Journal of
Research in Pharmacy

Research Article
www.jrespharm.com

Colorimetric analysis of phenols and flavanoids in pomegranate plant extract

Sujiya BALLA[1,2*], Nagasen DASARI [1,2], Sharon Priyanka PALINA[1,2], Sathis Kumar DINAKARAN [1,2], Lavanya PULIDINDI[3].

1 Adity Pharmacy College, Surampalem, Andhra Pradesh, India.
2 Jawaharlal Nehru Technological University Kakinada, Andhra Pradesh, India.
3 Department of Horticulture, Andhra Pradesh, India.

* Corresponding Author. sujiyab@gmail.com (S.B.); Tel. +91-833-294 26 78.

Received: 22 October 2023 / Revised: 16 January 2024 / Accepted: 18 January 2024

ABSTRACT: *Punica granatum* (Pomegranate) is widely accessible and used by natural health professionals for a range of medicinal purposes. The purpose of the current study to use a derivatization method in conjunction with FT-IR, colorimetry, and UV-Visible spectroscopy to determine the total phenolic and flavonoid content of various pomegranate sections. Pomegranate is used to treat ulcers, diarrhoea, Male infertility and so on. Currently, pomegranate is marketed in different novel drug formulations such as nanoparticles for local mucosal drug delivery, niosomes, hydrogels for chronic ınflammation, microemulsion for skin care and analytical techniques using HPLC, GC and GC-MS, LC-MS for carrying out the various quantitative and qualitative analyses are some examples of these developments. In this study, a part of pomegranate was extracted and evaluated by using Microscopical and Macroscopical evaluation, Fluorescence Analysis, FT-IR studies using derivatisation method and finally quantified the phenol and flavonoid content using UV-Visible Spectroscopy. Results of the study revealed that the pomegranate extract gives data about the linearity, precision, accuracy of peel and bark ethanolic and aqueous extract gives linearity 1-5µg/ml, Regression 0.998, LOD 0.691µg/ml, LOQ 1.32µg/ml. Through the appropriate selection of Solvents, we successfully developed a stable and effective method for the determination and estimation of phenol and flavonoid content by derivatization and FTIR studies. Method was developed, optimized and validated with its parameters.

KEYWORDS: Derivatization method; Flavonoid content; Phenolic content; UV-Visible spectroscopy; FT-IR; *Punica granatum* (Pomegranate).

1. INTRODUCTION

Punica granatum, the scientific name for the pomegranate fruit, is a Middle Eastern native tree that is now cultivated all over the world. It is appreciated by many due to its richness in nutrients and helpful phytoconstituents such as phenolics, flavonoids, alkaloids, ellagic acid, punicalagins and tannins. It is claimed to have antioxidant, anticancer, antimicrobial, anti-infective and anti-cardiovascular activities amongst many others. Due to its richness in phytoconstituents it was crowned the most health-promoting fruit in the super fruit group. Its extracts are used in many nutraceuticals as supplements in capsules[1,2]. The fruit of pomegranate is eaten as a delightful snack or incorporated in various products such as juice, jams and other delicacies. The other parts of the fruit such as the peel are rendered as waste and the other parts such as the leaves, roots and flowers are generally neglected. However, recently, these other unwanted parts have attracted so many researchers as to whether they contain any useful constituents such as the fruit itself. It is worth mentioning that the peel has been found to also contain powerful antioxidants[3, 4]. The leaves were found to contain some content but not as much as the peel. The studies have drawn attention as people around the world are looking for ways of developing sustainable habits and practices[5-8]. Many parts have been comparatively studied but we have noticed a research gap in the studies conducted earlier. There was no article or research work that focused on the study of the bark of the pomegranate plant. Therefore, we exploited this opportunity to increase the ever-growing well of knowledge in medicinal science[9-12]. Approximately 76% of the world's pomegranate is cultivated in India, Iran, China, Turkey and

How to cite this article: Balla S, Dasari N, Palina SP, Dinakaran SK, Pulidindi L. Colorimetric analysis of phenols and flavanoids in pomegranate plant extract. J Res Pharm. 2024; 28 (5): 1720-1741.

https://dx.doi.org/10.29228/jrp.2022.00
ISSN: 2630-6344

the USA. They all contain varying amounts of active constituents according to the environment that they are grown in[13-16].

2. RESULTS And DISCUSSION

A precise, accurate and economical method was made to produce the following results obtained.

2.1 Pharmacognostic evaluation

2.1.1 Powder microscopy

Microscopical evaluation for the pomegranate peel powder were performed. The result is as follows shown in Table 1.

Table 1. Powder Microscopy Parameters of the Powder in microns (μ)

PARAMETER	PEEL POWDER (μ)
Starch grains (length)	11
Calcium Oxalate crystal (length)	28
Trichomes (length)	6.5
Xylem vessels	32
Mucilage	40

2.1.2 Microscopical Analysis

Microscopical Analysis was performed for powder peel and the images were shown in Figure 1.

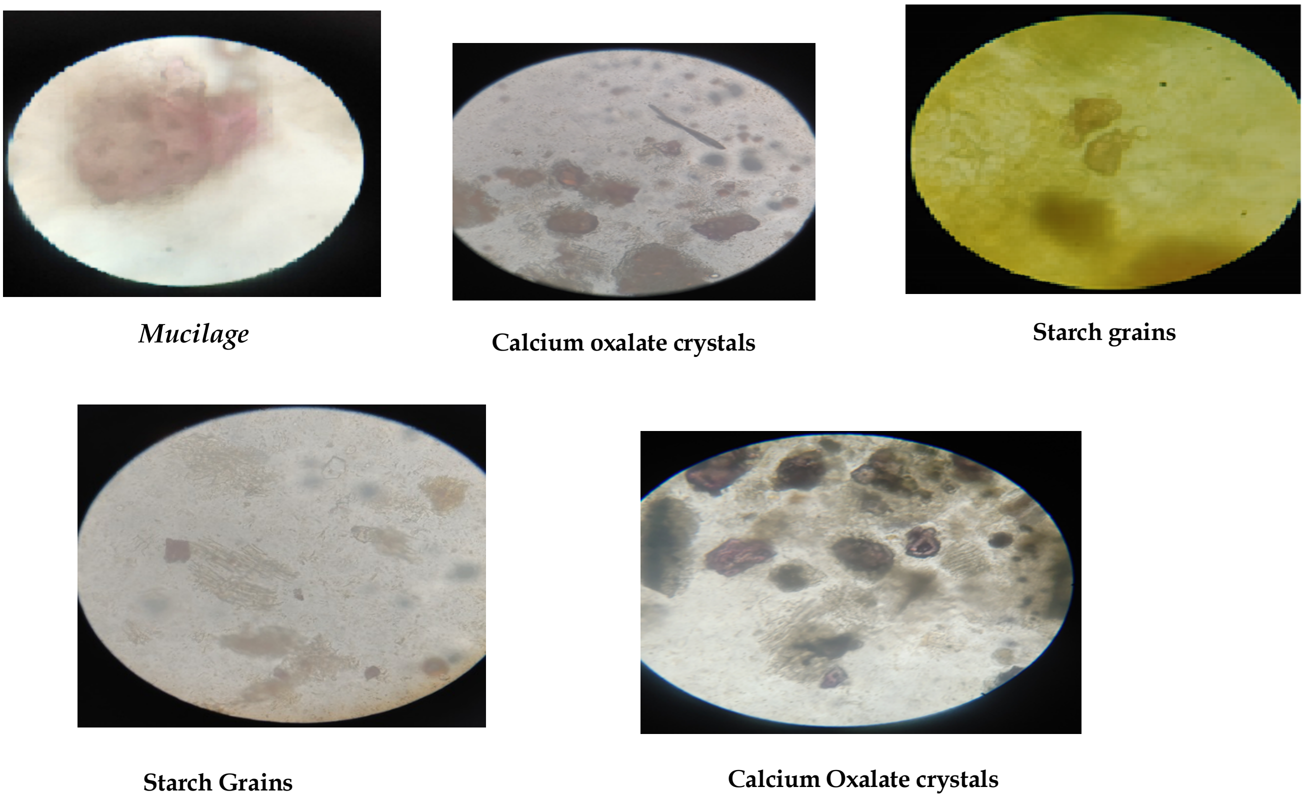

Figure 1. Powder microscopy results

2.1.3 Extraction yield

The percentage yield and the morphology of the extract are depicted in Table 2 below:

https://dx.doi.org/10.29228/jrp.2022.00
J Res Pharm 2024; 28(5): 1720-1741

Table 2. Percentage yield of the extracts and their physical appearance

	PERCENTAGE YIELD		*NATURE OF PLUCK OUT*	
EXTRACT (%w/w)	*ALCOHOL*	*WATER*	*ALCOHOL*	*WATER*
PEEL POWDER	64.9%	6.44%	Brown Sticky paste	Brown Crystalline powder

2.1.4 Proximate Analysis

Physicochemical properties like total ash value, moisture content of peel pluck out was analysed and the values are tabulated in Table 3.

Table 3. Physicochemical properties of the pluck out (values in %w/w)

	PEEL POWDER	
QUANTITATIVE PARAMETERS	*ETHANOL PLUCK OUT* *Values obtained (%w/w)*	*WATER PLUCK OUT* *Values obtained (%w/w)*
Total Ash Value	13.2	11.5
Acid Insoluble Ash	0.2	0.4
Water Soluble Ash	2.1	3.2
Sulphated Ash	1.1	3.2
Moisture Content	0.1	0.1
Alcohol Soluble Extract value	64.9%	6.44%
Water Soluble Extract Value	64.9%	6.44%

2.1.5 Fluorescence Analysis

Fluorescence analysis is a helpful tool for identifying the components of plant puck outs and gives an indication of their chemical makeup. It is also used to identify the adulteration of unprocessed pharmaceuticals. When the powder drug analysis was handled with different chemical reagents, observations were made in both visible and ultraviolet light. Table 4 provides the results of the samples' fluorescence analysis.

Aqueous extracts, alcoholic extracts of pomegranate peel Preliminary phytochemical tests to identify various chemical constituents. The existences of different Phytochemicals such as triterpenoids, steroidal glycoside, alkaloids, sugars, tannins, phenols, glycosides and flavonoids was analysed by known standard methods.

2.1.6 Preliminary phytochemical investigation

The results of the preliminary phytochemical investigations for the crude powder and the powder extracts showed the presence of the compounds as illustrated in the Table 5 below:

2.1.7 Preliminary thin layer chromatograpic studies

Secondary metabolites were analysed such as phenolics, flavonoids, tannins, saponins, glycosides and alkaloids using Thin Layer Chromatography (TLC). The results of the TLC for the crude powders and extracts are presented below in Figure 2 and results of TLC is tabulated in Table 6

Journal of Research in Pharmacy
Research Article

Table 4. Fluorescence analysis of the peel pluck out phytochemical analysis

S.NO	*REAGENT*	*Peel Alcoholic Pluck Out*			*Peel Aqueous Pluck*		
		Normal Light	*Shorter λ*	*Longer λ*	*Normal Light*	*Shorter λ*	*Longer Λ*
1	CH_3OH	Brown	Black	Pale Green	Brown	Black	Pale Green
2	50% H_2SO_4	Brown	Black	Pale Green	Brown	Black	Pale Green
3	50% HNO_3	Brown	Black	Pale Green	Brown	Black	Pale Green
4	50% HCl	Brown	Black	Pale Green	Brown	Black	Pale Green
5	5% NaOH	Brown	Black	Pale Green	Dark Brown	Black	Pale Green
6	1N methanolic NaOH	Brown	Black	Pale Green	Brown	Black	Pale Green
7	1N methanolic KOH	Brown	Black	Pale Green	Brown	Black	Pale Green
8	1N KOH	Brown	Black	Pale Green	Brown	Black	Pale Green
9	5% KOH	Brown	Black	Pale Green	Brown	Black	Pale Green
10	5% $FeCl_3$	Black	Black	Black	Black	Black	Black
11	Conc. HCl	Brown	Black	Pale Green	Brown	Black	Pale Green
12	Conc. H_2SO_4	Brown	Black	Pale Green	Dark Brown	Black	Dark Green
13	Ammonia	Brown	Black	Pale Green	Brown	Black	Pale Green
14	Conc. HNO_3	Brown	Black	Pale Green	Brown	Black	Pale Green

Table 5. Preliminary test results

Phytoconstituent	*PEEL*		*PEEL POWDER*
	Alcohol extract	*Water extract*	
Carbohydrates	+	+	+
Proteins & Amino acids	-	-	-
Flavonoids	+	+	-
Glycosides	+	+	+
Phytosterols	+	+	+
Saponins	+	+	+
Alkaloids	+	+	+
Phenolic compounds & Tannins	+	+	+

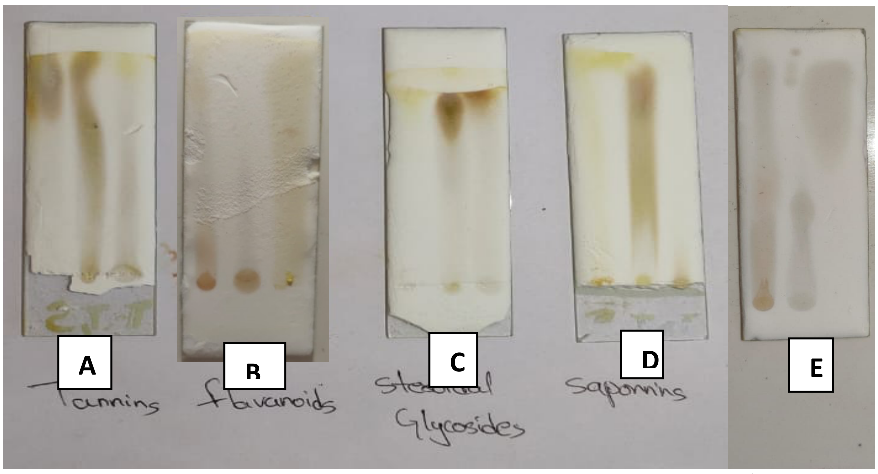

Figure 2. Images of TLC plates with different secondary metabolites **(Key: A:** Tannins, **B:** Flavonoids, **C:** Steroidal glycosides**, D:** Saponins, **E:** Phenols)

Table 6. TLC Results

Journal of Research in Pharmacy
Research Article

STANDARD	Rf VALUE OF STANDARD PEEL	PEEL PLUCK OUT ETHANOLIC	PEEL PLUCK OUT AQUEOUS
Phenol (Gallic Acid)	0.92	0.84	0.48
Tannins (Tannic acid)	0.94	0.88	0.69
Flavonoids (Quercetin)	0.98	0.54	0.54
Glycosides (Digoxin)	0.87	0.38	0.34
Saponins (Liquorice)	0.98	0.72	0.36

2.1.8 Determination of flavonoids and phenolic content by UV-Visible Spectroscopy

Linearity data for standard and samples are illustrated.

Table 7. Linearity data for standard and samples

FLAVONOID ANALYSIS				PHENOL ANALYSIS			
Standard		Sample		Standard		Sample	
Quercetin Linearity Data		Peel Ethanolic Pluck Out	Peel Aqueous Pluck Out	Gallic Acid Linearity Data		Peel Ethanolic Pluck Out	Peel Aqueous Pluck Out
Concentration (µg/ml)	Absorbance at 400 nm	Absorbance at 400 nm	Absorbance at 400 nm	Concentration (µg/ml)	Absorbance at 550 nm	Absorbance at 550 nm	Absorbance at 550 nm
1	0.086	0.043	0.060	1	0.119	0.057	0.051
2	0.128	0.072	0.111	2	0.149	0.107	0.068
3	0.175	0.098	0.187	3	0.178	0.167	0.085
4	0.215	0.122	0.252	4	0.201	0.233	0.098
5	0.255	0.151	0.306	5	0.229	0.291	0.116

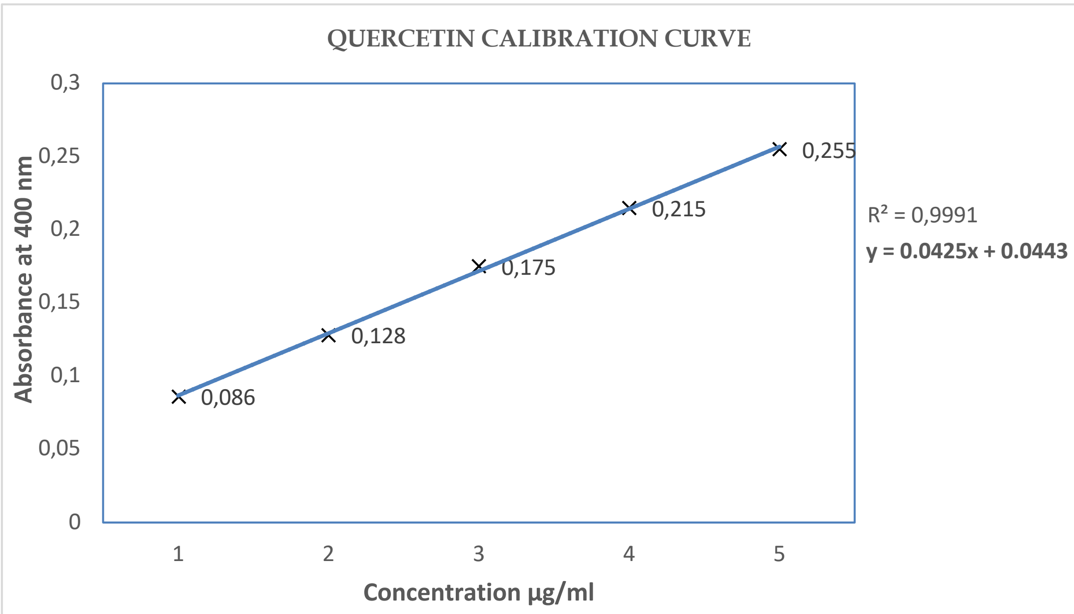

Figure 3. Linearity Plot for Quercetin (Standard)

https://dx.doi.org/10.29228/jrp.2022.00
J Res Pharm 2024; 28(5): 1720-1741

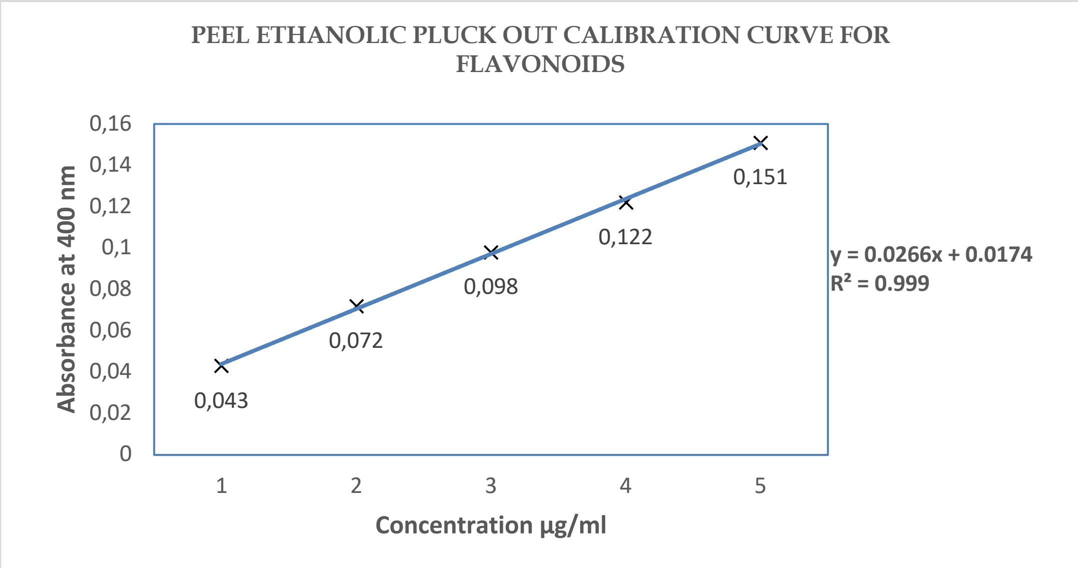

Figure 4. Linearity plot for peel ethanolic pluck out for flavonoids (Sample)

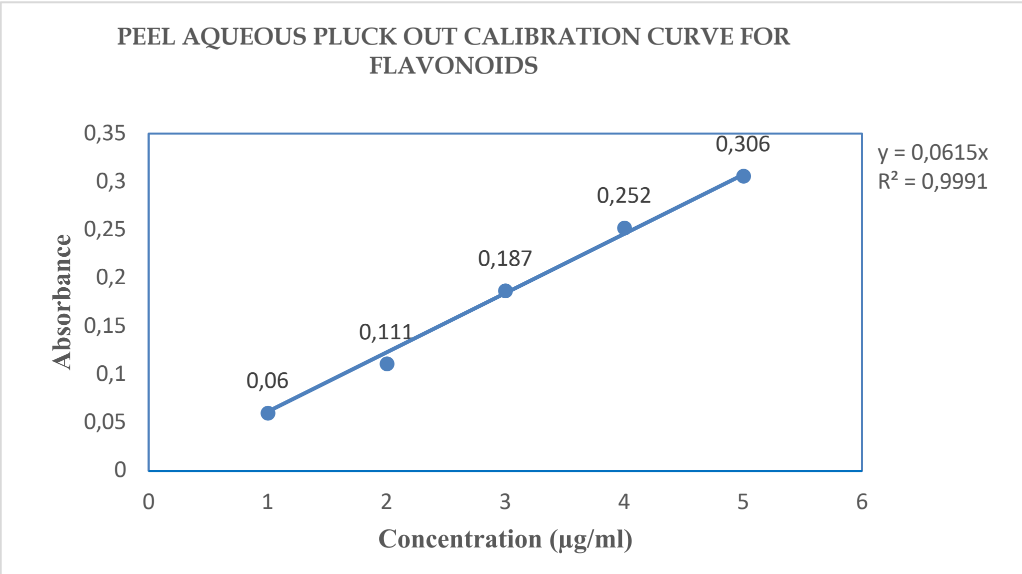

Figure 5. Linearity plot for peel aqueous pluck out for Flavonoids (sample)

Journal of Research in Pharmacy
Research Article

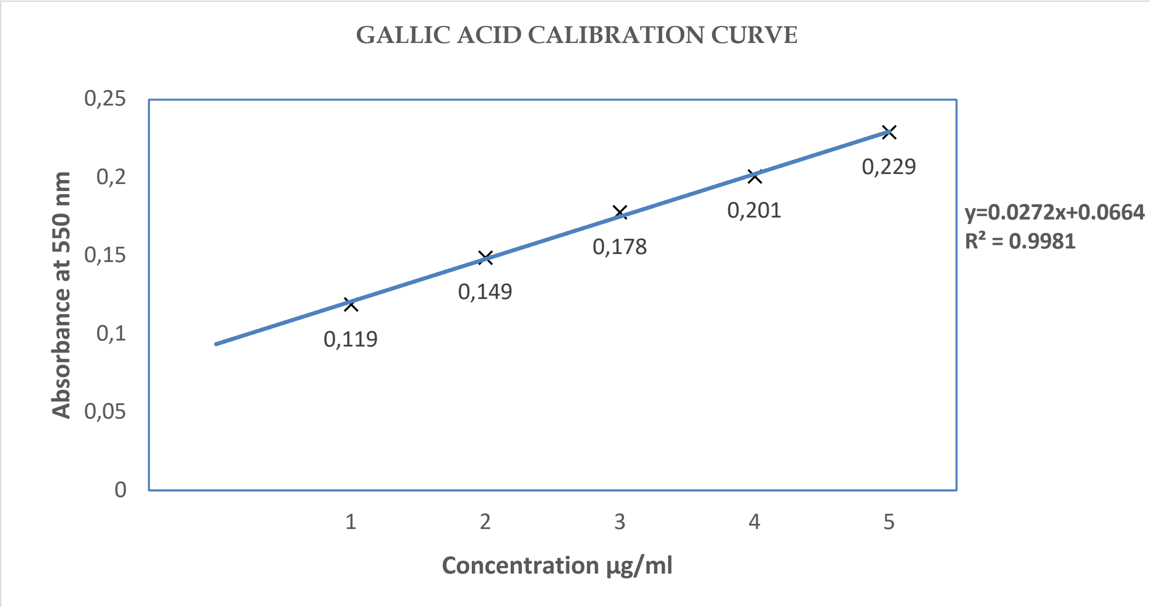

Figure 6. Gallic acid calibration curve (standard)

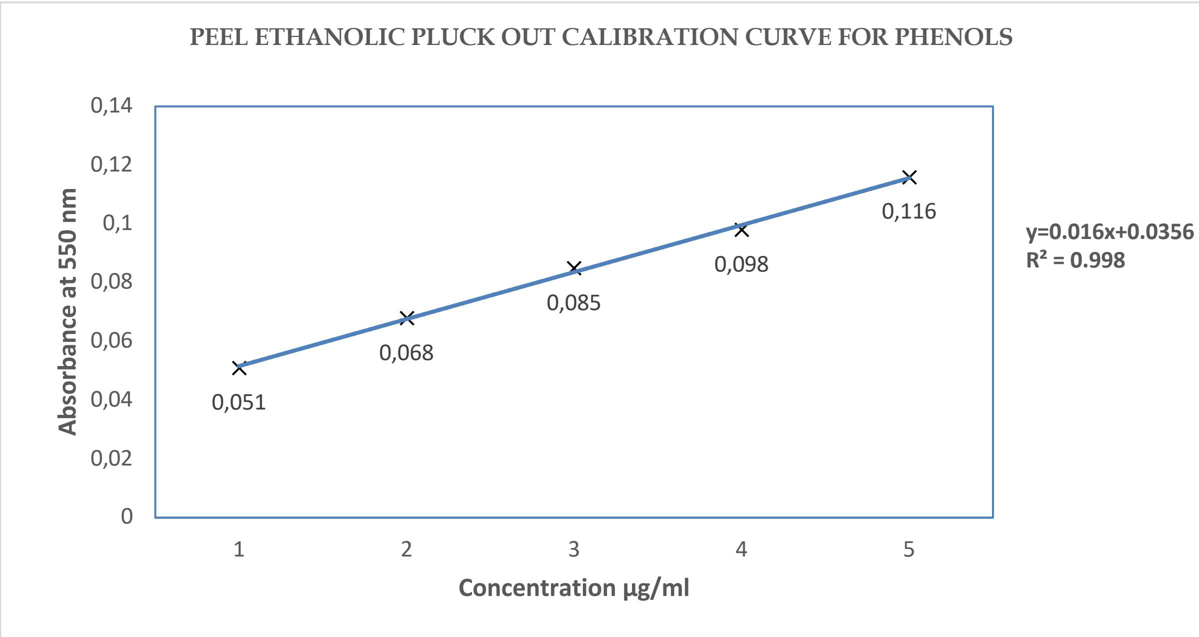

Figure 7. Linearity plot for peel ethanolic pluck out for phenols (Sample)

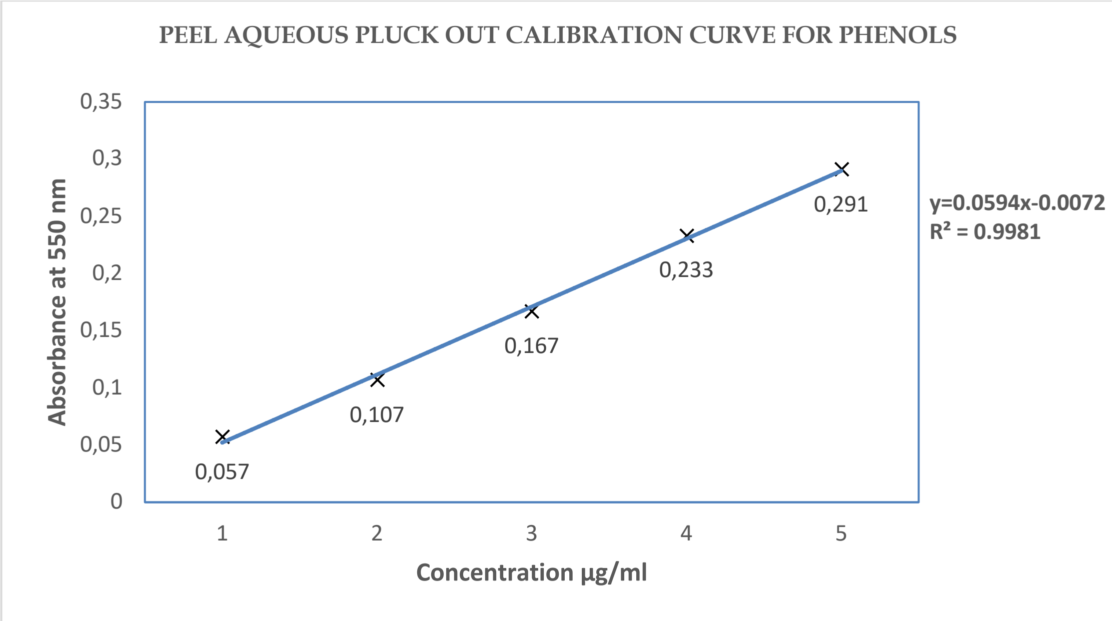

Figure 8. Linearity plot for peel aqueous pluck out for phenols (sample)

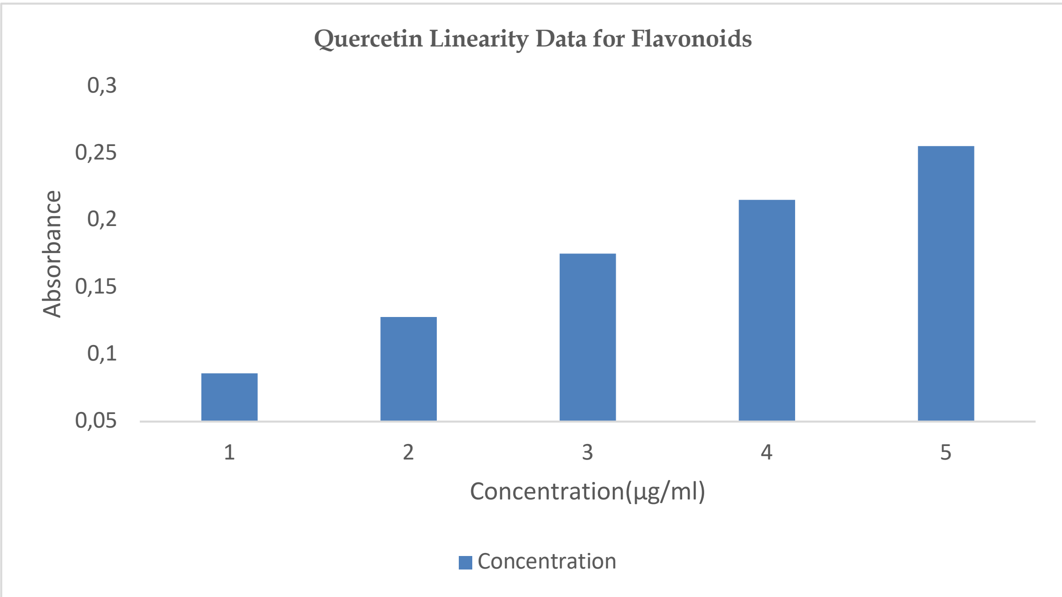

Figure 9. Quercetin linearity data for flavonoids (standard)

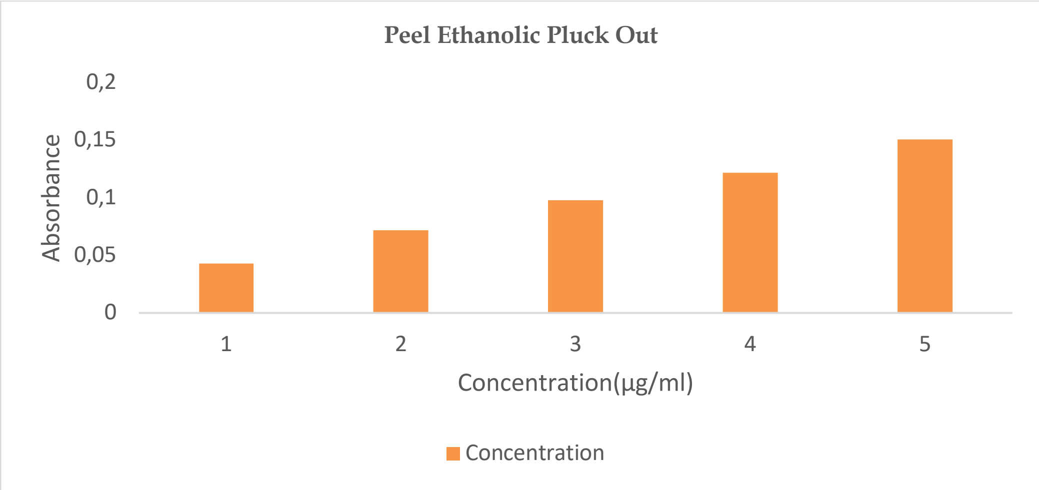

Figure 10. Peel ethanolic pluck out linearity data (sample)

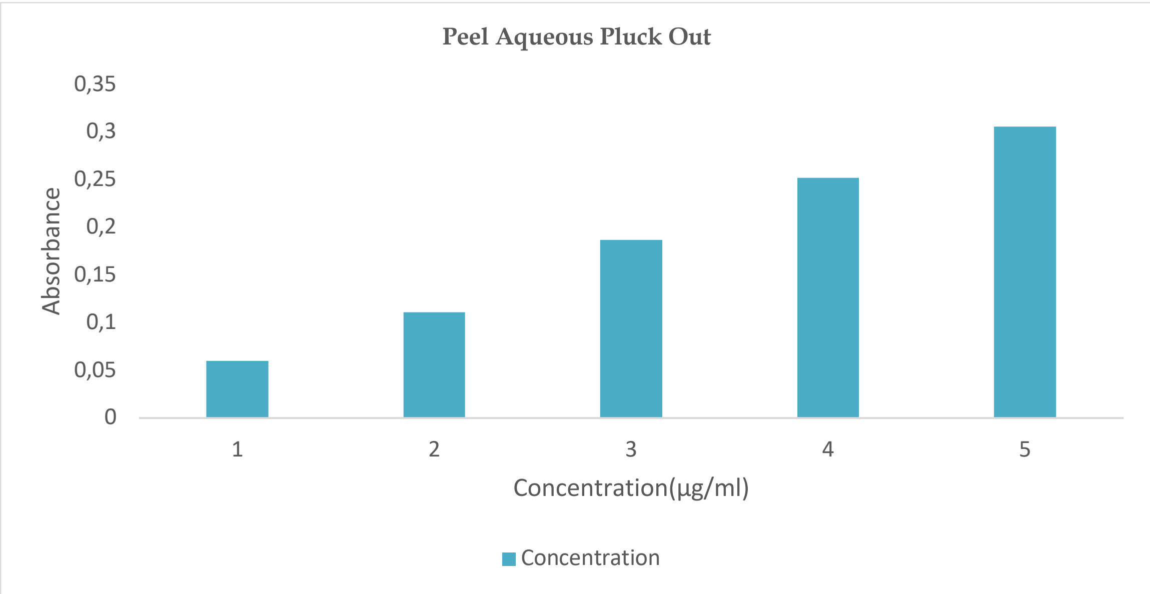

Figure 11. Peel aqueous pluck out linearity data (sample)

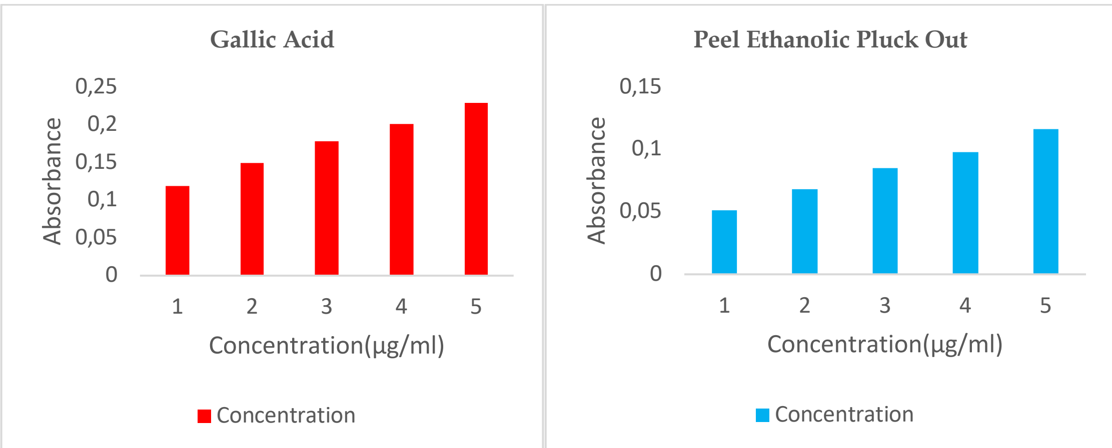

Figure 12. Gallic Acid (standard) and Peel Ethanolic Pluck Out Linearity Data (sample)

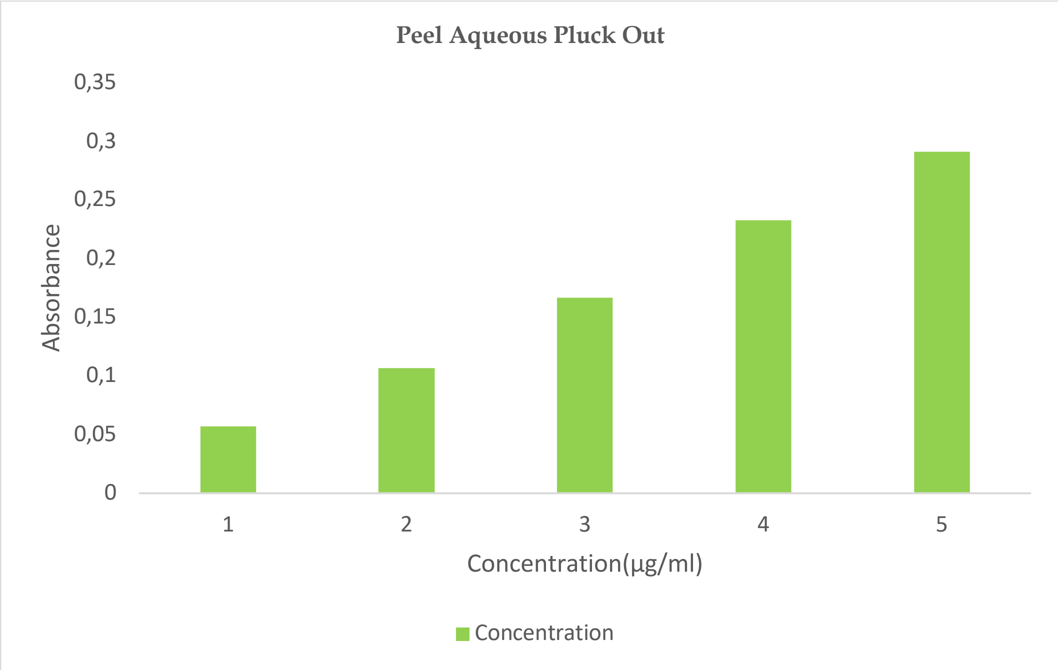

Figure 13. Peel aqueous linearity data for phenols (sample)

2.1.9 Beer-Lambert's Law Data

The results of Beer Lambert's law are shown in Table 8.

Table 8. Beer-Lamberts Law

FLAVONOID ANALYSIS				PHENOL ANALYSIS			
QUERCETIN (Standard)		PEEL ETHANOLIC PLUCK OUT	PEEL AQUEOUS PLUCK OUT	GALLIC ACID (Standard)		PEEL ETHANOLIC PLUCK OUT	PEEL AQUEOUS PLUCK OUT
Parameter	**Absorbance at 400 nm**	**Absorbance at 400 nm**	**Absorbance at 400 nm**	**Parameter**	**Absorbance at 550nm**	**Absorbance at 550nm**	**Absorbance at 550nm**
Linearity range	1µg/ml to 5 µg/ml	1µg/ml to 5 µg/ml	1µg/ml to 5 µg/ml	Linearity range	1µg/ml to 5 µg/ml	1µg/ml to 5 µg/ml	1µg/ml to 5 µg/ml
Correlation coefficient	0.9991	0.999	0.9991	Correlation coefficient	0.9981	0.998	0.9981
Slope (m)	0.0425	0.0266	0.0654	Slope (m)	0.0272	0.016	0.0594
Intercept (c)	0.0443	0.0174	0.0108	Intercept (c)	0.0664	0.0356	0.0072

2.2. Observation and inference

The graphs give straight lines and obeys Beer-Lamberts Law. Regression coefficient was found to be within the limits of accuracy.

2.2.1 Validation parameters

The validation parameters like accuracy, precision, LOQ and LOD are checked and the results are illustrated in Tables 12-16.

2.2.2 Accuracy data

Table 9. Accuracy data for flavonoids at 400nm

Concentration	Amount Spiked	Total Amount	PEEL ETHANOL PLUCK OUT Amount found	PEEL ETHANOL PLUCK OUT % Recovery	PEEL AQUEOUS PLUCK OUT Amount found	PEEL AQUEOUS PLUCK OUT % Recovery
2µg	1µg	3	2.98	99.33	2.95	98.33
3µg	1µg	4	3.99	99.75	3.93	98.25
4µg	1µg	5	4.97	99.40	4.94	98.80

Table 10. Accuracy data for phenols at 550 nm

Concentration	Amount Spiked	Total Amount	PEEL ETHANOL PLUCK OUT Amount found	PEEL ETHANOL PLUCK OUT % Recovery	PEEL AQUEOUS PLUCK OUT Amount found	PEEL AQUEOUS PLUCK OUT % Recovery
2µg	1µg	3	2.94	98.0	2.89	96.33
3µg	1µg	4	3.92	98.0	3.92	98.00
4µg	1µg	5	4.91	98.2	4.95	99.00

In conclusion, recovery % was found to be within the limits. This indicates that the method is accurate.

2.2.3 Precision

Table 11. Intra day precision data

SAMPLE CONCENTRATION	FLAVONOIDS PEEL ETHANOLIC PLUCK OUT	FLAVONOIDS PEEL AQUEOUS PLUCK OUT	PHENOLS PEEL ETHANOLIC PLUCK OUT	PHENOLS PEEL AQUEOUS PLUCK OUT
3µg/ml	**Absorbance**	**Absorbance**	**Absorbance**	**Absorbance**
1	0.098	0.187	0.167	0.085
2	0.095	0.185	0.166	0.084
3	0.096	0.182	0.165	0.086
4	0.098	0.186	0.166	0.085
5	0.097	0.187	0.165	0.085
6	0.095	0.185	0.167	0.085
Mean	0.0965	0.1853	0.166	0.085
SD	0.00125	0.0017	0.000894	0.00070
% RSD	1.2953	0.9174	0.5385	0.8235

Table 12. Inter day precision data

SAMPLE	FLAVONOIDS ABSORBANCE				PHENOLS ABSORBANCE			
	PEEL ETHANOLIC PLUCK OUT		PEEL AQUEOUS PLUCK OUT		PEEL ETHANOLIC PLUCK OUT		PEEL AQUEOUS PLUCK OUT	
S.NO	DAY - I	DAY - II	DAY – I	DAY – II	DAY – I	DAY – II	DAY – I	DAY – II
1	0.098	0.095	0.187	0.185	0.167	0.165	0.085	0.084
2	0.095	0.096	0.185	0.186	0.166	0.165	0.084	0.083
3	0.096	0.098	0.182	0.183	0.165	0.164	0.086	0.085
4	0.098	0.095	0.186	0.185	0.166	0.165	0.085	0.084
5	0.097	0.096	0.187	0.186	0.165	0.164	0.085	0.084
6	0.095	0.094	0.185	0.184	0.167	0.166	0.085	0.085
Mean	0.0965	0.0957	0.1853	0.1848	0.166	0.1648	0.085	0.0841
SD	0.001258	0.001247	0.0017	0.00107	0.00089	0.00075	0.00070	0.00075
%RSD	0.1258	0.1247	0.1700	0.1067	0.5385	0.4550	0.8235	0.8917

In conclusion, %RSD was found to be within the limits. So, the method was found to be precise.

Table 13. LOD and LOQ data

PARAMETERS	FLAVONOIDS ABSORBANCE (400nm)		PHENOLS ABSORBANCE (550nm)	
	PEEL ETHANOLIC PLUCK OUT	PEEL AQUEOUS PLUCK OUT	PEEL ETHANOLIC PLUCK OUT	PEEL AQUEOUS PLUCK OUT
LOD	0.895µg/ml	0.555µg/ml	0.491µg/ml	0.623µg/ml
LOQ	1.50µg/ml	1.25µg/ml	1.58µg/ml	1.75µg/ml

2.3 FTIR Spectral Data

FTIR was performed by Pressed Pellet Technique and the results are illustrated in form of figuresand tables in figures 14-17 and Tables 17-20

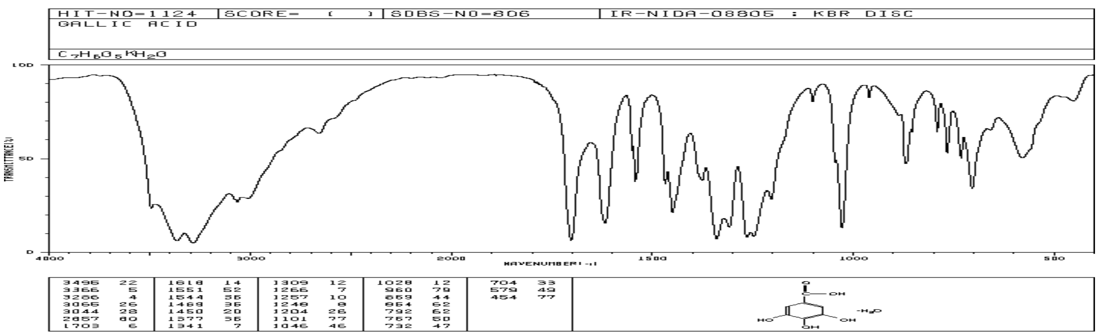

Figure 14. FTIR Spectra for Gallic Acid

Table 14. FTIR data for gallic acid

Wavenumber (1/cm)	Functional group for Gallic acid
3496, 3366, 3266 (broads)	Aromatic O-H Stretch
3044	Aromatic CH stretch
1703	Carboxylic C=O Stretching
1618	Aromatic C=C Bending
1248, 1028	Aromatic C-O stretch
704	Aromatic CH bending

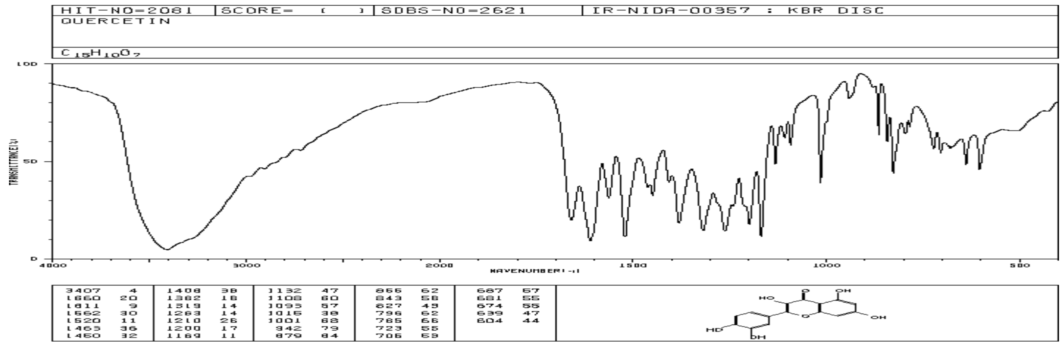

Figure 15. FTIR spectra for quercetin

Table 15. FTIR Spectra for Quercetin

Wavenumber (1/cm)	Functional group for Quercetin
3407 (broads)	Aromatic O-H Stretch
1660	C=O Stretching
1611	Aromatic C=C Bending
1362	CH bending
1169	Aromatic C-O stretch
1108, 1095	C-O stretching for C-O-C
723	Aromatic CH bending

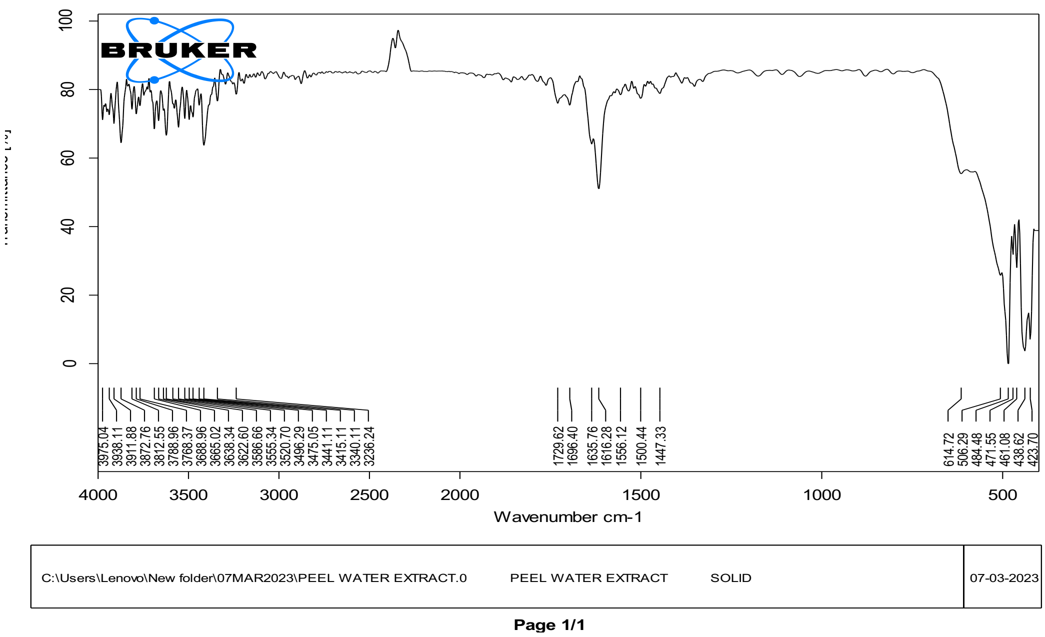

Figure 16. FTIR spectra for peel aqueous pluck out

Table 16.FTIR Spectra for Peel Aqueous Pluck Out

Wavenumber (1/cm)	Functional group for Gallic Acid
713	Aromatic CH bending
1672	C=O for aldehyde
1666	Aromatic C=C stretching
3500-3300	Phenolic OH

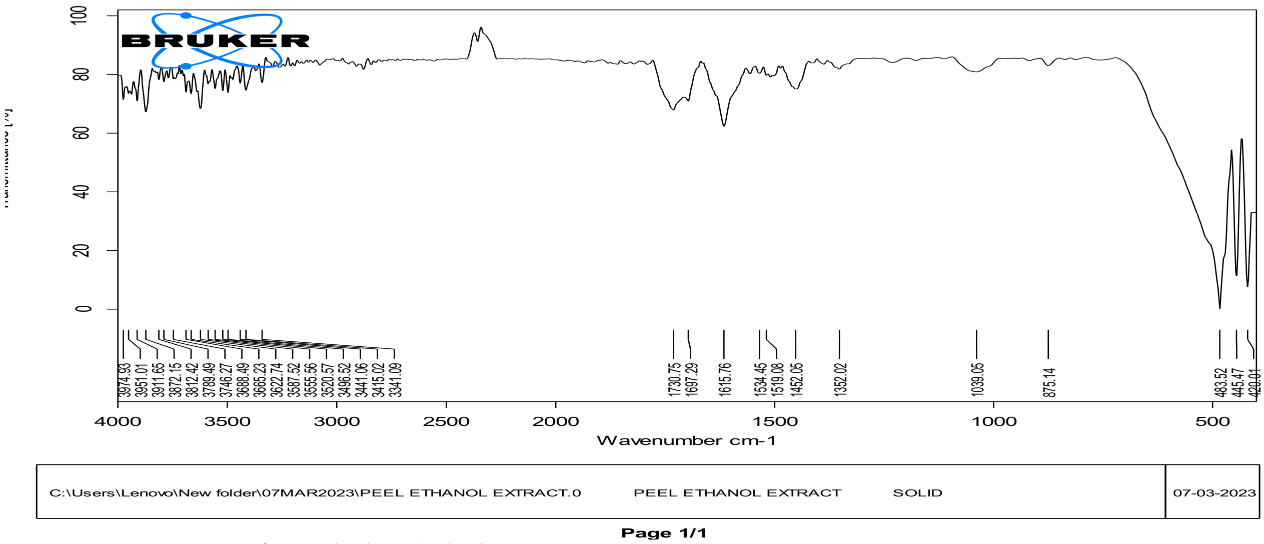

Figure 17. FTIR spectra for peel ethanol pluck out

Table 17. FTIR spectra for peel ethanol pluck out

Wavenumber (1/cm)	Functional group for Gallic Acid
1360	CH bending
1605	Aromatic C=C Bending
1656	C=O Stretching
3418 (Broad, s)	Aromatic O-H Stretch
3500-3300	Phenolic OH

3. CONCLUSION

The results indicate that the proposed method is simple, precise, and accurate. They comply with the Method Validation in line with ICH guidelines. Moreover, colorimeters are readily available and affordable. The table presents absorbance data for flavonoids and phenols extracted from peel samples using different solvents (ethanolic and aqueous) and extraction methods (pluck out). The absorbance measurements were taken at two wavelengths (400nm and 550nm), and the parameters such as linearity range, regression, slope, intercept, LOD (Limit of Detection), and LOQ (Limit of Quantification) were determined for each combination. The results indicate that the flavonoids and phenols extracted from peel samples show good linearity within the specified concentration ranges for both ethanolic and aqueous extracts, regardless of the extraction method. The regression values are consistently high, suggesting a strong correlation between concentration and absorbance at both wavelengths. The slopes and intercepts provide insights into the sensitivity and baseline values of the calibration curves. The LOD and LOQ values indicate the lowest concentrations that can be reliably detected and quantified, respectively, for each combination. The results are tabulated in Table 18.

Table 18. Summarized table

PARAMETERS	FLAVONOIDS ABSORBANCE (400nm)		PHENOLS ABSORBANCE (550nm)	
	PEEL ETHANOLIC PLUCK OUT	PEEL AQUEOUS PLUCK OUT	PEEL ETHANOLIC PLUCK OUT	PEEL AQUEOUS PLUCK OUT
Wavelength(nm)	400nm	400nm	500nm	500nm
Linearity Range	1-5µg/ml	1-5µg/ml	1µg/ml to 5 µg/ml	1µg/ml to 5 µg/ml
Regression	0.998	0.9855	0.998	0.9981
Slope	0.016	0.0654	0.016	0.0594
Intercept	0.0174	0.0108	0.0356	0.0072
LOD	0.895µg/ml	0.555µg/ml	0.491µg/ml	0.623µg/ml
LOQ	1.50µg/ml	1.25µg/ml	1.58µg/ml	1.75µg/ml

4. MATERIALS AND METHODS

4.1 Chemicals and reagents

Aluminium trichloride, Ethanol, Quercetin, Acetic acid, Tannic acid, Liquorice, Gallic acid, Digoxin, Ethyl acetate and Distilled water.

4.2 Morphological observations

The size, shape, colour, taste and odour of fruit peel, bark and leaves were observed with naked eye or with the help of microscope for morphological ıdentification.

4.3 Collection of plant material and preparation of extract

Fresh fruit of pomegranate were purchased from a market in the city of Kakinada, East Godavari district, Andhra Pradesh, in beginning of November, 2022 and the fruits were eaten and the peels were set aside. By soxhlation with ethanol and water, the extracts were made. The dried entire powder was placed in a thimble and stored in aa Soxhlet apparatus, where ethanol and water were used as individual solvents for the extraction process. The marc was finally dried. By evaporating the solvent, ethanol and aqueous extract were concentrated, and the resulting extracts were weighed. The different extracts physical properties and yield percentages were recorded. Prior to analysis, the dried extracts of all solvents were in a desiccator [15-17]. In research conducted by Marra et al., reported a comparison of 2 drying procedures amongst the “Wonderful” varieties of pomegranate cultivated in Southern Italy, South Africa and India. They tested components such as phenols, flavonoids, antioxidants and antioxidant activities by HPLC. In another research conducted by Karthikeyan et al., 2019 studied and reported about the antioxidant and antibacterial activity of the pomegranate peel. In an investigation led by Redha et al., in 2018 performed Analytical and Medicinal analysis of the peel extract as well as the juice of pomegranate. They used freeze-drying technique to dry the peel (rind) and the seeds (aril). Furthermore, the research work reported by Zhao et al., on flavanol and flavone changes in the pomegranate peel extracts during the growth of the fruit. The differences were done comparatively against four Chinese cultivars. This study was done using HPLC. In 2020 Fernanda machado chaves et al., reported a comparison between juice and the pomegranate peel extracts in prostate cancer DU-145 and PC-3 cell lines.

4.4 Processing of plant materials

Processing of the plant samples involved washing the gathered plant materials with distilled water and rinsing them with clean tap water. The peel, leaves, and bark were allowed to dry at room temperature(24-27ºC), without any additional light or moisture from the air. The remaining plant components were ground into a powder while a tiny amount of the air-dried samples was employed for

Macroscopic, Organoleptic, and Anatomical (transverse section) examinations. The entire and powdered plant samples were kept in airtight, light-resistant containers that were sealed with lids at room temperature. The materials were sieved in 60# and ground into fine and coarse powders for physicochemical, phytochemical, and chromatographic analyses. For the physicochemical and phytochemical investigations, shade- dried powdered samples were additionally used according to standard method.

4.5 Microscopical observations

4.5.1 Powder Microscopy

On a glass slide, a small amount of the powder was added together with a few drops of phloroglucinol and concentrated Hcl (1:1). Remove the excess reagents with tissue and then mount it with glycerine and cover it with a cover slip. Care must be taken to avoid air bubbles[17].

4.6 Determination of Moisture content (Loss on drying)

1.5g of the crude plant product (peel and bark) was weighed into a thin porcelain dish that was dried in a 100°C oven and cooled in a desiccator. The weight loss was documented[18].

4.7 Determination of ash values of a crude drug as values

Total Ash value is calculated by taking about 1g of the crude plant powder into a crucible dish and incinerate it until vapours cease to be evolved. Then this was cooled in a desiccator. Total Ash value was calculated with reference to the air-dried sample.

$$Total\ ash\ value = \frac{Z - X}{Y} \times 100$$

Where, X= weight of empty dish
Y= weight of the dish + ash (after Incineration)
By performing the procedures listed in the technique for determining the total ash value of a crude medication in reverse order, acid soluble ash is computed. The remaining ash form the plate was then washed into a 100ml beaker with 25ml of diluted Hydrochloric acid (HCl). An ashless filter paper was used to filter the solution. The filtration paper caught fire. The desiccator was cooled after that. Based on the weight of the air-dried sample of the crude drug, if this residue were computed as an acid-insoluble ash of the crude drug. [18].
Weight of Residue= 'a' g (Equation here, Acid Insoluble Ash)

'y' g of the air-dried drug gives $\frac{100\ x\ a}{y}$g of Acid-Insoluble Ash.

Acid Insoluble Ash value of the sample = $\frac{100\ x\ a}{y}$% .

Similar procedure was applied as that of acid-insoluble ash value but solubilization was done in water.

About 2g of the raw plant powder was weighed into the dish or crucible and burned until no more vapours were emitted to determine the sulphated ash method. After that, it was cooled, soaked with 1 cc of sulfuric acid, gently heated until no longer emitting white vapours, then burned at 800ºC for 25ºC until no longer producing black particles. A few drops of sulphuric acid were added once the product had cooled, and the combination was once again heated and ignited before being allowed to cool and be weighed. The procedure was performed two more times until the weight difference was no greater than 0.5mg.[19, 20].

4.8 Determination of acid insoluble extractives

A dry 250ml conical flask was filled with about 5g of the raw plant material after it had been weighed in a weighing bottle. A 100ml graduated flask containing 90% alcohol was used. After being corked, the flask was left for 24 hours while being periodically shaken, as in maceration. The mixture was filtered into a thin porcelain plate that had been weighed in order to determine the ash value. On a water bath, the mixture was evaporated to dryness, and the drying process was finished in a 100°C oven. It was weighed after cooling in the desiccator. Calculations were used to determine the extractive's weight percentage relative to the air-dried crude plant.

25ml of alcoholic extract = x g of residue

100ml of alcoholic extract = 4(x) g of residue

There is approximately 4(x) g of alcohol-soluble residue for every 5 g of air-dried medication. 80 (x) grams of alcohol are produced for every 100 grams of air-dried medication, with a reference content of 90% and no soluble residue. 80 (x) percent of the sample's extractive value is soluble in alcohol (90 percent).

Similar procedures were performed to determine water-soluble extractives, but instead of alcohol, chloroform water was utilised (chloroform acts as a preservative).

With steady stirring, 50 ml of 10% v/v nitric acid was heated with 2 g of coarse plant powder. Remainder rinsed in hot water after mixture was strained through fine cotton. 50ml of a 2.5 percent v/v sodium hydroxide solution was added before boiling this. Hot water was used to wash the strained mixture. Weighing the residue, the proportion of crude fibres was calculated. [21-23].

4.9 Fluorescence analysis

After being treated with various chemical and organic reagents, powdered medication was examined under ultraviolet light. Three factors—observation under long-wavelength UV (266 nm), short-wavelength UV (256 nm), and regular daylight—were taken into account. [24, 25].

Procedure: A beaker was filled with 2 g of powdered drug sample, which was then dissolved in 5 cc of methanol. The sample was put on a watch glass, and the colour and fluorescence were checked in a UV chamber. Similar processes and findings were reported with a variety of chemicals, including 50% H_2SO_4, 50% HNO_3, 5% NaOH, IN methanolic KOH, 1N methanolic KOH, 1N KOH, 5% KOH, 5% $FeCl_3$, 5% HCl, 5% H_2SO_4, 5% ammonia, and 5% HNO_3. Similar extracts were also put through a UV chamber, where fluorescence was seen and consistency was highlighted as a unique characteristic for identification. [26-28].

4.10 Phytochemical screening

All of the extracts (peels) underwent preliminary examinations to identify the various chemical components that were present. By using the normal standard techniques, the presence or absence of several phytoconstituents, such as carbohydrates, proteins, and amino acids, flavonoids, glycosides, phytosterols, alkaloids, phenolic compounds, and tannins, was determined. [29, 30].

4.10.1 Preliminary Thin Layer Chromatography

Phenols and flavonoids were qualitatively determined using thin layer chromatography. The TLC plates were treated to saturation with a formulated appropriate solvent. Before conducting the analysis, combine toluene, acetone, and formic acid (4.5:4.5:1). The extracts were spotted on a TLC plate, dissolved in the proper solvent, and dried in a hot air oven. After drying, locate spots on a chromatogram using the Folin-Ciocalteu reagent to carry out the resolution of extracts' components. Each spot's distance from the application point is measured, noted, and the Rf value is computed. [6, 31].

Table 19. Thin Layer Chromatography for Various Constituents

Phytoconstituents	Solvent system	Detection 1	Detection 2
Phenols	Acetone, toluene, Formic acid (4.5: 4.5: 1)	Folin Cio-calteu reagent and Sodium carbonate solution.	Iodine Chamber
Tannins	n-butanol, Acetic Acid, Water (4: 1: 5)	UV – Chamber	Iodine Chamber
Flavonoids	Toluene (30): Ethyl acetate (40): Glacial acetic acid (5).	1:1 mixture of potassium ferrocyanide and ferric	Iodine Chamber

		chloride, along with a UV lamp (254nm).	
Steroidal glycoside	Ethyl Acetate (100), Methanol (13.3), Water (10)	UV – Chamber	Iodine Chamber
Saponin	Chloroform (64): Methanol (50): Water (10	Vanillin Sulphuric acid	Iodine Chamber

4.10.2 quantification of flavonoid content by UV-Visible Spectroscopy

Using quercetin as a reference substance, the total flavonoid contents of the extracts were determined using the specified methodology. The following procedure was used to determine the total flavonoids: A mixture of 1 ml of an extract in methanol (10g/L) and 1 ml of aluminium chloride in ethanol (20g/L) was combined before being diluted to a final volume of 25mL with ethanol. After 40 minutes at 200°C, the absorbance of 400 nm was measured. 1ml of plant extract and 1 drop of acetic acid were combined to create blank samples, which were then diluted to 25ml. The same process was used to prepare the quercetin calibration curve in ethanolic solutions. ethanolic solutions were used to create the various quercetin concentrations. The overall flavonoid concentration in quercetin equivalents in plant extracts was calculated. A standard curve (1 to 5 g/ml; y=0.03524x+0.000093; r2 = 0.99410.0071; y is the absorbance; x is the solution concentration) was created using quercetin. For each gramme of powdered crude medication, the results were represented as milligrams of quercetin equivalents (GAE). [11, 32, 33].

4.10.3 Quantification of phenolic content by UV-Visible Spectroscopy

A modified Folin-Ciocalteu colorimetric method was used to conduct a spectrophotometric analysis of the total phenolic content. In each test tube, 0.125ml of all the extracts (1:10g/ml) were taken. Folin-Ciocalteu reagent was diluted to 0.125 ml in 1.5 ml of water, and the mixture was left to stand for 6 minutes. Each mixture contained 3ml of water, 1.25ml of sodium carbonate, 7 percent, and was left to stand for 90 minutes at room temperature. Using an Elico UV/Visible spectrophotometer, the absorbance was assessed at 550 nm following colour development. A standard curve (1 to 5 g/ml; y= 0.1071x+0.007829; r2= 0.9987 0.0016; x is the solution concentration) was created using gallic acid. The results were expressed as milligrams of gallic acid equivalents (GAE) per gramme of powdered medication for each gramme of powdered crude. [34-36].

4.10.4 FTIR Studies

FTIR was performed by Mull technique, Pressed pellet followed by thin film techniques.

4.10.5 Mull Technique

Sample type: solid, sample cell; Sodium chloride
Using the Nujol Mull Technique, sample a slurry. The sample is crushed in an agate mortar and pestle, combined with Nujol, and pounded into a thick paste. A spectrum is captured when a spot is applied between two sodium chloride and positioned in the instrument's IR beam path[20, 37, 38].

4.10.6 Pellet Technique

Sample type: solid, sample cell: Potassium Bromide (KBr).
The sample is dissolved in a solvent that is non-aqueous, non-reacting, and non-IR absorbing in the measurement range after being pressed in a die-dried sample film. On a sodium chloride(Nacl) plate, a drop of this solution is applied, and the solvent is evaporated until it is completely dry, leaving a thin sample layer [39].

4.10.7 Thin Film

Sample type: Solid, sample cell: NaCl plates.
Drying of sample on plate forced pellet using an evacuable die and hydraulic press, a one milligram sample to 100 milligram KBr is finely ground and crushed onto a transparent disc. Another disc is created similarly using only dry, KBr and is used as a guide[40].

4.10.8 Determination of flavonoids and phenolic content by UV-Visible Spectroscopy

Parameters fixation: The optimum conditions for the determination of the Phenols and Flavonoids were established via several preliminary experiments according to previous research work by various researchers[24].

Table 20. Standard and Sample Solution Preparation for UV-Visible Spectroscopy

S. No	Solution	PROCEDURE FOR SOLUTION PREPARATION				DETECTION	
		FLAVONOIDS		PHENOLS		Flavonoid	Phenol
		STANDARD	SAMPLE	STANDARD	SAMPLE		
1	Stock I	Dissolve 100mg of quercetin in 100ml of distilled water.	100ml of distilled water is used to dissolve 100mg of the ethanol and aqueous extract.	In 100ml of distilled water, 100mg of gallic acid is dissolved.	100ml of distilled water is used to dissolve 100mg of the ethanol and aqueous extract.	400nm	550 nm
2	Stock II	1ml of Stock I solution into 100ml volumetric flask	1ml of Stock I solution into 100ml volumetric flask	1ml of Stock I solution into 100ml volumetric flask	1ml of Stock I solution into 100ml volumetric flask	400nm	550 nm
3	Working	Pipette out 0.1ml, 0.2ml, 0.3ml, 0.4ml and 0.5ml (1 to 5µg/ml) into five different volumetric flasks + 0.1ml of 95% Ethanol + 0.1ml of 10% Aluminium chloride ($Alcl_3.6H_2O$) + 0.10ml of sodium acetate + 2.80ml of distilled water and allowed incubate for 40mins. After colour development make up to 10ml with distilled water	Pipette out 0.1ml, 0.2ml, 0.3ml, 0.4ml and 0.5ml (1 to 5µg/ml) into five different volumetric flasks + 0.1ml of 95% Ethanol + 0.1ml of 10% Aluminium chloride ($Alcl_3.6H_2O$) + 0.10ml of sodium acetate + 2.80ml of distilled water and allowed incubate for 40 mins. After colour development make up to 10ml with distilled water	Pipette out 0.1ml, 0.2ml, 0.3ml, 0.4ml and 0.5ml (1 to 5µg/ml) into five different volumetric flasks + 0.15ml of water + 0.125ml of Folin-Ciocalteu Reagent + 1.25ml of 7% Sodium carbonate and allowed incubate for 90 mins. After colour development make up to 10ml with distilled water	Pipette out 0.1ml, 0.2ml, 0.3ml, 0.4ml and 0.5ml (1 to 5µg/ml) into five different volumetric flasks + 0.15ml of water + 0.125ml of Folin-Ciocalteu Reagent + 1.25ml of 7% Sodium carbonate and allowed incubate for 90 mins. After colour development make up to 10ml with distilled water	400nm	550 nm

4.10.9 Preparation of 10% aluminium chloride solution: Weigh accurately 10 mg of aluminium chloride into 100ml volumetric flask and dissolve in distilled water and make up to the mark with the distilled water.
4.10.10 Preparation of sodium acetate solution: accurately weigh 24.6gm of Sodium Acetate into 100ml Volumetric flask and add 80ml of distilled water to it. Then adjust the P^H of the 5.2 with Glacial Acetic acid and allow the solution to cool overnight.
4.10.11 Preparation of 7% sodium carbonate solution: Weigh accurately 7gm of Aluminium chloride into 100ml volumetric flask and dissolve in distilled water and make up to the mark with the distilled water.

Table 21. Validation parameters of the developed method

S.NO	VALIDATION PARAMETERS	PROCEDURE	ACCEPTANCE CRITERIA
1.	**LINEARITY**	Calibration curve was constructed by using Concentration Vs Absorbance for 5 concentrations of standard and sample solutions. A line of best fit was taken, and the correlation coefficient, slope and y-intercept were calculated.	R^2 value should not be less than 0.98.
2.	**ACCURACY**	Recovery study was conducted at the level of 3 concentrations 2µg, 3µg, 4µg of the normal or target concentration.	The average percentage recovery should be between 99-101%
3.	**PRECISION**	Repeatability: Measuring 6 replicates of sample containing 3µg/ml. INTER-DAY PRECISION: It checked on 2 consecutive days by preparing 3µg/ml concentration and the absorbances were checked. %RSD was calculated.	%RSD: Not More Than 2% %RSD: Not More Than 2%
4.	**SPECIFICITY**	Absorbance of blank solution was measured and is found to very negligible -0.014.	--
5.	**LOD AND LOQ**	The limit of detection and limit of quantification Samples were calculated from the calibration curve, by using formula. LOD $=\frac{3.3\sigma}{S}$ LOQ $=\frac{10\sigma}{S}$ $\sigma = Standard\ deviation\ of\ responses.$ S = Slope of calibration curve.	--

Acknowledgements: I hereby acknowledge to my parents and all my teachers who made me stand in a position to teach many students. We gratefully acknowledge the Aditya Pharmacy College, affiliated to JNTU Kakinada for providing research facilities.

Author contributions: Concept – S.B., N.D.; Design – S.B., N.D; Supervision – S.B., N.D; Resources – S.B., N.D., S.P.,S.D.,L.P; Materials – S.B., N.D., S.P.,S.D.,L.P; Data Collection and/or Processing – S.B., N.D., S.P.,S.D.,L.P; Analysis and/or Interpretation – S.B., N.D.; Literature Search – S.B., N.D., S.P.,S.D.,L.P; Writing – S.B., N.D., S.P.,S.D.,L.P; Critical Reviews – S.B., N.D., S.P.,S.D.,L.P;.

Conflict of interest statement: "No conflict of interest" in the manuscript.

REFERENCES

[1] Alonso-Castro AJ, Villarreal ML, Salazar-Olivo LA, Gomez-Sanchez M, Dominguez F, Garcia-Carranca A. Mexican medicinal plants used for cancer treatment: pharmacological, phytochemical and ethnobotanical studies. J Ethnopharmacol. 2011;133(3):945-972. https://doi.org/10.1016/j.jep.2010.11.055.

[2] Bhattacharjee I, Chatterjee SK, Chatterjee S, Chandra G. Antibacterial potentiality of Argemone mexicana solvent extracts against some pathogenic bacteria. Mem Inst Oswaldo Cruz. 2006;101(6):645-648. https://doi.org/10.1590/s0074-02762006000600011.

[3] Dasari N, Balla S, Kondrapu PR, Gummadi R, Surada NR, Kondru UM, Pindiprolu SK.Targetıng Angiogenesis with fluphenazine-zinc oxide nanoconjugates: A potential mechanism for improving antipsychotic efficacy. Int J Appl Pharm. 2023; 15(5):339-343. https://doi.org/10.22159/ijap.2023v15i5.48317

[4] Aminabee S, Dasari SK, Rao KH, Sirisha V, Kumari VA, Dasari N, Balla S, Leelavati TS. Pharmacoeconomic analysis of biologic vs. biosimilar therapies in rheumatoid arthritis. Int J Chem Biochem Sci. 2023;24(4): 395-400 .

[5] Sun W, Shahrajabian MH. Therapeutic potential of phenolic compounds in medicinal plants-natural health products for human health. Molecules. 2023;28(4):1845. https://doi.org/10.3390/molecules28041845.

[6] Some S, Das S, Mondal R, Gangapodhay M, Basak GK. Medicinal plant extract mediated green synthesis of metallic nanoparticles: A review. Int J Plant Environ.2021; 7(2):119-132. https://doi.org/10.18811/ijpen.v7i02.02.

[7] Sinanoglou VJ, Zoumpoulakis P, Fotakis C, Kalogeropoulos N, Sakellari A, Karavoltsos S, Irini F. Strati. on the characterization and correlation of compositional, antioxidant and colour profile of common and balsamic vinegars. Antioxidants (Basel). 2018; 7(10): 139. https://doi.org/10.3390/antiox7100139.

[8] Siddiqi KS, Husen A. Recent advances in plant-mediated engineered gold nanoparticles and their application in biological system. J Trace Elem Med Biol. 2017;40:10-23. https://doi.org/10.1016/j.jtemb.2016.11.012.

[9] Shukla AK, Iravani S (Eds). Green synthesis, characterization and applications of nanoparticles. 2018. Elsevier.

[10] Noah NM, Ndangili PM. Green synthesis of nanomaterials from sustainable materials for biosensors and drug delivery. Sensors Int. 2022; 3: 100166. https://doi.org/10.1016/j.sintl.2022.100166.

[11] da Nóbrega Santos E, de Albuquerque Sousa TC, de Santana Neto DC, Viegas Brandão Grisi C, Cardoso da Silva Ferreira V, Pereira da Silva FA. Edible active film based on gelatin and Malpighia emarginata waste extract to inhibit lipid and protein oxidation in beef patties. LWT. 2022;154: 112837. https://doi.org/10.1016/j.lwt.2021.112837.

[12] Mastellone G, Marengo A, Sgorbini B, Scaglia F, Capetti F, Gai F, Peiretti PG, Rubiolo P, Cagliero C. Characterization and biological activity of fiber-type *Cannabis sativa* L. aerial parts at different growth stages. Plants (Basel). 2022 ;11(3):419. https://doi.org/10.3390/plants11030419.

[13] Lankatillake C, Huynh T, Dias DA. Understanding glycaemic control and current approaches for screening antidiabetic natural products from evidence-based medicinal plants. Plant Methods. 2019;15:105. https://doi.org/10.1186/s13007-019-0487-8.

[14] Kristi E,Tsiaka T, Ioannou AG, Mantanika V, Strati IF, Panderi I, Zoum P, Sinanoglou VJ. In vitro and ın silico studies to assess edible flowers' antioxidant activities. Appl Sci. 2022; 12(14): 7331. https://doi.org/10.3390/app12147331.

[15] Kamiloglu S, Tomas M, Ozdal T, Yolci-Omeroglu P, Capanoglu E. Bioactive component analysis. In: Innovative food analysis 2021; pp. 41-65, Academic Press. https://doi.org/10.1016/B978-0-12-819493-5.00002-9.

[16] Joshi DD. Herbal drugs and fingerprints: Evidence based herbal drugs. Springer Science & Business Media.

[17] Ghareeb M, Saad A, Ahmed W, Refahy L, Nasr S. HPLC-DAD-ESI-MS/MS characterization of bioactive secondary metabolites from *Strelitzia nicolai* leaf extracts and their antioxidant and anticancer activities ın vitro. Pharmacogn Res. 2021; 10:368-378. https://doi.org/10.4103/pr.pr_89_18.

[18] Alencar Fernandes FH, Nunes Salgado HR. Gallic acid: Review of the methods of determination and quantification. Crit Rev Anal Chem. 2016;46(3):257-265. https://doi.org/10.1080/10408347.2015.1095064.

[19] Fazio A, Iacopetta D, La Torre C, Ceramella J, Muià N, Catalano A, Carocci A, Sinicrop MS. Finding solutions for agricultural wastes: Antioxidant and antitumor properties of pomegranate Akko peel extracts and β-glucan recovery. Food Funct. 2018; 9: 6618-6631. https://doi.org/10.1039/C8FO01394B.

[20] El Sawi SA, Aly HF, Sleem A, El-Feky A. Potent therapeutic effects of polysaccharides ısolated from some edible plant wastes: Characterization and bioactivities.Egypt J Chem. 2022;65(131): 1401-1416 https://doi.org/10.21608/EJCHEM.2022.153901.6660.

[21] Chen G, Bu F, Chen X, Li C, Wang S, Kan J. Ultrasonic extraction, structural characterization, physicochemical properties and antioxidant activities of polysaccharides from bamboo shoots (*Chimonobambusa quadrangularis*) processing by-products. Int J Biol Macromol. 2018;112:656-666. https://doi.org/10.1016/j.ijbiomac.2018.02.013.

[22] Boual Z, Pierre G, Delattre C, Benaoun F. Mediterranean semi-arid plant *Astragalus armatus* as source of bioactive galactomannan. Bioact Carbohydr Diet Fibre. 2015;5(1):10-18. https://doi.org/10.1016/j.bcdf.2014.11.002.

[23] Baaziz I, Ghazouani L, Rjeibi I, Feriani An, Mnafgui K, Mufti A, Traikia M, Cref DL, Michuiad P, Pierre G, Cherif S. Structural characterization and cardioprotective effect of water-soluble polysaccharides extracted from *Clematis flammula.* Appl Sci. 2022; 12(21): 10818. https://doi.org/10.3390/app122110818.

[24] Chintamaneni PK, Nagasen D, Babu KC, Mourya A, Madan J, Srinivasarao DA, Ramachandra RK, Santhoshi PM, Pindiprolu SKSS. Engineered upconversion nanocarriers for synergistic breast cancer imaging and therapy: Current state of art. J Control Release. 2022;352:652-672. https://doi.org/10.1016/j.jconrel.2022.10.056.

[25] Ajila CM, Brar SK, Verma M, Tyagi RD, Godbout S, Valéro JR. Extraction and analysis of polyphenols: Recent trends. Crit Rev Biotechnol. 2011;31(3):227-249. https://doi.org/10.3109/07388551.2010.513677.

[26] Agrawal OD, Kulkarni YA. Mini-review of analytical methods used in quantification of ellagic acid. Rev Anal Chem.2020; 39(1):31-44 https://doi.org/10.1515/revac-2020-0113.

[27] Dinakaran SK, Sujiya B, Avasarala H. Profiling and determination of phenolic compounds in Indian marketed hepatoprotective polyherbal formulations and their comparative evaluation. J Ayurveda Integr Med. 2018;9(1):3-12. https://doi.org/10.1016/j.jaim.2016.12.006.

[28] Ye Z, Li T, Qing D, Sun Y, Chen H, Yu Q, Yan C. Structural elucidation and osteogenic activity of a novel heteropolysaccharide from *Alhagi pseudalhagi*. Int J Biol Macromol. 2021;171:185-197. https://doi.org/10.1016/j.ijbiomac.2020.12.189.

[29] Pindiprolu SKSS, Madhan J, Srinivasarao DA, Dasari N, Kumar CSP, Katta C, Sainaga Jyothi VGS. Therapeutic targeting of aberrant sialylation for prevention of chemoresistance and metastasis in triple negative breast cancer. J Drug Deliv Sci Technol. 2023; 86(20):104617. https://doi.org/10.1016/j.jddst.2023.104617.

[30] Ullah MW, Manan S, Khattak WA, Shahzad A, Ul-Islam M, Yang G. Biotemplate-mediated green synthesis and applications of nanomaterials. Curr Pharm Des. 2020;26(45):5819-5836. https://doi.org/10.2174/1381612824999201105164531.

[31] Kamiloglu S. Authenticity and traceability in beverages. Food Chem. 2019;277:12-24. https://doi.org/10.1016/j.foodchem.2018.10.091.

[32] Joshi DD.TLC: Herbal Drugs and Fingerprints. In: Herbal Drugs and Fingerprints, pp 29–48, Springer, India. https://doi.org/10.1007/978-81-322-0804-4_2.

[33] Jha N, Madasamy S, Prasad P, Lakra AK, Tilwani YM, Arul V. Physico-chemical and functional characterization of polysaccharide purified from mangrove *Rhizophora mucronata* leaves having potent biological activity. South Afr J Bot.2022; 147: 659-669. https://doi.org/10.1016/j.sajb.2022.02.036.

[34] Hashem NM, Hosny NS, El-Desoky N, Soltan YA, Elolimy AA, Sallam SMA, Abu-Tor EM. Alginate nanoencapsulated synbiotic composite of pomegranate peel phytogenics and multi-probiotic species as a potential feed additive: Physicochemical, antioxidant, and antimicrobial activities. Animals (Basel). 2023;13(15):2432. https://doi.org/10.3390/ani13152432.

[35] Grigelmo-Miguel N, Rojas-Graü MA, Soliva-Fortuny R, Martín-Belloso O. Methods of analysis of antioxidant capacity of phytochemicals. In: Fruit and Vegetable Phytochemicals: Chemistry, Nutritional Value, and Stability. Willey online, 2009. https://doi.org/10.1002/9780813809397.ch10

[36] Abdi G, Shokrpour M, Karami L, Salami SA. Prolonged water deficit stress and methyl jasmonate-mediated changes in metabolite profile, flavonoid concentrations and antioxidant activity in peppermint (*Mentha piperita* L.). Notulae Botanicae Horti Agrobotanici Cluj-Napoca. 2019; 47(1): 70-80. https://doi.org/10.15835/nbha47110952.

[37] Endou K, Iizuka K, Yoshii A, Tsukagoshi H, Ishizuka T, Dobashi K, Nakazawa T, Mori M. 8-Bromo-cAMP decreases the Ca2+ sensitivity of airway smooth muscle contraction through a mechanism distinct from inhibition of Rho-kinase. Am J Physiol Lung Cell Mol Physiol. 2004;287(4):L641-648. https://doi.org/10.1152/ajplung.00287.2003.

[38] Ulewicz-Magulska B, Wesolowski M. Total phenolic contents and antioxidant potential of herbs used for medical and culinary purposes. Plant Foods Hum Nutr. 2019; 74(1): 61–67. https://doi.org/10.1007/s11130-018-0699-5.

[39] Corrêa RCG, Garcia JAA, Correa VG, Vieira TF, Bracht A, Peralta RM. Pigments and vitamins from plants as functional ingredients: Current trends and perspectives. Adv Food Nutr Res. 2019;90:259-303. https://doi.org/10.1016/bs.afnr.2019.02.003.

[40] Amala M, Faujdar S, Patel S, Juvvala V, Dasari N. *Lumnitzera racemosa* Willd:An Insight into phytochemistry and pharmacology. J Basic Sci. 2023; 23(12): 352-405. https://doi.org/10.37896/JBSV23.12/2771.