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Analysis of antioxidant properties of *Azadirachta indica* in Urban and Coastal area

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Abstract

The study compared the total antioxidant properties of *Azadirachta indica* in Urban and Coastal area. The selected plant has varieties of phytochemicals such as Alkaloid, Flavonoid, Phenol, Saponin, Tannin and Terpinoid. It possess various activities such as radical scavenging, anti-microbial, insecticidal, anti-fungal and has the potential to prevent several diseases. The result accomplished that total alkaloid content in *Azadirachta indica* in urban area is $0.18 \pm 0.12\%$ and in Coastal area is $0.12 \pm 0.04\%$. Total flavonoid content of the studied plant in Urban area is 1.33 ± 0.27 mg/ml and in Coastal area is 1.29 ± 0.17 mg/ml. Total phenolic content of *Azadirachta indica* in Urban area is 0.33 ± 0.1 mg/ml and Coastal area is 0.25 ± 0.22 mg/ml. Total saponin content of *Azadirachta indica* in Urban area is 1.09 ± 0.11 mg/ml and in Coastal area is 4.95 ± 0.57 mg/ml. Total tannin content of *Azadirachta indica* in Urban area is 0.05 ± 1.15 mg/ml and in Coastal area is 0.03 ± 0.53 mg/ml. Total terpinoid content of *Azadirachta indica* in Urban area and Coastal area is 0.01 ± 0.41 mg/ml and 0.09 ± 0.04 mg/ml respectively.

Keywords: *Azadirachta indica*, antioxidant properties, antimicrobial activity, oxidative stress

Introduction

Azadirachta indica is a tree which belongs to the Meliaceae family. It is rich source of antioxidant properties. It is mostly found in tropical and semi tropical areas of India, Bangladesh, Pakistan and Nepal. *Azadirachta indica* tree which grown medium large and possess medicinal values. It grows faster with minimum rainfall. It is widely used in the Chinese, Indian medicinal field for the treatment of various diseases. Findings estimated that *Azadirachta indica* take part in scavenging free radical production and thereby preventing various harmful diseases. *Azadirachta indica* consist of various antioxidant such as alkaloids, flavonoids, saponins, phenols, tannin, terpinoids, etc.

The alkaloids are compounds which have effective role in the participation of free radical scavenging and they are phytochemicals and which possess anticancer us, antimalarial actions. It has great role in the human cell metabolism. Flavonoids are antioxidants which prevent the production of free radicals which cause damage to cells and tissues. Folin-ciocatea method is generally accepted method for the estimation of flavonoid content. Phenols are also other antioxidant it possess various functions such as improvement of vascular health, prevention of cancerous activities etc. Saponin are compound which gives soapy lather to the leaves of plants studies says that it reduces the cholesterol level and also the blood pressure level in blood thus it prevents all types of cardio vascular diseases. Tannin and terpenoids are also compounds which are effective in the prevention of ulser, diarrhea, and also have wound healing properties.

Materials and methods

Sample collection

The fresh *Azadirachta indica* leaf samples were collected from coasted and urban area. It is dried and powdered. The collected samples were packed in plastic bag for further steps.

Estimation of Alkaloid

5g of *Azadirachta indica* leaf powder is taken in a 250ml beaker and then 200ml of 20% acetic acid is added to the beaker and kept the beaker covered for four hours.

Then the volume of sample is minimized using water bath and add ammonium hydroxide solution into the beaker drop by drop till the precipitate occurs. Allow the precipitate to settle and then the precipitate is collected and filtrate then finally total alkaloid content is estimated.

Estimation of Flavonoid

The estimation procedure described by Zhishen *et al.*, 0.5ml of plant extract is taken in a test tube and 0.5ml of distilled water is added to the plant extract. Then 0.3ml of 5% NaNO₂ is added and then the extract is incubated for 6 minutes at 25°C. After incubation 0.3ml of 10% aluminium chloride is added and followed by 2ml of sodium hydroxide (4%) is added to the mixture. The absorbance is noted at 510nm. Quercetin is used as a standard

Estimation of Phenol

Folin – ciocalteu reagent method is used for the estimation of phenol. 1g of plant leaf powder is added to the 80% methanol and kept it until 20 minutes. Filtrate the mixture into a centrifuge collector and centrifuge the sample at 5000rpm for 10 minutes. Collect the supernatant and make it to a known volume using methanol. Then add 2ml of 20% sodium carbonate and kept it in the water bath for 5 minutes. After a white precipitate occurs centrifuge the mixture at 5000 rpm for 10 minutes. Absorbance is noted at 725nm.

Estimation of Saponin

Total saponin content is determined by the method described by Makkar *et al.*, [14]. Above 50ml of plant leaf extract is added to the distilled water of 250ml. Then 250ml vanillin reagent is added to the extract (800mg of vanillin in 100ml of 99.5% of ethanol). The 2.5ml of 72% sulphuric acid is added and then kept it for water bath at 60°C for 10 minutes and cool it with cold ice water. The absorbance is noted 544nm. Diosgenin is used as the standard.

Estimation of Tannin

50% methanol is added to 1g of plant leaf powder and kept it for 20-28 hours. Then centrifuge the extract and the supernatant is collected and 5ml of vanillin hydrochloride reagent is added. Read the absorbance at 500nm for 20 minutes and catechin is used as a standard.

Estimation of Terpinoid

10ml of methanol is collected in a test tube and then 0.8g of plant extract is filtered. Take filtrate of 5ml and add 2ml of chloroform and then 3ml of sulphuric acid is added to the mixture. Reddish brown precipitate is formed. Reading is noted in spectrophotometer. Methanol is used as blank and absorbance were analyzed in 538nm.

Result and discussion

The antioxidant properties of *Azadirachta indica* play a great role in medicinal aspects [4, 9]. It consist of antioxidants like alkaloids, tannins, phenols, terpinoids, saponins, and flavonoids. The total alkaloid content in urban area is $0.18 \pm 0.12\%$. It is higher than the coastal area, is $0.12 \pm 0.04\%$. The total flavonoid content also higher in urban area than the coastal area. That is $1.33 \pm 0.27\text{mg/ml}$ and $1.29 \pm 0.17\text{mg/ml}$ respectively. But the phenolic content were greater in coastal area is $0.25 \pm 0.22\text{mg/ml}$. phenolic content of *Azadirachta indica* in urban area is $0.33 \pm 0.1\text{mg/ml}$. The saponin content in urban area is $1.09 \pm 0.11\text{mg/ml}$ and in coastal area is $4.95 \pm 0.57\text{mg/ml}$. Coastal area shows higher presents of saponin content in *Azadirachta indica*. The tannin content is maximum in urban area than the coastal area. It is $0.05 \pm 1.15\text{mg/ml}$ and $0.03 \pm 0.53\text{mg/ml}$ respectively. Total terpinoid content of *Azadirachta indica* in urban area is $0.01 \pm 0.41\text{mg/ml}$ and in coastal area is $0.09 \pm 0.04\text{mg/ml}$. The terpinoid content of *Azadirachta indica* is greatly found in coastal area. The estimation of studied plant is discussed in C Egbuna *et al.*, 2015 [4].

Conclusion

From the present study, it could conclude that leaf of *Azadirachta indica* possess high antioxidant property. The plant extract contain significant amount of alkaloid, flavonoid, phenol, saponin, tannin, and terpinoid. Comparatively high yield of antioxidant were found in urban area. Thus there is a significant antioxidant activity in *Azadirachta indica*.

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Table 1: Antioxidant content of *Azadirachta indica* in urban and coastal area

Antioxidants	Urban area	Coastal area
Alkaloids	$0.18 \pm 0.12\%$	$0.12 \pm 0.04\%$
Flavonoid	$1.33 \pm 0.27\text{mg/ml}$	$1.29 \pm 0.17 \text{mg/ml}$
Phenol	$0.33 \pm 0.1 \text{mg/ml}$	$0.25 \pm 0.22 \text{mg/ml}$
Saponin	$1.09 \pm 0.11 \text{mg/ml}$	$4.95 \pm 0.57 \text{mg/ml}$
Tannin	$0.05 \pm 1.15 \text{mg/ml}$	$0.03 \pm 0.53 \text{mg/ml}$
Terpinoid	$0.01 \pm 0.41 \text{mg/ml}$	$1.09 \pm 0.04 \text{mg/ml}$

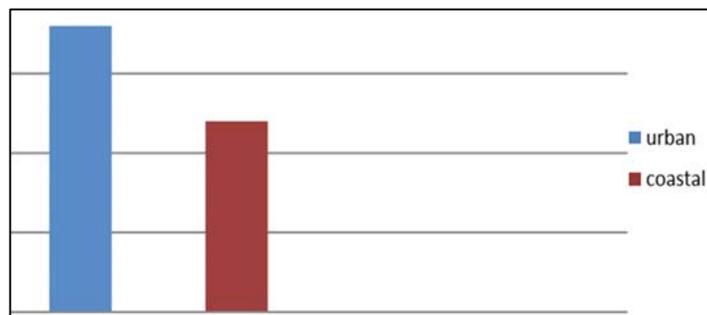


Fig 1: Total Alkaloid content (%) of *Azadirachta indica* in urban and coastal area
~ 360 ~

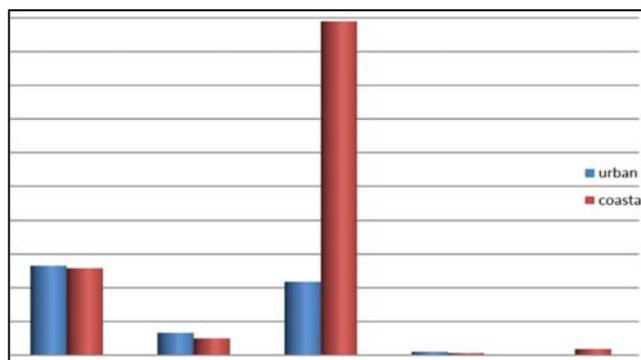


Fig 2: Total Flavonoid, Phenol, Saponin, Tannin, and Terpinoid content of *Azadirachta indica* in urban and coastal area

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