

**Original Article**

**ASSESSMENT OF PHYTOCHEMICALS AND *IN VITRO* ANTIOXIDANT, ANTI-INFLAMMATORY ACTIVITY OF *SARCOSTEMMA BRUNONIANUM* WIGHT AND ARN**

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Received: 15 Sep 2020, Revised and Accepted: 17 Nov 2020

**ABSTRACT**

**Objective:** *Sarcostemma brunonianum* Wight and Arn is a potential medicinal plant belonging to Asclepiadaceae. Bioactive constituents of the plant support the application of treating various ailments in the traditional system of medicine. The study aims to determine the presence of various phytoconstituents in stem, root, and flowers.

**Methods:** Hot percolation method was carried out to obtain crude extracts using different solvent systems from low polar-high polar solvents ranging from petroleum ether, chloroform, (mid-polar) ethyl acetate, methanol and water. Estimation of total phenols, tannins and *In vitro* antioxidant, anti-inflammatory activities were evaluated for the determination of potential pharmaceutical drugs.

**Results:** The results revealed the presence of some phytoconstituents such as phenols, tannins, glycosides, gums and mucilages. Ascorbic acid, BSA, Rutin and Gallic acid were used as the reference standard. The total phenolic content was found to be high in stem methanol extract  $440.84 \pm 69.99$  mg/g Gallic acid equivalent, whereas the tannin content was  $291.78 \pm 4.68$  mg/g GAE. The result proves that the *S. brunonianum* stem methanol extract possesses antioxidant and anti-inflammatory activities when compared to reference standards. *In vitro*, Nitric oxide scavenging activity of stem showed a maximum % of inhibition in methanol stem extract ( $24.39 \mu\text{g/ml}$ ) and anti-inflammatory activity maximum inhibition was found to be (55.56 %) in stem methanol and flower (53.62 %). The IC<sub>50</sub> (concentration required for 50% inhibition) was also calculated for the DPPH radical model.

**Conclusion:** This study results proclaims and justifies the role of folklore medicinal plant *S. brunonianum* in the treatment of inflammatory-related ailments and can be recommended for an effective drug.

**Keywords:** *Sarcostemma brunonianum*, Phytochemicals, Antioxidants, Anti-inflammatory

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DOI: <https://dx.doi.org/10.22159/ijcpr.2021v13i1.40819>. Journal homepage: <https://innovareacademics.in/journals/index.php/ijcpr>

**INTRODUCTION**

*S. brunonianum* a latex bearing climber commonly used as an emetic by local people of Eastern Ghats of Tamil Nadu [1]. The rural people of Maruthamalai hills, Coimbatore are using the plant stem is used as a medicine for snake bites, dyspepsia and various illness. Commonly, it is called Perumaattaan Kodi [2]. The plant has been traditionally used in different areas of India especially the roots are used in treating snakebite and taken as an infusion in dog bite cases [3]. *Sarcostemma* existing in warmer regions of the world like India, China, Pakistan, Srilanka, The perennial, fleshy, glabrous branched, milky latex secreting shrub posses various religious and pharmacological significances [4]. Several species of *Sarcostemma* genera resemble each other on similar morphological characters. The plant *S. acidum* is well used by the tribal people for asthma, swelling, fever, colds, dyspepsia, inflammatory infection and gastric problem [5]. The fresh plant material of *S. viminalis* is minced and mixed with margarine, then applied to wounded areas [6]. The composite mixture of bioactive materials synthesized by plants is responsible for metabolic functions, recently, these plant metabolites are referred to as phytochemicals [7].

The explorations of medicinal plants are considered as a relevant source in the production of new drugs is getting increased day by day. The demand for plant resources leads to research on bioprospecting fields. Free radicals and reactive oxygen species are the main cause of the troubling of physiological as well as metabolic activities [8]. Free radicals are any chemical species that are capable of existing with one or more unpaired electrons and are extremely reactive and highly unstable [9]; non-natural phenolic antioxidants such as (*BHT*) butylated hydroxytoluene, (*BHA*) Butylated hydroxyl anisole antioxidants were widely used as a food preservative and its consumption leads to affect various organs like kidney, liver, and skin on moderate to excessive level [10]. Many plants frequently

contain substantial amounts of antioxidants, including vitamins, carotenoids, flavonoids, phenols, and tannins, etc. and thus can be utilized to scavenge the unpaired electrons [11]. Various researchers are still in active practice to develop new drugs with efficient antioxidant activity.

In such a way, the study focused to utilize least utilized laticifer *Sarcostemma brunonianum* Wight and Arn to explore the photo components, to check antioxidant capacity and anti-inflammatory properties.

**MATERIALS AND METHODS**

**Collection and processing of the plant materials**

The fresh stem, root, and flowers of *S. brunonianum* were collected from the Maruthamalai forest of Western Ghats, Coimbatore District, Tamil Nadu state. The collected plant materials were identified, and they are authenticated by the Botanical Survey of India, Southern Regional Centre, Coimbatore. The collected plant materials were cleaned and shade dried, powdered and stored for further studies.

**Successive solvent extraction of plant parts**

The powdered plant material was extracted in the Soxhlet apparatus successively with petroleum ether, chloroform, ethyl acetate, methanol, and water. The different solvent extracts obtained were concentrated and then air-dried.

**Qualitative phytochemical screening**

The stem, root, and flower extracts of *S. brunonianum* were tested for the presence of major phytochemicals such as alkaloids, saponins, phenolic compounds, tannins, flavonoids, glycosides, phytosterols, fixed oils and fats, and gums and mucilages according to standard methods [12-15].

## Phenolic compounds

### Ferric chloride test

About 50 mg of the plant sample was dissolved in 5 ml of distilled water. To this, a few drops of 5% neutral ferric chloride solution was added. Phenolic compounds were indicated by the presence of a dark green color.

### Tannins

#### Potassium hydroxide test

The plant material (0.5 g) was added into 10 ml of freshly prepared 10% potassium hydroxide (KOH) in a beaker and shaken to dissolve. A dirty precipitate indicates the occurrence of tannin.

### Glycosides

#### Borntrager's test

50 mg of plant material was hydrolyzed with concentrated hydrochloric acid for two hrs on a water bath and filtered. About 2 ml of filtrate is added with 3 ml of chloroform and shaken. The chloroform layer was separated and a 10% ammonia solution was added to it. The pink color indicated the presence of glycosides.

### Fixed oils and fats

#### Saponification test

A few drops of 0.5 N alcoholic potassium hydroxide solutions were added to some samples along with a drop of phenolphthalein. The mixture was kept under a boiling water bath for 2 h. The formation of soap indicates the occurrence of fixed oils and fats.

### Gums and mucilage

#### Absolute alcohol test

About 100 mg of plant sample was dissolved in 10 ml of distilled water followed by 25 ml of absolute alcohol was added with continuous stirring. White precipitate indicates the existence of gums and mucilage.

### Quantification of total phenolic and tannin contents

The total phenolic content was determined according to the method described by [16]. Fifty ml triplicates of the extracts (20 mg/20 ml) were taken in the test tubes and makeup to the volume of 1 ml with distilled water. About 0.5 ml of Folin and Ciocalteu phenol reagent (1:1 with water) and 2.5 ml sodium carbonate solution (20%) were added successively in each tube. The reaction mixtures were vortexed and all the test tubes were kept under dark incubation for 40 min and the absorbance was recorded at 725 nm against the reagent blank. The investigation was done in triplicate and the results were expressed in gallic acid equivalents.

The tannin contents were estimated using the same extract after treatment with polyvinyl polypyrrolidone (PVPP) [17], 100 mg of PVPP was weighed into transferred into Eppendorf tube. About 1 ml of distilled water was added along with 1 ml of the sample extracts. The whole content was vortexed and kept in the freezer at 4 °C for 15 min. Next, the samples were centrifuged for 10 min at 4000 rpm at room temperature. Finally, the supernatants were collected. The supernatant contains only the simple phenolics other than the non-phenolic contents (the tannins were precipitated along with the PVPP). The total phenol content was measured and expressed as the content of non-tannin phenolics.

Tannins = Total phenols - Non tannin phenols

### In vitro antioxidant assays

#### Phosphomolybdenum assay

The antioxidant activity of samples was evaluated by the green phosphomolybdenum complex formation according to the method [18]. About 40 µL aliquots of a sample or ascorbic acid in 1 mmol dimethyl sulphoxide (standard) or distilled water (blank) were

added with 1 ml of reagent solution (0.6 M sulphuric acid (H<sub>2</sub>SO<sub>4</sub>), 28 mmol sodium phosphate, and 4 mmol ammonium molybdate) in each test tubes. All the test tubes were covered with foil and incubated under a water bath for about 90 min at 95 °C. Later than, the samples were cooled to room temperature, the absorbance of the mixture was measured at 695 nm against the reagent blank. The results reported (Total antioxidant capacity) are mean values expressed as mg AAE/g extract (Ascorbic Acid Equivalents per gram extract).

#### DPPH radical scavenging activity

The antioxidant activity of the extract was determined in terms of hydrogen donating or radical scavenging ability using the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH), according to the method of [19]. Various concentrations of sample extracts were taken and the volume was finalized to 100 µl with methanol. About 5 ml of a 0.1 mmol methanolic solution of DPPH was added to the aliquots of samples and standards (BHA, BHT, Rutin, and Quercetin) and shaken vigorously. The negative control was prepared by adding 100 µl of methanol in 5 ml of 0.1 mmol methanol solution DPPH. All the test tubes were incubated at 27 °C for 20 min. The absorbance was measured at 517 nm against the blank (methanol). Radical scavenging activity of the samples was expressed as IC<sub>50</sub> which is the concentration of the sample required to inhibit 50% of DPPH concentration.

#### Nitric oxide scavenging activity

The Nitric oxide scavenging activity was determined by the method [20], where sodium nitroprusside in aqueous solution at physiological pH, spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent. Nitric oxide scavengers compete with oxygen leads to a reduction in the production of nitrite ions. For the experiment, sodium nitroprusside (10 mmol) in phosphate-buffered saline (0.2 M, pH-7.4) was mixed with various concentrations of sample solution of various extracts or BHT and Rutin (standard) and incubated at room temperature for 150 min. The same reaction mixture without the sample was used as a negative control. After incubation, 0.5 ml of Griess reagent (1% sulfanilamide, 2% H<sub>3</sub>PO<sub>4</sub> and 0.1% N-(1-naphthyl), ethylenediamine dihydrochloride) were added. The absorbance of the chromophore formed was read at 546 nm against the blank (phosphate buffer). The scavenging activity (%) was calculated as:

$$\text{Radical scavenging activity (\%)} = [(A_0 - A_1) / A_0] \times 100$$

Where A<sub>0</sub> is the absorbance of the control and A<sub>1</sub> is the absorbance of sample extract/standard.

#### In vitro anti-inflammatory activity

##### RBC membrane stabilization method

The membrane-stabilizing activity assay was carried out with minor changes as previously described [21, 22]. The collected blood sample was mixed with an equal volume of sterilized Alsever solution (2% dextrose, 0.8% sodium citrate, 0.05% citric acid and 0.42% sodium chloride in water). The whole mixture was centrifuged at 3000 rpm and the packed cells were washed up with isosaline (0.85%, pH 7.2). The assay mixture with the sample extract (0.5 ml; 50–1000 µg/ml)/Diclofenac-sodium standard drug (0.5 ml; 50, 100, 200 µg/ml), 1 ml phosphate buffer (0.15 M, pH 7.4), 2 ml of hyposaline (0.36%) and 0.5 ml of HRBC suspension was added and incubated at 37 °C for 30 min and centrifuged for 20 min at 3000 rpm. The total hemoglobin content in the suspension was estimated using a spectrophotometer at 560 nm. The percentage of hemolysis produced in the presence of distilled water was taken as 100%. The RBC membrane stabilization or protection was calculated using the formula:

%Stabilization = 100 - [(A<sub>1</sub> (sample/standard)/A<sub>0</sub> (control)) × 100, where A<sub>0</sub> was the absorbance of the control reaction and A<sub>1</sub> was the absorbance in the presence of the sample/standard (Diclofenac-sodium).

### Statistical analysis

The results were expressed as mean±SD/SEM. The data were statistically analyzed using SPSS version 2.0 using one-way ANOVA followed by Duncan's test for antioxidant studies. Mean values were measured statistically significant when  $p < 0.05$ ,  $p < 0.01$  and  $p < 0.001$ .

### RESULTS

Qualitative phytochemical screening on *S. brunonianum* plant parts such as stem, root, and the flower was carried out to detect the presence of numerous bioactive compounds, both primary and few classes of secondary metabolites.

**Table 1: Result obtained from phytochemical screening of *S. brunonianum***

| Test                | Name of the test           | Observation               | Results |      |        |
|---------------------|----------------------------|---------------------------|---------|------|--------|
|                     |                            |                           | Stem    | Root | Flower |
| Phenolics           | Ferric chloride test       | Dark green color          | ++      | +    | +      |
| Tannin              | Potassium hydroxide test   | Dirty ppt                 | +       | ++   | -      |
| Glycosides          | Borntrager's test          | Pink color                | +       | +    | -      |
| Phytosterols        | Liebermann Burchard's test | An array of color changes | +       | +    | +      |
| Fixed oils and fats | Saponification test        | Formation of soap         | +       | +    | +      |
| Gums and mucilage   | Absolute alcohol test      | White ppt                 | +++     | +    | +      |

(+): indicates the presence of chemical compound, (-): indicates of absence of chemical compound. (+)<(++)<(+++): Based on the intensity of distinguishing color.

Phytochemical screening of the plant parts revealed the presence of flavonoids, tannins, saponins, carbohydrates, and Phytosterols are high in stem compared to root and flower.

#### Total phenol and tannin contents

The contents of phenols, tannins and flavonoids varied significantly concerning the solvent extracts used (table 2). The methanolic extract of the stem shows the highest contents of total phenol, flavonoid, and tannin. However, the water extract of the stem expressed a lower level of polyphenols compared to other parts.

#### In vitro free radicals scavenging activities

##### DPPH<sup>•</sup> scavenging activities of *S. brunonianum*

The DPPH radical scavenging activities of *Sarcostemma* extracts are shown in (fig. 1). In this assay, synthetic (BHT) and natural antioxidant (rutin) were used as a reference control. Methanol extracts showed superior activities compared to other solvent extracts; methanol extract of *S. brunonianum* stem (IC<sub>50</sub>: 19.71 µg/ml), root (IC<sub>50</sub>: 40.68 µg/ml) and water extract of the stem (IC<sub>50</sub>: 36.48 µg/ml) showed maximum DPPH radical scavenging activities which are comparable to the standards BHT (IC<sub>50</sub>: 5.19 µg/ml), and Rutin (IC<sub>50</sub>: 6.48 µg/ml). Overall, the free radical scavenging activity was found to be lower in ethyl acetate extracts.

##### Total antioxidant capacity

The total antioxidant capacity (TAC) of the plant extracts was determined using a phosphomolybdate assay (fig. 2). The assay activity is based on the reduction of molybdenum (VI) into molybdenum (V) in the presence of a reducing agent (antioxidant),

forming a green phosphomolybdate(V) complex, and evaluated spectrophotometrically at 765 nm. Fig. 2. Among different parts used, the methanol extract of the stem has shown a better antioxidant capacity (834.67 mg AAE/g extract) followed by ethyl acetate. This order of activity from polar aqueous to nonpolar petroleum ether extract is quite normal since the polar solvents have a much stronger ability to dissolve and extract polar phytochemicals. The lower antioxidant capacities of the flower extracts. Root part of methanol extract also showed higher antioxidant capacity (498.67 mg AAE/g extract) as compared to other solvent extracts. Thus, the antioxidant capacity observed from the extracts of *S. brunonianum* can be correlated with its free radical scavenging activity equivalent to that of natural antioxidant ascorbic acid.

##### Nitric oxide activity

Nitric oxide (NO) has a significant role in various types of inflammatory processes in the animal body. Scavenging NO activities is detrimental to human health. The scavenging activities of various samples on nitric oxide were analyzed and the percentage of scavenging activities of stem, bark and fruit of *S. brunonianum* are shown in (fig. 3). The Nitric oxide radical scavenging activity of stem, root and flower extracts of the plant showed greater % of inhibition, especially in methanol extract of the stem (24.39µg/ml), root extract (26.48µg/ml) followed by ethyl acetate stem extract (30.03µg/ml). The scavenging activities of all extracts were comparable with that of Rutin and BHT. The scavenging of nitric oxide assay has shown the relevant inhibitory concentration in the methanolic extract of the bark sample. The result indicates that the sample extracts might contain some compounds which can inhibit nitric oxide and offers scientific evidence for the utilization of the plant in the inflammatory condition.

**Table 2: Showing total phenol and tannin contents found in *S. brunonianum* plant parts**

| Parts  | Solvent extraction | Total phenols (GAE mg/g)    | Total tannins (GAE mg/g)    |
|--------|--------------------|-----------------------------|-----------------------------|
| Stem   | Petroleum ether    | 285.9477±18.53 <sup>c</sup> | 149.7778±8.49               |
|        | Chloroform         | 249.3464±12.53              | 230.2222±2.34 <sup>c</sup>  |
|        | Ethyl acetate      | 290.5229±16.44 <sup>b</sup> | 291.7778±4.68 <sup>a</sup>  |
|        | Methanol           | 440.8497±69.98 <sup>a</sup> | 264.2222±13.10 <sup>b</sup> |
|        | Water              | 166.9935±13.91              | 148±33.24                   |
| Root   | Petroleum ether    | 144.1176±18.53              | 151.7778±3.28               |
|        | Chloroform         | 192.4837±10.06              | 161.3333±1.33               |
|        | Ethyl acetate      | 199.6732±9.86 <sup>c</sup>  | 174.4444±1.38 <sup>c</sup>  |
|        | Methanol           | 365.6863±7.84 <sup>a</sup>  | 281.5556±4.91 <sup>a</sup>  |
|        | Water              | 72.87582±7.92               | 239.778±11.08 <sup>b</sup>  |
| Flower | Petroleum ether    | 34.3137±8.98                | 142.8889±3.90               |
|        | Chloroform         | 59.15±6.3 <sup>c</sup>      | 157.7778±2.77 <sup>c</sup>  |
|        | Ethyl acetate      | 171.5686±15.31 <sup>a</sup> | 170.8889±4.72 <sup>b</sup>  |
|        | Methanol           | 152.6144±10.8 <sup>b</sup>  | 180.4444±4.43 <sup>a</sup>  |
|        | Water              | 32.35294±5.89               | 141.7778±2.03               |

GAE-Gallic Acid Equivalents; Values are mean of triplicate determination (n=3)±standard deviation (SE); statistically significant at  $p < 0.05$  where <sup>a>b>c</sup> in each column.

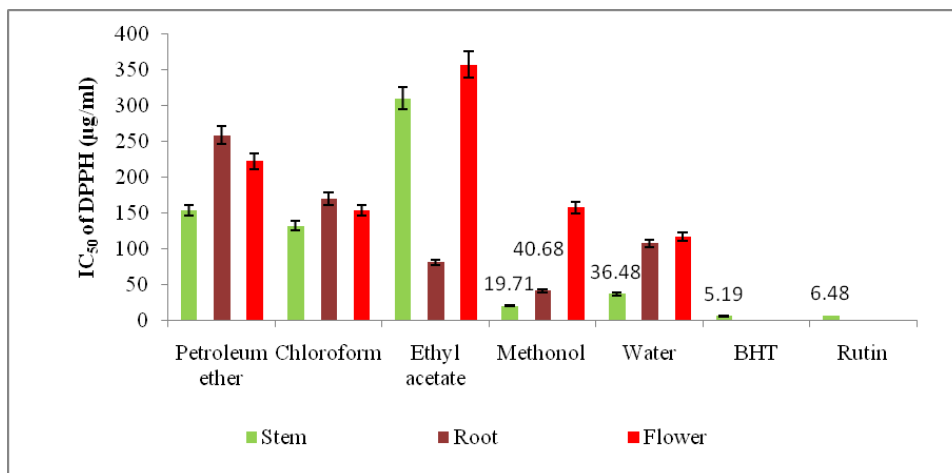


Fig. 1: DPPH scavenging activity

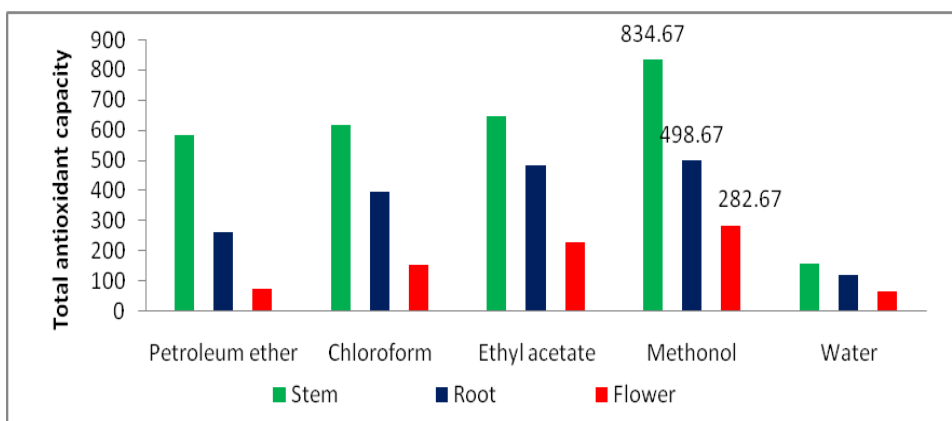


Fig. 2: Total antioxidant capacity

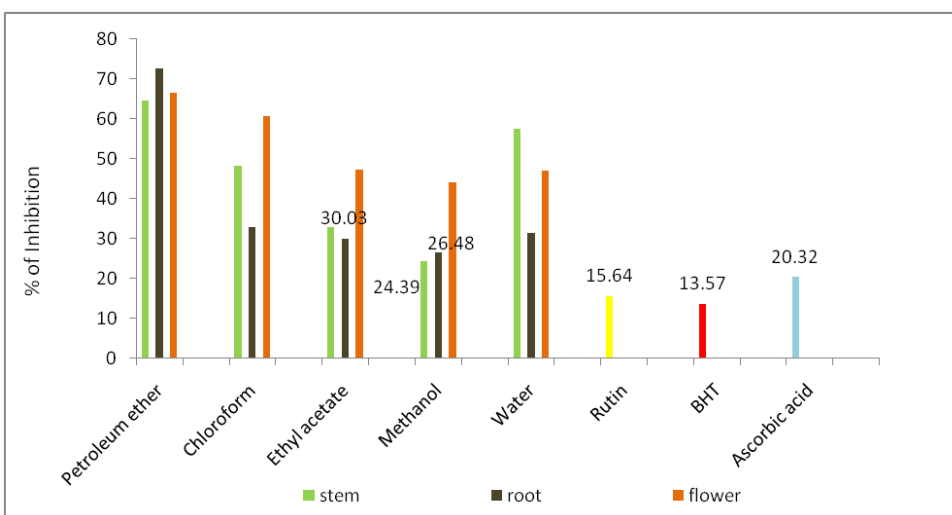


Fig. 3: Nitric oxide activity

**In vitro anti-inflammatory activity**

The methanol and ethyl acetate extract of the stem of *Sarcostemma brunonianum* were studied for *in vitro* anti-inflammatory activity by

the RBC membrane stabilization method. Fig. 4 five different kinds of extract were used among which stem methanol (55.56 %) and flower (53.62 %) have shown higher % of inhibition of RBC in a hypotonic solution.

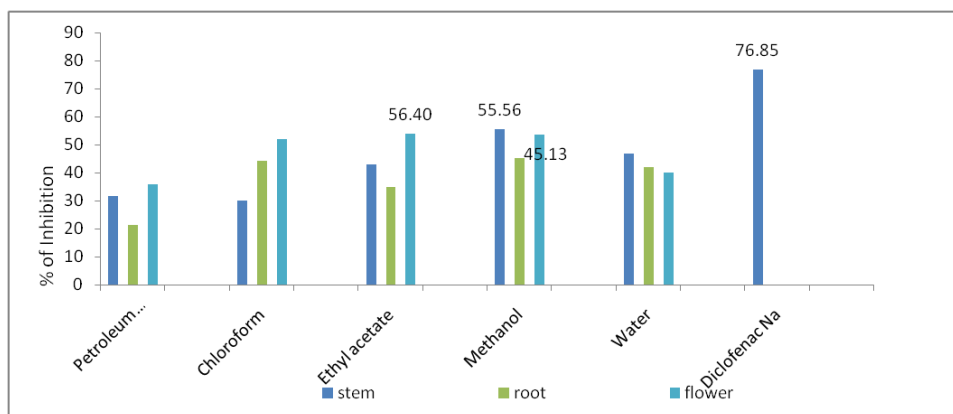


Fig. 4: Anti-inflammatory activity

All the results were compared with standard Diclofenac Sodium, which showed 76.85% protection. This activity may be due to the presence of phytochemical constituents present in the sample extract.

## DISCUSSION

The study provides a piece of strong evidence for the use of *Sarcostemma* in folkloric treatment as an anti-inflammatory agent. Therefore, the plant could be suggested as a natural source of membrane stabilizers and was capable of providing an alternative remedy for the management and treatment of inflammatory-related disorders and diseases [5]. For phenolic composition, results demonstrated that methanol was a better solvent in extracting polyphenols. Therefore, the polarity of the solvent will play an important role in increasing the solubility of phenols [23]. Then, a variation of phenol accumulation between plant parts should be related to their specific cells and tissues. The high presence of phenols in plant parts is attributed to the phenological organ growth, transport involved in polyphenols distribution, the interaction between plant parts and biosynthesis processes [24]. Observed antioxidants and the presence of the active phytochemicals in attributed to a good source of a pharmaceutical drug. Thus, the plant can be used as a source for antioxidant components as well as an alternative drug against Nonsteroidal anti-inflammatory drugs (NSAIDs). Discovering novel, effective and safe anti-inflammatory agents has remained a major thrust area in the field of developing alternatives to NSAIDs.

The test for the analysis of phytochemical compounds put forth a way to determine therapeutic drugs progressively. Preliminary phytochemical analysis made for the stem, root, and flower of *S. brunonianum* revealed the presence of alkaloids, saponins, phenolics, tannins, flavonoids, glycosides, phytosterols, and other bioactive compounds. Till date, phytochemical investigation and other studies on *S. brunonianum* has not yet been studied completely. Hence, the present study was focused to determine phytochemicals, to evaluate the antioxidant potential of plant extracts and It can be concluded that *S. brunonianum* is a therapeutically potential herb that can be attributed to its phytoconstituents and justifies its use in the folklore system of medicine. Besides, ethyl acetate and methanolic extracts also showed anti-inflammatory activity, which was quite comparable to the one observed for diclofenac at 10 mg/kg and the results were found statistically significant.

## CONCLUSION

Investigation on phytochemicals has indicated the presence of phenols, tannins, glycosides, gums and mucilages. This study has also shown the presence of phenolic compounds in *S. brunonianum* suggests that the antioxidant activity may be due to the polyphenol contents. Such kind of effective natural antioxidants can replace the synthetic ones that can be used in foods and cosmetics. Thus, the valuable source of *S. brunonianum* could be employed in varied

medicinal preparations to combat inflammatory diseases associated with oxidative stress and related disorders. The obtained results demonstrated that methanol was the most effective solvent to extract phytochemical compounds compared to petroleum ether, chloroform, ethyl acetate and aqueous. In conclusion, these results reveal that *S. brunonianum* has significant antioxidant and anti-inflammatory properties. An overall positive correlation between anti-inflammatory and antioxidant activity was observed.

## ACKNOWLEDGMENT

We are highly thankful to Phytochemistry Lab, Dept of Botany, Bharathiar University, DST-FIST, PURSE and SERB for providing all kind of support during this research.

## FUNDING

Nil

## AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

## CONFLICTS OF INTERESTS

The authors strongly declare that there is no conflict of interest.

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