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Comparative study on the phytochemical and free radical scavenging (antioxidant) activity of ethanolic extracts from different parts of *Annona squamosa* L. (Annonaceae).

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A B S T R A C T

The present study aimed at the evaluation of preliminary Phytochemical and free radical scavenging (Antioxidant) potential of extracts of different parts of *Annona squamosa* L. plant and their correlation with the total phenolic contents (TPCs). The free radical scavenging activity of ethanol extracts were assessed by 2, 2-diphenyl-1-picrylhydrazyl assay and TPCs by Folin-Ciocalteu method. The percentage radical scavenging activities of samples were compared to the standard butylated hydroxyl anisole (BHA) and catechol. The leaf extract shown marked quantity of phenolics and promising free radical scavenging (antioxidant) activity followed by seed and root extracts. IC_{50} values for the scavenging activities of DPPH exhibited by the ethanol extracts of leaf, seed and root were 80µg/mL, 790 µg/mL, and 980 µg/mL, respectively. The total phenolic content of extracts of *Annona squamosa*. L exhibited the leaf (0.114mg/g), seed (0.091 mg/g) and root (0.022mg/g).The reductive ability of the extracts was found to increase in a concentration dependent manner. The results suggested that the phenolic compounds in extracts are most likely to be responsible for the observed free radical scavenging antioxidant activity.

Keywords: *Annona squamosa* L, custard apple, antioxidant activity, 2, 2-diphenyl-1-picrylhydrazyl assay, total phenolic content, phytochemicals.

A R T I C L E I N F O

CONTENTS

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1. Introduction

Reactive oxygen species (ROS) are constantly formed in the human body by normal metabolic action and these are exert oxidative damaging effects by reacting with nearly every molecule found in living cells including nucleic acids, proteins, lipids or DNA and may involve in several chronic and degenerative diseases. [1] Free radicals are highly reactive compounds, they are chemical species associated with an odd or unpaired electron and can be formed when oxygen interacts with certain molecules. They are neutral, short lived, unstable and highly reactive to pair with the odd electron and finally achieve stable configuration. Once formed these highly reactive radicals can start a chain reaction they are capable of attacking the healthy cells of the body, causing them to lose their structure and function. Cells may function poorly or die if this occurs. [2] The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease. Plants are potential sources of natural antioxidants. It produces various antioxidative compounds to counteract reactive oxygen species (ROS) in order to survive. [3] ROS, which include free radicals such as superoxide anion radicals (O^{2-}), hydroxyl radicals (OH^{-}) and non free-radical species such as H_2O_2 and singled oxygen ($^1O_2$), are various forms of activated oxygen. These molecules are exacerbating factors in cellular injury and aging process. The majority of the antioxidant activity is due to the flavones, isoflavones, flavonoids, anthocyanin, coumarin, lignans, catechins and isocatechins. [4] Antioxidant-based drug formulations are used for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer.

The family (Annonaceae), is a large family which comprising about 130 genera over 2000species; the most important genera having a largest number of species are *Annona*, with 120 species, from genera, the species of *Annona squamosa* .L commonly known as custard apple is cultivated throughout India, mainly edible fruit. The plant is tradionally used for the treatment of epilepsy, dysentery, cardiac problem, worm infection, constipation, hemorrhage, antibacterial infection, dysuria, fever, ulcer Anti fertility, Anti tumor and Abortifacient properties.[5-8] Several activities have been studied on the plant of *Annona squamosa*. L like Antiovulatory ,[9] Ameliorate hyperthyroidism,[10] Anti cancer,[11] Insecticidal agent, [12] Hypoglycemic anti diabetic effect, [13-14] Free radical scavenging [15], Antimutagenic, [16] Anthelmintic,[17] licicidal,[19] Hepatoprotective, [20] Antigenotoxic,[21] Antiplasmodial, [22] Molluscicidal, [23] Analgesic, [24] and Antimicrobial activity.[25-26] Aporphine alkaloids, [27-28] Flavonoids, [29] Glycoside, [30] Terpine derivatives, [31] and Novel diazepine, Squamoline, [32] were isolated from this plant.The aim of the present study was to evaluate the preliminary Phytochemical and free radical scavenging (antioxidant) potential of ethanol extracts of *Annona squamosa* .L leaf, seed and root on antioxidant potential of medical importance.

2. Matirials and Methods

Sample collection:

The fully matured *Annona squamosa* .L leaf, seed and root were collected month of august-2013 from around Gulbarga University, Gulbarga, Karnataka, India. The leaf, seed and root were identified and authenticated voucher specimen no. **HGUG19,** at herbarium, Department of Botany, Gulbarga University, Gulbarga, Karnataka, India.

Preparation of plant extracts:

The leaf, seed and root of *Annona squamosa* .L was washed, air-dried and ground into a fine powder (200g) was successively extracted with ethanol solvent in a soxhlet extractor for 24 h. The three extract were filtered through a watman No-1 filter paper and evaporated to dryness under reduced pressure. The plant parts used for extraction, yield of extractions, and nature of extracts were tabulated in Table -1. All the extracts were stored at 0^0c in airtight containers until need for further studies.

Phytochemical screening:

All the crude extracts were subjected to phytochemical screening for the presence of alkaloids, terpenoids, carbohydrates, glycosides, flavonoids, saponins and phenols using the standard method of harborne [33] and their results are tabulated in table-2.

Determination of Antioxidant capacity:

DPPH Radical Scavenging Activity [34]

Initially different concentrations (100µg, 200 µg, 400µg, 600 µg and 800 µg) of test sample and Butylated hydroxyl anisole (BHA) were taken in different test tubes. 1 milliliter of 0.1mM methonolic solution of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) was added to these tubes. The volume was adjusted to 5 ml by adding methanol and shaken vigorously. The tubes were allowed to stand at Room temperature for 30 min. The control was prepared as above without any extract. The absorbances of the sample were measured at 517 nm. Radical scavenging activity was calculated using the following formula.

Radical scavenging activity (%) = $A_{control} - A_{sample} / A_{control} \times 100$

Where $A_{control}$ is the absorbance of the control (DPPH + methanol) and A_{sample} is the absorbance of the sample (DPPH + methanol + sample).

Determination of IC_{50} values:

IC_{50} values were calculated from the linear regression of the percentage antioxidant activity against concentrations of extracts used. IC_{50} values were defined as the concentrations of samples required for the conversion of the half of the DPPH radicals to their more stable molecular counterparts 2, 2-diphenyl-1-picrylhydrazines.

Quantitative estimation of phenolic compounds:

Quantitative estimation of phenolic compounds by Folin-ciocalteu method (Malick and singh.1980). 500mg of the plant material was homogenated using pestle and mortar in 80% ethanol. The homogenized solution was centrifuged at 10,000 rpm for 20 minutes. The supernatant was retained and the extraction was repeated with the residue for 5-7 times. All the supernatants were mixed and evaporated to dryness. The residue thus obtained was dissolved in 5ml of distilled water and used for the estimation of total phenols. 1ml of the extract was mixed with 1ml of Folin-Ciocalteu reagent and 2ml of Sodium carbonate solution. Shaken the tube and heated in a boiling water bath for 1 min. and then cooled under running tap water. Diluted the solution to 25ml by adding distilled water and measured the absorbance at 650nm. With the help of standard curve obtained using different concentrations of catechol, calculated the amount of phenol present in the sample.

3. Result and Discussion

DPPH Radical Scavenging Activity:

The stable DPPH radical model is a widely used, relatively quick and precise method for the evaluation of free radical scavenging activity. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. Antioxidant on interaction with DPPH both transfer electron or hydrogen atom to DPPH and thus neutralizing its free radical character and convert it to 1-1,diphenyl-2- picryl hydrazine and the degree of discoloration indicates the scavenging activity of the drug. The reduction capacity of DPPH radical is determine by the decrease in its absorbance at 517 nm induced by antioxidants. [35] The decrease in absorbance of DPPH radical caused by antioxidants because of the reaction between antioxidant molecules and radical progress which results in the scavenging of the radical by hydrogen donation. It is visually noticeable as a change in color from purple to yellow. Hence DPPH is usually used as a substance to evaluate the antioxidant activity. [36,37] Table- 3 shown DPPH radical scavenging activity of standard BHA selected parts of *Annona squamosa*.L. The extracts of *Annona squamosa* .L leaf was showed highest DPPH scavenging activity and compared with BHA as standard IC_{50} value is 28 µg/ml. Antioxidant activity of *Annona squamosa* .L extracts were showed in table-4 following order: leaf (80 µg/ml) > *seed* (790 µg/ml) > root (980 µg/ml).

Total phenolic content:

Many plant extracts have been reported to have multiple biological effects, including antioxidant properties due to their phytoconstituents including phenolics. The antioxidant activity of phenolics is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides. The extract of *Annona squamosa* .L leaf showed highest total phenolic content and it was 0.114mg/g calculated as Catechol equivalent of phenols was detected. Table- 4 showed the total phenolic content of ethanolic extracts of *Annona squamosa*. L exhibited the following order: leaf (0.114mg/g) > seed (0.091 mg/g) > root (0.022mg/g).

Table 1: The plant part used, yield of extractions, and nature of ethanol extracts of *Annona squamosa*.L

Sl. No.	Part of the plant	% of yielded ext	Nature of ext
1.	Leaf	1.89	Greenish gummy
2.	Seed	1.72	White oily mass
3.	Root	1.86	Brownish gummy

Table 2: Preliminary Phytochemical screening of ethanol extracts of *Annona squamosa*.L

Sl. No.	Part of the plant	Secondary metabolites					
		Ster	Alka	Flavo	Phen	Glyco	Sap
1.	Leaf	+	+	+	+	+	-
2.	Root	+	+	-	+	+	+
3.	Seed	+	-	-	+	+	-

+ Indicate presence, - Indicate absent

Table 3: Results of DPPH radical scavenger activity of ethanol extracts of *Annona squamosa*. L

Sl. No.	Conc. (µg/ml)	% of radical scavenging activity.			
		Leaf ext	Seed ext	Root ext	Standard
1.	100	49.89	31.29	21.11	57.22
2.	200	53.39	35.88	26.47	65.86
3.	400	60.39	41.79	30.19	69.36
4.	600	65.86	46.71	34.79	72.21
5.	800	69.36	50.10	37.85	78.99

Table 4: Results of IC_{50} and TPCs value of ethanol extracts of *Annona squamosa*. L

Sl.No	Extracts of plant	IC_{50} (µg/mL.)	TPCs (mg/g of extract)
1.	Leaf	80	0.114mg/g
2.	Seed	790	0.091 mg/g
3.	Root	980	0.022mg/g
4.	Standard	28	-

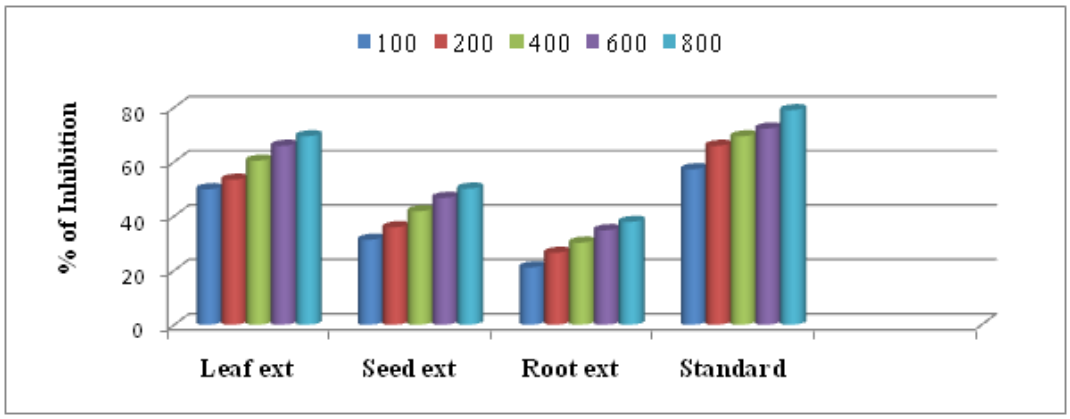

Figure 1: Results of DPPH radical scavenger activity of ethanol extracts of *Annona squamosa*. L

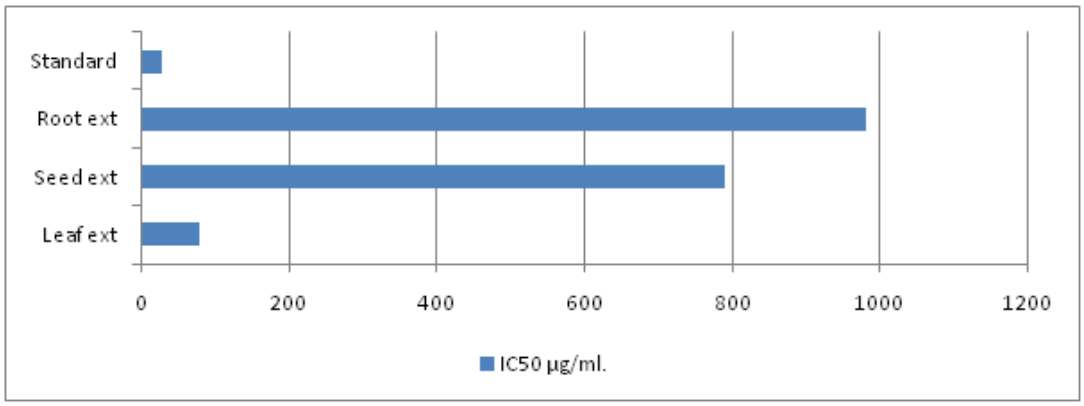

Figure 2: Results of IC_{50} of ethanol extracts of *Annona squamosa*. L

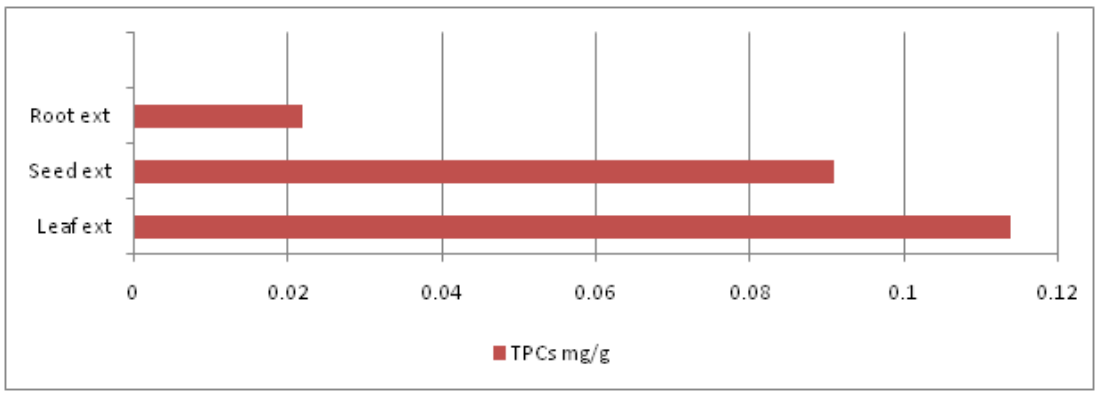

Figure 3: Results of TPCs value of ethanol extracts of *Annona squamosa*. L

4. Conclusion

The objective of the study was to determine the free radical scavenging potential of the whole plant, *Annona squamosa* .L showed in fig no-1, 2 and 3 and also to provide a comparative analysis between the leaf, seed and root ethanol extracts of the plant as a free radical scavenger to specify the extract with a better scavenging potential.

According to the findings, the ethanol leaf extract showed in fig no-2 and 3, the free radical scavenging activity and the highest amount of phenolics as compared to the seeds and root. As we know that free radicals are important contributors to several severe pathological conditions, the findings suggest that the extracts of the whole plant is equally useful as a source of natural antioxidants with subsequent health benefits. Hence, further investigation is required to isolate and elucidate the active principles, and to evaluate pharmacological properties.

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