogen radical to become a stable diamagnetic molecule. DPPH radicals act with appropriate reducing agents, then depriving colour stoichometrically with the number of electrons depleted which is measured spectrophotometrically at 517 nm26. As shown in table 1, Bauhinia purpurea of hydroalcoholic and aqueous extracts strongly scavenged DPPH radical with the IC50 being 111.81 and 153.03 µg/ml, respectively.

Table 1: Free radical scavenging capacity of hydroalcoholic and aqueous extracts of Bauhinia purpurea

<table>
<thead>
<tr>
<th>Conc. (µg/ml)</th>
<th>DPPH Scavenging %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hydroalcoholic</td>
</tr>
<tr>
<td></td>
<td>Extract</td>
</tr>
<tr>
<td>50</td>
<td>32.67±1.06</td>
</tr>
<tr>
<td>100</td>
<td>46.18±0.93</td>
</tr>
<tr>
<td>150</td>
<td>61.47±0.57</td>
</tr>
<tr>
<td>200</td>
<td>75.28±0.68</td>
</tr>
<tr>
<td>250</td>
<td>86.91±0.48</td>
</tr>
<tr>
<td>IC50</td>
<td>111.81</td>
</tr>
</tbody>
</table>

Values are mean ± SEM of six determinations.
The scavenging was found to dose dependent. The standard drug ascorbic acid scavenged DPPH radical was found to be 93.42. The hydroalcoholic extracts exhibited highest scavenging property compared to aqueous extracts.

3.1.2 Total phenolic content of Bauhinia purpurea

The hydroalcoholic and aqueous extract of Bauhinia purpurea was evaluated for investigation of the total phenolic content concentrations in extracts. Standard curve of gallic acid was calculated and plotted in distilled water for determining absorption data (Table 2).

Table 2: Determination of total polyphenol content of Bauhinia purpurea

<table>
<thead>
<tr>
<th>Extract</th>
<th>Total polyphenol content (GAE mg/gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroalcoholic</td>
<td>85.34±1.21</td>
</tr>
<tr>
<td>Aqueous</td>
<td>71.29±1.18</td>
</tr>
</tbody>
</table>

Data expressed as gallic acid equivalent (GAE) mg per gm of the extract, Values are mean ± SEM of triplicate determinations.

From this Beer’s law range and regression coefficient is determined. The linear equation of gallic acid was found to be $y = 0.0383 x + 0.0021$ (Fig 1).

Fig 1: Calibration curve of gallic acid in distilled water

The results of the total phenolic content of the extracts examined, using Folin-Ciocalteu method, are depicted in table 2. The total phenolic content in extracts, expressed as gallic acid equivalents. The total phenolic content of hydroalcoholic and aqueous extract of Bauhinia purpurea were 85.34 and 71.29 GAE mg/gm, respectively. The hydroalcoholic extracts exhibited highest amount of total polyphenol content compared to aqueous extracts.

3.1.3 Total flavonol content of Bauhinia purpurea

The concentration of flavonoids in hydroalcoholic and aqueous extract of Bauhinia purpurea were determined spectrophotometrically using aluminum chloride. The content of flavonoids was expressed in terms of quercetin equivalents.

Standard curve of quercetin was calculated and plotted in distilled water for determining absorption data. From this Beer’s law range and regression coefficient is determined. The linear equation of quercetin was found to be $y = 0.0382 x + 0.0097$ (Fig 2). The content of flavonoids identified in the tested extracts is shown in table 3. The concentrations of flavonoids in hydroalcoholic and aqueous extract of Bauhinia purpurea were 62.41 and 45.37 QE mg/gm, respectively. The hydroalcoholic extracts exhibited highest amount of flavonoids content compared to aqueous extracts.

Table 3: Determination of total flavonol content of Bauhinia purpurea

<table>
<thead>
<tr>
<th>Extract</th>
<th>Total flavonol content (QE mg/gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroalcoholic</td>
<td>62.41±0.64</td>
</tr>
<tr>
<td>Aqueous</td>
<td>45.37±0.58</td>
</tr>
</tbody>
</table>

Data expressed as gallic acid equivalent (GAE) mg per gm of the extract, Values are mean ± SEM of triplicate determinations.

Fig 2: Calibration curve of quercetin in distilled water

It is well documented that plant flavonoids and phenols in general, are greatly effective free radical scavenging and antioxidants. Polyphenol and flavonoids are used for the prevention and cure of various diseases, which are mainly associated with free radicals. The phenolic compounds have been recognized as antioxidant and have been known to show medicinal activity as well as for exhibiting physiological functions. It has been reported that compounds such as the flavonoids, which contain hydroxyl, are responsible for the radical scavenging effects of most plants. The mechanism of action of the flavonoids is through scavenging or chelating processes. It is well known that plant phenolics, in general are highly effective in free radicals scavenging, and they are antioxidants.

The findings of total polyphenol and flavonol content of hydroalcoholic and aqueous extract of Bauhinia purpurea supports the study of DPPH scavenging capacity of extracts.
3.1.4 Reducing power assay of Bauhinia purpurea

The absorbance value of ascorbic acid was considered to be 100% antioxidant activity. The higher the absorbance of the reaction mixture, the higher would be the reducing power. Table 4 revealed that the antioxidant activity of hydroalcoholic and aqueous extract of Bauhinia purpurea. The reducing power of the hydroalcoholic and aqueous extract of Bauhinia purpurea were found to be 39.11% and 26.43%, respectively.

**Table 4: Antioxidant activity of Bauhinia purpurea extracts**

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Absorbance at 700 nm</th>
<th>Antioxidant activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic acid</td>
<td>0.749±0.03</td>
<td>100.00</td>
</tr>
<tr>
<td>Hydroalcoholic extract</td>
<td>0.293±0.02</td>
<td>39.11</td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>0.198±0.04</td>
<td>26.43</td>
</tr>
</tbody>
</table>

Values are mean ± SEM of triplicate determinations

The reducing power of ascorbic acid was found to be higher than hydroalcoholic and aqueous extracts. It has been reported that the reducing power of substances is probably because of their hydrogen donating ability. The hydroalcoholic extract of Bauhinia purpurea might, therefore, contain high amount of reductions than aqueous extract. The result indicates that extracts act as electron donors and could react with free radicals to convert them into more stable products and then terminate the free radical chain reactions. The findings indicate that antioxidant activity was produced due to the presence of polyphenol compounds.

The reducing power assay is generally used to estimate the ability of an antioxidant to donate an electron which is an important mechanism of phenolic antioxidant action. Earlier many researchers reported that phenolic and flavonol contents of certain plant extracts is directly proportional to the antioxidant activity of extracts. The finding of hydrogen-donating activity as well as total polyphenol and flavonol content implies that antioxidant activities of hydroalcoholic extract are maximum compared to aqueous extracts. Hence, here reducing power assay justify that hydroalcoholic extract of Bauhinia purpurea contain the maximum amount of the total polyphenol and flavonol. The various researchers used plant extracts containing antioxidant as free radical scavengers to prevent inflammation and arthritis.

From the results of antioxidant activity, it can be concluded that hydroalcoholic extracts of Bauhinia purpurea produces higher antioxidant activity compared to aqueous extract and could alleviate the number of oxidative stress induced inflammation and arthritis. The above study was done only for proper selection of extracts of Bauhinia purpurea, to isolate the polyphenol and flavonoids fractions from extract expressing maximum antioxidant activity. Hence the hydroalcoholic extract of Bauhinia purpurea was selected for the isolation of polyphenol and flavonoids compounds by column chromatography.

3.2 Isolation of compound from hydroalcoholic extract of Bauhinia purpurea

The hydroalcoholic extract of Bauhinia purpurea was subjected to column chromatography and fractions were eluted with the gradient polarity of solvent namely hexane, dichloromethane, ethanol and methanol. The eleven different fractions were collected and dried. The fraction FBP1, FBP2 and FBP3 were containing waxy material; the fractions FBP4, FBP6, FBP7 and FBP11 were powder but quantity was very little. The yield of fraction FBP5, FBP8, FBP9 and FBP10 were 210 mg, 320 mg, 350 mg and 260 mg, respectively. These four fractions were further analyzed for phytochemical screening to determine the nature of isolated fractions.

3.3 Preliminary phytochemical analysis of isolated fraction of hydroalcoholic extract of Bauhinia purpurea

The phytochemical investigation of FBP5 of Bauhinia purpurea leaves revealed the presence of alkaloids, glycosides and carbohydrates. The FBP8 and FBP10 indicate the presence of tannins & phenolic compounds and flavonoids. However, the FBP9 exhibited the presence of alkaloids, carbohydrates, tannins & phenolic compounds and flavonoids (Table 5).

**Table 5: Preliminary phytochemical analysis of isolated fraction of hydroalcoholic extract of Bauhinia purpurea**

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>FBP5</th>
<th>FBP8</th>
<th>FBP9</th>
<th>FBP 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Tannins &amp; Phenolic compounds</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Proteins and Amino acids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fixed Oils and Fats</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

(+) Present , (-) Absent

The secondary metabolites impart various pharmacological activities namely anti-inflammatory, analgesic, anti-arthritis, anti-diabetic, hepatoprotective etc. The fractions obtained from the hydroalcoholic extract of Bauhinia purpurea exhibited various types of secondary metabolites. Many investigations have proven that varieties of flavonoid molecules possess anti-inflammatory activity on various animal models of inflammation. Especially, some flavonoids were found to inhibit chronic...
Inflammation of several experimental animal models. Thus, it may be valuable to continuously evaluate the anti-inflammatory and anti-arthritis activity of flavonoids, not only for establishing anti-inflammatory and anti-arthritis mechanisms, but also for developing a new class of anti-inflammatory agents\(^\text{30}\). The FBP8, FBP9 and FBP10 containing polyphenol and flavonoids compound and these organic substances impart chief role in anti-inflammatory and anti-arthritis activity. Hence this result supports to evaluate the anti-inflammatory and anti-arthritis activity of the FBP8, FBP9 and FBP10.

### 3.4 In vivo assays

The in vitro studies of the FBP8, FBP9 and FBP10 isolated from Bauhinia purpurea leaves extracts indicate the presence of flavonoids and polyphenol. Therefore in vivo activities were performed with FBP8, FBP9 and FBP10.

#### 3.4.1 Anti-inflammatory studies

**Table 6: Effect of isolated fraction from Bauhinia purpurea leaves extract on carrageenan induced paw oedema**

<table>
<thead>
<tr>
<th>Group</th>
<th>Paw volume after induction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 hr</td>
</tr>
<tr>
<td>Control</td>
<td>0.26±0.28</td>
</tr>
<tr>
<td>Indomethacin (10 mg/kg)</td>
<td>0.24±0.53</td>
</tr>
<tr>
<td>FBP8 (25 mg/kg)</td>
<td>0.28±0.72</td>
</tr>
<tr>
<td>FBP9 (25 mg/kg)</td>
<td>0.22±0.62</td>
</tr>
<tr>
<td>FBP10 (25 mg/kg)</td>
<td>0.25±1.21</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM, n = 6 in each group. *P<0.05 compared to control group

**3.4.1.1 Carrageenan-induced oedema**

The effect of the FBP8, FBP9 and FBP10 isolated from Bauhinia purpurea on carrageenan-induced paw oedema is presented in Table 6. The animals administered only distilled water, the subplantar injection of carrageenan produced a local oedema that increased progressively from 0.26±0.28 ml after the first hour to reach a maximum within 5 h. The administration of fraction FBP8, FBP9 and FBP10 (25 mg/kg) revealed significant (P<0.05) reduction in oedema in the rats compared with the same time of the distilled water treated group. Indomethacin (10 mg/kg) produced a significant (P<0.05) decrease in oedema at the 2 hour compared with the same time of the distilled water treated group. The decrease order of oedema in the rats for fractions were FBP9 > FBP8> FBP10.

Inflammation comprises activation of various enzymes, extravasation of fluid, release of mediators, migration of cells, tissue breakdown and repair. Additionally, the three main vascular effects namely vasodilatation and proliferated vascular flow, enhanced vascular permeability and leucocytes passage to the injured tissues leads to be acute inflammatory. The anti-inflammatory drugs inhibits the inflammatory process by using several different experimental model\(^\text{31,32}\). The present study creates the anti-inflammatory activity of the isolated compound of Bauhinia purpurea leaves in a number of experimental rat models, which represent different phases of inflammation. The isolated compound namely FBP8, FBP9 and FBP10 exhibited anti-inflammatory effect on carrageenan-induced paw oedema. The FBP9 isolated from Bauhinia purpurea produces maximum anti-inflammatory effect compared to other isolated compounds. Carrageenan-induced oedema is a model of acute inflammation used in the study of NSAIDS drugs. The model is suitable for evaluating the antioedematous effect of natural products and is believed to be biphasic. The first phase which occurs within an hour is believed to involve the release of serotonin and histamine while the second phase which occurs after one hour has been attributed to prostaglandin and the continuity between the two phases is provided by kinin. That the isolated compound produced marked anti-inflammatory effect 2h post-carrageenan injection suggests that its anti-inflammatory activity may involve the inhibition of prostaglandin synthesis and cyclooxygenase products since the carrageenan inflammatory model basically reflects the action of prostaglandins. The isolated compound prevented formation of exudate and leucocytes mobilization induced by intraperitoneal injection of carrageenan. The carrageenan-induced leucocytes migration assay has been adjudged as an excellent acute and sub-acute model for the measurement of fluid extravasation, leucocytes migration and other biochemical parameters which accompany inflammatory stimuli. Production of exudate in this model is related to local release of histamine, kinins and synthesis of prostaglandins. Migration of leucocytes would not be directly related to cyclooxygenase products, but the process is inhibited by non-steroidal anti-inflammatory compounds indicating that many mechanisms may be implicated in its control. The inhibitory effect of the isolated compound on the intraperitoneal formation of exudate and leucocytes mobilization is probably due to the inhibition of prostaglandins. This possibility is reinforced by the

UK J Pharm & Biosci, 2017: 5(1); 53
fact that the isolated compound remarkably inhibited paw oedematous process which is believed to be mediated by prostaglandins31. Moreover, it is reported that the flavonoids display anti-inflammatory activity by the inhibition of prostaglandin synthesis31.

3.4.1.2 Cotton pellet-induced granuloma model

The effect of FBP8, FBP9 and FBP10 isolated from Bauhinia purpurea was studied at the doses of 25 mg/kg per body weight. The results revealed that the isolated fraction of Bauhinia purpurea shows dose dependent inhibition of weight of both wet and dry cotton pellets. The mean number of decrease in weight of both wet and dry cotton pellets for rats, which received 25 mg/kg body weight of the isolated compound was significant ( p < 0.05) lower than those in the control rats (Table 7). The FBP8, FBP9 and FBP10 demonstrated 27.47%, 29.73% and 19.17% inhibition, respectively in weight of wet cotton pellets. The FBP8, FBP9 and FBP10 demonstrated 43.04%, 47.83% and 32.87% inhibition, respectively in weight of dry cotton pellets. The FBP9 was found to be most effective compared to other isolated compound. The standard drug exhibited 35.00% and 65.15% inhibition in weight of wet cotton pellets and dry cotton pellets, respectively.

<table>
<thead>
<tr>
<th>Group</th>
<th>Wet weight (mg)</th>
<th>%Inhibitions</th>
<th>Dry weight (mg)</th>
<th>%Inhibitions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>302.21±1.35</td>
<td>--</td>
<td>82.14±2.18</td>
<td>--</td>
</tr>
<tr>
<td>Indomethacin (10 mg/kg)</td>
<td>196.43±1.58*</td>
<td>35.00</td>
<td>28.62±1.63*</td>
<td>65.15</td>
</tr>
<tr>
<td>FBP8 (25 mg/kg)</td>
<td>219.17±1.63*</td>
<td>27.47</td>
<td>46.78±1.94*</td>
<td>43.04</td>
</tr>
<tr>
<td>FBP9 (25 mg/kg)</td>
<td>212.35±1.48*</td>
<td>29.73</td>
<td>42.85±2.08*</td>
<td>47.83</td>
</tr>
<tr>
<td>FBP10 (25 mg/kg)</td>
<td>242.64±1.73*</td>
<td>19.71</td>
<td>55.14±1.56*</td>
<td>32.87</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM, n = 6 in each group. *P<0.05 compared to control group.

Generally the cotton-pellet granuloma is employed to assess the transudative and proliferative components of the chronic inflammation. The transude correlates with the moist weight of the pellets while dry weight of the pellet correlates with the amount of granulomatous tissues. The proliferation of macrophages, neutrophils and fibroblast are responsible for granuloma formation, and leads to chronic inflammation. Indomethacin diminishes the granuloma dimension by deterring the granulocyte infiltration, impeding the production of collagen fibers and quashing mucopolysaccharides34. The isolated fractions of Bauhinia purpurea showed significant anti-inflammatory activity in cotton pellet induced granuloma and thus found to be effective in chronic inflammatory conditions, which reflected its efficacy in inhibiting the increase in the number of fibroblasts and synthesis of collagen and mucopolysaccharides during granuloma tissue formation. Consequently, the pattern of anti-inflammatory activity displayed by the isolated fractions was similar to that of indomethacin. The flavonoids impart anti-inflammatory activity, and phytochemical study of isolated fractions justifies the above statement. The outcome of the study endorses the anti-inflammatory activity of isolated fraction was due to presence of flavonoids.

3.4.2 Anti-arthritis activity

3.4.2.1 Adjuvant Induced Chronic Arthritis

The anti-arthritis models proposed that mycobacterial infections can trigger autoimmune arthritis, predominantly through T-cell mediated responses. Arthritis was induced in rats by injecting dead mycobacteria in liquid paraffin. There was a significant increase in rat paw volume in FCA injected control rats when compared to the standard and fraction treated rats. The rats treated with isolated fractions at the dose of 25 mg/kg exhibited significant reduction in rat paw edema volume compared to control group. Table 8 demonstrated the effect of FBP8, FBP9 and FBP10 isolated from Bauhinia purpurea on Freund's adjuvant induced arthritis. After 28 days, it was found that FBP8, FBP9 and FBP10 significantly displays dose dependent inhibition in paw thickness i.e. the chronic inflammation induced by adjuvant shows decrease in paw thickness. The decreased in paw thickness after administration of FBP8, FBP9 and FBP10 were 0.31±0.51, 0.26±0.28 and 0.36±0.48 ml, respectively. Standard drug indomethacin significantly decreased the paw thickness i.e. 0.23±0.91 ml after induction of Freund's adjuvant. The FBP9 was found to be most effective compared to other isolated compound.

3.4.2.2 Hematological parameters

The administration of FBP8, FBP9 and FBP10 isolated from Bauhinia purpurea on Freund's adjuvant induced arthritis animals enhanced the levels of RBC and Hb compared to control animals (Table 9). The WBC count and ESR were significantly reduced after administration FBP8, FBP9 and FBP10 compared to control animals. Rheumatoid arthritis is a widespread autoimmune disorder; the FCA incited arthritis model is pondered as one of the prominent animal models of rheumatoid arthritis. The systemic
inflammation for arthritis was induced in experimental animals by inoculating with FCA (which was prepared by suspending heat-killed *Mycobacterium butyricum* in liquid paraffin at a dose of 10 mg/ml *Mycobacterium butyricum* in paraffin oil).

The progression of arthritis was confirmed in our study by scoring total arthritis lesions. The inflammation induced by FCA is primarily due to edema formation and cellular influx. The investigation of paw oedema is according to the grapevine simple, inclined and rapid procedure to evaluate the degree of inflammation, and assess the therapeutic effects of drugs. The adjuvant-induced arthritis rats developed a chronic swelling in several joints with influence of inflammatory cells, erosion of joint cartilage and bone destruction and remodeling which have close similarity to human rheumatoid arthritis. These inflammatory changes eventually result in the complete destruction of joint stability and mobility in the arthritis rats. Also, soft tissue swelling around the ankle joints was appeared during the progress of arthritis in FCA injected rats, which was considered as oedema of the exacting tissues.

The progression of arthritis was confirmed in our study by scoring total arthritis lesions. The inflammation associated with AIA is mainly dependent on prostaglandin E2 generated by cyclooxygenases. Besides, the role of cytokines like TNF-α and IL-1 has also been implicated in this model. The outcomes indicate that the FBP8, FBP9 and FBP10 treated arthritis animals showed decreased inflammation of joints. Therefore, the anti-arthritis action of isolated fractions may be mediated by prostaglandin and/or cytokine inhibition. These results are also in line with reports that anti-inflammatory action of *Bauhinia purpurea* leaves has been attributed to polyphenolic component. The inhibition of lipid peroxidation, capillary permeability and fragility, and enzymes such as phospholipase A2, cyclooxygenase, and lipoxygenase may be due to tannins and polyphenols components.

In present study, arthritis control rats showed a reduced RBC count, reduced Hb levels, and an increased erythrocyte sedimentation rate (ESR). All these symptoms indicate an anemic condition. The FBP8, FBP9 and FBP10 treated groups showed a significant recovery from the induced anemia. The significant increase in leukocyte count in adjuvant induced arthritis rats may be due to the stimulation of immune system against the invading antigens and significant decrease in FBP8, FBP9 and FBP10 treated groups showed its immunomodulation effect. This clearly indicates the anti-arthritis activity of *Bauhinia purpurea* isolated fractions. This study confirmed that flavonoid fraction obtained from *Bauhinia purpurea* leaves extract are

**Table 8:** Effect of isolated fraction from *Bauhinia purpurea* leaves extract on FCA induced paw volume

<table>
<thead>
<tr>
<th>Group</th>
<th>Paw volume after induction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
</tr>
<tr>
<td>Control</td>
<td>0.21±0.32</td>
</tr>
<tr>
<td>Indomethacin (10 mg/kg)</td>
<td>0.27±0.54</td>
</tr>
<tr>
<td>FBP8 (25 mg/kg)</td>
<td>0.22±0.14</td>
</tr>
<tr>
<td>FBP9 (25 mg/kg)</td>
<td>0.26±0.28</td>
</tr>
<tr>
<td>FBP10 (25 mg/kg)</td>
<td>0.23±0.39</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM, n = 6 in each group. *P<0.05 compared to control group

**Table 9:** Effect of isolated fraction from *Bauhinia purpurea* leaves extract on hematological parameters

<table>
<thead>
<tr>
<th>Group</th>
<th>RBC (millions/cmm)</th>
<th>WBC (thousands/cmm)</th>
<th>Hb (gm/dL)</th>
<th>ESR (mm/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.25±1.24</td>
<td>23.17±0.84</td>
<td>8.31±1.53</td>
<td>35.26±1.32</td>
</tr>
<tr>
<td>Indomethacin (10 mg/kg)</td>
<td>8.12±0.48*</td>
<td>9.63±1.41*</td>
<td>13.74±0.58*</td>
<td>2.31±0.67*</td>
</tr>
<tr>
<td>FBP8 (25 mg/kg)</td>
<td>7.32±1.32*</td>
<td>11.18±1.06*</td>
<td>12.05±0.45*</td>
<td>4.24±1.37*</td>
</tr>
<tr>
<td>FBP9 (25 mg/kg)</td>
<td>7.53±0.69*</td>
<td>10.23±0.92*</td>
<td>12.48±0.34*</td>
<td>3.52±0.83*</td>
</tr>
<tr>
<td>FBP10 (25 mg/kg)</td>
<td>6.48±0.54*</td>
<td>11.78±0.49*</td>
<td>11.18±1.24*</td>
<td>5.57±0.61*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM, n = 6 in each group. *P<0.05 compared to control group
responsible for its anti-arthritis activity and the effects observed are attributable due to the presence of flavonoids in the plant.

It has been validated by various researchers that medicinal plants demonstrating relations between anti-inflammatory/anti-arthritis and phenol or flavonoid content. Several researches suggest that the combination of secondary metabolites with flavonoids compounds produces synergistic pharmacological activity. Although no record of chemical constituents isolated and characterized from Bauhinia purpurea was found, and the methods used for the identification of phytochemical constituents are preliminary in nature. The anti-inflammatory and anti-arthritis effects recorded for isolated fractions of Bauhinia purpurea in this study, caused by the total polyphenol and flavonoids constituents present in the plant. The phytochemical study of isolated compound justifies the above statement. The fractions FBP8 and FBP10 containing flavonoids and polyphenol compounds, while the FBP9 indicates the presence of other phytoconstituents along with flavonoids and polyphenol. The FBP9 demonstrated maximum anti-inflammatory and anti-arthritis activity, it suggest the synergistic anti-inflammatory and anti-arthritis activity of isolated fractions. The results obtained in this study established the anti-inflammatory and anti-arthritis actions for the isolated fractions. However, the mechanism of these actions is uncertain, and the flavonoids and polyphenol imparts chief role for the anti-inflammatory and anti-arthritis activity.

4 Conclusion
The outcomes from the present study displayed that the fractions of hydroalcoholic extracts of Bauhinia purpurea comprised of polyphenol and flavonol, and prominently inclined their antioxidant properties. The fractions FBP8, FBP9 and FBP10 demonstrated significant anti-inflammatory and anti-arthritis activity, and suggesting that polyphenol and flavonoids is the main contributor for anti-inflammatory and anti-arthritis activity of the fractions. The in vitro antioxidant activity and phytochemical results scientifically supported the anti-inflammatory and anti-arthritis activity of the fractions might be due to presence of flavonoids and polyphenol compound. Further studies are carried for the possible mechanism and the characterization of the bioactive component responsible for anti-inflammatory and anti-arthritis activity.

5 Conflict of interests
The authors declare that there is no conflict of interests regarding the publication of this paper.

6 Author’s contributions
AS carried out literature review and experimental work of the present study. VDT was responsible for statistical work and calculations in addition to manuscript proofing. RKS carried out discussion of the present study. All authors read and approved the final manuscript.

7 Acknowledgements
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8 References


