Exploring discarded pea peels as potential source of phytochemicals and evaluation of antifungal potential of pea peel extract

Maryam Zahra [1], Aeysha Sultan [1*], Khalid Mahmood [2], Noreen Sajjad,[3] Farhana Amman[4]

[1]Department of Chemistry, Division of Science and Technology, University of Education Lahore, Pakistan
[2]Department of Chemistry, Gordon College Rawalpindi, Pakistan
[3]Department of Chemistry, University of Lahore, Lahore, Pakistan
[4]Department of Chemistry, Sargodha Campus, Sargodha, Pakistan

***Corresponding author's email**: ayesha.sultan@ue.edu.pk

DOI: 10.53992/njns.v8i3.145

Abstract

The rapid increase in global waste generation necessitates innovative strategies for waste valorization. Inspired by recent reports that indicate the potential of waste as source of fine chemicals, this research paper focused on harnessing phytochemicals and cellulose from pea peels (PPs) and evaluating their antifungal properties. Different protocols were used to quantify 11the amount of carbohydrates, proteins, ascorbic acid, flavonoids, and chlorophyll from the discarded pea peels. Our findings indicated that PPs contain 20% proteins, 24.52% chlorophyll, 70% flavonoids, 12% ascorbic acid and 70% carbohydrates. Our findings were found to be consistent with those reported in literature; however, the quantitative analysis of ascorbic acid for PPs is being reported for the first time by us. This phytochemical profile of PPs indicates that this waste is full of useful phytochemicals and can be explored as source of nutraceutical and fine chemicals.

PPs were also used for cellulose extraction and for this purpose three different strategies that yielded different results. One strategy that was devised by us yielded superior yield that that reported in literature with added advantage of lesser consumption of chemical reagents and facile protocol involved.

The methanolic and aqueous PPs extract were also screened for its antifungal potential against two fungal strains: *Penicillium* and *Aspergillus niger*. Our findings indicate that extracts showed significant antifungal potential at higher concentration. However, aqueous extract showed superior activity.

The findings of this word offer dual solution to pressing issues: waste valorization and natural antimicrobial development. The study's outcomes can revolutionize waste management practices, encouraging the utilization of kitchen waste for valuable compounds. Simultaneously, the development of natural antimicrobial agents contributes to the fight against fungal infections and food spoilage without harming the environment.

Keywords: Pea Peels, waste valorization, phytochemicals, cellulose, antifungal activity

Introduction:

Due to more stringent regulations and demand for renewable chemicals and fuels, manufacturing industries are increasingly moving towards higher sustainability to

improve cost effectiveness and meet consumer demand. One area of current research that has received a lot of attention in recent years as a possible alternative is the valorization of waste. [1]

The waste valorization involves conversion of discarded items into highly beneficial items such as chemicals [2], materials [3], and fuel. Although valorization is not a new concept [5]; it has not been given its due importance until recent times when the primary and natural resources are rapidly decreasing due to their rapid consumption associated with ever increasing human population. [6]

With the valorization of waste, we can avoid many problems such as climate change, energy crisis, resource scarcity and pollution. Valorization of waste is gaining ground in today's due to its two-fold approach; it converts waste into useful items and reduces the volume of waste that is being continually dumped on earth crust. [7]

Many valorization techniques have the potential to meet industrial demands by converting waste into valuable products. A recent review by Ruiz et al. highlighted the various benefits of continuous flow processes, especially for biomass and food waste valorization, which requires reaction control, efficient reaction cycles, higher yield production, ease of scale-up, and no separation of catalysts.[8] Microwave heating is a green valorization approach by which extremely strong biopolymers and amorphous compounds can be decomposed without involving huge input of energy, pressure or extreme temperature conditions.

Another valorization method involves pyrolysis of biomass at high temperatures in the absence of oxygen for fuel production. Previously the pyrolysis approach was used to produce char, now it is being used to produce usable small molecules from very strong biopolymers. Pyrolysis is also currently being used to produce some modern materials such as carbon nanotubes and grapheme-type materials that have many advantages. [9,10]

Bioconversion is another method of valorization using biological microorganisms for complex waste degradation and fuel production. Bioconversion has been under intensive research for the past few years, and it is the most important use in the field of "the possibility of artificial control of metabolic pathways of microorganisms" to develop processes that increase product production. [11,12]

Household waste contains most of the kitchen waste such as vegetable and fruit peels and seeds, etc. Vegetable and fruit waste is present in very high quantities, they have a very high moisture ratio, due to which microbes are produced in them and unpleasant odors spread. This causes many diseases. This waste carries many basic and important chemicals within itself. Fruits and vegetable peels, seeds, stems, leaves, pulp, and roots contain many valuable compounds such as antioxidants, oils, fatty acids, lipids, proteins, vitamins, carbohydrates, and carotenoids. These bioactive elements find extensive applications in pharmaceutics, cosmetics, biofuels, bio plastics, for storing food and in food flavors. [13]

The Pisum Sativum, a pod plant, also commonly called pea, is a fruit but known as a vegetable. They are eaten in large quantities everywhere and therefore the amount of their peels discarded as waste is also significant. Various studies reveal that they contain high amounts of chlorophyll proteins, carotenoids, vitamins, carbohydrates, flavonoids, and antioxidants. Sucrose is a carbohydrate that

is found in large quantities in PPs, which we can also extract from peels by different ways. This sucrose can then be used in various fields such as bio plastics etc. Other nutrients also perform many functions in our body such as improving the immune system against diseases, keeping the skin fresh, losing weight, etc. Powder of PPs is used in soups and snack crackers. [15,16]

Considering the importance of waste Valorization and the potential importance of PPs, the work was designed to extract and estimate chlorophyll, carbohydrates, protein, and flavonoids from the pea peels. The cellulose from the peels was isolated and the PPs_extract was screened for antifungal activity against *penicillium and Aspergillus niger*.

Materials and Methods

Sample preparation.

The Discarded PPs were collected, washed three times with distilled water and dried in shade for 3-4 days. The dried sample was ground to fine powder which was then preserved in sealed pack for further testing.

Estimation of chlorophyll

Arnon protocol (1949) [17,18] was used to determine the percentage amount of chlorophyll. In the samples. Briefly 0.5g sample was homogenized with 10ml of 80% acetone with the help of piston and mortar. The extract thus obtained was then centrifuged for 20 minutes at 8000 rpm and 4°C temperature. The absorbance of supernatant at 645 nm (for chlorophyll A), 663 nm (for chlorophyll B), and 480 nm for carotenoids was recorded on UV/Visible spectrophotometer.

Estimation of protein content

Protein estimation was carried out by following Bradford protocol (1976) [19,20]. Briefly, 0.2g sample mixed with 5ml phosphate buffer (pH 7) was centrifuged for 15 minutes at 15000 rpm and 4°C temperature. The supernatant was separated and to 1 mL of this supernatant was added 5ml Bradford reagent. The sample mixture has shaken, and absorbance was noted at 595 nm on the spectrophotometer.

Estimation of carbohydrates

The estimation of carbohydrates was carried out using reported protocol [21,22].

Briefly, a 2ml aliquot of solution (made by homogenizing 0.5g sample into 5ml distilled water) was mixed with 1ml of 5% phenol solution in a test tube. To this aliquot 5ml of concentrated H2SO4 was added immediately and the solution was allowed to stand for 10 min. Afterwards, the mixture was vortexed for 1 minute and placed in a water bath for 20 minutes for color development. The absorbance was recorded at 490nm on spectrophotometer for the estimation of carbohydrates.

The following formulae were used for the estimation of chlorophyll.

$$Chlorophyll\ A = \frac{[12.7(A663) - 2.69(A645)V]}{1000 \times \mathrm{W}}$$

$$Chlorophyll\ B = [22.9(A645) - 4.68(A663)] \times \frac{V}{1000} \times \mathrm{W}$$

$$Total\ Chlorophyll = Chlorophyll\ A + Chlorophyll\ B$$

$$Carotenoids = [A480 + 0.114(A663) - (0.638 - A645)] \times \frac{V}{1000} \times \mathrm{W}$$

Where,

V= total volume of extract (ml)
W= weight of plant sample
A= Absorbance

Estimation of flavonoids content

Flavonoids determination was the method reported by Ejikeme et al.[24] and Boham and Kocipai [25]. Briefly, 1g of sample added to 10ml of 80% methanol, the resulting solution was then centrifuged for 20min at 8000rpm and 4°C temperature supernatant.

The percentage yield of flavonoids was calculated by the following formula [26]

Percentage yield of flavonoids was calculated by the following formula26:

$$\%\text{ age of Flavonoids} = \frac{\text{weight of Flavonoids}}{\text{weight of sample}} \times 100$$

Estimation of vitamin C

A reported protocol was used for the estimation of ascorbic acid [27,28] Briefly, 1g sample was crushed with 4 ml oxalic-EDTA solution (mixture of 0.05M oxalic Kocipai [25] acid and 0.2M EDTA solution). To the filtrate, 0.5 ml of orthophosphoric acid, 1ml of H_2SO_4 (5%), 2ml of ammonium molybdate (5%) and 3 ml of distilled water was added, and the resulting solution was allowed to stand for 20 minutes followed by recording absorbance at 760 nm on spectrophotometer.

Extraction of cellulose

Cellulose was extracted from PPs by employing a reported procedure. [29,30]. Briefly, 1g of dried and crushed pea peels sample was treated with 2M NaOH for 1 hour at 1210C. The solution thus obtained was filtered and the residue was subjected to the same procedure 3 times. The residue was later treated with AcOH (40ml of 85% acetic acid) and conc HNO3 (3 ml) 3 hr. at 1500C. After cooling to ambient temperature, EtOH (50ml) was added to the mixture and centrifuged for 20 min at 6000rpm and 4oC. Centrifugation was repeated with H2O (50 ml). The white colored residue thus obtained was dried in oven at 370C.

To explore the most effective and economical protocol for extraction of cellulose from PPs, we designed two different strategies that yielded different but interesting results.

Strategy 1

Sample (1g) was suspended in MeOH (10ml of 80%) for 24 hrs. at rt and later centrifuged for 20 min at 4000rpm at 40C. The residue washed thrice with EtOH (10ml of 80%) and solid thus obtained dried in oven for 48 hr. the dried solid was heated and stirred at 121C in presence of 2M NaOH solution for 1 hour. Mother liquor was discarded and the solid thus obtained was subjected to the same procedure twice again. The solid obtained after alkali treatment was then subjected to acid treatment by heating it with AcOH (40ml of 85% acetic acid) and conc HNO3 (4 ml) nitric acid) for 3 hours at 1200C. The resulting solid was cooled to rt, EtOH (50 ml) was added to induce precipitation and the resulting mixture was centrifuged for 20 min at 4000rpm and 40C. The solid thus obtained was washed with EtOH and again centrifuged with H2O (50 mL) to yield white crystals of cellulose that were dried in oven at 370C.

Strategy 2

Pretreated sample (1g) was heated in presence of 2M NaOH solution for 90 min at 150°C. The resulting reaction mixture was filtered, and the same procedure was repeated twice again. The solid residue thus obtained was dried at 65°C. Acid treatment was carried out by treating solid with

AcOH (20ml of 50%) and conc HNO3 (1 ml) for 5 hr. at 130°C. The mixture was cooled to ambient temperature and allowed to stand for 24hours at rt. EtOH (20ml) was added to the solution to induce precipitation and resulting solution was allowed to stand for additional 2 hr. the reaction mixture was filtered, and residue was suspended in EtOH (15 min) for 1 hr. followed by filtration to afford cellulose as white solid. The solid thus obtained was dried in oven at 37C and weighed to calculate yield.

Antifungal activity of PPs extract

Preparation of pea peels extract

Methanolic extract of pea peels was obtained by refluxing 5g of finely ground sample with 250 ml of MeOH using a Soxhlet apparatus. The extract thus obtained was preserved in a sealed container for further use [31,33]

Evaluation of antifungal activity

Nutrient media was prepared by dissolving nutrient agar (2.8 g) in 100ml of distilled H2O taken in a conical flask. The flask along with its contents were covered with cotton and sterilized in an autoclave for 90 min at 151°C [34].

The prepared nutrient media was poured onto sterilized Petri dishes under laminar air flow. The prepared petri dishes were sealed with scotch tape and kept at ambient temperature for 24 hours in an incubator to check for any contamination before using them for streaking [35].

The prepared petri plates free from contamination were then streaked with Penicillium and Aspergillus niger under laminar flow. Sample was loaded in the wells created by using micro-tips on the streaked petri dishes. Dermosporin (Antimicrobial drug) was used as standard, and all samples were applied in triplicate. After sample loading, the petri dishes were incubated for 1 week at ambient temperature. Antifungal activity was calculated by measuring the zone of inhibition of samples and the standard [36,39].

Results and Discussion

Preliminary phytochemical screening

PPs are wasted in huge quantities every year and discarded as waste. There is a huge amount of literature that indicates presence of many beneficial ingredients inside pea pods;[15,16] however, not much has been done on exploring the potential of pea peels. This is the first report of complete phytochemical profiling of PPs.

Estimation of chlorophyll content

Chlorophyll is a pigment which gives green color to plants, and it is very beneficial like it reduces aflatoxins which are carcinogenic compounds, boost immune system, weight loss, and clear skin, neutralize body odor, increase body energy, have anti-aging property due to antioxidant activity and improve red blood cells quality.

The percentage of chlorophyll A in pea peels was estimated to be 15.52% and Chlorophyll B was estimated to be 9%. The total chlorophyll content was estimated to be 24.52%. Holasova etal (2009) reported a total chlorophyll content to be 23.06mg/100g in pea pods [40].

Estimation of flavonoids

Flavonoids possess antioxidants, anticancer, anti-inflammatory, antiviral, cardio protective, and neuro protective and anti-microbial activities. The total flavonoids content in PPs was found to be 70%; the value is consistent with that reported in literature for pea pods [41,42].

Estimation of protein content

Proteins are the building blocks of the body made up of different amino acids. The body requires protein for the maintenance and growth of cells and tissues. Our dietary requirements for protein changes throughout life. Enzymes are proteins that causes biochemical reactions in cells, some hormonal proteins act as a messenger, mostly proteins provide structure to cells and tissues, maintenance of blood and other body fluid pH, boost immune system, transport and store essential nutrients and provides energy to body.

Kocipai [25]. Briefly, 1g of sample added to 10ml of 80% methanol, the resulting solution was then centrifuged for 20min at 8000rpm and 4°C temperature supernatant. The mixture was then centrifuged for 20min at 8000 rpm and 4°C temperature; supernatant was discarded, and the obtained residue was washed with ethanol (3×10ml of 80%). Solid sample was transferred to a crucible and left for drying in an oven for 8-10 hours at 37°C temperature.

The protein content in pea peels was calculated to be 20%; the literature indicates 17.76% in pea shells [43].

Estimation of ascorbic acid

Vitamin C is a very essential nutrient which acts as an antioxidant and is also involved in regulation of blood pressure, prevents iron deficiency, lowers risk of heart diseases, reduces uric acid level, and prevents gout attacks. The percentage of ascorbic acid determined in pea peels was 12%.

Estimation of carbohydrates

Carbohydrates are present in almost every type of food. These biomolecules play a very crucial role in human physiological processes, such as: serving as source of energy, maintaining blood glucose level, splitting of fatty acids for stopping ketosis, and are involved in biological recognition process.

Pea peels are rich in carbohydrates concentration as literature explains peels have more than 70% carbohydrates and experimental work also correlates with literature, found percentage of carbohydrates in pea peas is 79.05% [44].

Estimation of cellulose percentage

Cellulose is a complex polysaccharide consisting of hundreds to thousands of C, H and O. It is formed by more than 3,000 units of glucose. It is a major part of plant's cell wall whose function is to keep plant stiff and upright. The human body can't digest it, but it is important in diet as Fiber, but animals like horses, sheep, goat, and cow digest it that's why they get the required amount of energy from grass. Cellulose has many other vast applications like paper manufacturing and clothing. We separated cellulose from pea peels by using a reported protocol which yielded 16.38% cellulose which was less than that reported in literature.

In our attempts to optimize the %yield of cellulose, two modified strategies were devised. The first strategy involved using the same protocol as reported in literature with a difference that instead of centrifugation, cellulose was separated by filtration and this strategy yielded 19.09% cellulose. Whereas the second strategy involved using less volume of acids (AcOH and HNO3 and solvents) and filtration was used for cellulose separation and this strategy afforded cellulose in best %age (32.66%) as compared to reported yield with added advantage of less consumption of expensive chemicals. The second strategy has the potential to be explored for extraction of cellulose in higher yields from various other food wastes as well.

Cellulose percentage calculated by following formula:

$$\%\ Cellulose = \frac{Final\ weight\ of\ extracted\ cellulose}{total\ weight\ of\ raw\ material} \times 100$$

Figure 1: (a) Green pee peals sample before treatment (b) Extracted Cellulose.

The FTIR spectrum of the extracted cellulose was compared with commercial Cellulose and the IR spectrum of both samples was found to be the same (Figure 2).

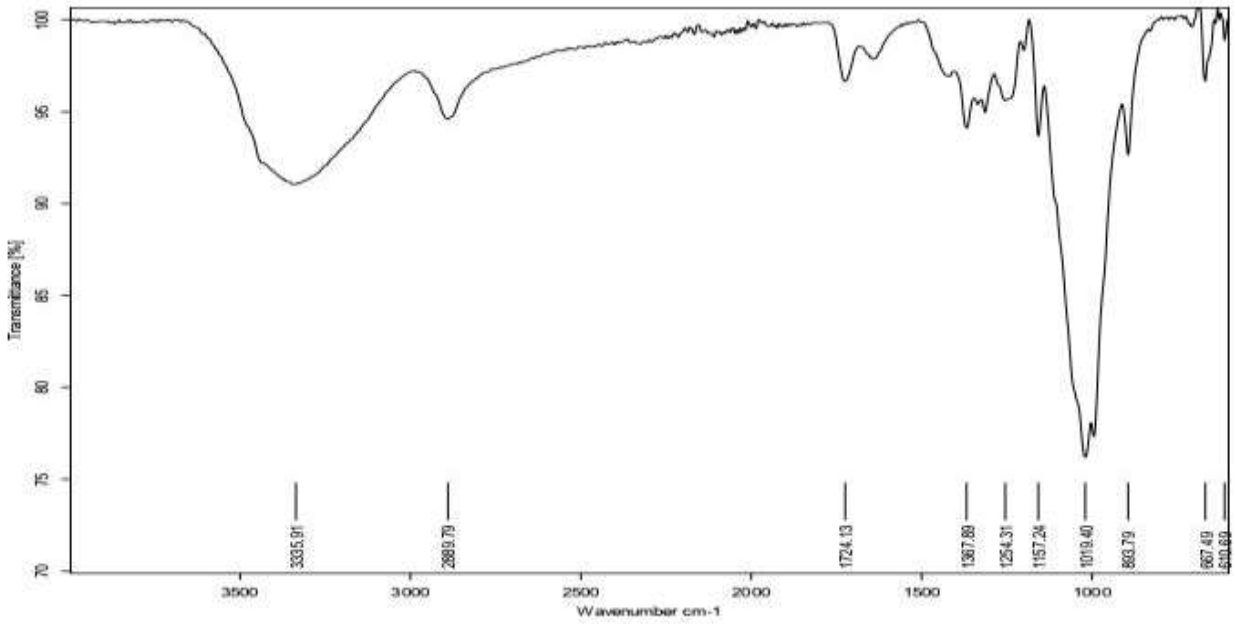

Figure 2: FTIR spectrum of cellulose extracted from pea peels.

Antifungal activity of pea peels extract

Plant pathogens like bacteria, viruses, and fungi harms plant and reduce crops' yield thereby affecting the economy from agricultural sector; amongst these pathogens, fungi are the most common one. Therefore, searching for agents that can reduce/eliminate the impact of fungal pathogens is of crucial importance. In our studies towards exploring the potential of PP as antifungal agents, we employed aqueous as well as methanolic extract of PP. Both extracts were used against two fungal strains (Aspergillus Niger & Pencilium). Initially lower concentrations

(20µL, 40µL, 60µL, 80µL and 100µL) were employed and later concentrations were increased (200µL, 400µL, 600µL, 800µL and 1000µL) to get optimum activity from extracts. antifungal activity was checked in terms of zone of inhibition after 7 days of incubation at 37°C; Dermasporin (antibiotic) was used as standard /control.

The findings from the preliminary studies indicate that both extracts showed similar activities against both fungal strain; however, aqueous extract showed superior activity at higher concentration. The minimum inhibitory concentration was found to be 100µL; below this concentration no inhibitory activity of either of the two extracts was observed. Another important aspect that was observed was that methanolic extract showed its activity after 8 days of incubation whereas aqueous extract showed significant activity after 3 days of incubation.

The results from antifungal activity are displayed in table 1.

Sr.	Concentrations	Zone of inhibition (mm)			
		Aspergillus Niger		*Penicillium*	
		Methanolic Extract	**Water Extract**	**Methanolic Extract**	**Water Extract**
1	20µL	4.00	5.00	3.50	4.00
2	40µL	5.00	8.00	4.00	6.00
3	60µL	7.50	9.00	7.00	8.50
4	80µL	9.00	11.00	8.50	10.00
5	100µL	10.00	13.00	9.50	12.00
6	200µL	17.50	24.00	15.00	21.50
7	400µL	20.00	28.00	18.00	25.00
8	600µL	27.00	31.00	23.00	28.00
9	800µL	30.00	35.00	25.00	31.00
10	1000µL	32.00	39.00	28.00	34.00
11	Dermasporin	42.00			

Conclusion

The presented work was concerned with detection and quantification of phytochemicals in discarded PPs. Our findings suggest that PP discarded as waste contains significant quantities of protein, carbohydrates, ascorbic acid. Therefore, this waste can be used as nutraceuticals. Cellulose was also extracted from PP and the methanolic as well as aqueous extracts of PP were screened for antifungal potential against two fungal strains. The results suggest that aqueous extract showed antifungal potential at higher concentrations; however, at lower concentrations, the antifungal activity was not pronounced much.

This study has yielded interesting as well as promising results and we believe that in-depth analysis of phytochemicals from PP can prove to be sustainable source of fine chemicals.

References

1. Nerea J-M., Irene E., Fernando B., Luis M G., Carmen A-A., Valorization of selected fruit and vegetable wastes as

bioactive compounds: Opportunities and challenges, 2019.
2. Eun J C., Ly T P T., Younho S. Yoon G L., Hyeun-Jong B., Bioconversion of biomass waste into high value chemicals, Bioresource Technology, 2020, 298, 122386.
3. B.S.S. Phanisankar.; N. Vasudeva Rao., J.E. Manikanta., Conversion of waste plastic to fuel products, 2020, 33, 5190-5195
4. Kabongo, J.D. (2013). Waste Valorization. In: Idowu, S.O., Capaldi, N., Zu, L., Gupta, A.D. (eds) Encyclopedia of Corporate Social Responsibility. Springer, Berlin, Heidelberg.
5. Maia. M M., Ayano. S F., The Impact of Population Growth on Natural Resources and Farmers' Capacity to Adapt to Climate Change in Low-Income Countries, June 2021,
6. Kabongo, J.D. (2013). Waste Valorization. In: Idowu, S.O., Capaldi, N., Zu, L., Gupta, A.D. (eds) Encyclopedia of Corporate Social Responsibility. Springer, Berlin, Heidelberg.
7. Rick A D., A. Carol S K L., King M C., Tsz H K., Rafael L., Advances on waste valorization: new horizons for a more sustainable society., 2013,
8. Shin Y F., Yi H C, Wai Y C., Noor H K, Tengku N B T I.; Christian S., Wanxi P., Pau-Loke S., Su S L.; Progress in waste valorization using advanced pyrolysis techniques for hydrogen and gaseous fuel production, 2021, 320, 124299.
9. Yousaf A., Jianzhao Z., Weifeng S., Jingzheng R., Innovative valorization of biomass waste through integration of pyrolysis and gasification: Process design, optimization, and multi-scenario sustainability analysis, 2023, 282, 128417.
10. K.C.S., Irini A., Samir K K., Bioconversion of waste-to-resources (BWR-2021): Valorization of industrial and agro-wastes to fuel, feed, fertilizer, and biobased products, 2022, 347, 126739.
11. Shraddha C., Bhoomika Y., Anusha A., R.D. T., Jonathan W.C. W., Patrick D., Bioconversion of organic wastes into value-added products: A review, 2022, 244, 126398
12. Minaxi S., Zeba U., Vijai K G., Rajeev B., Valorization of fruits and vegetable wastes and by-products to produce natural pigments, 2021,
13. Verma, N., Bansal, M. C., and Kumar, V. (2011). "Pea peel waste: A lignocellulosic waste and its utility in cellulase production by *Tricoderma reesei* under solid state cultivation," *BioRes.* 1505-1519.
14. Entesar H., Shalini G. R., Vidya R. S., Vasudha S., Utilization of pea pod powder for formulation of instant pea soup powder, 2020,
15. Mona M H M., Mohammed A E-M., Heba I G., Mohammad Y A., Nora H M E-W., Eman M H.; Galila A H A.; Pea peels as a value-added food ingredient for snack crackers and dry soup, 2021,
16. Pramod N. K., Sanjay P. G., Ranjeet S. M., Anupreet T., Estimation of Chlorophyll Content in Young and Adult Leaves of Some Selected Plants, 2015, 5, 306-310
17. Madain P-P., Jorge L C-A., Avisai S-A., Abiel A-G., Federico G-M., Elias E-G., Yvon V., Carlos R-R., Ruben G-C., Optical Method for Estimating the Chlorophyll Contents in Plant Leaves, 22 February 2018, *Sensors* 2018, *18*(2), 650.
18. Bradford, MM. A rapid and sensitive for the quantization of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72: 248-254. 1976.
19. Stoscheck, CM. Quantitation of Protein. Methods in Enzymology 182: 50-69 (1990).
20. H. Weinmann., Determination of Total Available Carbohydrates in Plants, 1947, 22(3): 279–290

21. R. P. Murphy., A method for the extraction of plant samples and the determination of total soluble carbohydrates, 1958, 9, 714-717
22. Firoj A. T., Harinath N. M., Susmita S. B., Ankita S. P., Namdeo R. J., Suresh G. K., Estimation of Total Carbohydrate content by Phenol Sulphuric acid Method from Eichhornia crassipes (Mart.) Solms, 2020, ISSN 0974-4169,
23. C. M. Ejikeme, C. S. Ezeonu, and A. N. Eboatu, "Determination of physical and phytochemical constituents of some tropical timbers indigenous to Niger Delta Area of Nigeria," European Scientific Journal, vol. 10, no. 18, pp. 247–270, 2014.
24. Boham, B.A. and Kocipai, A.R. Flavonoids and Condensed Tannins from Leaves of Hawaiian Vaccinium vaticulatum and V. calycinium. Pacific Sci. 48:458-463, 1994.
25. Samidha K., Vrushali K., Vijaya P., Estimation of Phenolic content, Flavonoid content, Antioxidant and Alpha amylase Inhibitory Activity of Marketed Polyherbal Formulation, 2014, 4(09), 061-065
26. Satya P. A., Meenakshi M.;Preeti J., Photometric Methods for the Determination of Vitamin C, Analytical Sciences volume 14, (1998)
27. M.M. Rahman K., M.M. Rahman., M.S. Islam., S.A. Begum., A Simple UV-spectrophotometric Method for the Determination of Vitamin C Content in Various Fruits and Vegetables, 2006, 6, 388-392
28. Leila K., Javier A L-P., Hamid R., Seyed O R S., Andrius J., Said A-H., Roya R R S., Eva N K., Extraction of sugarcane bagasse arabinoxylan, integrated with enzymatic production of xylo-oligosaccharides and separation of cellulose, 2021,
29. Williene F D P B., Guilherme D., Laura N D A., Marcela P S P., Rosane F B., Flavio C C.; Aldo E J., Chemical treatment of sugarcane bagasse and its influence on glyphosate adsorption, 13 May 2022
30. J. Kim, K. Choi, D.S. Chung., Sample Preparation for Capillary Electrophoretic Applications, 2012, 3, 701-721

a. Zygler, M. Słomińska, J. Namieśnik.; Soxhlet Extraction and New Developments Such as Soxtec, 2012, 2, 65-82

31. Dipa A., Soxhlet Extraction: Principle, Extraction procedure, and Apparatus, 2022,
32. Jacobus Nicolaas Eloff., Avoiding pitfalls in determining antimicrobial activity of plant extracts and publishing the results, 2019, 106.
33. Mukhrizah O., Hwei S L., Christophe W., Teng J K., Kaun H L.; Kang N T., Optimal methods for evaluating antimicrobial activities from plant extracts, 2011, 84, 161-166.
34. Acharya T., Streak Plate Method: Principle, Procedure, Uses, 2022
35. Mounyr B., Moulay S., Saad K I., Methods for *in vitro* evaluating antimicrobial activity, 2016, 6, 71-79.
36. J.L. Ríos., M.C. Recio., Medicinal plants and antimicrobial activity, 2005, 100, 80-84
37. Duncan W., Pierre T., Rene J. B., Robert P. R., Antifungal activity of medicinal plant extracts; preliminary screening studies, 2008, 115, 140-146
38. M. Holasová.; R. Dostálová.; V. Fiedlerová.; J. Horáček.; Variability of Lutein Content in Peas (Pisum sativum L.). in Relation to the Variety, Season and Chlorophyll Content, 2009, 27
39. Lilia B F.; Fatma C.; Fatma K.; Mohamed K.; Soumaya Z-E.; Imen K.; Semia E C.; Dhouha G-A.; Antioxidant and antimicrobial activities of polyphenols extracted from pea and broad bean pods wastes, 2022
40. Luigi C., Luana I., Anna G., Sonia L., Yelko R-C., Alfonso N., Michela G., Alberto R., Chemical Composition of Green Pea (*Pisum sativum* L.) Pods

Extracts and Their Potential Exploitation as Ingredients in Nutraceutical Formulations, 2021
41. Amita B., Vikram., Savita S. Veenu S., Darshan P., Chemical Constituents (Proximate, Minerals, Bioactive Compounds) of Pea [*Pisum sativum* (L.)] Shells, 26-05-2023
42. Gloria Urbano, Ph.D.a,*, María López-Jurado, Ph.D.a , Sławomir Frejnagel, Ph.D.b , Elena Gómez-Villalvaa , Jesús M. Porres, Ph.D.a , Juana Frías, Ph.D.c , Concepción Vidal-Valverde, Ph.D.c , Pilar Aranda, Ph.D.a, Nutritional assessment of raw and germinated pea (Pisum sativum L.) protein and carbohydrate by in vitro and in vivo techniques, 2004