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Studies on Extraction of Flavonoids from the Peel of *Pisum Sativum*

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Abstract

Pisumsativum is commonly known as Pea plant and belongs to Fabaceae family. It is an herbaceous annual plant. Pea pods not only yield an adequate quality of dietary fibre but provide a substantial amount of proteins, sugars, and minerals. Globally, significant quantities of pea residue are generated, an enormous amount of which is used as animal feed. The pea pods consist of an appreciable amount of polyphenols, including phenolic acids such as 5-caffeoylquinic acid and flavanols like catechin and epicatechin. In this experiment, qualitative, quantitative and purification of phytochemicals are studied. In qualitative analysis, saponins, terpenoids, flavonoids and quinines are present based on observation of colour. In quantitative analysis, water extract and ethanolic extracts showed best result of quercetin and kaempferol and their concentrations were found to be 14.0 µg/ml and 11.5 µg/ml. In purification process, quercetin(water extract) and kaempferol(ethanolic extract) concentrations were found to be 17.0 µg/ml and 12.5 µg/ml.

Keywords: *Pisumsativum*, quercetin, kaempferol, extraction, purification

INTRODUCTION

Pisum sativum, a member of the *Fabaceae* family, is an herbaceous annual plant with a climbing hollow stem that can grow up to 2–3.5 m long [1]. A pea is usually green, although it can also be golden yellow or purple. It is a pod-shaped vegetable that is frequently produced as a winter vegetable crop. Plant the seeds as soon as the soil temperature exceeds 11°C (50.8°F), with the plants developing best at temperatures ranging from 13.4 to 18.6°C (56.2 to 65.58°F). They do not survive in the summer heat of temperate and lowland tropical zones but perform well in cooler, higher-altitude tropical zones [2]. Several cultivars achieve maturity 60 days after planting. The pod is a seed container made up of two sealed valves that break at the seam between them. Pea seeds are green, spherical, and smooth. The average pea weighs between 0.13 and 0.37 grams [3]. The outer pod contains about 34–40% of the pea's weight. Pea pods not only yield an adequate quality of dietary fibre but provide a substantial amount of proteins, sugars, and minerals. Globally, significant quantities of pea residue are tossed or used to feed the livestock and as fertilizers. These wastes are profoundly inclined to microbial spoilage and thusly create significant issues to the environment. So that, these wastes ought to be figured out how to be used advantageously. Nowadays, many studies are performed to utilize these wastes to reduce the environmental pollution and get some medicinal benefits. These peels are a significant source of bioactive chemicals, which are mostly used as antioxidant and anticancer medicines. To stimulate appropriate reuse of these wastes for various uses in medicine, it is vital to reveal the biological activities of these peels, reap the benefits of their waste products, and also examine their chemical makeup. The pea pods consist of an appreciable amount of polyphenols, including phenolic acids such as 5-

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caffeoylquinic acid and flavanols like catechin and epicatechin [4].

MATERIALS AND METHODS

Chemicals

Ethanol, Methanol, Chloroform, Sodium hydroxide (NaOH), Sulphuric acid (H_2SO_4), Potassium acetate (CH_3CO_2K), Aluminium chloride ($AlCl_3$), Silica gel and Distilled water.

Source of Plant Material

Peel of peas are collected from vegetable market, Meerut, Uttar Pradesh. These peels are minced, cleaned and dried under sunlight. Finally, a very fine powder is made from dried peels. The plant materials are shown in Figure 1.

Figure 1. Peel of Pea and its powdered form.

Preparation of Plant Extract

One-grampowder is taken in conical flask and25 ml of organic solvents (ethanol, methanoland water) is added in each flask separately. Soak the samples up to 24 hrs and then perform filtration using Whatman paper No.1 through funnel [5]. Finally, plant extracts are prepared. The plant extracts are shown in Figure 2.

Figure 2. Plant extracts.

Determination of Phytochemicals

Qualitative Analysis

- *Saponins:* Take1 ml plant extract in a test tube and add 1 ml water, foamformation indicates the presence of saponins [6].
- *Terpenoids:* Take 0.5 ml plant extract in a test tube, add 1 ml chloroform and 2ml H_2SO_4, appearance of red/brown colour indicates the presence of terpenoids [7].
- *Flavonoids:* Take 2ml plant extract in a test tube and add 1 ml 2N NaOH, appearance of yellow colour indicates the presence of flavonoids [8].
- *Quinones:* Take 1 ml plant extract in a test tube and add 1 ml H_2SO_4, appearance of red colour indicates the presence of quinones. The test samples are shown in Figure 3.

Figure 3. Qualitative analysis.

Quantitative Analysis

- *Quercetin:* It was estimated by Aluminium chloride method. Take 0.5 ml plant extract in a test tube and add 1.5 ml methanol, 0.1 ml Aluminium chloride (10% in water), 0.1 ml Potassium acetate (10% in water) and finally 2.8 ml of distilled water [9]. After 30 min incubation period, observed the OD value using colorimeter at 415 nm.

Figure 4. Quantitative analysis.

- *Kaempferol:* Take 1 ml plant extract in a test tube and add 1 ml methanol. After 30 min incubation period, observed the OD value using colorimeter at 400 nm [10]. The test samples are shown in Figure 4.

Purification

Afterthedeterminationprocess, those plant extracts are purified using column chromatography. Some silica gel is taken in the column and it becomes wet with the help of methanol solvent [11]. Once it started getting absorbed, plant extracts (water, ethanol and methanol) are added in each case respectively. Finally, the purified samples are collected from column for the estimation of quercetin and kaempferol. The column chromatography is shown in Figure 5.

Figure 5. Column chromatography.

RESULTS AND DISCUSSION

Qualitative Analysis

In this analysis, we have studied some phytochemicals of this plant material with various solvent extracts based on colour observations [12] and the results are shown in Table 1.

Table 1. Qualitative analysis of phytochemicals.

S.N.	Plant Extracts	Phytochemicals			
		Saponins	***Terpenoids***	***Flavonoids***	***Quinones***
1	Water	+	+	+	-
2	Ethanol	+	+	+	+
3	Methanol	-	+	+	+

where '+' means present and '-' means absent.

Quantitative Analysis

In this analysis, we have studied quercetin and kaempferol phytochemicals of this plant material with various solvent extracts [13]. In this process, water extract and ethanolic extracts showed best result of quercetin and kaempferol and their concentrations were found to be 14.0µg/ml and 11.5µg/ml. The results are shown in Table 2.

Table 2. Quantitative analysis of phytochemicals.

S.N.	Phytochemicals	Plant Extracts		
		Water	***Methanol***	***Ethanol***
1	Quercetin (µg/ml)	14.0	5.5	9.0
2	Kaempferol (µg/ml)	3.0	10.0	11.5

Purification

In this process, we have studied about purification of quercetin and kaempferol by using column chromatography with the help of silica gel [14]. These results are better than quantitative analysis of phytochemicals due to the adsorption of impurities of plant extracts by silica gel [15]. In this process, quercetin(water extract) and kaempferol (ethanolic extract) concentrations were found to be 17.0 µg/ml and 12.5 µg/ml. The results are shown in Table 3.

Table 3. Purification analysis of phytochemicals.

S.No	Phytochemicals	Plant Extracts		
		Water	***Methanol***	***Ethanol***
1	Quercetin (µg/ml)	17.0	6.5	10.5
2	Kaempferol (µg/ml)	4.0	12.0	12.5

CONCLUSION

In this experiment, qualitative, quantitative and purification of phytochemicals are studied. In qualitative analysis, saponins, terpenoids, flavonoids and quinines are present based on observation of colour. In quantitative analysis, water extract and ethanolic extracts showed best result of quercetin and kaempferol and their concentrations were found to be 14.0 µg/ml and 11.5 µg/ml. In purification process, quercetin(water extract) and kaempferol(ethanolic extract) concentrations were found to be 17.0 µg/ml and 12.5 µg/ml.

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