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Phytochemical Investigation and in Vitro Antioxidant Activity of Citrus Sinensis Peel Extract

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Abstract*: The present study was undertaken to analyse the photochemical constituents both qualitatively and quantitatively in the peel extract of Citrus sinensis. The studies showed that the major phyto constituents present in the methanolic extract was hesperidin and nobiletin. The DPPH and hydroxyl radical scavenging activity of the methanolic extract was found to be better than the other extracts used in the study. Finger printing assay of the extract was carried out using HPTLC and we report the presence of two flavonoids - nobelitin and hesperidin. Keywords: C.sinensis peel, HPTLC, antioxidant, phenolic compound, phyto constituents.*

Keywords: Phytochemical Investigation

I. INTRODUCTION

Citrus sinensis or sweet orange originated from East Asia, but is consumed all over the world as an excellent source of vitamin C, a powerful natural antioxidant that builds the body immune system. The main phytoconstituents present in orange fruit are D-limonene (90%), citral, citronella, nootkaton, sinesal, n-nonanal, n-decanal, ndodecanal, linalyl acetate, genanyl acetate, citronlyl acetate and anthranil acid methyl ester [1]. The peel of Citrus fruit have abundant source of flavonones and many polymehtoxulated flavones which are very rare in other plants [2]. The chief flavonoids found in Citrus species are limonene, hesperidin, narirutun, arinigin and eriocitrin etc [3]. In our study we have found some phytochemicals like nobelitin and hespiridin when we conducted a finger-printing analyses using HPTLC. The antioxidant nature of the extract was also assessed.

II. MATERIALS AND METHODS

Collection and authentication of drug: The fruits of C.sinensis were purchased from a local market of Thanjavur and the fruits were peeled out and the sample were cleaned by tap water and dried in shadow dry. Finally the dried fruit peels cut into pieces and grained for further research work. Preparation of extract: The fruits peel powder (10g) of C.sinensis was weighed and soaked in 100 ml of different solvents like aqueous, acetone, butyl alcohol, chloroform, Methanol, Petroleum ether : Hexane: Ethyl acetate and Chloroform: acetic acid for 72 hrs in 250C separately. The extracts were filtered and concentrated for were stored for further researct

Phytochemical studies: Qualitative analysis The preliminary phytochemical investigations of different solvent extracts of C. sinensis was analyzed as per the standard protocols, and determined the different constituents of phytochemicals monitored like carbohydrate, tannin, saponins, flavanoids, anthocyanin and β cyanin, quinones, phenol, coumarins, protein and amino acids and alkaloids were measured by standard procedures [4]

2.1 Quantitative analysis of Phytochemicals:

1. Estimation of Alkaloids [5]

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.

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2. Determination of Saponin [6]

To 20g of the peel extract was added 100ml of 20% aqueous ethanol and heated in a hot water bath at 550C 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 [7]

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 [8]

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.

Isolation and purification

Methanolic crude extract of 10g was subjected to column chromatography, alumina used as the stationary phase. Methanol was used to isolate the fraction (F1), by increasing the polarity of eluting agent as Ethyl acetate and Hexane (40:60 v/v) respectively (F2). The major fraction of F2 was further subjected to HPTLC analysis.

Identification of active Phenolic compounds by HPTLC

The HPTLC finger printing analysis used to identify the compounds by the following steps

Application of sample:

The column isolated faction (F2) applied in different concentration like 2µl, 4µl, 6µl and 8µl were loaded as 6mm length in the silica gel 60F254 (Merck) TLC plate using Hamilton syringe and CAMAG LINOMAT IV instrument.

Development of Spot

After the application of sample the TLC plate allow to dry and kept in saturated solvent chamber with mobile phase of water:formic acid: acetonitrile (49.5:0.5:60) for the development of mobile phase as ascending mode.

Scanning:

The developed plate was fixed in scanner stage of CAMAG TLC SCANNER 2 and scanning at UV254nm. The peak table, peak display and peak densitogram recorde

Derivation:

The developed plate undergoes with spraying solution of phenolic compounds and allows drying at 1000C in hot air oven.

Documentation of Photo:

The developed plate was allowed to dry the solvent by using hot air. Then the plate was kept in photodocumentation chamber with CAMAG REPROSTAR 3 and the image captured at white light, UV 254nm and UV 366 nm.

Fingerprint:

The HPTLC fingerprint of C.sinensis peel was recorded by using computer aided printing machine.

$$\text{Rf Value} = \frac{\text{Distance traveled by the sample from the base line}}{\text{Distance traveled by the solvent from the base line}}$$

Antioxidant Assays 1.

DPPH free radical scavenging activity Assay[9] 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:

$$\% = [(Ao - A1)/Ao] \times 100,$$

where Ao was the absorbance of the control and A1 was the absorbance in the presence of the sample of extract.

Hydroxyl radicals Scavenging activity assays [10]

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.

$$\% \text{ Inhibition} = A0 - A1 / A0 \text{ x} \times 100$$

Where A0 was the absorbance of the control (without extract) and A1 was the absorbance of the extract or standard.

Table 1. Phytochemical analysis of secondary metabolites in the peel extract of Citrus sinensis in various solvents and Graph 1. represent the total quantity of phytochemical compounds.

Table 1: Phytochemical analysis of secondary metabolites in the peel extract of Citrus sinensis in various solvents

s.no	Test	Aqueous	Acetone	Butyl Alcohol	Chloroform	Methanol	PHEA	CA
1	Carbohydrate	-	-	-	+	-	-	+
2	Tannins	-	-	-	-	-	-	-
3	Saponins	+	+	+	+	+	+	+
4	Flavanoids	+	+	+	+	+	+	+
5	Anthocyanin & β Cyanin	+	+	-	-	+	-	-
6	Quinones	-	+	-	-	+	-	-
7	Phenol	+	+	+	+	+	+	+
8	Coumarins	-			-		-	-
9	Protein & Aminoacids	-	-	-	-	+	-	-
10	Alkaloids	+	+	+	+	+	+	+
11	Steroids	-	-	-	-	-	-	-

III. DISCUSSION

The phytochemical analysis of the peel extract of Citrus sinensis showed the presence of alkaloids, flavonoids, saponin and phenolic compounds etc. The quantitative analyses showed the presence of very high levels of flavonoids and phenols in the methanolic extract. The HPTLC finger printing showed the presence of two major components viz., nobelitin and hesperidin. This high content of secondary metabolites suggests its role as a potential antioxidant. The free radicals are chemical species which contains one or more unpaired electrons that are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability. Radical scavenging activities

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are very important due to the deleterious role of free radicals in biological systems. In this study, it is evident that the peel extract of C. sinensis possess effective antioxidant activity

It is probably due to the presence of phytochemicals like flavonoids, alkaloids and phenols [11,12].

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[13,14]. The results suggest that C. sinensis methanoic extract is capable for scavenging free radicals so, it is able to prevent the initiation of free radical-mediated chain reactions by stabilizing reactive species before they can participate in deleterious reactions.

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