Analysis of pharmacognostical standardization, antioxidant capacity and separation of phytocompounds from five different vegetable peels using different solvents

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ABSTRACT

Vegetables are one of the most preferred food commodities and can be consumed either raw or as processed due to their health-promoting nutrients. In the present work, analysis of pharmacognostical standards, antioxidant capacity, and separation of phytocompounds through thin layer chromatography (TLC) from cabbage, cauliflower, pea, carrot, and potato peels were carried out. Microscopic analysis revealed the presence of wood fibers, trichomes, crystals, and annular xylem vessels in the vegetable peels. Physicochemical analysis showed that all the vegetable peel samples which were analysed have low (7.08%-10%) moisture content. The total ash content of vegetable peels varied in cauliflower peels (1.95±0.58) to the peels of pea (19.86±1.9). The content of acid insoluble ash varied from 1.46±0.63 to 3.09±0.59 in cauliflower and pea. Potato peel has the lowest water-soluble ash content (1.16±1.90) as compared to other peels. The highest pH value was found in the peels of pea (7), while the lowest pH was found in the peels of cabbage (4). Among all extracts, the petroleum ether extract has shown the greatest yield (5.6±0.45). The fluorescence analysis showed various colours like green, brown, pale green, and yellow under different chemical treatments. Different types of pri-secondary metabolites were detected in small, moderate, and high amounts and notified to provide numerous health benefits to humans. In case of DPPH assay, aqueous extract of cauliflower has shown the low value of IC₅₀ (24.82 µg/ml) in comparison to standard, suggested the higher antioxidant activity of the extract. Among all the extracts, aqueous and methanol extracts of cauliflower have shown the better reducing and total antioxidant activity in comparison to standard. TLC profiling of methanolic extract of cabbage and cauliflower peels revealed the presence of different compounds of varying Rf values. Above results indicate that the food waste consists of valuable components and may be utilized as noticeable and cheap source in pharmaceuticals for the treatment of several life-threatening diseases.

Introduction

Due to the change in diet habits and increasing population, processing and production of horticultural crops, mainly vegetables, have been remarkably noticed as growing tool to fulfil the demands (Schreinemachers et al., 2018). Several studies suggested that vegetables are low in calories and rich in selected minerals, antioxidants, and fibres. It has been found in some of the researches that vegetables are rich in potassium and have relatively low sodium content. Due to all these

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amazing benefits, vegetables hold a unique contribution in a healthy diet (Chauhan et al., 2021). Recent studies suggest that diseases like gastric cancers and cardiac problems are protected in a better way by including vegetables in our diet. The anti-inflammatory and antiproliferative capacities of the phytochemicals and antioxidants makes them effective for the prevention of cancer and inflammation (Gazdik et al., 2008; Sarkar et al., 2022). The phytochemicals present in vegetable peels can also be utilized as food additives, colouring agents, biopesticides, fragrances, flavours, agrochemicals, and pharmaceuticals (Saha et al., 2012). But now a days, the scenario is changing and the agroindustrial wastes, mainly the vegetable peels, have started gaining more attention than previous days because they have potential to provide multiple benefits to the society in the field of medicine. However, the main obstacle, which has stopped the promotion of uses of vegetable peels in the developed nations, is no proper evidence of documentation. Therefore, the aim of present study was to evaluate the pharmacognostical standards, antioxidant activity, and presence of different bioactive phytoconstituents of the five different vegetable peels.

Material and Methods

Collection of plant materials
Vegetables including Cabbage (Brassica oleracia var. Capitata), Cauliflower (Brassica oleracea var. Botrytis), Pea (Pisum sativum), Carrot (Daucus carota subsp. Sativus) and Potato (Solanum tuberosum L.) were obtained from the local wholesale market and their inedible part such as peels were separated with a peeler or knife. Then the vegetable peels were collected, washed, and shade dried, respectively.

Preparation of plant extracts
The shade dried samples were powdered with the help of grinder. Twenty-five grams of each sample was macerated sequentially using 100 ml of different solvent [petroleum ether (PET), chloroform (CH), methanol (ME), and water (AQ)]. Each extract of vegetable peels was air dried by the help of rotatory evaporator. After drying, the extracts were kept in the desiccator for one or two days and then were kept in the air tight containers at 5°C for further use (Sharma and Janmeda, 2017).

Organoleptic and microscopic study
Different vegetable peel samples were examined morphologically and various microscopic characters were determined after the staining of samples as described by Janmeda and Sharma (2013).

Physicochemical analysis
Physicochemical parameters such as moisture content, total ash content, acid insoluble ash content, water soluble ash content, pH of 1% and 10% solution and extractive value were determined by the method of Mushtaq et al. (2014). Fluorescence was observed at different wavelengths of UV-Visible light as reported by Sharma and Janmeda (2013).

Phytochemical evaluation
Different phytochemicals from the different samples of vegetable peels were determined by using the standard methods (Saxena et al., 2013; Banu and Cathrine, 2015).

Determination of in vitro antioxidant activity

2, 2-Diphenyl-1-picrylhydrazyl scavenging activity (DPPH)

DPPH assay was determined by using the protocol of Chaudhary and Janmeda (2022) with slight modifications. To one ml (0.2-0.5 mg/ml) of sample and ascorbic acid (standard), 4 ml of DPPH solution (25 mg/ml) which was prepared in methanol was added. The solutions were shaken and allowed to incubate in dark for 30 min. After 30 min, the absorbance of the solution was recorded at 517 nm using methanol as blank by the help of the below mentioned formulae:

\[
\text{Inhibition concentration (\%)} = \left( \frac{\text{Absorbance of control}}{- (\text{Absorbance of test sample})} \right) \times 100
\]

Ferric reducing antioxidant power (FRAP)
The reducing power of a sample was determined by using the FRAP assay (Benzie and Strain, 1999). Briefly, the FRAP reagent was prepared by mixing the acetate buffer (300 mM, pH 3.6), a solution of 10 mM TPTZ in 40 mM HCl, and 20 mM FeCl₃ at 10:1:1 (v/v/v). A potential antioxidant can reduce the ferric ion to the ferrous ion and resulted in the formation of blue coloured complex, whose absorbance was increased at 593 nm.

Total antioxidant capacity determination (TAC)
Phosphomolybdate method was applied to determine the total antioxidant capacity (TAC) of
the different samples (Prieto et al., 1999). An aliquot of 0.4 ml (mg/ml) of each sample was mixed with 4 ml of reagent (4 mM ammonium molybdate, 0.6 M sulphuric acid, 28 mM sodium phosphate) solution. Then the mixture was shaken and was incubated at 95°C for 90 min in the water bath. Finally, the absorbance of the sample was recorded at 765 nm against the blank sample.

**Thin layer chromatography (TLC)**

TLC of the selected extracts was carried out with various solvent phase by using silica gel. For TLC analysis, Silica gel 60 F254 TLC (Merck, Germany) plates were utilized. The marking on the plate were made with the help of soft pencil. Glass capillaries were utilized to load the 1-μl of sample on TLC plate and then the plate was allowed to run in the presence of different solvent system. When the solvents reached to a certain height on the TLC plates, we removed the plate from the TLC chamber and allowed it to dry. Then the bands were observed in iodine chamber and on UV transilluminator. The movement of the analyte was expressed by its retention factor (R_f) values which was calculated by the help of below mentioned formulae (Gujjeti and Mamidala, 2013).

\[
R_f = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent from TLC plates}}
\]

Where, R_f is retention factor

**Statistical analysis**

All the assays and test were performed in triplicates and their outcomes were expressed as mean ± standard deviation (SD).

**Results and Discussion**

**Organoleptic evaluation**

Organoleptic evaluation and characteristic features of powdered drug of all five samples of vegetable peels are listed in Table 1 and Figure 1. The quality of vegetable peels mainly comprises of five primary attributes, 1) taste, 2) odour, 3) adulteration, 4) colour, 5) texture and the examination of these primary characteristics is generally very useful in the development of new products and in determining the product standards (Shewfelt, 1993).

**Powder microscopic analysis**

Powder microscopy is used to determine the specific microscopic characters after staining it with different staining solutions. The adulterants can be detected by doing a comparative study with authenticated sample (Amponsah et al., 2014).

**Cabbage peel**

The very fine powder of vegetable peels was mounted in glycerine and was stained with iodine and phloroglucinol. Microscopic analysis revealed the presence of wood fibers, trichomes, crystals, and annular xylem vessels from the cabbage peel as shown in Figure 2.

**Cauliflower Peel**

Powder microscopic analysis of cauliflower peel revealed the presence of different types of fibers and crystals as shown in Figure 3.

**Pea Peel**

The powder of pea peel shows the presence of fibers, wood fibers, different types of crystal, and xylem vessels as shown in Figure 4.

**Carrot Peel**

The microscopic analysis of powder revealed the presence of parenchyma, fibers, trichomes, and calcium oxalate crystals from the carrot peel as shown in Figure 5.

**Potato Peel**

Powder microscopic analysis revealed the presence of wood fibers, simple fibers, trichome, crystals, and spiral xylem vessel as shown in Figure 6.

**Physicochemical properties of different vegetable peels**

The physicochemical parameters of five different vegetable peels were determined in order to detect any type of adulteration and improper handling of plant material. Lower content of moisture represents the higher stability and less chances of microbial growth that ultimately increases the shelf life of product (Alam and us Saqib, 2015). Results showed that all the vegetable peel samples which were analysed have low moisture content as shown in Table 2. One of the other parameters is ash content that gives information regarding the presence of organic, inorganic and any other impurities in the sample (Alam and us Saqib, 2015). The total ash content of vegetable peels varied from 1.95±0.58 in cauliflower peels to 19.86±1.9 in the peels of pea as shown in Table 2. The results of acid insoluble (Table 2) and water soluble ash content (Table 2) of different vegetable
Table 1: Organoleptic Characters of different vegetable peel powder

| Samples     | Taste         | Odour        | Adulteration | Colour       | Texture |
|-------------|---------------|--------------|--------------|--------------|---------|
| Cabbage     | Bitter        | Characteristic| Nil          | Moss green   | Rough   |
| Cauliflower| Sweet and Sour| Characteristic| Nil          | Bronze       | Rough   |
| Pea         | Bitter        | Characteristic| Nil          | Moss green   | Rough   |
| Carrot      | Sour          | Characteristic| Nil          | Sage green   | Fibrous |
| Potato      | Sour          | Characteristic| Nil          | Cider colour | Smooth  |
| Powder drug | Cabbage       | Cauliflower  | Pea          | Carrot       | Potato  |
| Color       | Brown         | Brown        | Light brown  | Dark brown   | Brown   |

Odour Characteristic

Figure 1: Morphological features and characteristics of cabbage, cauliflower, pea, carrot, and potato vegetable peels.

Fluorescence analysis of different vegetable peel powder
The fluorescence analysis is utilized as a tool to determine the constituent and chemical nature of the herbal drug. The observations of fluorescence analysis of cabbage, cauliflower, pea, carrot, and potato are presented in Table 4, and 5.

Phytochemical screening
The phytochemical screening of different extracts of vegetable peels is shown in Table 6, and 7. This screening helps in determining the presence of various pharmacologically active compounds (Pandiyan and Illango, 2022). The results of present study revealed that protein, carbohydrate, cardiac glycosides, steroids, terpenoids, fats and oils are present in these vegetable peels. These secondary metabolites help in providing the defence mechanism to plant, and in turn provide numerous health benefits to humans (Sharma et al., 2022).
Figure 2: Powder microscopic analysis of cabbage peel. a. wood fibers, b. trichomes, c. crystals, d. annular xylem vessels.

Figure 3: Powder microscopic analysis of cauliflower peel. a. fibers with lumen, b. fibers, c. & d. crystal.

Figure 4: Powder microscopic analysis of pea peel. a. & b. fibers, c. different types of crystals, d. wood fibers, e. xylem vessels

Figure 5: Powder microscopic analysis of carrot peel. a. parenchyma, b. fibers, c. trichomes, d. calcium oxalate crystal.

Figure 6: Powder microscopic analysis of Potato peel. a. wood fibers, b. fibers, c. trichomes, d. crystals, e. spiral xylem vessels.
Table 2: Physicochemical properties of different vegetable peels

| Peel Powder | Moisture content % | Total ash content % | Acid insoluble ash content % | Water soluble ash content % | pH 1% | pH 10% |
|-------------|-------------------|---------------------|-----------------------------|-----------------------------|-------|--------|
| Cabbage     | 7.86              | 4.6±2.88            | 2.45±1.40                   | 4.1±2.62                    | 5.56±0.42 | 4.0±0.10 |
| Cauliflower | 7.52              | 1.95±0.58           | 1.46±0.63                   | 1.81±0.51                   | 6.36±0.20 | 4.9±0.10 |
| Pea         | 7.08              | 19.86±1.90          | 3.09±0.59                   | 16.31±1.88                  | 5.91±0.10 | 5.7±0.10 |
| Carrot      | 8.30              | 8.91±2.30           | 2.41±0.15                   | 3.52±1.98                   | 5.30±0.10 | 4.8±0.10 |
| Potato      | 10.00             | 3.06±1.88           | 1.62±1.02                   | 1.16±1.90                   | 5.9±0.9  | 4.5±0.10 |

Note: Mean ± SD

Table 3: Preliminary phyto-profile of different vegetable peel extracts

| Vegetable samples | Solvent | P.I. | B.P. of solvents (°C) | Colour          | Consistency | Nature | % yield ± SD |
|--------------------|---------|------|-----------------------|-----------------|-------------|--------|--------------|
| Cabbage            | PET     | 0.0  | 60-80                 | Olive green     | Sticky      | Solid  | 1.69±0.1     |
|                    | CH      | 4.1  | 61.2                  | Fern green      | Sticky      | Solid  | 2.9±0.51     |
|                    | ME      | 5.1  | 64.2                  | Olive green     | Sticky      | Semi-solid | 3.7±0.11     |
|                    | AQ      | 9.0  | 100                   | Greenish brown  | Dry         | Solid  | 3.9±0.21     |
| Cauliflower        | PET     | 0.0  | 60-80                 | Army green      | Sticky      | Solid  | 2.08±1.11    |
|                    | CH      | 4.1  | 61.2                  | Sacramento green| Sticky      | Solid  | 2.1±0.13     |
|                    | ME      | 5.1  | 64.2                  | Fern green      | Sticky      | Semi-solid | 3.12±0.12    |
|                    | AQ      | 9.0  | 100                   | Army green      | Dry         | Solid  | 4.01±0.13    |
| Pea                | PET     | 0.0  | 0.0                   | Moss green      | Dry         | Solid  | 5.6±0.45     |
|                    | CH      | 4.1  | 4.1                   | Crocodile green | Dry         | Solid  | 2.01±1.12    |
|                    | ME      | 5.1  | 5.1                   | Fern green      | Sticky      | Semi-solid | 4.5±0.19     |
|                    | AQ      | 9.0  | 9.0                   | Army green      | Dry         | Solid  | 4.9±1.1      |
| Carrot             | PET     | 0.0  | 60-80                 | Brick red       | Sticky      | Solid  | 1.19±0.2     |
|                    | CH      | 4.1  | 61.2                  | Brick red       | Dry         | Solid  | 1.09±0.3     |
|                    | ME      | 5.1  | 64.2                  | Brick red       | sticky      | Semi-solid | 2.01±0.1     |
|                    | AQ      | 9.0  | 100                   | Brownish red    | sticky      | Solid  | 1.1±0.22     |
| Potato             | PET     | 0.0  | 0.0                   | Ivory brown     | Dry         | Solid  | 0.3±0.6      |
|                    | CH      | 4.1  | 4.1                   | Tortilla brown  | Dry         | Solid  | 0.67±0.4     |
|                    | ME      | 5.1  | 5.1                   | Ivory brown     | Sticky      | Semi-solid | 3.2±1.1      |
|                    | AQ      | 9.0  | 9.0                   | Dark brown      | Dry         | Semi-solid | 4.08±1.9     |

Note: PET: Petroleum ether, CH: Chloroform, ME: Methanol, AQ: Aqueous

Antioxidant potential of vegetable peels

DPPH scavenging assay

DPPH assay measured the antioxidant potential of plant extracts which reduces the DPPH free radicals to hydrazine with the change of violet colour to yellow colour and reduction in absorbance at 517 nm in a concentration dependent manner (Hossen et al., 2021; Chaudhary and Janmeda, 2022). The inhibitory concentration of different extracts like PET extract, CH extract, ME extract and AQ extracts of cabbage (CB), cauliflower (CA), pea (PE), carrot (CT) and potato (PT) is listed in Table 8. The IC₅₀ values of DPPH assay was in the following order: CAAQ>ST>PTAQ>CBAQ> CTAQ> PEME>PEAQ> CBME>CACH>CBPET> CTME> PTME>PECH>PTCH>CAPET>PTPET>CPEP>CTCH. Among all extracts, CH extract of CT has shown the highest IC₅₀ value whereas the AQ extract of CA has shown the low value of IC₅₀ in comparison to standard, suggested the higher antioxidant activity of the extract. Kalpna et al. (2011) reported the IC₅₀ value of 200 µg/ml and 380 µg/ml from the acetone and methanol extract of Solanum tuberosum whereas Biswas et al. (2021) reported the
Figure 7: Sequential extracts of different vegetable peels. (a.-e.) petroleum ether extract, (f.-j.) chloroform extract, (k.-o.) methanol extract, (p.-t.) Aqueous extract of cabbage, cauliflower, pea, carrot, and potato.

Table 4: Fluorescence characteristics of cabbage, cauliflower and pea peels

| Reagents used  | Cabbage |  | Cauliflower |  | Pea |  |
|---------------|---------|---------------|-------------|---------------|-------------|
|               | Visible | High UV (366 nm) | Low UV (254 nm) | Visible | High UV (366 nm) | Low UV (254 nm) | Visible | High UV (366 nm) | Low UV (254 nm) |
| HCl           | Light green | Purple | Dark army green | Reddish brown | Purple | Dark army green | Reddish brown | Purple | Dark army green |
| H<sub>2</sub>SO<sub>4</sub> | Light green | Purple | Light green | Brown | Black | Light green | Brown | Black | Light green |
| HNO<sub>3</sub> | Light green | Purple | Light green | Black | Violet | Light green | Black | Violet | Light green |
| Picric acid   | Light green | Purple | Green | Light green | Light brown | Green | Light green | Light brown | Green |
| Ethyl acetate | Light green | Purple | Dark green | Charcoal black | Black | Dark green | Charcoal black | Black | Dark green |
| Glacial acetic acid | Light green | Purple | Dark brown | Light green | Brown | Dark brown | Light green | Brown | Dark brown |
| Methanol      | Light green | Purple | Dark green | Light green | Purple | Dark green | Light green | Purple | Dark green |
| Chloroform    | Light green | Purple | Dark green | Light brown | Purple | Dark green | Light brown | Purple | Dark green |
| Water         | Light brown | Purple | Army green | Reddish orange | Purple | Army green | Reddish orange | Purple | Army green |
| Benzene       | Dark brown | Purple | Dark black | Dark brown | Black | Dark black | Dark brown | Black | Dark black |
| NaOH          | Light green | Purple | Dark green | Reddish yellow | Brown | Dark green | Reddish yellow | Brown | Dark green |
| FeCl<sub>3</sub> | Light green | Purple | Dark green | Light green | Purple | Dark green | Light green | Purple | Dark green |
| NH<sub>4</sub>OH | Light green | Purple | Dark green | Light green | Purple | Dark green | Light green | Purple | Dark green |
| Iodine        | Light green | Purple | Dark green | Light green | Purple | Dark green | Light green | Purple | Dark green |
| Powder        | Brown | Purple | Greenish brown | Reddish brown | Purple | Greenish brown | Reddish brown | Purple | Greenish brown |
Table 5: Fluorescence characteristics of peels of carrot and potato

| Reagents used | Carrot | Potato |
|---------------|--------|--------|
|               | Visible | High (366 nm) | Low UV (254 nm) | Visible | High (366 nm) | Low UV (254 nm) |
| HCl           | Dark brick red | Purple | Blackish brown | Dark brick red | Fluorescent purple | Blackish brown |
| H₂SO₄         | Dark brick red | Dark purple | Blackish brown | Dark brick red | Dark purple | Blackish brown |
| HNO₃          | Brown red | Dark purple | Blackish brown | Brown red | Dark purple | Blackish brown |
| Picric acid   | Brown red | Dark purple | Dark brown | Brown red | Dark purple | Dark brown |
| Ethyl acetate | Brown red | Purple | dark green | Brown red | Purple | Fluorescent dark green |
| Glacial acetic acid | Brown red | Purple | Dark green | Brown red | Purple | Dark green |
| Methanol      | Brick red | Purple | Dark brown | Brick red | Purple | Dark brown |
| Chloroform    | Brick red | Purple | Dark brown | Brick red | Purple | Dark brown |
| Water         | Reddish brown | Purple | Blackish brown | Reddish brown | Purple | Blackish brown |
| Benzene       | Reddish brown | Purple | Blackish brown | Reddish brown | Fluorescent purple | Blackish brown |
| NaOH          | Reddish brown | Purple | Blackish brown | Reddish brown | Fluorescent purple | Blackish brown |
| FeCl₃         | Green | Purple | Green | Fluorescent green | Fluorescent green | Fluorescent green |
| NH₄OH         | Light brown | Purple | Green | brown | Cream | Green |
| Iodine Powder | Light brown | Purple | Green | Light brown | Fluorescent purple | Green |

Table 6: Phytochemical screening of cabbage, cauliflower and pea

| S.No | Test                  | Cabbage | Cauliflower | Pea |
|------|-----------------------|---------|-------------|-----|
|      | PET CH ME AQ          | PET CH ME AQ |
|      | **Proteins**          |          |             |     |
| 1.   | Millon’s test         | -        | -           | +   |
| 2.   | Sulphur containing protein | -        | -           | +   |
|      | **Carbohydrates**     |          |             |     |
| 3.   | Fehling’s test        | -        | -           | +   |
| 4.   | Benedict’s test       | -        | -           | +   |
|      | **Fats and oil**      |          |             |     |
| 5.   | Filter paper test     | -        | -           | +   |
|      | **Alkaloids**         |          |             |     |
| 6.   | Mayer’s test          | -        | -           | -   |
| 7.   | Tannic acid test      | +        | -           | +   |
|      | **Flavonoids**        |          |             |     |
| 8.   | Sulphuric acid test   | ++       | +           | -   |
| 9.   | Alkaline reagent test | +        | ++          | ++  |
|      | **Phenol and tannins**|          |             |     |
| 10.  | Ferric chloride test  | -        | -           | +   |
| 11.  | Nitric acid test      | -        | -           | +   |
|      | **Cardiac glycosides**|          |             |     |
| 12.  | Legal’s test          | -        | -           | ++  |
| 13.  | Keller-killiani test  | +        | ++          | ++  |
|      | **Steroids**          |          |             |     |
| 14.  | Salkowski test        | -        | -           | -   |
| 15.  | Foam test             | -        | -           | ++  |
| 16.  | Olive oil test        | -        | -           | +   |

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Table 7: Phytochemical analysis of carrot and potato

| S.No. | Test                  | Carrot |      |      |      | Potato |      |      |      |
|-------|-----------------------|--------|------|------|------|--------|------|------|------|
|       |                       | PET    | CH   | ME   | AQ   | PET    | CH   | ME   | AQ   |
| 1.    | Millon’s test         | ++     | -    | +    | +    | -      | -    | -    | ++   |
| 2.    | Sulphur containing    | -      | ++   | -    | +    | ++     | +    | -    | -    |
|       | protein               |        |      |      |      |        |      |      |      |
| 3.    | Fehling’s test        | -      | +    | -    | -    | -      | -    | -    | -    |
| 4.    | Benedict’s test       | -      | -    | +++  | +    | -      | -    | ++   | ++   |
| 5.    | Filter paper test     | -      | -    | -    | -    | -      | -    | -    | -    |
| 6.    | Mayer’s test          | +      | +    | ++   | +    | -      | -    | -    | -    |
| 7.    | Tannic acid test      | +      | +    | +++  | ++   | +      | +    | +    | +    |
| 8.    | Sulphuric acid test   | -      | -    | -    | -    | -      | -    | -    | -    |
| 9.    | Alkaline reagent test | -      | +    | ++   | ++   | +      | ++   | ++   | ++   |
| 10.   | Ferric chloride test  | -      | -    | +    | +    | -      | -    | -    | -    |
| 11.   | Nitric acid test      | -      | -    | +    | +    | -      | -    | -    | +    |
| 12.   | Legal’s test          | -      | -    | -    | +++  | -      | -    | ++   | +    |
| 13.   | Keller-killiani test  | +      | +    | -    | +    | -      | -    | -    | -    |
| 14.   | Salkowski test        | -      | -    | +    | +    | -      | -    | -    | -    |
| 15.   | Foam test             | -      | -    | +    | +    | +      | -    | -    | -    |
| 16.   | Olive oil test        | +      | -    | -    | +    | ++     | +    | -    | -    |
| 17.   | Salkowski test        | +      | +    | +    | +    | +      | +    | -    | -    |
| 18.   | Hydrochloric acid test| ++     | -    | +    | +    | -      | ++   | -    | ++   |

PET: Petroleum ether, CH: chloroform, ME: Methanol, AQ: aqueous

Table 8: IC50 values of DPPH, FRAP, and TAC assay of different vegetable peel extracts

| Different vegetable peel samples | DPPH Values (µg/ml) | FRAP Values (µMFe(II)/g) | TAC Values (µg/ml) |
|---------------------------------|---------------------|--------------------------|--------------------|
|                                 | PET     | CH     | ME     | AQ     | ST    | PET     | CH     | ME     | AQ     | ST    | PET     | CH     | ME     | AQ     | ST    |
| Cabbage                         | 49.28   | 85.76  | 43.31  | 29.28  | 26.7  | 44.2    | 51.2   | 52.2   | 42.2   | 30.8  | 78.9    | 75.8   | 36.2   | 34.3   | 28.3  |
| Cauliflower                     | 69.792  | 84.69  | 46.29  | 24.82  | 26.7  | 50.22   | 43.22  | 41.2   | 39.22  | 30.8  | 74.8    | 58.2   | 24.4   | 31.2   | 28.3  |
| Pea                             | 80.312  | 61.11  | 31.87  | 34     | 26.7  | 61.2    | 89.34  | 48.3   | 41.2   | 30.8  | 59.2    | 87.32  | 49.3   | 35.9   | 28.3  |
| Carrot                          | 62.35   | 92.75  | 51.6   | 31.23  | 26.7  | 58.2    | 62.5   | 49.2   | 48.7   | 30.8  | 68.5    | 81.8   | 55.9   | 35     | 28.3  |
| Potato                          | 79.1    | 64.2   | 56.11  | 28.3   | 26.7  | 71.22   | 42.33  | 45.2   | 44.22  | 30.8  | 94      | 91.3   | 36.66  | 35     | 28.3  |

Note: ST: standard, PET: petroleum ether, CH: chloroform, ME: methanol, AQ: aqueous
Table 9: TLC analysis of methanolic extract of cabbage and cauliflower

| Vegetable samples | Solvent      | Ratio | No. of spots | No. of spots | No. of spots | Total Spots | RF Value |
|-------------------|--------------|-------|--------------|--------------|--------------|-------------|----------|
|                   |             |       | Visible | LC | UV |             |          |
| Cabbage           | M:n-H:EA    | 1:3:1 | 2        | 3 | 2 | 3           | 0.38, 0.56, 0.81 |
| Cabbage           | C:M         | 8:2   | 1        | 2 | 2 | 2           | 0.12, 0.56 |
| Cabbage           | DCM:M       | 8:2   | 1        | 2 | 2 | 2           | 0.66, 0.79 |
| Cauliflower       | C:M         | 8:2   | 0        | 2 | 2 | 2           | 0.51, 0.53 |
| Cauliflower       | DCM:M       | 8:2   | 0        | 3 | 3 | 3           | 0.88, 0.34, 0.65 |
| Cauliflower       | M:n-H:EA    | 1:3:1 | 0        | 0 | 0 | 0           | 0         |

M: methanol, nH: n-Hexane, EA: ethyl acetate, LC: iodine chamber, and UV: ultraviolet

DPPH radical scavenging activity of 13.34 ± 0.11 mg AAE/g DW from the peel of Solanum tuberosum. The IC\textsubscript{50} value of methanol and acetone extract was found to be 859 μg/μL and 76 μg/μL in the case of carrot peel (John et al., 2017).

**FRAP assay**

FRAP assay is based on the reduction capability of an antioxidant to reduce ferric ion into ferrous (Chaudhary and Jammeda, 2022). Results of FRAP activity of different extracts of all five vegetable peels are shown in Table 8 and Figure 9. The reducing power of the sample was found to be in the following order: ST>CAAQ>CAME>PEAQ>CBAQ>PTCH>CACH>CBPET>PTAQ>PTME>PEME>CTAQ>CTME>CAPET>CBCH>CBME>CTPET>PET>CTCH>PTPET>PECH. Among all extracts, the AQ and ME extract of CA showed the better antioxidant activity than the other solvent system but it was lower than the standard BHT.
Nguyen et al. (2016) reported the reducing power of hexane, water, ethanol, and methanol extracts of a carrot peel and it was found to be 0.31, 4.82, 8.88, and 15.31 mg TE/g dry weight. FRAP assay revealed the 18.61 mmol/100g of antioxidant activity in case of potato peels extract (Rowayshed et al., 2015). Though antioxidant activity of vegetables is influenced by geographical area, cultivar, harvest and storage time but variability can be seen in content between fresh vegetables and its by-products. The by-products of cabbage and cauliflower contain 20 and 15 times more reducing ability than the peels of potato and pea i.e., 20 ± 0.22 mM. Similarly, the peels of carrot have 5-30 times higher antioxidant potential and higher reducing ability than their edible parts (John et al., 2017).

**TAC Assay**

TAC assay is based on the antioxidant activity of plant extract on the reduction of Mo(VI) to Mo(V) and subsequent generation of green coloured complexes of phosphate/Mo(V) at acidic pH. Results of TAC activity of different extracts of all five vegetable peels was found to be in the following order: CAME>ST>CBAQ>CCHQ>CTAQ>CTAQ>CTAQ>PEAQ>CBME>PEME>CTME>CACH>PEPET>CTPET>CAPET>CBCA>CBPET>CAPET>CAPET>CAPET>PTCH>PTPET>PTPET (Table 8 and Figure 10). Among all extracts, the IC$_{50}$ value of AQ extract of CA and CB was found to be low which indicated the higher antioxidant activity of this extract in comparison to standard.

**Thin layer chromatography**

The observations from thin layer chromatography analysis of methanolic extract of cabbage and cauliflower are listed in Table 9. TLC of methanolic extract of cabbage revealed the presence of 3 compounds with $R_f$ values of 0.38, 0.56, and 0.81 respectively in a solvent phase of Methanol: n-Hexane: Ethyl acetate (1:3:1) as shown in Figure 8. In another solvent system i.e., Chloroform: Methanol (8:2), and Dichloromethane: Methanol (8:2), two spots were observed with $R_f$ value of 0.12, 0.56, 0.66, and 0.79 respectively (Figure 9 and 10). TLC of methanolic extract of cauliflower revealed the presence of 2 spots of $R_f$ value 0.51, and 0.53 (Figure 11) in solvent phase of Chloroform: Methanol (8:2). Three spots with $R_f$ value of 0.34, 0.65, and 0.88 were observed in a solvent system of Dichloromethane: Methanol (8:2) as shown in Figure 12. and no spot was observed in the case of Methanol: n-Hexane: Methanol (1:3:1) respectively. These $R_f$ values provide valuable information regarding the isolation of these phytochemicals in the isolation process by using an appropriate solvent system for further pharmacological applications.

**Conclusion**

In the present work, different pharmacognostical standardization parameters and antioxidant assays were applied to determine the quality, safety, and antioxidant potential of the five different vegetable peels. The results obtained from the present study would be useful in determining the crude extract of different peels as a potent source of antioxidants. These are economic and natural sources of antioxidants that can be utilized for the prevention of different human ailments. TLC profiling of phytochemicals showed the good separation and on the sensitivity. However, further studies are needed on isolation, identification, and characterization of specific phytocompound before it can be utilized as a novel source of antioxidant. This opens the scope for the future application of vegetable waste for different therapeutic purposes.

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Conflict of interest
The authors declare that they have no conflict of interest.
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