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EXPLORING THE ANTIOXIDANT POTENTIAL OF ARGYREIA IMBRICATA: A BIOCHEMICAL ANALYSIS

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ABSTRACT: Reactive oxygen species (ROS) play a substantial role in the pathophysiology of a variety of diseases. An excess of the ROS produced by metabolic activities, such as superoxide radical anion(SOR), hydrogen peroxide(H2O2), hydroxyl radical(OH), and nitric oxide(NO), causes inflammatory responses. Antioxidants protect cells from cellular damage caused by reactive oxygen species. The most popular "antioxidants" are vitamins A(retinol), C(L-ascorbic acid), β-carotene, minerals like selenium, and naturally occurring polyphenols. Antioxidants are present in the diet, and some people additionally take antioxidant supplements. The ingestion of natural antioxidants will reduce the risk of cardiovascular disease, diabetes and other diseases of cancer associated with ageing. Nature has abundant source of antioxidant availability present in this study we have investigated Argyreia imbricata leaves aqueous extract(AIAE) for antioxidant activity and lipid peroxidation assay. The aqueous extract showed the maximum antioxidant activity, as measured by total antioxidant activity(TAA) of 1.32µg/ml and ferric reducing antioxidant power of 16.36µg/ml. Furthermore, aqueous extract has shown the greatest DPPH radical scavenging activity with an IC50 value of 33.16µg/ml, ABTS radical cation decolourisation assay with a value of 20.85µg/ml, nitric oxide scavenging assay with a value of 26.89µg/ml and maximum inhibition of lipid peroxidation. In conclusion, the AIAE leaves was analysed for antioxidant activities, in which the extract exhibited significant activity. In conclusion, this biochemical study highlights the antioxidant and lipid peroxidation properties of A.imbricata leaves extract, suggesting its potential as a natural therapeutic agent in combating oxidative stress-related disorders.

Key words: [Reactive oxygen species, antioxidants, Argyreia imbricate, lipid peroxidation.]

1. INTRODUCTION

Reactive oxygen species (ROS) have been shown in studies to play a substantial role in the pathophysiology of a variety of diseases. Most living things have ROS defense mechanisms, both enzymatic and non-enzymatic. In healthy ecosystems, these mechanisms can maintain a reactive balance (Pietta et al., 1998). It is imperative to emphasize the useful role ROS play in the initiation and progression of inflammation. An excess of the ROS produced by metabolic activities, such as superoxide radical anion (SOR), hydrogen peroxide (H_2O_2), hydroxyl radical (OH), and nitric oxide (NO), causes inflammatory responses (Souza et al., 2017).

The most popular "antioxidants" are vitamins A (retinol), C (L-ascorbic acid), E (tocopherol), β-carotene, minerals like selenium, and naturally occurring polyphenols. Antioxidants protect cells from cellular damage caused by reactive oxygen species. Vitamins and β-carotene in foods like fruits and vegetables contain conjugated double bonds and antioxidant functional groups. Antioxidants are present in the diet, and some people additionally take antioxidant supplements (Lee et al., 2018).

Antioxidants are compounds which inhibit the oxidation by removing the free radical intermediates. Antioxidants may be natural or synthetic. Synthetic antioxidants are compounds with phenolic structures of various degrees of alkyl substitution, whereas natural antioxidants are from plant source rich in various phytochemical molecules such as vitamins, flavonoids, quinones, coumarins, alkaloids, amines, betalains, terpenoids, phenolic acids, lignins, stilbenes, tannins and other metabolites, which are rich in antioxidant activity. Studies have shown that many of these antioxidant compounds possess anti-inflammatory, anti-atherosclerotic, antitumor, anti-mutagenic, anti-carcinogenic, antibacterial and antiviral activities. The ingestion of natural antioxidants will reduce the risk of cardiovascular disease, diabetes and other diseases of cancer associated with ageing. The natural plant based antioxidants play an important role in themaintenance of human health (Lee et al., 2018) Therefore, the present study was undertaken to evaluate and compare the antioxidative activities of A. imbricata by FRAP, DPPH, ABTS, TAA, Nitric oxide assay (Souza et al., 2017).

A. imbricata is well known that the therapeutic usage of plant sources is time immemorial, medicinal plants are commonly distributed all over the world, but, notably in tropical countries. Since the past, medicinal plants are continuously explored for their therapeutic potentials. According to the World Health Organization, plants are one of the best sources for a variety of drugs, and approximately 25% of present-day medicines are

derived directly or indirectly from higher plants. Of-course, traditional medicines occupy a prime position in the primary healthcare need of a significant proportion of world's population, particularly in developing countries. Importantly, usage of medicinal plants in Asian countries indicates a longstanding tradition of human relationship with environment (Sebastin et al., 2019).

With over 220 species, Argyreia is one of the major genera in the Convolvulaceae family,found across Asia, including India. Our study focused on Argyreia imbricata, a plant prevalent in southern India at altitudes up to 300 m MSL. The flowering and fruiting season is August – December for this dicotyledonous plant. A huge white woolly climber. Asymmetrical, strigose leaves 8–12cm long, with obtusely sharp, rounded, or subcordate base. 3cm petiole; 5cm peduncle; Small bracteoles and bracts It has little flowers with a short pedicellate calyx lobe anda 2cm long pink corolla. 5mm berry, reddish, thickly hairy (Sebastin et al., 2019).

An anti-diabetic action of Argyreia imbricata was demonstrated in a streptozotocin- induced diabetes model in Wistar albino rats, according to earlier investigations. This characteristic was observed in petroleum ether, chloroform, ethyl acetate, and methanol extracts. Studies on the inhibition of amylase and glucosidase were used to evaluate the in vitro anti-diabetic activity of all extracts (Sebastin et al., 2021).

2. Materials and methods:

Materials Chemicals required:

Biuret reagent[Sodium hydroxide solution, potassium sodium tartarate, Copper sulphate, potassium iodide], BSA ,Gallic acid, Methanol , Acetate buffer , TPTZ [2,4,6- tripyridyl –S- triazine], HCL [Hydrochloric acid], $Fecl_3$ [Ferric chloride], DPPH [2,2 – diphenyl -1- picryl hydrazyl], ABTS [2,2'-azino-bis-3-ethylbenzothiazoline-6,sulfonic acid], Potassium per sulphate, Aspirin, Thiobarbituric acid, Acetic acid, $NaNo_2$ (sodium nitrite), 1-butanol.

Methods:

Collection of plant:

The whole plant of A. imbricata was collected from Tiptur, the rural area located near Tiptur, Tumakuru District, Karnataka, India.

Preparation of powdered material and extraction:

The A. imbricata were collected, washed and shade dried. The dried leaves were powderedusing a mixer and used for extraction. The aqueous leaves extract was prepared. 10gm of leaves powder was dissolved in 100ml of distilled water. Then kept in magnetic stirrer for 24 hours with magnetic bead at 800 rpm. Then centrifuged the sample extract for 20 min at 8000 rpm. The extracts were kept in the refrigerator until they were used.

Figure.1. Argyreia

Figure.2. Dried A. imbricata leaves powder

Figure.3. Preparation of aqueous extract of dried A. imbricata leaves powder

Protein Estimation by Biuret Method:

The protein content was determined according to the Biuret method. Different proportions (25 and 50 µl) of aqueous extract was diluted to 50µl with distilled water. Then 3ml of Biuret reagent is added to each test tube and incubated for 10 minutes at room temperature. Absorbance was measured at 540 nm. The protein content was determined using bovine serum albumin (BSA) as a standard (Plummer D.T., 1988).

3. Antioxidant activities:

Total antioxidant activity (TAA)

Total antioxidant activity was determined using modified phosphomolybdenum technique according to the method of (Prieto et al.,. 1999). The assay is based on the reduction of Mo (VI) to Mo (V) by sample compound and formation of green coloured phosphate/Mo (V) complex at acidic (pH-4.0) 0.1ml of extract from the varying concentrations (100-500 µg/ml) was added to 1ml reagent solution (0.6M H_2SO_4, 28mM sodium phosphate and 4mM ammonium molybdate) the mixture was incubated at 95°C for 90 minutes and absorbance was measured at 695 nm after cooling the samples. Total antioxidant capacity was expressed as GAE.

FRAP (ferric reducing antioxidant power)

According to (Benzi et al., 1996), the antioxidant capacity of AIAE was estimated colorimetrically. The method is based on reduction of Fe^{3+}-tetra (2-pyridyl) pyrazine (TPTZ) complex to Fe^{2+}-tripyridyltriazine formed by action of electrons donating antioxidant at low pH. FRAP reagent was prepared by addition of 300mM sodium acetate buffer, 10ml TPTZ dissolved in 40mM Hcl and 20 mM $FeCl_3$ in the ratio 10:1:1. 1000µl of standard was added to1ml ofFRAP and the

mixture was incubated at 37° C for 30 min. Absorbance was recorded at 593 nm against blank. The values of FRAP was expressed as GAE for varying concentration of the extract (100-500 µg /ml)

ABTS radical scavenging assay

According to (Seeram et al., 2006) Free radical scavenging activity of plant extracts was determined by ABTS by radical decolourization assay. In brief ABTS (2.2 Azino-Bis-3-ethyl benzthiazoline-6-sulfonic acid cation radical) was produced by the reaction between 7 mM ABTS in water and 2.45mM potassium persulphate in water (1:1). This reaction mixture was stored in dark at room temperature for 16 to 20 hours. This ABTS solution was then diluted with methanol to get absorbance of 0.7 at 734 nm. 5 microlitre of plant extract was added to 3.995 ml of ABTS solution and incubated for 30 min and absorbance was measured at 734 nm. The results were expressed as percent scavenging effect of plant extracts.

DPPH radical scavenging activity

The antioxidant activity of the plant extract was examined on basis of the scavenging effect on the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical activity as described by (Braca et al., 2002) ethanolic solution of DPPH 0.05mM (300 µl) was added to 40µl of extract of different concentrations (100-500µg/ml). After 5 min absorbance was measured colorimetrically at 517nm against extract blank. The radical scavenging activity of the plant extract expressed as percent inhibition was calculated according to the following equation:

Percent inhibition of DPPH radical = (absorbance control – absorbance test) / absorbance control × 100

Nitric oxide radical scavenging activity

Nitric oxide is generated in biological tissues by specific Nitric oxide synthases, which metabolises Arginine to Citrulline with the formation of Nitric oxide via 5 electron oxidative reaction .The compound Sodium nitroprusside is known to decompose in aqueous solution at physiological (pH-7.2) producing Nitric oxide under aerobic conditions, Nitric oxide reacts with oxygen to produce stable products (Nitrate And Nitrite), the quantities which can be determined using Griess reagent. 2ml of 10mM Sodium nitroprusside dissolved in 0.5ml Phosphate buffer saline 9 (pH-7.4) is mixed with 0.5 ml of sample at various concentrations (100-500µg/ml). The mixture is incubated at 25°C. After 150 min of incubation 0.5ml of the incubated solution is withdrawn and mixed with 0.5 ml Griess reagent [(1ml Sulfanilic acid reagent)(0.335 In 20% Glacial acetic acid at room temperature for 5min with 1ml of Naphthylethylenediamine Dichloride(0.1% w/v)].The mixture is then incubated at room temperature for 30 min and its absorbance pouring into a cuvette is measured at 546nm. The assay was performed according to method described by (Sreejayan and Rao et al., 1997).

Isolation of erythrocytes

Blood was collected from healthy volunteers, mixed with ACD (acid citrate dextrose) 85 mmol/l sodium citrate, 71 mmol/l citric acid; 111 mmol/l dextrose at the ratio of 6:1 (blood/ACD, v/v) and centrifuged at 2000rpm for 10 minutes at 37°C platelet–rich plasma (supernatant) and erythrocytes (pellet) were obtained. The erythrocytes were washed two to three times with 10 mmol/l PBS (pH-7.4). RBC suspension (2% haematocrit) was prepared from the RBC pellet using 10 mmol/l PBS having 1 mmol/l glucose and the same was used for the assays.

4. Results and discussion:

Biuret method

Biuret test is a protein estimation method. Different volume (25µl and 50µl) aqueousextract was taken as unknown samples. As the result, concentration of 25µl of the given unknown sample was 250µg/ml and concentration of 50µl of the given unknown sample was 500µg/ml respectively, (Fig. 4).

Total antioxidant activity

Total antioxidant activity is a quantitative measure of the antioxidant capacity of the extract and it is reported in Gallic acid equivalents (GAE). The total antioxidant capacity of AIAE was taken. The total antioxidant activity of aqueous extract increases steadily as extract concentration increases from 100-500µg/ml. The GAE value was found to be 1.32±0.09µg/ml GAE at 500µg/ml aqueous extract respectively. The results were tabulated in Table. 1.

Concentration (µg)	TAA (GAE)	FRAP (GAE)
100	0.92±0.02	12.97±0.29
200	1.08±0.91	13.82±0.2
300	1.17±1.01	14.73±0.15
400	1.25±0.78	15.07±0.10
500	1.32±0.09	16.36±0.2

Table. 1. GAE value for Anti-oxidant activity of A. imbricata leaves extract.

Ferric Reducing Antioxidant Power Assay (FRAP)

The results revealed a considerable antioxidant capacity in terms of FRAP measured as GAE of AIAE, which enhanced steadily with increasing concentrations from 100-500 µg / ml of samples. The antioxidant activity of AIAE was observed. Aqueous extract showed the antioxidant power activity. The GAE value was found to be 16.36±0.2 µg/ml GAE at 500µg/ml of aqueous extract respectively. The results are tabulated in Table. 1.

DPPH radical scavenging activity

We investigated the extract's radical scavenging properties by measuring changes in the absorbance of the 1,1-diphenyl-2-picrylhydrazyl radical at 517nm to learn more about the mechanisms of its antioxidative activities. The DPPH radicals were scavenged by AIAE in dose dependent manner (100-500 µg/ml). The % inhibition of free radical (DPPH) scavenging activity at 500µg/ml of concentration, the AIAE leaves displayed radical scavenging activity with an IC_{50} of 33.16 µg/ml, respectively, (Fig. 5).

ABTS radical cation decolorization assay

The extract scavenged the ABTS radical in a concentration-dependent manner (100-500 µg/mL). The aqueous extract exhibited activity as a radical scavenger. The outcomes of the free radical scavenging activity of (ABTS) expressed as % inhibition. The results indicated that the AIAE had an IC_{50} of 20.85µg/ml, respectively, (Fig. 6), at a concentration of 500 µg/ml.

Nitric oxide scavenging assay

The aqueous extract scavenged the Nitric oxide radical (100-500 µg/ml). The outcomes of the free radical scavenging activity of nitric oxide expressed as % inhibition. The results indicated that the AIAE had an IC_{50} of 26.89µg/ml, respectively (Fig. 7), at a concentration of 500µg/ml.

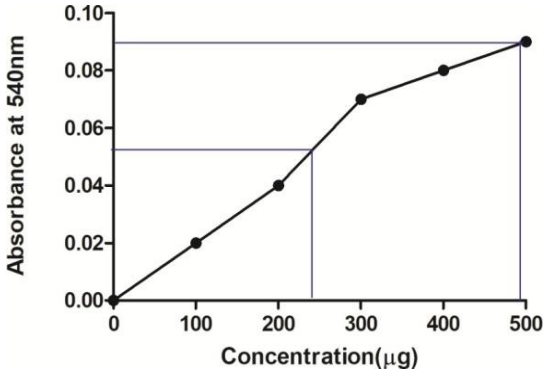

Figure. 4 Biuret method of AIAE. Biuret reagent was incubated with different concentrations of AIAE at room temperature for 10min. Absorbance was measured at 540nm

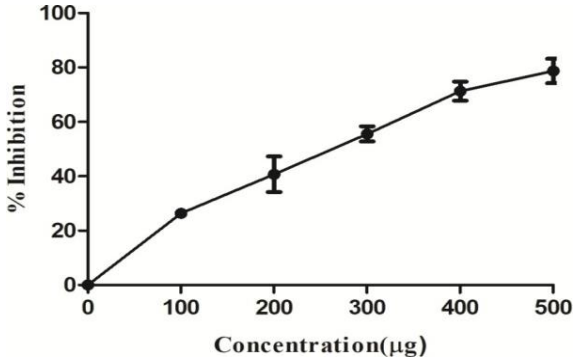

Figure. 5. DPPH radical scavenging activity of AIAE. DPPH was incubated with different concentrations of AIAE at 37°C for 30 minutes. The absorbance was read at 540nm.

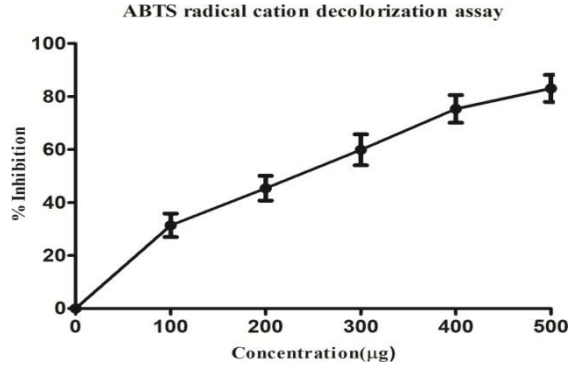

Figure. 6. ABTS radical scavenging activity of AIAE. ABTS was incubated with different concentrations of AIAE at room temperature for 30mins.

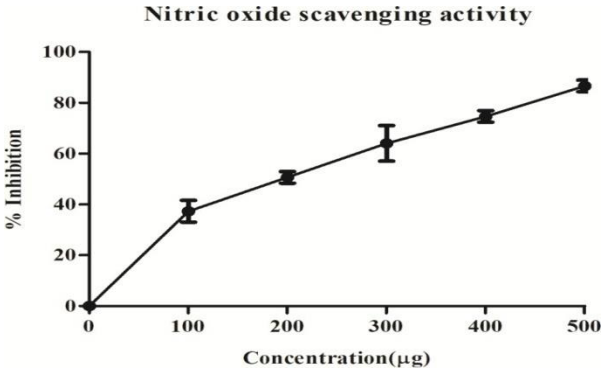

Figure. 7. Nitric oxide radical scavenging activity of AIAE. The reaction was initiated by adding Griess reagent and incubated at 37°C for 30 min. Absorbance was monitored colorimetric ally at

Discussion

In our project work we have prepared aqueous extract for A. imbricata leaves. To explore the various processes responsible for antioxidant activities, aqueous extract of A. imbricata leaves extract was tested for antioxidant activities such as TAA, FRAP, DPPH, ABTS, and Nitric oxide scavenging activity. In this study, the total antioxidant capacity of AIAE was found to be 1.32±0.09µg /ml. The aqueous extract exhibited significant total antioxidant activity. Earlier study using G. combogia seed extracts (methanol, ethyl acetate and acetone), the methanol extract showed highest TAA at a concentration of 92.63µg/ml (Sumachirayu CK et al, 2020). In FRAP assay, antioxidant capacity is measured by the reduction of the ferric ion complex TPTZ. In the presence of antioxidants, the Fe3+/ferricyanide combination can bereduced to Fe2+/ ferrocyanide. As a result, the binding of the ligand to Fe2+ produces a highly vivid navy-blue colour. As a result, the quantity of reduced iron may be measured and associated with the number of antioxidants by detecting the production of Perl's Prussian blue at 593 nm. AIAE exhibited ferric-reducing antioxidant power in this study. The AIAE showed the ferric reducing antioxidant power at

16.36±0.2µg/ml expressed as gallic acid equivalents (GAE). Likewise, (Uddin et al., 2014) study has shown the ferric reducing antioxidant power activity of methanol extract of Argyreia argentea (Roxb) was 150.83±4.26µmol ascorbic acid/gm. The capacity of plant extracts to donate hydrogen atoms was tested by decolorizing a methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). DPPH in methanol creates a violet/purple colour that fades to shades of yellow in the presence of antioxidants. The DPPH radical scavenging activity of AIAE was determined. The present study's findings indicated that aqueous extract of the leaves exhibited the radical scavenging activity with IC_{50} value of 33.16µg/ml. Similarly, (Mahendra et al., 2016) study has shown DPPH radical scavenging activity in Argyreia osyrensis Roth plant extract. In ABTS method, antioxidants which are capable of donating electrons, can convert the blue-green ABTS radical solution into a colourless form. The potential of AIAE to scavenge ABTS was shown to have an IC_{50} of 20.85µg/ml. Similarly, (Prashith kekuda et al., 2018) showed the maximum ABTS scavenging activity in methanol extract of Argyreia cuneata (wild) Ker Gawl plant. The leaf methanol extract was more effective in scavenging ABTS radicals with IC_{50} value of 9.34µg/ml followed by flower methanol extract (IC_{50} value 14.98µg/ml) and stem extract (IC_{50} value 22.4µg/ml). In nitric oxide method, scavenging activity of AIAE was determined. The aqueous extract of the leaves exhibited the radical scavenging activity with IC_{50} value of 26.89µg/ml. This is lined with the finding of (Lee et al., 2010), who found that the ethyl acetate of H. porphyrae (30.1%) and the 80% of methanol extract of O. unicellularis (49.3%) exhibited significantly higher nitric oxide radical scavenging effects than those of the commercial antioxidants. In this study, the aqueous leaves extract was found to have the highest percentage of radical scavenging activity.

CONCLUSION:

In conclusion, the AIAE leaves was analyzed for antioxidant activities, in which the extract exhibited significant activity. This study focuses on the pharmacological discovery process for preventing cell damageby oxidants.

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