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PHYTOCHEMICAL INVESTIGATION AND IN VITRO ANTIOXIDANT ACTIVITY OF COCCINIA GRANDIS FRUIT EXTRACT

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ABSTRACT:

The present study was undertaken to analyse the photochemical constituents both qualitatively and quantitatively in the fruit extract of Coccinia grandis. The DPPH, hydroxyl radical and hydrogen peroxide scavenging activity of the methanolic and ethyl acetate extract were found to be better than the other extracts used in the study.

Keywords: C.sinensis peel, HPTLC, antioxidant, phenolic compound, phyto constituents.

Oxygen is the most essential component for the survival of all living beings on earth. During the process of oxygen utilization in normal physiological and metabolic processes approximately 5% of oxygen gets univalent reduced to oxygen derived free radicals like hydrogen peroxide, hydroxyl, nitric oxide and superoxide radicals.
Free radicals are produced in large amount during metabolic disease conditions like diabetes, hypertension, atherosclerosis, urolithiasis, ulcers etc. This free radicals attack DNA, protein molecules, enzymes and cells leading to change in genetic material and cell proliferation (Cancer). Several anti oxidants of plants origin are experimentally proved and used as effective protective against oxidative stress [1].

Plants which contains carotenoids, flavonoids and Tannins can be utilized to scavenge the excess free radicals from human body [2].
Coccinia grandis (C. grandis) (L) Voigt is one such plant with medicinal properties. It belongs to the Curcubitaceae family commonly known as gourd melon and pumpkin family. It is a dioecious, perennial and herbaceous climber, with glabrous stems, tuberous roots and axillary tendrils. There predominantly found in the tropics and they are mostly annual vines. Its leaves are arranged alternately along the stems; the shape of the leaves varies from heart to pentagon shaped. (up to 10 cm wide and long). The upper surface of the leaf is hairless, whereas the lower is hairy. There are 3–8 glands on the blade near the leaf stalk. Tendrils are simple. Flowers are large, white and star-shaped. The calyx has five subulate, recurved lobes, each 2–5 mm long on the hypanthium; peduncle 1–5 cm long. The corolla is white, campanulate, 3–4.5 cm long, deeply divided into five ovate lobes [3]. The fruit is red, ovoid to elliptical, 25–60 mm long, 15–

35 mm in diameter, glabrous, hairless on stalks. The roots and stems are succulent, tuberous and most likely facilitate the plant to survive prolonged drought.

MATERIALS AND METHODS:

The fruits of C.grandis were peeled out and the sample were cleaned by tap water and dried in shadow dry. The fruits peel was powdered and used for the assay. About (10g) of C.grandis was weighed and soaked in 100 ml of water and Methanol.

1. Estimation of Alkaloids [4]

The sample (5g) was weighed into a beaker 200ml of 10% acetic acid in ethanol was added and covered to stand for 4 hrs. This was filtered and concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop-wise to the extract until precipitate was complete. The precipitate was collected and washed with dilute ammonium hydroxide. It was filtered and the residue which is the alkaloid, which was dried and weighed.

2. Determination of Saponin [5]

To 20g of the peel extract was added 100ml of 20% aqueous ethanol and heated in a hot water bath at 55 °C for 4hrs with continuous stirring. It was filtered and the residue re-extracted with another 200ml of 20% ethanol. This combined extract was reduced to 40ml over water bath at about 900C. It was transferred into a 250ml separating funnel and 20ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered. Ether layer was discarded. Purification process was repeated and 60ml of n-butanol was added. The combined n-butanol extract were washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation of the samples were dried in the oven to a constant weight. Saponin content was calculated as percentage.

3. Quantification of Total Phenolic content [6]

Folin-Ciocalteu Method: To 0.5ml of extract was added 2.5ml of a 1/10 aqueous dilution of Folin-Ciocalteu reagent mixed with 2ml of Disodium Carbonate (7.5%), after 5 minutes incubated at room temperature for 120minutes. The optical density read at 765nm by UV Spectrometer.

4. Estimation of Total flavonoid Content [7]

Aluminium Chloride Method: To 1ml of peel extract was added 1ml of 2% methanolic AlCl3.6H2O and was incubated for 10 minutes under room temperature. The optical density was taken at 430nm.

Antioxidant Assays:

1. **DPPH free radical scavenging activity Assay [8]**

   Extracts at concentrations (0.25 - 0.5 µg/ml) were added, at an equal volume of methanolic solution of DPPH. The mixture was allowed to react at room temperature in the dark for 30 minutes. Ascorbic acid was used as standard controls. Three replicates were made for each test sample. After 30 minutes, the absorbance (A) was measured at 518 nm and converted into the percentage antioxidant activity using the following equation:

% Inhibition = [(Ao - A1)/Ao] × 100, where Ao was the absorbance of the control and A1 was the absorbance in the presence of the sample of extract.

2. Hydroxyl radicals Scavenging activity assays [9]

Reaction mixture contained 60 µl of 1.0 mM $FeCl_2$, 90 µl of 1mM 1,10-phenanthroline, 2.4 ml of 0.2 M phosphate buffer, 150 µl of 0.17 M H_2O_2, and 1.5 ml of extract at various concentrations. The reaction was initiated by the addition of H_2O_2. After incubation at room temperature for 5 min, the absorbance of the mixture at 560 nm was measured with a spectrophotometer. The hydroxyl radicals scavenging activity was calculated.

% Inhibition = A0 − A1 / A0 x × 100 Where A0 was the absorbance of the control (without extract) and A1 was the absorbance of the extract or standard.

3. Hydrogen Peroxide scavenging activity assay [10]

A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer. Different concentrations of the plant extracts (0.025 - 0.05 µg/ml) in distilled water were added to a hydrogen peroxide solution (0.6 ml, 40 mM). The absorbance of hydrogen peroxide at 230 nm was determined after ten minutes against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging by the extracts and a standard compound was calculated as follows:

% Scavenged $[H_2O_2] = [(A_o - A_1)/A_o] \times 100$
Where A_o was the absorbance of the control, A1 was the absorbance in the presence of the sample of extract and standard.

Results and Discussion:

Qualitative and quantitative analysis of the plant extract was carried out and the bioactive secondary metabolites were determined.
Compounds such as carbohydrates, lipids, phytochemicals, pharmaceutics and pigments are present in the fruit extract of C. grandis. The results of the qualitative analysis show the presence of a number of bioactive compounds as shown in Table 1.

Table 1: Phytochemical Analysis Of Coccinia Grandisfruit Extract

S. No	Particulars	Phytochemical content
1.	Carbohydrates	++
2.	Tannins	++
3.	Flavonoids	++
4.	Steroids	++
5.	Cardiac glycosides	+
6.	Terpenoids	+
7.	Alkaloids	++
8.	Saponins	++
9.	Phenols	+
10.	Protein	+

++ = Presence, + =Moderate

Table 2: Total Metabolites In Coccinia Grandis Fruit Extracts

S. No	Name of the Parameter	Aqueous Extract	Methanolic Extract
1	Alkaloid mg/kg	1.32	2.65
2	Total Flavonoids mg/kg	2.58	3.65
3	Total Saponin mg/kg	1.01	1.21
4	Total Phenols mg/kg	0.98	1.42

The amounts of primary and secondary metabolites were analyzed and represent in Table 2. Phytochemical of estimation of C. grandis methanolic fruit extract showed higher flavonoids (3.65mg/kg) and alkaloids (2.65mg/kg) content than in aqueous extract. Flavonoids are a group of polyphenolic compounds which may influence the radical scavenging, inhibition of hydrolytic and oxidative enzymes and can act as anti-inflammatory agent. The other compounds such as, Phenols and Saponin were present in moderate quantity. Maximum amount of secondary metabolites were found in methanolic extract of C. grandis fruit extract but lower concentration were determined in aqueous extract. Besides, the phytochemical analyses that shown better results were subsequently followed further compound identification, properties and all other biochemical research.

EFFECT OF ANTIOXIDANT ACTIVITY:

In vitro antioxidant activity of the fruit extract of C. grandis was investigated in the present study by total antioxidant, DPPH, hydroxyl radical scavenging and metal chelating assays. The main function of the antioxidants is to neutralize the free radicals, which routinely produced in the biological system. Reactive oxygen species (ROS) readily combine and oxidize biomolecules such as carbohydrates, proteins and lipids and thus making them inactive with subsequent damage to cells, tissues and organs.

2, 2- Diphenyl-1-Picrylhydrazyl (DPPH) assay fruit extract of C. grandis:

DPPH radical scavenging ability is widely used as an index to evaluate the antioxidant potential of medicinal plants. In in vitro antioxidant studies (Figure 1) of the five different extracts of C. grandis of concentrations (20 – 200 μg/ml) was measured with ascorbic acid as the standard. The radical scavenging effect was found to increase with increasing concentrations of the extract. The control showed a maximum activity of 71.88 %. While the maximum activity in the plant extract was seen in the methanolic extract which was 56.2%at 200 μg/ml concentration and found to

increase as the concentration increases. The methanolic extract at all concentrations showed higher levels on inhibition compared to the other extracts ($P<0.01$) which ranged from 34.5% - 56.2%. Figure 1 shows comparative analysis of the different extracts at concentrations from 20 – 200 (µg/ml).

The study shows that the extract of C. grandis fruit exhibits the proton- donating ability and could serve as free radical inhibitors or scavengers. Antioxidant activity measured using DPPH accounts partially for the bound and insoluble antioxidants. Antioxidant activity and nutritional labeling data including vitamins, fibers, and minerals will aid in the interpretation of clinical results obtained as a source than can be tested in biological models for chronic disease. Thus it is important to know the antioxidant content is the knowing of basic nutritional information such as the protein, fiber, fat, mineral and vitamin contents.

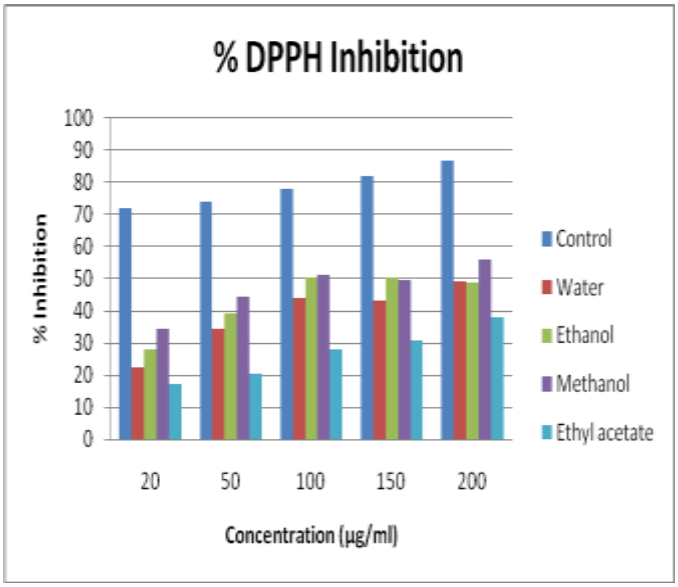

Fig 1: Percentage inhibition of DPPH by Coccinia grandis extracts

Hydroxy l radical scavenging assay of C. grandis fruit extract:

The scavenging activity was determined to be increased with the increase in the concentration of extract from 20 to 200µg/ml.The hydroxyl radical is the neutral form of the hydroxide ion (HO^-). Hydroxyl radicals are highly reactive and consequently short-lived. The OH scavenging activity of C. grandis fruit extracts was assessed by its ability to compete with salicylic acid for OH radicals in the OH generating/detecting system was performed.

Among the five different concentration 20 µg/ml of C. grandis fruit, the methanol extract showed the maximum inhibition however, the aqueous extract also showed comparable values. It was seen that lower concentration - 20µg/ml and the highest concentration tested 200µg/ml the activities were markedly significant in methanol, ethanol and aqueous extracts.
The scavenging effect of OH was investigated using the Fenton reaction and the results shown as the % inhibition rate in Figure 2. The observed difference in scavenging activity could be attributed to the concentration difference in main antioxidant components in various C. grandis fruit extracts.

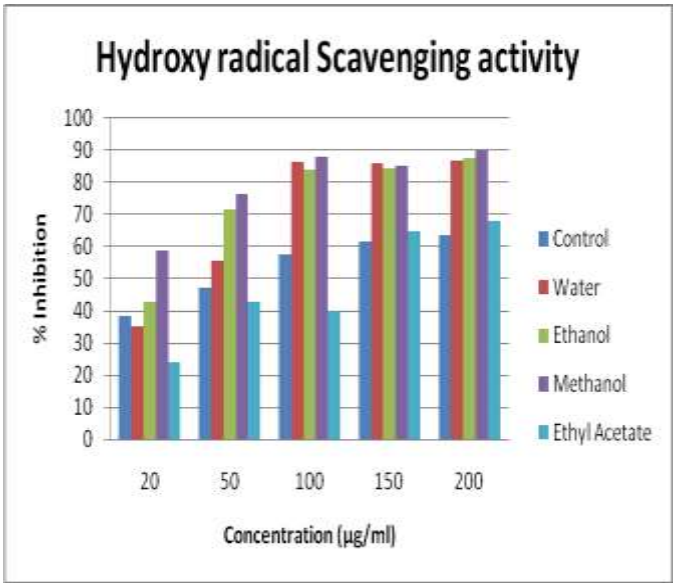

Fig 2: Hydroxyl radical scavenging assay by Coccinia grandis extracts

Hydrogen Peroxide scavenging assay of C. grandis fruit extract:

As shown in figure 3 C. grandisfruit extract also demonstrated hydrogen peroxide decomposition activity in a concentration dependent manner. The extracts in the range of 20-200 µg/ml was determined with ascorbic acid as standard. The standard ascorbic acid showed higher inhibition percentage at 200µg/ml when compared to the other concentrations. The methanolic extract showed maximum inhibition at all concentrations tested when compared to the other extracts.

Scavenging activity of H_2O_2 by the extract may be attributed to their phenolics, which can donate electrons to H_2O_2 thereby neutralizing it into water. The H_2O_2 is an evidence to scavenged, either directly or indirectly via its reduction product hydroxyl radical. In this method, when a scavenger is incubated with hydrogen peroxide, the decay or loss of hydrogen peroxide can be measured spectrophotometrically.

The different extracts of C. Grandis fruit also demonstrated hydrogen peroxide decomposition activity in a concentration dependent manner with 20-200 µg/ml was determined with the standard ascorbic acid. While all the extracts showed very similar inhibition percentages methanolic extract was found to be the best and it was found to be very close to the standard.

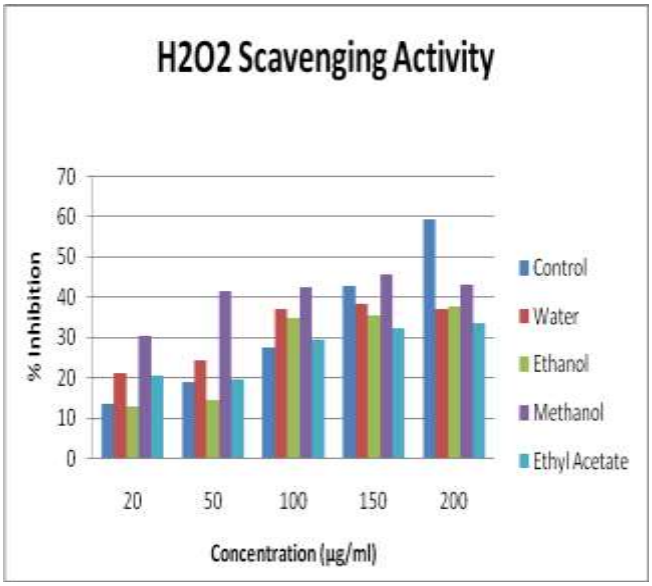

Fig 3: H_2O_2 scavenging assay by Coccinia grandis extracts

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