

Phytochemical screening, antioxidant and anti-inflammatory activity of *Urginea indica* (Roxb) kunth, hyacinthaceae

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Abstract

Industrialization is very fast growing during the human civilization. During this industrialization more pollution is generated and due to the pollution, free radicals are generated. This free radical can damage the most of the cell of the animals and human. This may cause the various diseases in human and animal. Beside that there are various problems related to the inflammation. In search of the plants we find one of the important plant *Urginea indica* (Roxb) which was one of the rare plant in the region of Maharashtra region as well as in India. We have obtained the plant from the Rajapur region of Maharashtra, India. The leaves are screened for the antioxidant activity by the DPPH, nitric oxide. This plant was studied for the phytochemical activity of the leaves and flowers. We also study the anti-inflammatory activity of the leaves by antioxidant and anti-inflammatory protocol. The result shows that the leaf of the plant shows the excellent antioxidant activity and the leaves and flowers contain various phytochemicals. The leaves extract shows the excellent anti-inflammatory activity. *Urginea indica* (Roxb) Kunth leaves shows the good antioxidant, anti-inflammatory activity.

Keywords: *Urginea indica* (Roxb) kunth, phytochemical, antioxidant activity, anti-inflammatory activity

Introduction

The oxidative stress is the main reason of the many diseases during this the certain detoxification of prooxidants takes place and this happens due to the electron transport in mitochondria [1]. Mostly due to environmental effects the Iosis increased or the antioxidant activity is decreased which causes an increase in ROS. Free radicals and ROS are constantly generated in the environment due to pollution, which causes the damage to tissue and various diseases. This leads to the extensive problem [2]. There are many synthetic drugs involving free radicals scavenging activity. They protect oxidative damage with many drugs [3]. There are many alternative drugs for antioxidants in the market, natural antioxidants in various food supplements and traditional medicines [4]. There are many plant or plant extract acts as antioxidants and this antioxidant can be used for the protection against the various oxidants and different diseases. Inflammation is one of the important phenomena when a cut wound or the infection due to the bacteria, fungi, viruses involved in the body or react with blood [5, 6]. Third inflammation process responsible for the cell death, tissue injury, cancer, degeneration and ischemia [7, 9]. This more or less results into the immune response or innate immune [10]. This immune system gives the better response due to cancer cells, various cells including macrophages and dendritic cells. This activity due to the B and T cells which are responsible dendritic cells for eradicating pathogens and cancer and specific receptors for the antibodies. There are number of the inflammatory mediators are synthesized by various ways

and of the different types pro- and anti-inflammatory mediators [1, 13].

Plants have been the basis of traditional medicines from time immemorial throughout the world and continue to provide new targets for remedies for many afflictions of mankind. The past couple of decades have seen considerable change in opinion regarding ethno pharmacological therapeutic applications of phytochemicals. A great deal of effort therefore still focuses on identifying and using these phytochemicals as source of novel therapeutic molecules. India is a country of the agriculture; it has a good history of the agriculture. There are number of crops taken in India with different varieties but along with these there are certain plants grow in the forest. The most common plant grow in the forest is *Urginea Indica* (Roxb) Kunth. This plant having the contingent on the existence of discrete diagnostic characters. *Urginea indica* (Roxb) Kunth is a perennial bulbous geophyte native to India [14-18]. This is also called as the sea onion or in India it was also called as Indian Squall, it grows in forest and sandy soil. It grows in hilly area also. It has very good medicinal value along with pharmaceutical and biological applications. They have good cardiotonics, good digestive and used in the diseases like skin ailments, leprosy, rheumatism, dropsy, asthma. The Bulb extract having the anticancer and properties hypoglycemic given by Ghani 2003 [21]. These bulbs also having the properties like isolation of glycoproteins, glycosides. They also show certain insecticidal and antimicrobial properties, they also show the other properties like the good antibacterial

Agent, good antifungal agent etc ^[21].

Materials and methods

Chemicals

Gallic acid/BHT is used as the standard for the antioxidant activity. This is purchased from S.D. Fine limited Mumbai, India. Ascorbic acid was obtained from the Merck Specialties Pvt. Ltd., Mumbai, India. HPLC grade methanol, acetonitrile and acetic acid purchased from Merck limited. All chemicals used were analytical grade.

Plant material and extract preparation

The plant material was collected from the Forest from the Konkan region. This was identified and authenticated by Dr. Arun Chandore, Abasaheb Marathe College, Rajapur, tehsil of Ratnagiri District, Maharashtra, India, and specimen kept in the Rajapur. Abasaheb Marathe College as specimen sample. (Ref. No. ACS/2233).

The plant material was collected from the forest of Konkan region of Maharashtra, India. The leaves collected are washed with distilled water three to four times then dried at 40 °C for 2 h, then it was shade dried for the 6-30 days in shade. Then grind to the powder, after grinding into fine powder dried at the room temperature, then stored in the air tight container. This method is based on the extraction using the polar solvent. This method was given in the reference 1. According to this method the 50 g of the powder was taken

in round bottom flask, 100 mL of the 70 % methanol added and kept for reflux about 6 hrs. Then cooled at room temperature and filtered. The methanol layer was distilled off to get methanol extract. The residue was washed with distilled water, then repeat the same procedure for the petroleum ether, chloroform and ethyl acetate. Different extract in the form were collected as Methanol extract powder, Petroleum ether extract powder.

Phytochemical Studies

In this study determination of the various phytochemicals were carried out. The phytochemicals such as saponins, glycosides, steroids flavonoids, tannins, alkaloids along with total phenolic content was studied ^[22].

Extraction and phytochemical investigation

The leaves of the plant were collected. Material was shade dried. After shade dried, plant dried into the oven at 35 °C for 5 hours. Then fine grind into the fine powder. The obtained powder was dried in oven at 40 °C, then dried material cools at room temperature. Then they are packed in bottle in nitrogen atmosphere. The obtained powder was extracted with solvent of the different polarity such as hexane, acetone, dichloromethane ethanol and methanol. The solvents are evaporated to get the fraction, the evaporation carried over the Rota evaporator, the powder obtained stored as separate fraction and stored properly.

Table 1: Phytochemical Analysis of Leaves of the Plant

| Group | Test | Hexane Extract | Ethanol Extract | Methanol Extract | DCM extract | Water Extract |
|---------------------------------------|---|----------------|-----------------|------------------|-------------|---------------|
| Carbohydrate | Molisch's test | - | + | + | - | + |
| | For Reducing Sugars | - | + | + | - | + |
| | Tests for Monosaccharides | - | - | + | - | + |
| | Tests for Non-Reducing Sugars | - | - | + | - | + |
| Alkaloids | Dragendroff's test: 2-3 ml of the test solution | - | - | - | - | - |
| | Mayer's test | - | - | - | - | - |
| | Hager test | - | - | - | - | - |
| | Hager test | - | - | - | + | + |
| Test for steroids | Salkowski Reaction | - | - | - | - | - |
| | Liebermann-Burchard Reaction | - | - | - | + | - |
| | Lieberman Reaction | - | - | - | - | + |
| Tests for Glycosides | General Test | - | + | - | - | + |
| | Test for Cardiac Glycosides | - | - | + | - | + |
| | Legal Test | - | - | + | - | + |
| | Test for Deoxysugars | - | - | + | - | + |
| | Liebermann's test | - | + | + | - | + |
| Test for Saponin Glycosides | Test for Coumarin Glycosides | - | + | + | + | + |
| | Foam Test | - | - | + | - | + |
| | Hamolytic Test | + | - | + | - | + |
| Test for tannins and phenolic content | ferric chloride | - | - | - | + | - |
| | lead acetate | - | - | - | + | - |
| | gelatin | - | - | - | + | - |
| Test for Protein | Biuret test | - | - | - | + | - |
| | Million's test | + | - | - | + | - |
| | Xanthoprotein test tyrosine or tryptophan | - | - | - | + | - |
| | Precipitation test 5 % of mercuric chloride | - | - | - | - | - |
| Test for amino acid | 5 % mercuric chloride | - | - | - | - | - |
| | 5 % cupric sulphates 5 % lead sulphates | + | - | - | - | - |
| | Ninhydrin of amino acids | + | + | - | + | + |
| Test for flavonoids | Shinoda test | - | - | - | - | - |
| | Ferric chloride test | + | + | - | + | + |
| Test for Vitamins | Test for vitamin A | + | - | - | + | - |
| | Test for vitamin C | - | + | - | - | + |
| | Test for vitamin D | + | - | - | + | - |
| Test for Triterpenoids | Test for Triterpenoids | + | - | - | - | - |

Total phenolic Content (TPC) of the plant *Urginea indica* (Roxb) Kunth was studied using known protocol [23]. Various concentrations of the Gallic acid was used for the plotting the standard curve. The total phenolic content was expressed as mg of gallic acid equivalent /gm for dry weight of the extract

Flavonoid content of the plant *Urginea indica* (Roxb) Kunth leaves carried using the known method [24]. The flavonoid content was expressed in as mg quercetin equivalents/gm dry weight of extract

Tanin content of leaves of *Urginea indica* (Roxb) Kunth was estimated by butler method [25, 26]. This Tanin content was expressed in mg rutting equivalents/gm dry weight of extract.

Ascorbic acid content of *Urginea indica* (Roxb) Kunth was determined using the method described in the research papers. These were expressed as $\mu\text{g/gm}$ dry weight.

Standardization Polyphenol content of *Urginea indica* (Roxb) Kunth using the gallic acid as standard. The reverse phase separation of gallic acid from the other constituent by using Jasco HPLC. The specification of the UV 800 detector with injection 20 μL loop and using the column (250 mm x 4.6 mm have the inner diameter 5 μm particle size) at 30 $^{\circ}\text{C}$. The mobile phase water: acetonitrile: acetic acid as 80:10:10, the flow rate 1.0 mL/min and wavelength for absorption 280 nm.

Reducing power assay

Reducing power was studied on the basis of their antioxidant principle for the coloured complex with potassium ferricyanide with TCA and FeCl_3 studied using [27] then it was plant extract 1mL having the various concentration 25, 50, 100, 150, 200 and 250 $\mu\text{g/mL}$ with potassium ferricyanide and phosphate buffer pH 7.6 and incubated for 40 $^{\circ}\text{C}$ for 10 min. TCA was added 10 % and then centrifuge for 4000 rpm for 15 min. The supernatant liquid was separated to this FeCl_3 (0.1%) added. Then absorbance was measured 740 nm. The higher the absorbance higher the reducing reagent.

Total antioxidant capacity of the phenol and phenolic content of the *Urginea indica* (Roxb) Kunth was studied using the spectrophotometer method of concentration 50-500 $\mu\text{g/mL}$ [28]. Experiment was carried in the triplet expressed as μg .

DPPH radicle scavenging assay Ability leaves *Urginea indica* (Roxb) Kunth powder the was studied the BHT, and Ascorbic acid 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicle was method given Shimada *et al.* Addition of 0.1 mM DPPH solution in various concentration (10, 25, 50, 75, 100 and 150 $\mu\text{g/mL}$) of the plant extract ascorbic acid in tris-HCl buffer (50 mM, pH-7.4) and absorbance 520 nm. The mixture of methanol and extract served as blank. This can be calculated absorbance of extract / ascorbic acid treated against the blank. IC50 values for polyphenol extract was recorded.

Nitric acid radical scavenging assay

Leaves *Urginea indica* (Roxb) Kunth the polyphenolic was calculated different 50-500 $\mu\text{g/mL}$ scavenging activity was determined [29]. Various concentrations of polyphenolic extract was added with 10 mM sodium nitropruside and incubated at 30 $^{\circ}\text{C}$ at 2.5 h for the Griess reagent tubes and absorbance 610 nm. The blank solution contains mixture 0.5 mL extract. IC50 values and percent inhibition was calculated.

Hydrogen peroxide scavenging assay of hydrogen peroxide scavenging activity leaves *Urginea indica* (Roxb) Kunth of the leaves 50-500 $\mu\text{g/mL}$ was studied using hydrogen peroxide scavenging activity [30] a solution of hydrogen peroxide (2 mmoles / liter) with phosphate buffer of pH 7.4 with polyphenol extract 25, 50, 75, 100, 125 and 150 $\mu\text{g/mL}$ and add 0.9 mL of H_2O_2 and phosphorus phosphate 3.6 mL and leaves extract 0.7 mL. The absorbance measured at 280 nm buffer was used as standard with hydrogen peroxide [31, 32].

Reducing power assay

We have determined the capacity of the polyphenolic extract of leaves *Urginea indica* (Roxb) was measured using the conversion of the Fe^{3+} to Fe^{2+} using the various concentration such as 25, 50, 100, 150, 200 and 250 $\mu\text{g/mL}$ in this case the concentration increases as concentration is increased for 250 $\mu\text{g/mL}$ for it was 0.854. This the reducing power assay depend upon the presence of the reduction this can be free radical activity this activity was based on the conversion of Fe^{3+} to Fe^{2+} of the potassium ferrocyanide

The antioxidant activity (Total Capacity)

Total antioxidant capacity of the polyphenol extract of the leaves of leaves *Urginea indica* (Roxb) shows the 0.168 to 0.480 at the graded concentration of the 50 to 600 $\mu\text{g/mL}$. The plant shows the good total antioxidant activity of the plant with the scavenging activity [33].

DPPH radical scavenging assay

The DPPH radical scavenging activity of the leaves *Urginea indica* (Roxb) at different concentration was calculated with reference to the ascorbic acid.

The radical scavenging activity was found to be maximum dose of 250 $\mu\text{g/mL}$ by extract with an IC50 value of 15 ± 0.07 μg while the ascorbic acid showed IC50 value of 18 ± 0.08 $\mu\text{g/mL}$.

The effect was the concentration dependent the free radical activity was determined using the DPPH. DPPH is stable nitrogen centered free radical.

This can be used to trap the free radical very easily. The observed results are highly effective; this plant extract shows good antioxidant activity [34].

Antioxidant Activity (AA)

Free radical scavenging activity (DPPH)

Radical scavenging activity of the various plant extracts was measured by 1-1-diphenyl-2-picryl-hydrazil (DPPH) [35]. This is measured at 520 nm. The standard used was ascorbic acid and BHT. The results are tabulated in the observation table.

DPPH Scavenging Test: Quantitative measurement of radical scavenging property was carried out in a universal bottle. The reaction mixture contained 50 µl of test samples

(or 80% MeOH as blank) and 5 mL of a 0.004% (w/v) solution of DPPH in methanol. Using given standard protocol [36].

Table 2: Antioxidant activity of leaves powder with Extract

| Conc. mg/mL | BHT | Ethanol | Water | Methanol |
|-------------|-------|---------|-------|----------|
| 0.05 | 49.14 | 48.67 | 37.53 | 40.47 |
| 0.1 | 52.94 | 48.45 | 38.53 | 44.90 |
| 0.2 | 54.78 | 53.26 | 43.50 | 23.80 |
| 0.3 | 62.89 | 57.18 | 42.00 | 37.90 |

Determination of Anti-inflammatory Activity

Study of anti-inflammatory activity (In-vitro models): *Urginea indica* (Roxb) Kunth, leaves extract and flower extract was screened.

For anti-inflammatory activity by using inhibition of albumin denaturation technique which was studied according to Muzushima and Kabayashi with slight modification at the doses of 200 mg/kg [37-45].

Table 3: Anti-inflammatory activity of leaves powder

| | Dose (mg /kg) | Absorbance value (Mean + SE) | Inhibition of denaturation (%) |
|----------------------|---------------|-------------------------------|--------------------------------|
| Control | 5mg/ kg | 0.097 | ---- |
| Standard (Ibuprofen) | 100mg/kg | 0.19 | 94 |
| Ethanol | 200mg/kg | 0.18 | 86 |
| water | 200mg/kg | 0.16 | 80 |
| Methanol | 200mg/kg | 0.18 | 86 |
| n-Butanol | 200mg/kg | 0.14 | 76 |
| Ethanol | 200mg/kg | 0.17 | 82 |

Results and discussion

The Phytochemistry of the plant indicates that presence of the various phytochemicals such as alkaloids, flavonoids, tannins, spanning are present. The total phenolic content present was 156.67 mg Gallic acid equivalent/g dry weight in the leaves. The flavonoid content present in the leaves *Urginea indica* (Roxb) extract was 8.898, 7.934 mg of quercetin equivalent/g dry weight of the given sample. The tannin present in the leaves *Urginea indica* (Roxb) was studied using typical protocol. It was found that 27.60 mg rutin equivalent/g dry weight. Ascorbic acid was found in the extract of the leaves *Urginea indica* (Roxb) is 8.74 µg/g dry weight of extract. The phytochemical analysis of leaves *Urginea indica* (Roxb) have ellagic acid, ascorbic acid (Vitamin C), mannitol, corilagin, chebul agi acid, and other many chemicals. The polyphenols are very important constituent for the plant which can shows the scavenging of free radicals. The ethanol extract of the leaves of the plant has very good antioxidant activity. These plants having the very good polyphenols present in the powder of leaves powder. The plant extract shows very good anti-inflammatory activity.

Conclusion

We have study the antioxidant and anti-inflammatory activity of the leaves *Urginea Indica* (Roxb) Kunth. We have observed that the different extract shows the excellent antioxidant activity, as well as the anti-inflammatory activity. The various extract shows the presence of the various phytochemical present in the leaves extract. This leaves may be use as best antioxidant and anti-inflammatory.

Conflict of interest

Authors do not have any conflict of interests to declare.

Funding

No funding received from any agency

Acknowledgements

Authors are thankful to Principal Rayat Shikshan sansthas Karmaveer Bhaurao Patil College, Vashi, Navi Mumbai for providing the necessary Support. We are also thankful to Rayat Shikshan Sanstha Satara, for their Support.

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