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ESTIMATION OF TOTAL PHENOLIC AND FLAVONOID CONTENT OF SOME CUCURBIT FRUIT PEELS AND *IN-VITRO* EVALUATION OF THEIR METHANOLIC EXTRACTS FOR ANTIOXIDANT POTENTIAL

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Peel, Free radical scavenging activity, TPC, TFC, FRAP

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ABSTRACT: Objective: To analyze In vitro antioxidant activity, total phenolic and flavonoid content of methanolic extract of some cucurbit fruit peels. **Methods:** Phytochemical screening of extracts was carried out. Aluminium chloride colorimetric method was used to estimate TFC (total flavonoid content), and TPC (total phenolic content) was measured by Folin-ciocalteu method. Two different assays- DPPH free radical scavenging and FRAP were used for the determination of antioxidant activity. **Results:** The total phenolic content was found to be maximum in Cucumis melo *i.e.* 42.64 ± 0.85 mg GAE/gdw, and minimum in *Cucumis sativus* i.e. 8.52 ± 0.74 mg GAE/gdw. Total flavonoid content was found to be maximum in *Cucumis melo i.e.* 4.71 ± 0.67 mg QE/gdw and minimum in *Citrullus lanatus i.e.*, 0.74 ± 0.31 mgQE/gdw. Results of DPPH Scavenging activity and FRAP assay revealed that all the peel samples had a significant antioxidant potential, where *Cucumis melo* show the highest antioxidant potential. **Conclusions:** Results of the present study showed that the selected fruit peels of cucurbit family have bioactive compounds of commercial importance. All the samples showed antioxidant activity, indicating that they can be used as a new source of natural antioxidant compounds and can be used for therapeutic purposes.

INTRODUCTION: Plants are used medicinally in different countries since ancient and are a source of potent and powerful drugs. Since time immemorial people have tried to find medications to alleviate pain and cure different illnesses [1]. Plants generally contain secondary metabolites like phenolics, flavonoids, glycosides, coumarins, saponins, terpenoids and alkaloids *etc*. which reveal their specific characteristic properties and attribute to their pharmacological properties [2]. The phyto-constituents have a major potential for developing phyto-medicines which are considered to be generally safe.

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Cucurbits are vegetable crops belonging to the family Cucurbitaceae, which primarily comprised species consumed as food worldwide. Cucurbits are excellent fruits in nature, having a composition of all the essential constituents required for the good health of humans [3]. Consumption of bottle gourd has been associated with a number of health benefits and can be regarded as a natural protector against diseases [4]. The large-scale industrial processing waste can be used as feed and fertilizers, but the household peel waste generated from the fruits and vegetables is generally thrown in the garbage.

Studies show that waste material such as peels produced from vegetables and fruits can be used successfully as a major source of phytochemicals and antioxidants. Studies have revealed the presence of a wide range of secondary metabolites, including flavonoids, triterpenoid, saponins, and

phenolic acids in gourd vegetables possessing distinct biological activities [5].

Gourd vegetables such as *L. siceraria*, *L. cylindrica*, *Luffa acutangula,* and *Cucurbita pepo* have been reported for the presence of secondary metabolites such as alkaloids, flavonoids, glycosides, steroids, and saponins, *etc*. which possess antioxidant activity [6]. An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons or hydrogen from substances to an oxidizing agent. Oxidation reactions can produce free radicals. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidative reactions [7].

Antioxidants are often reducing agents such as thiols, ascorbic acid, or polyphenols; various methods are used for analysing the antioxidant capacity. Ferric Reducing / Antioxidant Power (FRAP) method measures the ability of antioxidants to reduce ferric iron [8]. It is based on the reduction of the complex of ferric iron and 2, 3, 5 - triphenyl -1, 3, 4 - triaza - 2 -azoniacyclopenta-1, 4-dienechloride (TPTZ) to the ferrous form atlow pH. This reduction is monitored by measuring the change in absorption at 593 nm, using a diode-array spectrophotometer [9]. The DPPH method measures the ability of antioxidants to reduce 2, 2- diphenylpicrylhydrazyl (DPPH). The present study was carried out on the selected plants of the family Cucurbitaceae - *Citrullus lanatus, Cucumis sativus, Lagenaria siceraria, Momordica charantia,* and *Cucumis melo.*

MATERIALS AND METHODS:

Sample Collection and Processing: Fresh fruit samples were collected from the local market of Jaipur at different times. All samples were free from microbial and physical damage. The samples were cut, and the peels were separated from the flesh. The peels were shade dried at room temperature for 10 days. The dried peels were grounded in powdered form. The powered sample was stored in an airtight container and was kept to be used for further phytochemical analysis.

Sample Extraction: The dried powered of peels were extracted by cold percolation method using methanol as a solvent. 10 g of the dried powder was taken in a conical flask having 100 ml methanol and kept in an orbital shaker at 120 rpm for 24 h.

After 24 h, the extracts were filtered through Whatman filter paper no.1 for removal of peel particles and evaporated under vacuum.

Chemicals: 1, 1 - Diphenyl - 2 - picrylhydrazyl (DPPH), 2, 4, 6 - tri (2 - pyridyl) - s - triazine (TPTZ), folin-ciocalteu reagent, gallic acid, quercetin, and ascorbic acid were purchased from Sigma Aldrich (Steinheim, Germany). All other chemicals used in this study were of analytical reagent grade.

Determination of Total Phenolic Contents in the Plant Extracts: TPC (The total phenolic content) was determined by the Folin-Ciocalteau method [10, 11]. The reaction mixture was prepared by mixing 0.5 ml of methanolic solution (1 mg/ml) of extract, 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in water, and 2.5 ml 7.5% $NaHCO_3$. The mixture was allowed to stand for 15 min at 45 °C, and the phenols were determined by the spectrophotometric method. The absorbance was determined at λ_{max} = 765 nm. The samples were prepared in triplicate, and the mean value of absorbance was obtained. Blank was concomitantly prepared, with methanol instead of extract solution. The standard curve was prepared using the standard solution of Gallic acid in methanol in the range 100-1000 µg/ml. The total phenolic content was expressed in terms of gallic acid equivalent (mg of GAE/g of dry weight), which is a common reference compound.

Determination of Total Flavonoid Concentrations in the Plant Extracts: The concentrations of TFC (total flavonoid content) was determined using Aluminum chloride spectrophotometric method [12] with slight modifications. Plant extracts (0.5 ml) were dissolved with 1.5 ml of methanol, 0.1 ml of 10 % aluminium chloride, 0.1 ml of 1 M potassium acetate, and 2.8 ml of distilled water and incubated for half an hour at room temperature. The absorbance of the reaction mixture was measured at 415 nm. All experiments were prepared in triplicate, and the mean value of absorbance was obtained, and values were expressed in mean ± standard deviation. The standard curve was

prepared using the standard solution of quercetin in methanol. Total flavonoid content of the extracts was expressed in milligrams of quercetin equivalents /gdw.

Determination of Antioxidant Activity:

FRAP Assay (Reducing Ability Assay): FRAP assay method of Benzie and Strain, 199613 is used with slight modified for determination of the total antioxidant activity in the extract of plant part. The stock solutions included 300 mM acetate buffer (0.3 M acetic acid and 0.3 M sodium acetate), pH3.6,10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM HCI and 20 mM $FeCl_3$. $6H_2O$ (ferric chloride) solution. The fresh working solution was prepared by mixing 25 mL acetate buffer, 2.5 mL TPTZ, and 2.5 mL $FeCl_3$. $6H_2O$. The temperature of the solution was raised to 37 °C before use. 100 µl each of methanolic peel extract was allowed to react with 2900 µl of the FRAP solution for 30 min in the dark condition. Readings of the coloured product (ferrous tripyridyltriazine complex) were taken at 593 nm. The standard curve was linear between 100 and 1000 µM $FeSO_4$. Results are expressed in mM Fe (II)/g dry mass.

DPPH Radical Scavenging Activity: The DPPH radical scavenging activity of the extracts were evaluated by 1, 1 - diphenyl 2 - picryl - hydrazil (DPPH) using the method given by Bhat and Karim[14]. An aliquot (100 µL) of peel extract was mixed with 3.9 ml of 0.1 mM DPPH methanolic solution. The mixture was vortexed thoroughly and kept in the dark for 30 min. The absorbance was measured at 515 nm, against a blank of methanol. The radical's scavenging activity was calculated using;

$$(Abcontrol - Absample / Acontrol) \times 100$$

Where, Abcontrol is the absorption of the DPPH solution and Ab sample is the absorption of the DPPH solution after the addition of the sample. A linear graph of concentration *vs* percentage inhibition was prepared, and IC_{50} values were calculated. The antioxidant activity of each sample was expressed in terms of IC_{50} (defined as the amount of concentration required to inhibit DPPH radical formation by 50%), calculated from the inhibition curve.

Statistical Analysis: All experimental results were carried out in triplicate and were expressed as the average of three analyses with Standard Deviation. The IC_{50} values were also calculated by linear regression analysis.

RESULTS AND DISCUSSION: Plants contain many phytochemicals that are useful sources of natural antioxidants such as phenolic diterpenes, flavonoids, tannins, and phenolic acid [15]. Studies have reported that the total phenol content is a good indicator of antioxidant capacity of the plant. The total phenolic content varied in the peels. The total phenolic content was significantly varied in order of *C. melo*> *Citrullus lanatus*> *L. siceraria*> *Momordica charantia*>*C. sativus*. The total phenolic content is maximum in *Cucumis melo* 42.64 ± 0.85 mgGAE/gm DW and minimum in *Cucumis sativus* 8.52 ± 0.74 mgGAE/gm DW. Flavonoids are a widely distributed group of phenols that act as effective antioxidants [16]. Total flavonoid content ranges from 0.74 ± 0.31 mgQE/gm DWto 4.71 ± 0.67 mg QE/gm DW.

Cucumis melo showing the highest flavonoid content. Total phenolic and total flavonoid contents are shown in **Table 1.** The DPPH method is used to estimate the radical scavenging activity of antioxidant compounds. DPPH is stable at room temperature and produces a violet solution in a solvent.

Antioxidant compounds cause the discoloration of violet color to a yellow color indicating the scavenging activity of the added samples. Free radical scavenging activity for DPPH radical was expressed as IC_{50} value (the concentration required to scavenge 50 % of DPPH). *Cucumis melo* shows the highest antioxidant potential by followed by *Citrullus lanatus* while least antioxidant potential was reported to be in *Cucumis sativus*. The radical scavenging activity of all the peel extract increases with an increase in the concentration.

The differences in the flavonoid structures and their substitutions influence the phenoxyl radical stability, thereby affecting the antioxidant properties of the flavonoids [17]. FRAP assay measures the reducing potential of an antioxidant reacting with a ferric tripyridyltriazine (Fe^3+-TPTZ) complex and producing a colored ferrous tripyridyltriazine (Fe2+-TPTZ) [18] the peel extract of *Cucumis melo* shows the highest FRAP value.

Studies show that cucumber peel possesses bioactive compounds and has good antioxidant and antimicrobial activity [19].

The polyphenol anti-oxidant capacity has been taken into account as one of the outstanding mechanisms of action in inhibiting mutagenesis and cancer initiation by means of their capacity to scavenge ROS, activate antioxidant enzymes, prevent carcinogen-induced DNA adduct formation, enhance DNA repair and reduce overall oxidative DNA injury [20]. Results of DPPH and FRAP assays are shown in **Table 2** and **Fig. 1**.

TABLE 1: TOTAL PHENOLIC AND FLAVONOID CONTENT IN THE PEELS OF CUCURBITS

Name of Plant	Total Phenolic Content (mgGAE/gm DW)	Total Flavonoid Content (mgQE/gm DW)
Citrullus lanatus	36.48±0.25	3.21±0.43
Cucumis sativus	8.52±0.74	0.74±0.31
Lagenariasiceraria	30.45±0.52	2.81±0.55
Momordica charantia	28.52±0.42	2.11±0.54
Cucumis melo	42.64±0.85	4.71±0.67

Each value in the table is represented as mean ± SD (n=3), mg GAE/gm DW: milligram gallic acid equivalent per gram dry weight, mgQE/gm DW: milligram quercetin equivalent per gram dry weight.

TABLE 2: ANTIOXIDANT ACTIVITY OF METHANOLIC EXTRACTS OF DIFFERENT CUCURBITS PEELS

Name of Plant	DPPH (IC_{50} mg/ml)	FRAP (mMg-1DW)
Citrullus lanatus	0.17	6.21±0.84
Cucumis sativus	0.4	1.24±0.21
Lagenariasiceraria	0.2	5.21±0.55
Momordica charantia	0.24	3.41±0.54
Cucumis melo	0.12	8.21±0.57

IC_{50} value is defined as the amount of antioxidant necessary to decrease the radical concentration by 50%. Each value is expressed as mean ± SD. (Standard Deviation). FRAP values are indicated as weight (g) of mMFeSO4 in 100 g of the plant extracts.

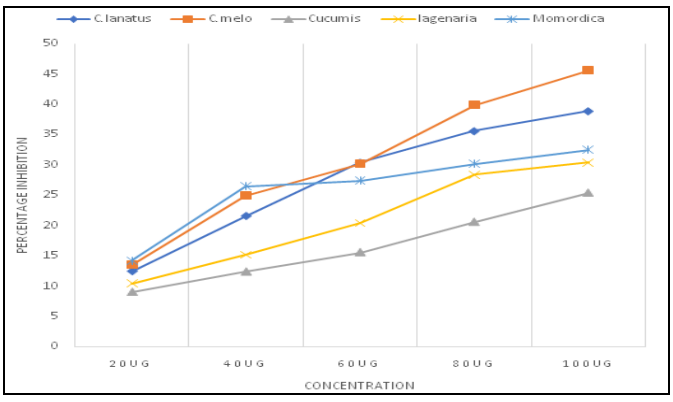

FIG. 1: ANTIOXIDANT ACTIVITY OF DIFFERENT EXTRACTS AT DIFFERENT CONCENTRATION

CONCLUSION: The findings of the study reveal that the peel extract of different cucurbits shows significant antioxidant properties. The phenolic content, flavonoid content, and antioxidant activity were highest in muskmelon peel, although other cucurbit peels also show significant phytochemical constituents. The peels are the low-cost source for different value-added compounds and are a cheap source of antioxidants, so there is a scope of future utilization of the peel for many therapeutic purposes.

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CONFLICTS OF INTEREST: Nil

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