

NEW-VISTA IN FINDING ANTIOXIDANT AND ANTI-INFLAMMATORY PROPERTY OF CRUDE PROTEIN EXTRACT FROM *SAUROPUS ANDROGYNUS* LEAF

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ABSTRACT

Aim. This study describes antioxidant and anti-inflammatory properties of *Sauropus androgynus* leaf dialysed protein extract. Free radicals are implicated for many diseases including diabetes mellitus, arthritis, cancer, ageing etc. In the treatment of these diseases, antioxidant therapy has gained utmost importance. The main objective of the present study was to reveal the antioxidant and anti-inflammatory potentiality from *S. androgynus* leaf dialysed protein extract.

Material and methods. The antioxidant and anti-inflammatory properties of *S. androgynus* studied using different models viz. hydroxyl radical scavenging, DPPH radical scavenging, reducing power assay, superoxide radical scavenging activity by alkaline DMSO and phosphomolybdenum antioxidant assays and *in vitro* anti-inflammatory activity by inhibition of protein denaturation, membrane stabilisation test models carried out.

Results. Antioxidant activity was estimated in dose dependent manner showed hydroxyl radical (55.62%), DPPH (50%), reducing power (0.286 Abs), alkaline DMSO (72.51%) and phosphomolybdenum (0.198 Abs) activity high at 50 µg/ml concentration compared to standard curcumin (62.31%), curcumin (56.61%), tannic acid (0.54 Abs), curcumin (75.38%) and vitamin E (0.15 Abs) respectively. In-vitro anti-inflammatory activity by hypotonic induced model showed maximum protection (74.17%) compared to standard Acetylsalicylic acid (86.88%) at 100 µg/ml concentration and also in protein denaturation model protected protein denaturation maximum (83.60%) compared to standard Diclofenac (86.82%) at 100 µg/ml concentration respectively.

Conclusion. The antioxidant property usually studied related to the polyphenols and flavonoids present in the extract but present finding concluded that *S. androgynus* giving hint even potential proteins can also show responsible action effective against free radical mediated disease.

Key words: antioxidant, anti-inflammatory, neutrophil, lysosomal, erythrocyte

INTRODUCTION

The term 'antioxidant' refers to the activity of numerous vitamins, minerals and phytochemicals which provide protection against the damage caused by ROS

[Khilfi et al. 2006]. Antioxidants interfere with the oxidative processes by scavenging free radicals, chelating free catalytic metals and by acting as electron donors

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[Gulcin et al. 2005]. Free radicals play an important role in various pathological and xenotoxic effects so antioxidant may have protective role in these pathological conditions. Living organisms have antioxidant defense systems that protects against oxidative damage by removal or repair of damaged molecules [Sun et al. 1998]. Polyphenolic compounds are ubiquitous in foods of plant origin, and thus they constitute an integral part of the human diet. Interest in polyphenols has greatly increased recently because these phytochemicals are known to suppress rates of degenerative processes such as cardiovascular disorders and cancer. Some of these potential health benefits of polyphenolic substances, have been related to the action of these compounds as antioxidants, free radical scavengers, quenchers of singlet and triplet oxygen and inhibitors of peroxidation [Manukumar et al. 2013].

Sauropus androgynus is a member of the family Phyllanthaceae. This plant is spread in India, Sri Lanka, Thailand, Laos, Malaysia, Indonesia and almost in all countries of Southeast Asia. *Sauropus androgynus* is commonly called as multivitamin plant, tropical asparagus, star gooseberry, sweet leaf bush, chakkerumuni. It is also a rich source of antioxidants. In Karnataka it is called as Chakramuni soppu and is used in making many delicacies and leaves are eaten raw as it has a nutty taste [Nahak and Sahu 2010]. It is a cheap source of dietary proteins and has earned the name of *multigreen* for its high vitamin content. *Sauropus androgynus* can be useful as a dye in food colouring a delicious hot weather green vegetable, widely considered being one of the most prolific, nutritious and appetizing of all green-leaved vegetables. The leaves have about 6-10% protein content. The roots and leaves are sometimes used as medicine. The leaves and roots are used to relieve fever and treat urinary problems. The juice from its leaves is dropped into the ear as a remedy for earache.

There are many important reasons to screen for novel alternative antioxidant substances from natural sources mainly plants. The toxicity and side effects of the drugs presently used in health care and medicine being a major area of concern. The generation of drugs in plenty from natural sources with more efficacy, low cost of production and low or negligible side effects has become a prime focus of the pharmacological industry [Manukumar and Madhu 2013]. Biologically active

peptides, proteins and phenolic compounds are either naturally produced by enzymatic digestion, fermentation, germination or enzymatic hydrolysis. In recent years, there is a growing interest to identify and utilize anti-oxidative compounds in many natural sources, such as soy protein [Moure et al. 2000]. The traditional Chinese medicine theory believes that black soybean has been used as a component in ancient medicines to treat diabetes, hypertension, anti-aging, cosmetology, blood circulation and so on [Cho et al. 2001] because of its active peptide compounds. Adipogenesis inhibitory peptide was isolated and identified from black soybean protein hydrolysate [Kim et al. 2007].

When cells in the body are damaged by microbes, physical agents or chemical agents, the injury is in the form stress. The response to the stress of tissue damage is called as inflammation. It is a defensive response that is characterized by redness, pain, heat, and swelling and loss of function in the injured area by producing reactive oxygen species (ROS). Thus free radicals are important mediators that provoke or sustain inflammatory processes and consequently, their neutralization by antioxidants and radical scavengers can attenuate inflammation.

Inflammation is a normal protective response to tissue injury caused by physical trauma, noxious chemical or microbial agents and body response to inactivate or destroy the invading organisms, to remove the irritants and set the stage for tissue repair. The lysosomal enzymes released during inflammation produced a variety of disorders. The extracellular activity of these enzymes is said to be related to acute or chronic inflammation. Stabilisation of lysosomal membrane is important in limiting the inflammatory response by inhibiting the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extra cellular release or by stabilising the lysosomal membrane [Vadivu and Lakshmi 2008]. HRBC or erythrocyte membrane is analogous to the lysosomal membrane [Chou 1997] and its stabilisation implies that the extract may as well stabilize lysosomal membranes. "Thus finding a natural agent as a remedy to treat both inflammation and free radical damage is upcoming new area of research".

"PROTEINS are valued by the food manufacturer for their functional properties and for their nutritional

value. Value of protein was done by researcher on soybean seed coat protein and identified and named as 'Bowman-Birk inhibitor (BBI)', having a molecular weight (MW) of 8 kD, proved as a cancer chemopreventative and anticarcinogenic agent by Sessa and Wolf [2001]. Similarly earlier report for the red gram seed coat dialysed protein showed antioxidant property [Manukumar and Madhu 2013]". Again we are investigating the *S. androgynus* to see having protein as an antioxidant or not.

There is limited published work focused on plant *S. androgynus* leaf proteins and less study about proteins having antioxidant character or not. With this drawback the objective was designed to study the *S. androgynus* leaf proteins has antioxidant and anti-inflammatory property or not for the first time for this plant by aqueous ammonium sulphate precipitation method and for dialysed fraction.

MATERIAL AND METHODS

Chemicals

DPPH, BSA, Ferric chloride, DMSO, Pottasium ferricyanide, SDS, Acetyl salicylic acid, Bradford reagent, NBT, Curcumin, Tannic acid, Commasie blue, Deoxyribose, Alsever's solution, TEMED were purchased from Sigma-Aldrich and Ferrous sulphate, Ammonium persulphate, Sulphuric acid, Sodium phosphate, Ammonium molybdate, Aetone, Dipotassium hydrogen phosphate, Potassium dihydrogen phosphate, Sodium chloride, Ammonium per sulphate and Methanol were of analytical grade.

Plant sample collection and preparation

The fresh leaves of *Sauropus androgynus* were collected from local agriculture field and washed with 70% ethanol solution followed by distilled water. After complete soaking of water, the leaves were dried for four days at 37°C for four days. The dried leaves were finely powdered by grinder and passed through the 1 mm mesh and obtained powdered was stored in an air tight container at 4°C until used.

Aqueous extraction of crude protein from *S. androgynus*

About 5 gm of finely powdered of *S. androgynus* leaf material was soaked in a 100 mL of doubled

distilled water for 4 h at 30°C on an orbital shaker. After time of incubation filtered the sample extract by using a No 1. Whatmann filter paper. The resulting filtrate was subjected to 65% ammonium sulphate precipitation and centrifuged the precipitated sample at 10 000 rpm for 15 minutes and pellet was dissolved in a minimum amount of distilled water. To remove the salts from the sample, dialysis was done against doubled distilled water at 4°C by using 2.5 kDa cutoff dialysis bag until the salts are removed. Finally lyophilize the sample to concentrate.

Estimation of protein concentration

The protein concentrations of the samples were determined by Bradford's method. The crude protein content of the *S. androgynus* extract was estimated according to Bradford's method [Bradford 1976].

Antioxidant activity

The antioxidant activity of ambient water extract of *S. androgynus* was studied in different model system using hydroxyl radical scavenging, DPPH free radical scavenging, Reducing power assay, Superoxide radical scavenging activity by alkaline DMSO and Phosphomolybdenum antioxidant assays.

Hydroxyl radical scavenging assay

Hydroxyl radicals were generated by a Fenton reaction system and the scavenging capacity towards the hydroxyl radicals was measured by using a deoxyribose method [Manukumar and Madhu 2013]. The reaction mixture containing deoxyribose 2.8 mM (100 µl), FeCl₃ (100 µl), EDTA (100 µl), ascorbic acid (100 µl) and H₂O₂ 1 mM (100 µl) were mixed with various concentrations of extract in phosphate buffer (KH₂PO₄-K₂HPO₄) 20 mM, pH 7.4 in 1 ml final volume. Incubation was carried out for 1 h at 37°C and the reaction stopped by addition of 1 ml 1% (w/v) thiobarbituric acid (1 gm in boiling water & cool then add) and the mixture was boiled for 20 min, cooled and add 1 ml acetone. Measured the absorbance at 535 nm spectrophotometrically. Curcumin was used as positive control. Phosphate buffer 20 mM, pH 7.4 was used as a blank was used as blank and the sample solution without deoxyribose as sample blank. The inhibition ratio was calculated from the following equation. The percent hydroxyl radical scavenging activity

of extracts was determined accordingly in comparison with the negative control.

$$\text{Scavenging activity (\%)} = (A_0 - (A_1 - A_2)/A_0) \times 100$$

where: A₀, A₁, and A₂ are the absorbance's of the blank, extract (or Curcumin) and the sample blank, respectively at 532 nm.

DPPH free radical scavenging assay

The scavenging activity of DPPH free radicals developed according to the method reported by Manukumar and Thiribhuvan [2014]. 50 µl of the extract in methanol was mixed with 1 ml of 0.135 mM DPPH in methanol solution and 450 µl of 50 mM Tris-HCl buffer (pH 7.4). Methanol (50 µl) was used as the experimental control. After 30 min of incubation at room temperature, the reduction in the number of DPPH free radicals was measured, read the absorbance at 517 nm, Curcumin was used as standard. The percent inhibition was calculated from the following equation:

$$\% \text{ inhibition} = \frac{[\text{absorbance of control} - \text{absorbance of test sample}]}{[\text{absorbance of control}]} \times 100$$

Ferric-reducing antioxidant power (FRAP) assay

The FRAP (Ferric-reducing antioxidant power) of grain extracts was determined according to the method of Oyaizu [1986] with some modifications. One ml of each sample was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide (K₃Fe (CN)₆). The mixture was incubated at 50°C for 20 min and then 2.5 ml of trichloroacetic acid (10%) was added to the mixture, which was centrifuged at 1000 rpm for 10 min. The upper layer solution (2.5 ml) was mixed with distilled water (2.5 ml) and FeCl₃ (0.5 ml, 0.1%) and the absorbance was measured at 700 nm, Tannic acid was used as standard. A higher absorbance indicates a higher reductive capability.

Superoxide radical scavenging activity by alkaline DMSO method

In this method with slight modification of Govindarajan et al. [2003], superoxide radical is generated by the addition of sodium hydroxide to air saturated dimethyl sulfoxide (DMSO). The generated superoxide

remains stable in solution, which reduces nitro blue tetrazolium (NBT) into formazan dye at room temperature and that can be measured at 560 nm. Briefly, to the reaction mixture containing 1 ml of alkaline DMSO (1 ml DMSO containing 0.1 ml of 5 mM NaOH) and 0.3 ml of the extract in various concentrations, added 0.1 ml of NBT (1 mg/ml) to give a final volume of 1.4 ml. The absorbance was measured at 560 nm. The percentage of super oxide radical scavenging by the extracts and standard curcumin compound were calculated as follows:

$$\% \text{ superoxide scavenging activity} = \frac{\text{test absorbance} - \text{control absorbance}}{\text{test absorbance}} \times 100$$

Phosphomolybdenum antioxidant assay

Total antioxidant activity of plants extract was evaluated by Phosphomolybdenum antioxidant assay method described by Prieto et al. [1999]. Total antioxidant capacity was measured by spectrophotometric method. The different concentrations of the extracts ranging from 10-50 µg/mL were combined in eppendorf tube with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a thermal block at 95°C for 90 min. After cooling to room temperature; the absorbance of the crude protein extract of *S. androgynus* solution of each was measured at 695 nm against blank. The antioxidant activity was compared with the standard vitamin-E.

Anti-inflammatory activity

Human erythrocyte suspension. The whole blood was collected from a healthy volunteer had not taken any NSAIDs for 2 weeks prior to the experiment and collected in Alsever's solution contained vacutainer. The blood was washed three times with 0.9% saline and centrifuged simultaneously for 10 minutes at 3000 rpm. The packed cells were washed with 0.9% saline and a 10% v/v suspension made using isotonic phosphate buffer which was composed of 154 mM NaCl in 10 mM Sodium Phosphate Buffer at pH 7.4 used as stock erythrocyte or RBC suspension.

Hypotonic solution-induced hemolysis. The membrane stabilising activity of the extract was assessed according to the method described by Manukumar and

Thribhuvan [2014] with slight modifications. The test sample consisted of stock erythrocyte (RBC) suspension 0.50 ml mixed with 5 ml of hypotonic solution (50 mM NaCl in 10 mM Sodium Phosphate Buffered saline at pH 7.4) containing extract different concentration (10, 25, 50 & 100 µg/ml). The control sample consisted of 0.50 ml RBC suspension mixed with hypotonic buffered solution alone. The standard drug Acetylsalicylic acid was treated at 100 µg/ml concentration. The experiment was carried out in triplicate. The mixtures were incubated at 10 minutes at room temperature, centrifuged for 10 minutes at 3000 rpm and absorbance of the supernatant was measured spectrophotometrically at 540 nm. The percentage inhibition of hemolysis or membrane stabilisation was calculated by the following equation.

$$\% \text{ inhibition of hemolysis} = 100 \times [A_1 - A_2 / A_1]$$

where: A₁ – absorbance of hypotonic buffered solution alone, A₂ – absorbance of test /standard sample in hypotonic solution.

Anti-denaturation property by heat-induction.

The protein denaturation inhibition activity of *S. androgynus* was determined as like Mizushima and Kobayashi [1968]. 450 µl of 5% BSA was incubated with test sample having different concentrations (or) Diclofenac (100 µg/ml). All tubes were adjusted to pH 6.3 and incubated at 37°C for 20 min followed by 54°C for 3 min and then cooled. 2.5 ml of PBS was added and the absorbance was measured at 416 nm spectrophotometrically. The protein denaturation inhibition was expressed in percentage and calculated by the following formula:

$$\% \text{ of inhibition} = \frac{100 - (\text{OD1 control} - \text{OD2 test})}{\text{OD1 control}} \times 100$$

where: OD₁ – test sample unheated, OD₂ – test sample heated, OD₃ – control sample heated.

Statistical analyses

Assays were performed in triplicate, and the results were expressed as mean values with standard deviations (SD).

RESULTS AND DISCUSSION

Protein confirmation by SDS-PAGE

Dialysed *S. androgynus* leaf protein concentration was determined by Bradford method. Also total phenol assay experiment exploring its positive result may be due to the presence of bound phenolics to the protein or it is from protein due to because of bulky positive amino acid groups are suspected. Dialysed protein was loaded to SDS-PAGE to confirm presence of protein in sample and its profile showed comparing the standard markers used range from 14.3-97.4 kDa molecular weights A-F (A = 97.4 kDa (Phosphorylase b), B = 66.0 kDa (BSA), C = 43.0 kDa (Ovalbumin), D = 29.0 kDa (Carbonic anhydrase), E = 20.1 kDa (Soybean trypsin inhibitor), F = 14.3 kDa (Lysozyme)). Standard marker used as per [Manukumar and Madhu 2013] to compare the sample proteins. And the crude dialysed sample protein was run along with the standard, showing about six bands. Compare to standard, presence of very low and high molecular weight proteins in the sample and having two proteins can be seen dominantly.

Antioxidant activity

Hydroxyl radical scavenging activity. Figure 1 showed hydroxyl radical scavenging property for dialysed *S. androgynus* leaf protein sample. The earlier report for water extract [Senthamarai and Baskar 2012] showed 26.08% of inhibition for *S. androgynus* plant by soxhlet method. Comparison to the previous report for anti-oxidant activity of sample *S. androgynus*,

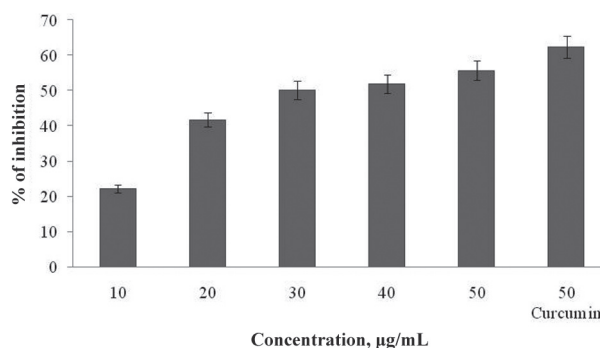


Fig. 1. Hydroxyl radical scavenging activity of *S. androgynus* leaf protein extract

present dialysed leaf protein extract, showed good source of natural anti-oxidants. Anti-oxidant activity of the sample showed dose dependent manner inhibition range from 22.28-55.62% at concentration 10-50 µg/ml compared to standard curcumin showed 62.31% of inhibition at 50 µg/ml concentration. However, percentage of scavenging potential is different for method of extraction with in the same plant sample was reported but an active component separated from source is also applicable than method to be noted.

DPPH free radical scavenging activity

From the above study, the *S. androgynus* leaf proteins are able to reduce the stable, purple-colored radical, DPPH, into yellow-coloured DPPH-H reaching over 50% of the reaction. DPPH assay for dialysis protein extract had highest scavenging activity inhibition at 50 µg/ml (50%) compared to standard curcumin (56.61%) at 50 µg/ml concentration (Fig. 2). Our finding approach method for extract applicable and stand to prove method highly effective in extracting potent scavenging components. The extracting meth-

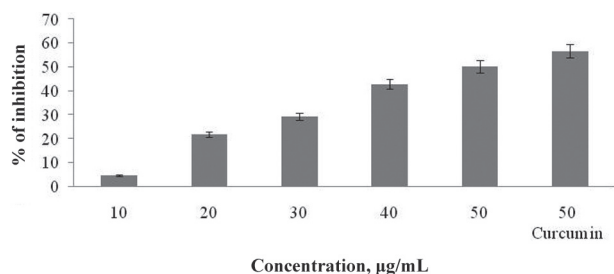


Fig. 2. DPPH free radical scavenging activity of *S. androgynus* leaf protein

od had a positive effect on DPPH scavenging activity. Earlier study reported that the DPPH scavenging activity of aqueous extract as 48.48% at 200 µg/ml concentration [Nahak and Sahu 2010], while the present extract gave 50% at 50 µg/ml. The comparatively higher value of DPPH activity of the present extract may be due to the high active content of scavenging component of proteins than phenolic components can be commentable according to earlier report.

Total reductive potential

The aqueous dialysis protein extract had moderate level of reductive potential as measured by ferric ion reduction in dose dependent manner (Fig. 3). When compared to standard curcumin sample showed less

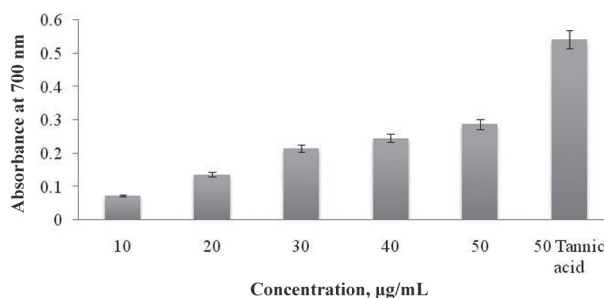


Fig. 3. Reducing power of *S. androgynus* leaf protein

potential for FRAP activity. The reducing capacity of a compound may be considered as an important indicator of its antioxidant activity [Hsu et al. 2006]. In this assay, ferric ions are reduced to ferrous ions and with it change in colour of the reaction mixture from yellow to bluish green. Higher colour intensity, greater the absorbance and higher reducing activity. The vegetative part of *S. androgynus* earlier study showed presence of significant level of components responsible for FRAP activity [Rattanasena 2012]. The highest activity was shown by standard than test sample. Method of extract affected in negative way on FRAP activity.

Superoxide scavenging activity

The scavenging activity of the extract against superoxide radical generated in NaOH-alkaline DMSO-NBT system, resulting in the formation of the blue formazan was studied in this research. Superoxide scavenger capable of reacting inhibits the formation of a red dye formazan. The inhibition of formazan formation was reflected through the dialysed protein sample, scavenging activity range from 32.43-72.51% inhibition at concentration 10-50 µg/ml compared to standard curcumin had activity about 75.38% of inhibition at 50 µg/ml concentration. There was less report to superoxide scavenging activity available for *S. androgynus* leaf polyphenol and protein sample.

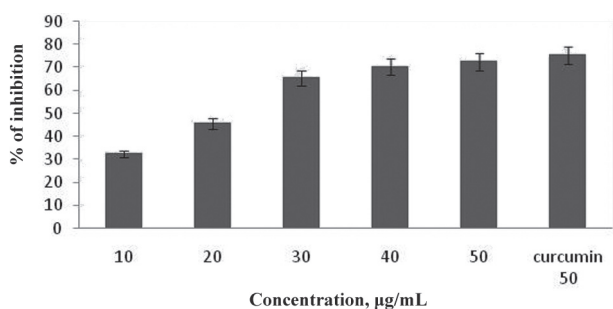


Fig. 4. Superoxide activity of *S. androgynus* dialysed leaf protein

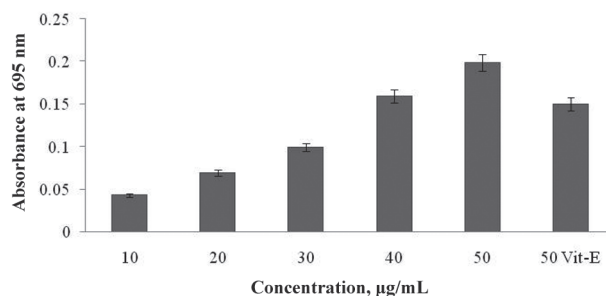


Fig. 5. Phosphomolybdenum antioxidant activity of *S. androgynus* leaf protein

Present study for protein extract after dialysis application showed nearly best activity compared to standard curcumin. Superoxide can cause oxidation or reduction of solutes depending on their reduction potential. Both aerobic and anaerobic organisms possess superoxide dismutase enzymes for defense process in the plant and animal, which catalyse the breakdown of superoxide radical [Shirwaiar et al. 2007]. In our study, alkaline DMSO used for superoxide generation indicates that *S. androgynus* is a potent superoxide scavenger (Fig. 4).

Phosphomolybdenum antioxidant activity

The total antioxidant capacity of *S. androgynus* dialysed leaf protein fraction was measured spectrophotometrically by the phosphomolybdenum method, which is based on the reduction of Mo(VI) to Mo(V) by the sample analyte and the subsequent formation of green phosphate/Mo(V) compounds with a maximum absorption at 695 nm [Miladi and Damak 2008]. Electron transfer occurs in this assay which depends upon the structure of the antioxidant [Prieto et al. 1999]. The phosphomolybdenum method usually detects antioxidants such as ascorbic acid, some phenolics, tocopherols and carotenoids. In this present study *S. androgynus* protein fraction also detected and showed antioxidant property. The total antioxidant activity of dialysed protein fraction was compared with the standard antioxidant vitamin E and the results graphically presented in Figure 5. The results indicated that dialysed protein fraction had the highest total antioxidant activity at 50 µg/ml (0.198) compared to standard vitamin E (0.150) at 50 µg/ml concentration exploring significance assay for the protein extracted for antioxidant a assay.

In-vitro anti-inflammatory activity

Erythrocyte membrane stabilisation activity of *S. androgynus* leaf protein. The present finding demonstrated that the extract of *S. androgynus* dialysed leaf protein extract has the capacity to stabilize red blood cell membrane against stress, which indicates the ability of the protein extract to prevent hemolysis or rupture of RBCs or stabilisation the RBCs membrne. The membrane stabilisation activity of *S. androgynus* dialysed sample is presented in Table 1. Erythrocytes were effectively protected by *S. androgynus* protein sample in dose dependent manner. The maximum inhibition of hypotonic induced hemolysis by dialysed protein was found 74.17% of protection to the membrane damage, which was compared to an antioxidant reference compound Acetylsalicylic acid, which also showed 86.88% of membrane stabilisation at 100 µg/ml concentration

Table 1. Effect of *S. androgynus* leaf protein extract membrane stabilization on hypotonic solution induced hemolysis of erythrocyte membrane

Sl No.	Concentration µg/mL	Percentage of	
		protection	damage
1	10	42.17 ±0.05	57.90 ±0.01
2	25	62.85 ±0.02	37.15 ±0.01
3	50	69.84 ±0.01	30.12 ±0.00
4	100	74.13 ±0.01	25.82 ±0.00
5	Std acetylsalicylic acid at 100 µg	86.88 ±0.00	13.14 ±0.01

respectively. Erythrocyte membrane stabilisation by an dialysed protein may stabilize lysosomal membranes because there is a close similarity between erythrocyte membrane and lysosomal membrane [Gandhisan et al. 1991]. Lysosomal membrane stabilisation is an important in reducing the inflammatory response by protecting the tissue from the inflammation and injury, caused by the lysosomal constituents such as activated neutrophils, bactericidal enzymes and the extracellular activity of these lysosomal constituents is said to be related to acute or chronic inflammation [Sadique et al. 1989]. The significant membrane stabilising activity of the leaf protein extract of *S. androgynus* is due to the presence of potent functional protein content of the extract (not yet reported). Several reports have been showed that herbal drugs are capable to facilitate the stabilisation of red blood cell membrane and possess anti-inflammatory activity and cereal grains, seed coat, honey, fruits seed-juice, containing polyphenols also showed responsible action in membrane stabilisation [Manukumar and Madhu 2013, Manukumar and Thribhuvan 2014].

Protein denaturation inhibitory activity from *S. androgynus* leaf protein. The inhibitory effect of *S. androgynus* leaf dialysed protein at different concentrations on protein denaturation is graphically illustrated in Table 2. Dialysis sample exhibited dose dependent inhibition of denaturation of protein BSA. The maximum protein denaturation protection from the protein sample was found 83.60%, this result was compared to the standard compound diclofenac, which showed 86.82% protein denaturation inhibition

Table 2. Effect of *S. androgynus* leaf protein extract anti-denaturation property

Sl No.	Concentration µg/mL	Percentage of	
		anti-denaturation	denaturation
1	10	32.76 ±0.03	67.24 ±0.04
2	25	42.64 ±0.02	57.36 ±0.02
3	50	63.92 ±0.01	36.08 ±0.05
4	100	83.60 ±0.02	16.40 ±0.01
5	Std diclofenac at 100 µg	86.82 ±0.00	13.18 ±0.01

at 100 µg/ml concentration respectively. Several anti-inflammatory drugs have shown dose dependent ability to inhibit thermally induced protein denaturation [Grant et al. 1970]. Denaturation of proteins is a well documented cause of inflammation in conditions like rheumatoid arthritis [Mizushima and Kobayashi 1968]. Thus protection against protein denaturation, which was the main mechanism of action of NSAIDS postulated by Mizushima and Kobayashi [1968] before the discovery of their inhibitory effect on cyclooxygenase by Vane [1971] may play an important role in the antirheumatic activity of NSAIDS.

CONCLUSION

In this study, anti-oxidant and anti-inflammatory activity of *S. androgynus* leaf extracted dialysed protein sample results were illustrated. The study result may suggest the potential use of *S. androgynus* for application in cosmetic and/or therapeutic products due to their high level of antioxidant activities or presence of functional protein in action compare to phenolic compounds. These reports provide a basic scientific evidence to support its traditional medicinal uses. In this study might suggest a possible use of natural anti-inflammatory agent. The result of dialysis extract *S. androgynus* shows anti-inflammatory activities and it shown dose dependent activities. The results support the traditional use of this plant in inflammatory conditions and suggest the presence of biologically active components which may be worth further investigation and elucidation. This edible plant may be used as alternative to synthetic chemicals particular because of their safety.

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