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ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITY IN FRESH AND DRY LEAVES OF *IPOMOEA OBSCURA* (L.) KER-GAWL

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ABSTRACT

Ipomoea obscura (Convolvulaceae) traditionally used to set bones of livestock's in rural areas of Kalaburagi district. In the present work aqueous extract of Ipomoea obscura fresh and dry leaves are evaluated for the phytoconstituents, antioxidants and anti-inflammatory activities. Results revealed that the phytoconstituents such as flavonoids 356.9±0.00, phenols 345.7±0.00, tannins 263.8±0.00, alkaloids 245.6±0.00 and glycosides 0.825±0.00mg/100g in fresh leaves whereas flavonoids, 486.9±0.00, phenols 436.8±0.01, tannins 236.7±0.01, alkaloids 274.3±0.00 and glycosides 1.023±0.00mg/100g in dry leaves extract respectively. The fresh leaves extract exhibited more potential antioxidant activity than dry leaves extract and these values are comparable of standards. Reducing power assay of fresh leaves extract showed highest reducing power capacity than dry leaves extract as well as standards. Anti-inflammatory activity showed that HRBC stabilization activity and albumin denaturation activity of dry leaf extract was found higher than fresh leaf extract as well as standard Dichlorofenac at different concentrations. The study concludes that the aqueous extract of both fresh and dry leaves showed the potential source of antioxidant as well as anti-inflammatory activities, this natural property is due to the presence of various phytoconstituents. The results of the present study justify the effective use of leaves to prepare crude drugs in folklore medicine.

KEY WORDS

Ipomoea obscura, leaves, Phytoconstituents, DPPH, Nitric oxide, H_2O_2 and Anti-inflammatory.

INTRODUCTION

Antioxidants act in different ways by preventing free radical formation, by scavenging free radicals, by preventing the propagation of the oxidative chain reaction, by being part of the redox antioxidant network, or by regulating gene expression. Antioxidants and trace elements has the ability to scavenge and neutralize the free radicals [1]. Free radicals are types of Reactive Oxygen Species (ROS), which include all highly reactive, oxygen-containing molecules. All these free radicals are capable of reacting with membrane lipids, nucleic acids, proteins and enzymes and other small molecules, resulting in cellular damage. Plants are rich source of free radical scavenging molecules such as vitamins, phenolic acids, flavonoids and other metabolites which are rich in antioxidant activity [2]. Polyphenols are a large and diverse class of compounds many of which occur naturally in a wide range of food and plants [3]. Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity [4]. The alkaloids, phenolic compounds, tannins, flavonoids have been associated with various degrees of antioxidant [5]. Flavonoids are class of secondary plant metabolites with

significant antioxidant and chelating properties [6]. The leaves of medicinal plants have various health-promoting effects on human. These leaves may be suitable singly or in combination as therapeutic agents and are important raw materials for manufacturing traditional and modern medicines [7]. **Inflammation** is a complex process, which is frequently associated with pain and involves occurrences such as the increase of vascular permeability, increase of protein denaturation and membrane alteration. Protein denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when denatured. Denaturation of proteins is well-documented cause of inflammation[8]. ***Ipomoea obscura*** commonly known as 'Laksmana' in Ayurveda, belongs to the morning glory family Convolvulaceae[9]. It is effectively used against dysentery, is applied to open sores and pustules. It is small climbing vine with small chordate leaves and acuminate apex, corolla composed of five fully fused petals. Plant grows on fences or low ground cover as substrate in disturbed areas [10]. The dried and powdered leaves are used to treat aphthae. The leaf sap is used to treat fits of insanity, a paste of the leaves, combined with the leaves of *Argyreia mollis* and alcohol is applied to open sores and pustules. Decoction of root is drunk against dysentery. It is native to Tropical Asia, Tropical East Africa, Mascarene Islands, throughout Malaysia to northern Australia and Fiji [11]. The phytochemical analysis of the medicinal plants is important and have commercial interest in both research institutes and pharmaceuticals companies for the manufacturing of the new drugs for the treatment of various diseases[12]. *I obscura* effectively used to set bones of livestock's in rural areas of Kalaburagi district particularly (Lambani tribe). The antioxidant and anti-inflammatory property of any plant extract plays an important role in treating diseases and disorders. So the main objective of the present study was to evaluate the antioxidant activity of aqueous extract of fresh and dry leaves in *Ipomoea obscura*.

MATERIALS AND METHODS

Extraction of plant materials

Plants collected from the various taluka's of Kalaburagi district were identified using various floras available in the Department of P.G. Studies and Research in Botany Gulbarga University, Kalaburagi. The leaves are shade dried for 15-20 days and powdered using electronic blending machine. The dry leaf powder of ***Ipomoea obscura*** (100gm) was extracted with 500 ml water (Aqueous) by Soxhlet extraction for 24 hours and fresh material was extracted using pestle and mortar. The extract obtained was stored in a refrigerator used for the antioxidant activity.

Chemicals

DPPH, Butylated hydroxyl anisole, Ascorbic acid, Phloroglucinol, Sodium nitroprusside, Hydrogen peroxide, Trichloron acetic acid and Potassium ferric cyanide were purchased from Shree Venkatesh chemicals Kalaburagi.

The quantitative estimation of phytoconstituents.

Estimation of Flavonoids (Swain and Hill 1959)

Vanillin Reagent: 1g of crystalline Vanillin is dissolved in 100ml of 70% con H_2So_4.

Procedure: 0.1 and 0.2ml extract is taken in the test tube diluted to 2ml with distilled water and to this 4ml Vanillin reagent was added rapidly after 15 minutes the brick red colour is read at 599nm using spectrophotometer against reagent blank. The standard curve is plotted using different concentrations of Phloroglucinol as the standard flavonoid. The amount of flavonoid present in each sample was calculated with the help of standard graph.

$$\text{Flavonoids in mg/100gm} = \frac{\text{Graphical value}}{\text{Wt. of the plant material}} \times \frac{\text{volume of total extract}}{\text{volume taken for reading}} \times 100$$

Estimation of total Phenols (Bray and Thorpe (1964).

Reagents required: 80% ethanol, Folin-Ciocalteu's reagent and 20% Na_2CO_3 Soln.

Catechol standard 100mg of Catechol is dissolved in100ml distilled water in a volumetric flask and further diluted to ten times to obtain a working standard.

Procedure: Take 0.1 and 0.5 ml aliquots and their final volume is made up to 3ml with distilled water. To these tubes, 0.5 ml Folin-Ciocalteu's reagent (FCR) and after 3 min, 2 ml of 20% (w/v) Na_2CO_3 were added and mixed thoroughly. All these tubes were incubated in a boiling water bath for a minute and cooled. The blue colour appeared (due to molybdenum complex) absorbance is measured at 650 nm in a spectrophotometer against the blank reagent. A standard curve is plotted using 1% Catechol as phenol.

$$\text{Phenols in mg/100gm} = \frac{\text{Graphical value}}{\text{Wt. of the plant material}} \times \frac{\text{volume of total extract}}{\text{volume taken for reading}} \times 100$$

Estimation of Alkaloids (Horn Borne 1973)

Reagents: 20% Acetic acid in ethanol (200ml), Concentrated NH_4OH.

Procedure: 5g of the sample was weighed in to 250ml beaker and 200ml of 20% Acetic acid in ethanol was added and stored for 4 hours. This content was filtered, and the extract was concentrated by using water bath to one quarter of the original volume. Then concentrated NH_4OH was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle, and the precipitation was collected and weighed.

Amount of alkaloid = $W_2 - W_1$, Where W_1- weight of the empty filter paper and
W_2- weight of filter paper along with ppt.

Estimation of Tannins (Shreelalitha S. J 2016)

The estimation of Tannins was carried out by following the method of (Shreelalitha S. J 2016) with little modification. The working standard solution were pipetted out in a series of test tubes ranging from 0.1,0.2,0.3,0.4 and 0.5ml. A volume of 0.2ml of the leaf extract is added into another test tube and 0.8ml of distilled water is added to make up the volume to 1ml in all the tubes following which 1ml of ferric chloride and potassium ferricyanide each is added to all the test tubes and mixed well. The absorbance was measured at 700nm spectrophotometrically.

Estimation of Glycosides (Pravate K Parhi *et al.* (2013)

Reagent required: Ethanol (70%), (Na2HPo4), (12.5%) Lead acetate and Methanol.

Procedure: 25g of powder plant material was mixed with 200ml of 70% ethanol and kept on rotator shake at 300 rpm for 6 h at room temperature. Filter it and add 500ml of distilled water followed by 100ml of 12.5% lead acetate (to precipitate tannins, resins, and pigments). Later the volume made up to 800ml with distilled water and keep it on shaker for 10min at 300rpm. To this, add 200ml of (4.77%) Disodium hydrogen phosphate (Na2HPo4) solution is added to ppt excess of Pb ions. The above solution is than filtered and evaporated to dryness. Calculate the % of glycoside using the following formula.

$$\text{\% of Glycosides} = \frac{\text{Weight of dried extract x 100}}{\text{Weight of plant material}}$$

Antioxidant activity

DPPH radical scavenging activity

The DPPH (2, 2 diphenyl-1-picrylhydrazyl) radical scavenging activity was carried out following the procedure of Vishnuvathan et al. (2017). 2ml of various concentrations such as 0.2, 0.4, 0.6, 0.8, 1 and 2mg of (test sample) in aqueous extract were prepare to which 1ml of 0.1 mM of DPPH in methanol solution is added. After 30 minutes of incubation period, the absorbance was measured at 517nm. The free radical scavenging activity of each sample was determined by comparing its absorbance with that of a blank solution(Control). The Ascorbic acid and Butylated hydroxyl anisole were used as standards. The DPPH radical scavenging activity was calculated using the following equation. % of inhibition = (Ac – As)/Ac×100. Where Ac is the absorbance of the control and As is the absorbance of test the sample.

Nitric oxide radical scavenging activity

Nitric oxide scavenging activity was carried out following the procedure of Vishnuvathan *et al*. (2017)**.** 0.5ml of 0.1M PBS (pH7.4) is added to the 2ml of 10 mM

Sodium nitroprusside and mixed well. To this mixture various concentrations of plant extract such as 0.2, 0.4, 0.6, 0.8, 1 and 2mg were added and incubated for 160 min at 30^0C. After incubation period, its absorbance was measured at 546 nm. The nitric oxide radical scavenging activity of each sample was determined by comparing its absorbance with that of a blank solution. Ascorbic acid and Phloroglucinol taken as standards. The percentage of inhibition of nitric oxide radical by extracts was calculated by using following formula. % of inhibition = Ac – As)/Ac × 100. Where Ac is the absorbance of the control and As is the absorbance of the test sample.

Hydrogen peroxide assay

Hydrogen peroxide radical scavenging activity was carried out following the procedure of Vishnuvathan *et al*. (2017) with little modification. A solution of H_2O_2 (30 mM) is prepared in distilled water. The plant extract of different concentrations such as 0.2, 0.4, 0.6, 0.8, 1 and 2mg in 2ml phosphate buffer (0.1 M, pH 7.4) mixed well and 0.5 µl of H_2O_2(30 mM) solution is added. After 10 minutes the absorbance of the reaction mixture was recorded at 230 nm. The hydrogen peroxide scavenging activity of each sample was determined by comparing its absorbance with that of a blank solution. The % of inhibition of hydrogen peroxide radical by extracts was calculated by using following formula, H_2O_2 scavenging activity (%) = (Ac – As) /Ac×100. Where Ac is the absorbance of the control and as is the absorbance of the sample.

Reducing power assay

Reducing power assay of the extract was evaluated according to the protocol of Lila et al. (2012). The 1ml of different concentrations of aqueous extract such as 0.2, 0.4, 0.6, 0.8, 1 and 2 mg were mixed with 0.1M phosphate buffer (pH6.6) and potassium ferricyanide (1ml, 1%), and the mixture was incubated at 50°C for 20 min. Next 2ml of Trichloro acetic acid (10%) is added to the reaction mixture, and then centrifuged at 10000 RPM for 10 min. The upper layer of the solution (1ml) is mixed with distilled water ((1ml) and ferric chloride (150 µl, 0.1%), and the absorbance was measured at 700nm against the blank sample (Control). The test was performed in triplicates and results are recorded.

***In-vitro* anti-inflammatory activity of plant extracts were evaluated by following methods.**

The Human red blood cell (HRBC) membrane stabilization method (Mahadevan *et al*.2016).

Blood sample (2ml) was collected from a volunteer in a heparinized tube and washed with phosphate buffered saline twice and centrifuged at 3000 rpm for 10 min. Then, RBC was suspended in normal saline and taken in a tube (0.5 ml) with 0.5 ml of extract and 0.5 ml hypotonic solution and incubated for 30 min at room temperature. Then, the contents were centrifuged at 1500 rpm for 10 min and the supernatant was collected and the absorbance read at 560nm. Based on the absorbance of extract and control, the membrane stabilization effect was calculated.

Inhibition of albumin denaturation method (Sunder *et al*. 2015).

The reaction mixture was consisting of plant extracts and 1% aqueous solution of bovine albumin fraction pH, of the reaction mixture was adjusted with 1N HCL. The extracts were incubated at 37°C for 20 min and then heated to 51°C for 20 min, after cooling the samples the turbidity was measured by spectrophotometrically at 660nm. The experiment was performed in triplicate. Percentage inhibition of protein denaturation was calculated as follows,

$$\% \text{ inhibition} = \frac{\text{Abs control} - \text{Abs Sample}}{\text{Abs control}} \times 100$$

2. Statistical analysis

The antioxidant activity was performed in triplicates. The results were expressed as mean ± Standard error mean. [Significant value P<0.001] using one-way ANOVA (Graph Pad Instat3) and Microsoft excel.

3. Results and Discussion

In the present study aqueous extract of fresh and dry leaves are subjected to the quantitative estimation of phytoconstituents, antioxidant and anti-inflammatory activity results are tabulated as follows,

Table No. 1. The quantitative estimation of phytoconstituents.

S No	Phytoconstituents	Fresh material extract	Dry material extract
1	Flavonoids	356.9±.0.01mg/100g	486.9±.0.00mg/100g
2	Phenols	345.7±0.00mg/100g	436.8±.0.01mg/100g
3	Tannins	263.6±0.01mg/100g	236.7±.0.01mg/100g
4	Alkaloids	245.8±0.00mg/100g	274.3±.0.00mg/100g
5	Glycosides	0.825±0.00mg/100g	1.023±0.00mg/100g

Mean ± Standard error mean, Significant value P <0. 001.

Fig 1. DPPH radical scavenging activity

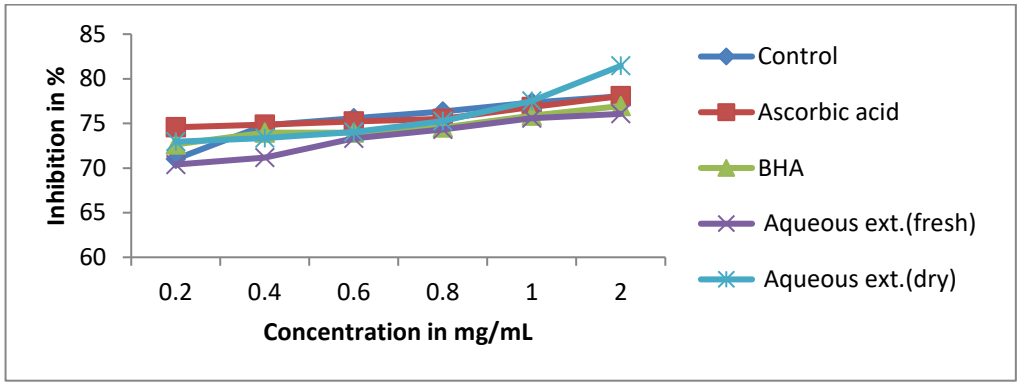


Fig 2. Nitric oxide radical scavenging activity

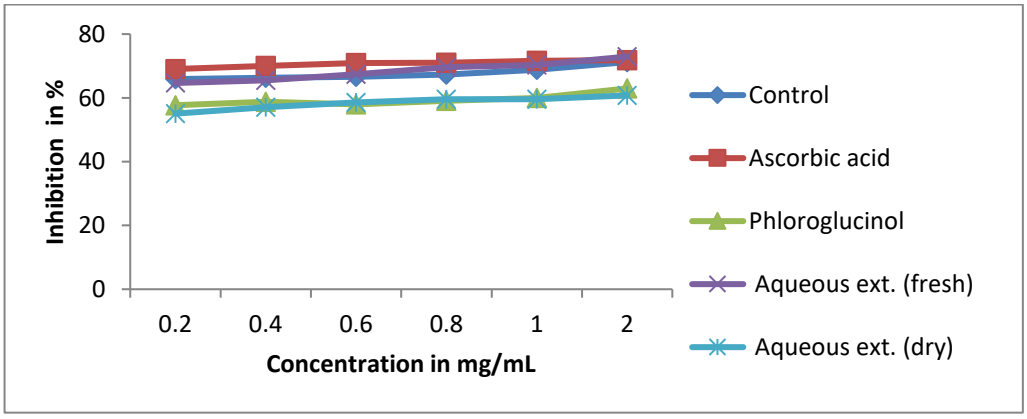


Fig 3. Hydrogen peroxide assay

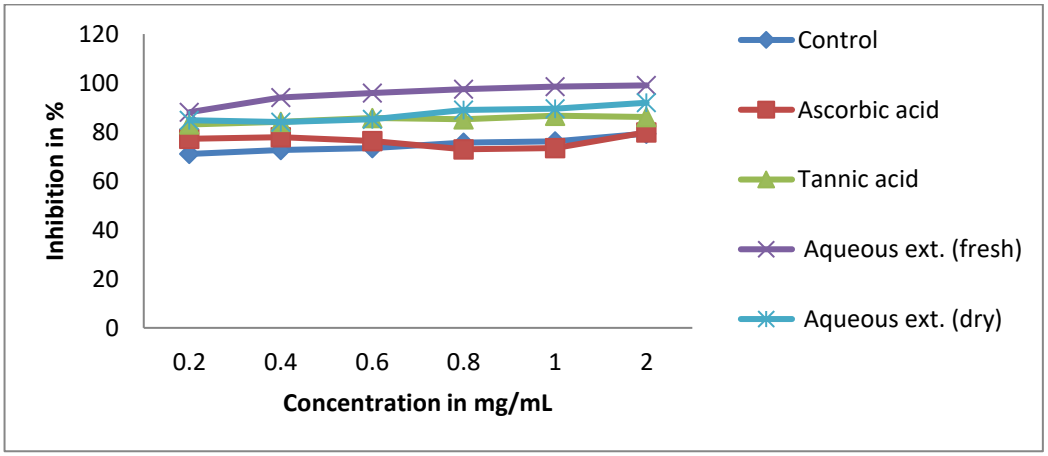


Fig 4. Reducing power assay

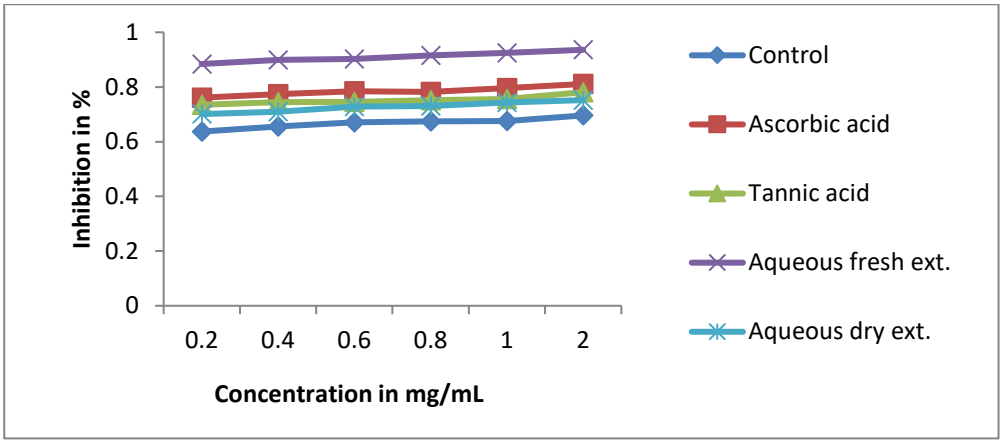


Fig 5. Inhibition of Albumin denaturation method

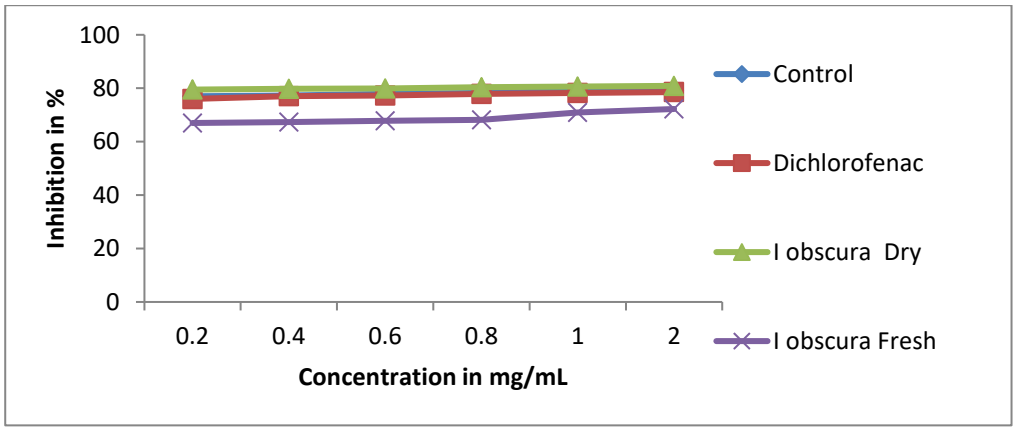
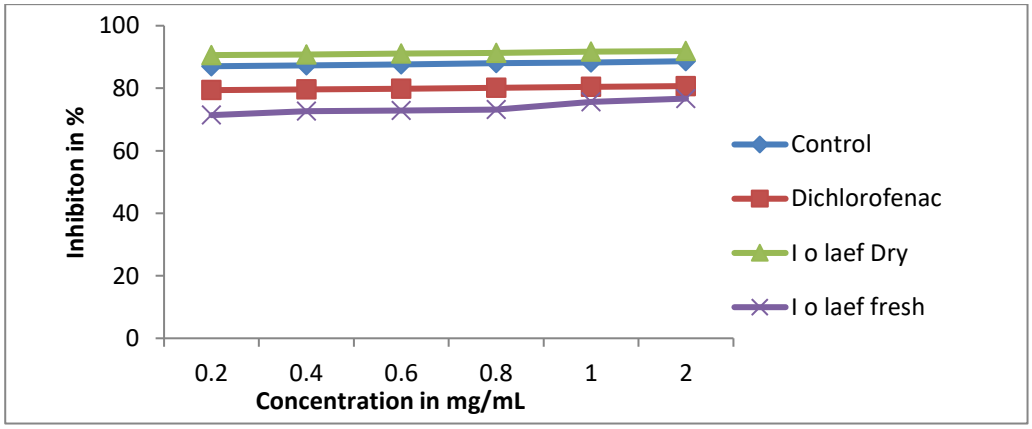


Fig 6. HRBC Membrane stabilization method



The results of quantitative estimation of phytoconstituents such as flavonoids 356.9±0.01, phenols 345.7±0.00, tannins 263.8±0.0, alkaloids 245.6±0.00 and glycosides 0.825±0.00mg/100g in fresh leaves extract whereas flavonoids, 486.9±0.00, phenols 436.8±0.01, tannins 236.7±0.01 alkaloids 274.3±0.00 and glycosides 1.023±0.00mg/100g in dry leaves extract respectively.

The results of antioxidants showed that the DPPH radical scavenging activity of the dry leaves extract found more effective than fresh leaves extract at different concentrations. The Butyl hydrated anisole and ascorbic acid taken as standards in Fig 1. Nitric oxide

radical scavenging activity showed that the fresh leaves extract found more effective than dry leaves extract as well as Phloroglucinol and ascorbic acid in Fig 2. Hydrogen peroxide radical scavenging activity of both fresh and dry leaves extract found higher than ascorbic acid in Fig 3. Reducing power assay of fresh leaves extract showed higher reducing power capacity than dry leaves extract and Butyl hydrated anisole and ascorbic acid at different concentrations in Fig 4.

Anti-inflammatory activity showed that HRBC stabilization activity of dry material is found higher than fresh as well as standard dichlorofenac and albumin denaturation activity is also found higher in dry extract as compare to fresh extract and standards in Fig 5. And Fig 6.

In the present study aqueous extract of fresh and dry leaves were subjected to the antioxidant activity and quantitative estimation of phytoconstituents. The results showed that the aqueous extract of fresh and dry leaves exhibited strong antioxidant activity at different concentrations. The presence of various secondary metabolites like phenols alkaloids, flavonoids, tannins and glycosides are directly responsible for the potential antioxidant activity of the plant.

Similar studies are conducted by Saravana Prabha P, Gopalakrishnan V. K (2015) on the successive ethanolic extract of *Ipomoea obscura* (L.) leaf. And T. P. Hamsa1 and Girija Kuttan (2011). On Anti-inflammatory and anti-tumor effect of *Ipomoea obscura* (L).

Further various methods are employed to screen and study drugs, chemicals, herbal preparations that inhibit the inflammation. HRBC membrane is similar to the lysosomal membrane. During inflammation, histamine from damaged tissues makes capillaries more permeable and lysosomes od damaged cells release their enzymes which help breakdown damaged tissue but may also cause destruction of nearby healthy tissue. J.N. DHARSANA and DR. SR. MOLLY MATHEW (2015). There is a strong need for effective antioxidant in order to prevent the free radicals implicated diseases which can have serious effects on the cardiovascular system, through lipid peroxidation or vasoconstriction Biswakanth Kar, RB Suresh Kumar, Indrajit Karmakar, Narayan Dolai, Asis Bala1, Upal K Mazumder, Pallab Haldar (2012). Dry weight from plant always give less error from plant while they are still fresh. In dry part no further enzymatic or metabolic alteration of natural plant product would become possible further. All compounds can be recovered in a natural unaltered form, while green leaves due to presence of chloroplast and active metabolic and protein synthesizing machinery there remains a possibility of formation of new compounds or secondary metabolites in responses to light and other factors **(**Vaidya *et.al. (*2013). Plant–derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio resources of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, pharmaceutical intermediates and chemical entities for synthetic drugs Daniel and Krishnakumari (2015).

CONCLUSION

In our study the aqueous extract of fresh and dry leaves found rich in phytochemicals, antioxidant and anti-inflammatory activity. Fresh leaves exhibited more potential antioxidant activity that of dry leaves extract whereas dry leaves exhibited more potential anti-inflammatory activity compare to fresh leaves extract. The results of quantitative estimation showed this plant is rich in phytoconstituents. Further similar studies are required to explore more pharmacological activities of this plant.

Conflict of interests

Declared none.

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