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, Sri Lanka, The perennial, fleshy, glabrous branched, milky latex secreting shrub possesses 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 Folien and Ciocâlteu 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 pyrrolidone (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 (H₂SO₄), 28 mmol sodium phosphate, and 4 mmol ammonium molybdate) in each test tube. 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 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 IC50 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 Greiss 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% H₃PO₄, and 0.1% N-(1-naphthyl), ethylenediamine dibydrochloride) were added. The absorbance of the chromophore formed was read at 546 nm against the blank (phosphate buffer). The scavenging activity (%) was calculated as:

Radical scavenging activity (%) = \[\frac{[(A0 - A1) / A0] \times 100}{\}\]

Where A0 is the absorbance of the control and A1 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 (0.05%, pH 7.2). The assay mixture with the sample extract (0.5 ml; 50–1000µg/ml) /Diclofenac-sodium standard drug (0.5 ml; 50, 100, 200µg/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 at3000 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 – [(A1 (sample/standard)/A0 (control))] \times 100,

where A0 was the absorbance of the control reaction and A1 was the absorbance in the presence of the sample/sample (Diclofenac-sodium).
Statistical analysis

The results were expressed as means±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 |
|---------------------|---------------------------|----------------------|---------|
| Phenolics           | Ferric chloride test      | Dark green color     | ++      |
| Tannin              | Potassium hydroxide test  | Dirty ppt            | +       |
| Glycosides          | Borntrager’s test         | Pink color           | +       |
| Phytosterols        | Libermann 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** radical 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$_{50}$: 19.71 µg/ml), root (IC$_{50}$: 40.68 µg/ml) and water extract of the stem (IC$_{50}$: 36.48 µg/ml) showed maximum DPPH radical scavenging activities which are comparable to the standards BHT (IC$_{50}$: 5.19 µg/ml) and Rutin (IC$_{50}$: 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 are 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 (26.49µg/ml) followed by ethyl acetate stem extract (50.03µg/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           | 149.7778±8.49           |
|              | Chloroform         | 249.346±12.53            | 230.222±2.34            |
|              | Ethyl acetate      | 290.52±16.44             | 291.7778±4.68           |
|              | Methanol           | 440.8947±9.68            | 264.222±13.10           |
|              | Water              | 166.9935±13.91           | 149±3.24                |
| Root         | Petroleum ether    | 144.1176±18.53           | 151.7778±3.28           |
|              | Chloroform         | 192.4837±10.06           | 161.333±1.33            |
|              | Ethyl acetate      | 199.6732±9.86            | 174.444±1.38            |
|              | Methanol           | 365.6863±7.84            | 281.555±4.91            |
|              | Water              | 72.8758±2.92             | 239.777±11.08           |
| Flower       | Petroleum ether    | 34.3317±8.98             | 142.889±3.90            |
|              | Chloroform         | 59.15±5.3               | 157.7778±2.77           |
|              | Ethyl acetate      | 171.56±15.31             | 170.889±4.72            |
|              | Methanol           | 152.61±10.8             | 180.444±4.43            |
|              | Water              | 32.52±4.58              | 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 <>/>=< in each column.
**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.
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 related to their specific cells and tissues. The high presence of variation of phenol accumulation between plant parts should be 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 related to their specific cells and tissues. The high presence of variation of phenol accumulation between plant parts should be 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 related to their specific cells and tissues. The high presence of variation of phenol accumulation between plant parts should be 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 related to their specific cells and tissues. The high presence of variation of phenol accumulation between plant parts should be 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 related to their specific cells and tissues. The high presence of variation of phenol accumulation between plant parts should be 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].

**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.

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**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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