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ORIGINAL ARTICLE

Fractionation and *In vitro* Evaluation of *Prunus persica* Pulp Extract against Urolithasis

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KEYWORDS

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ABSTRACT: The peach tree "*Prunus persica*" is widely cultivated in India, and its fruits which have been used for centuries in traditional Indian medicine, are also known as the Queen of the Fruits. *Prunus persica* has immense therapeutic potential, widespread use in folklore and ancient systems of medicine for its anti-urolithic and other medicinal properties, and is included in the Pashanbheda group of herbs mentioned in Ayurveda for the treatment of kidney stones. Moreover, with growing interest in phytopharmaceuticals in the present world, there always remains an urge for a comprehensive understanding of the phytochemicals responsible for the medicinal activity of herbal drugs, giving rise to the importance of conducting bioassay-guided fractionation. In this study, the anti-urolithiasis activity of different fractions of *Prunus persica* fruit extracts was evaluated via Nucleation, Aggregation, and Growth assays, and fraction III showed the highest effects in vitro. This study provides a pharmacological basis for using *Prunus persica* fruit extract in treating kidney stones.

INTRODUCTION

Kidneys are known to maintain and retain a stable internal environment called homeostasis. The primary role of kidneys is the excretion of waste from the blood, concentrating urine, and maintaining electrolyte balance [1, 2]. Nephrolithiasis, sometimes called urolithiasis or kidney stone disease, is the most common medical condition globally. At some point in their lives, 1 to 15% of people are expected to get kidney stones, and both the prevalence and incidence of kidney stones are rising globally. As per the composition of minerals that make up kidney stones, there are primarily five different types of stones, including calcium oxalate (65.9%), carbapatite (15.6%), urate (12.4%), magnesium ammonium phosphate (2.7%) and brushite (1.7%) [3]. According to a recent study, there are currently 1 in 17 Chinese individuals who have kidney stones, with a prevalence of 5.8% (6.5% for males and 5.1% for women) [4]. Over the last few decades, urolithiasis has become more common everywhere. With a recurrence incidence of 50% within 5-10 years and 75% within 20 years, urolithiasis is frequently a chronic, and recurrent disease [5]. The most common causes for the occurrence of urinary stones are related to insufficient drainage of urine, the existence of foreign bodies in the urinary system, microbial attack, excessive consumption of foods rich in protein and oxalate, aberrancies related to vitamins like a lack of vitamin A and too much vitamin D in the body. Due to these ailments occurrence, various metabolic defects such as hyperparathyroidism, cystinuria, gout, and intestinal malfunction occurred [6]. Besides these factors, certain medications also increase the risk of stone formation, such as nasal decongestants (ephedrine, guaiphenesin), potassium-sparing diuretics (triamterene),

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HIV protease inhibitors (indinavir), and anticonvulsants (felbamate, topiramate), etc [7].

Nature is acting as a reservoir of a diversity of medicinal resources that not only hold tremendous potential to fight a wide range of ailments, either chronic or acute but also maintain the ecosystem between living things. The use and popularity of medicinal plants from ancient times to the current era as remedies to various ailments can be evidenced by the dependency of about 80 % of the population in developing nations globally [8].

With the frequent discovery of new diseases and drug resistance in the modern age, the demand for and hunt for new agents from natural sources keeps growing. *Prunus persica* is a member of the Rosaceae family and the Amygdyloideae subfamily. It is often attributed to "Aaru" and is eaten widely worldwide; in English, it is frequently called "Peach." *Prunus persica* fruits are soft to mature, and they're also called the "Queen" of fruits (second most popular after the apples) [9]. They can be eaten fresh and have a somewhat short shelf life. Typically, peaches are less robust than drupe fruits. They can also be eaten dry, just like apricots and plums. Regarding nutrients and cost, peaches can be consumed fresh, dry, or frozen. Peaches also have an amazing flavor and taste, placing them among the world's most consumed fruits. It possesses various pharmacological activities such as anti-inflammatory, antioxidant, antiviral, anti-tumor, anti-allergic, antimalarial, anti-photoaging, analgesic properties, and spasmogenic activity [10]. In this study, we evaluate the impact of hydroethanolic extract of *Prunus persica* pulp and different fractions of it on urolithiasis.

MATERIALS AND METHODS

Collection, identification, and authentication of the plant material

The Fresh Fruit of *Prunus persica* Linn. (peach) was collected from Pithoragarh, Uttarakhand in June 2021. The authentication of the fruits was done at the Raw Materials Herbarium Museum, National Institute of Science Communication, and Information Resources (NISCAIR), New Delhi.

Fluorescence analysis

Approximately, 1-2 mg of the powdered pulp of *Prunus persica* was taken and observed under a microscope for physical characteristics and under short-wavelength UV light (254 nm) and long-wavelength UV light (366 nm) for fluorescence properties of the powder [11]. Observations were also done with various reagents such as 1 N sodium hydroxide (aqueous), 5% ferric chloride, 1 N sodium hydroxide (alcoholic), 1 N hydrochloric acid, etc., as shown in Table 1.

Physicochemical analysis

The physicochemical evaluation was conducted as per the previous report published by Mushtaq et al. (2014), which involved ash values (total, water-soluble, and acid-insoluble), etc [12].

Determination of total phenolic content

Stock solutions (1mg mL^{-1}) were made by dissolving pulp extract in deionized water. After that, 1 mL of the pulp extract solution was reacted with 1 mL of Folin–Ciocalteu reagent, and 6 min later 8% Na_2CO_3 solution (5 mL) and deionized water (3 mL) were added to the reaction mixture. The mixtures were incubated for an hour in the dark, and then the OD was recorded at 750 nm by UV-visible spectrophotometer [13, 14]. The standard curve was generated from five dilutions (2 µg mL^{-1} - 10 µg mL^{-1}) of Gallic acid (GA).

The total phenolic content was determined by using the following equation:

$$\text{Total phenolic content} = (C \times V\ m^{-1}) \quad (1)$$

Where C represents the GA concentration obtained from the calibration curve (mg mL^{-1}),

V represents the volume of pulp extract (mL),

m represents the weight of pulp extract (g).

The total phenolic content was reported as milligrams of GA equivalent to one gram of pulp extract (mg GAE g^{-1}). The experiment was conducted in triplicate.

Determination of total flavonoid content

The total flavonoid content of the pulp extract was determined by a modified aluminium chloride ($AlCl_3$)

colorimetric assay. The accurately weighed 2 g of pulp was added in 60 mL of water and boiled in a water bath for 25 minutes, filtered, concentrated, and dried below 50°C in an oven. 1 mg mL^{-1} stock solution of pulp extract was made in deionized water. Pulp extracts 0.5 mL) were mixed with 2 mL of deionized water, followed by the addition of 0.18 mL of a 5% solution of sodium nitrite. To the mixture, 10 minutes later 0.20 mL of 10% $AlCl_3$ was incorporated and left for another 15 minutes at room temperature. Following this, 2 mL of a 5% sodium hydroxide solution and a sufficient volume of deionized water were added to make the final volume of 8 mL. The mixtures were thoroughly mixed, and incubated for 20 minutes, and the optical density (OD) was recorded at 510 nm in comparison to a blank for the reagent. To plot the standard curve, five dilutions of 2 µg mL^{-1} - 10 µg mL^{-1} were created from a 1mg mL^{-1} stock solution of rutin (RU) [13].

The total flavonoid content was determined by using the following equation:

Total flavonoid content= ($C \times V\ m^{-1}$) (2)

Where C represents the RU concentration obtained from the calibration curve (mg mL^{-1}),

V represents the volume of pulp extract (mL),

m represents the weight of pulp extract (g).

The total flavonoid content was reported as milligrams of RU equivalent to one gram of pulp extract (mg RE g^{-1}). The experiment was conducted in triplicate.

Extraction of plant material

Accurately weighed 200 g of pulp powder was subjected to maceration with solvent (water: methanol (1:3)) for 24 h, which was followed by refluxing for 5 h, concentrating the mother extracts on a rotary evaporator, and then separation into different portions (preparation of sub-fractions).

Preliminary phytochemical screening

Various phytochemical analyses were conducted to detect the presence of bioactive phytoconstituents such as terpenoids, steroids, phenolics, glycosides, flavonoids, and alkaloids in the various fractions of *Prunus persica* pulp powder [15].

In vitro anti-urolithic studies

The *in vitro* inhibition of calcium oxalate (CaOx) crystal generation by Nucleation assay, Aggregation assay, and Growth assay (oxalate depletion assay) of various extract-based fractions were performed to evaluate antiurolithic activity. The antiurolithic efficacy of various extract-derived powdered pulps of *Prunus persica* was studied for their ability against Nucleation, aggregation, and Growth assays in vitro. The effect of the hydroethanolic extracts of the powdered pulp of *Prunus persica* was also seen in addition to the fractions for comparison [16, 17].

Nucleation assay

The nucleation assay, as described by Patel et al. (2012) [18], was used to investigate the impact of *Prunus persica* pulp aqueous extracts on calcium oxalate (CaOx) crystal formation. The solution containing sodium oxalate (7.5 mM) and calcium chloride (5 mM) in Tris-sodium chloride buffer-pH 6.5 (0.5M of Tris and 150 mM of NaCl). Stock solutions of the sample (extract fractions) and standard (Cystone) at a concentration of 1 mg mL^{-1} were made in deionized water and different dilutions of standard and samples were made in the range of 200-1000 µg mL^{-1}. The analysis was performed without inhibitors (normal control group) and with inhibitors (standard control group, and test control groups of extract and fractions). 1 mL of each concentration of standard and samples (five concentrations from 200 µg mL^{-1} - 1000 µg mL^{-1}) were mixed with 3 mL of calcium chloride following incorporation of 3 mL sodium oxalate solution. In the control set, 1 mL of deionized water was incorporated. The tubes were incubated for 30 min at 37 °C followed by taking the OD at 620 nm using a UV-visible spectrophotometer [18].

Percentage inhibition of nucleation was determined by using the following Equation –

$$\% \text{ inhibition} = 1-[OD_{(\text{test or std})}/OD_{(\text{control})}] \times 100 \quad (3)$$

Aggregation assay

Evaluation of the extract's ability to prevent aggregation in the presence of the standard and test samples was performed as per the method reported previously by Bawari et al. 2018 [19]. An equal volume of 50 mM

solution of both sodium oxalate and calcium chloride solutions was mixed and kept for a period of 1 h in a water bath (60°C). Then it is cooled at 37°C for 24 h, which led to the development of calcium oxalate monohydrate (COM) crystals. These COM crystals were collected by centrifugation and dried at 37°C. A fresh buffer pH 6.5 of Tris-sodium chloride (Tris- 0.05 M and sodium chloride 0.15 M) was carefully made at the time of analysis. A slurry of COM crystals was prepared in the Tris-sodium chloride buffer (0.8mg mL^{-1}). The experiment was performed without the inhibitor (control) and with the inhibitor (standard, extracts, and fractions). 1 mL of different dilutions of standard and sample (extract fraction) solutions (five concentrations from 200 µg mL^{-1} - and 1000 µg mL^{-1}) were incorporated into 3 mL calcium oxalate solution, mixed by vortexing, and kept at 37°C for about 30 min. OD of the final reaction mixtures was read at 620 nm using a UV-visible spectrophotometer [19]. The percentage inhibition of aggregation was determined using Equation 3.

Oxalate depletion assay

The effect of the standard and test samples (extract fractions) on the CaOx crystals growth was evaluated by the oxalate depletion assay as reported previously by Bawari et al. 2018 [19]. A buffer containing Tris-HCl (10 mM) and sodium chloride (90 mM) with pH 7.2 was made in which 4 mM sodium oxalate and 4 mM calcium chloride solutions were prepared. To this, 30µL slurry (prepared of COM crystals at a concentration of 1.5mg mL^{-1} in 50 mM sodium acetate buffer pH 5.7) was added. The amount of oxalate depletion from the mixture was then recorded at 214 nm for 10 min to calculate the development of CaOx crystals. The addition of 1 mL of each standard and sample concentration (200µg mL^{-1} to 1000µg mL^{-1}) in the reaction mixture was used to measure the impact on crystal growth and changes in the OD were again recorded with a UV-visible spectrophotometer (Shimadzu 1900i) at 214 nm for 600 seconds [19]. The percentage inhibition of crystal development was determined by using Equation 3.

RESULTS AND DISCUSSION

Fluorescence analysis

The fluorescence characteristics of the powdered pulp of *Prunus persica*, represented in Table 1, were unique and played a significant role in establishing its identity. Fluorescence properties arise owing to the presence of specific phytoconstituents in a particular herb of interest and are exclusive properties of the herb, thus making them an essential parameter for pharmacognostic identification.

Table 1. Fluorescence Analysis of the powdered pulp of *Prunus persica.*

Treatment	Visible light	UV light		Components present
		254 nm	366 nm	
Pulp-Powder as such	White	Whitish- Pink	-	Starch and Anthocyanins
Pulp-Powder + 1N NaOH (aqueous)	Dark brown	Whitish- Pink	Dark green	Quinic acid
Pulp-Powder + 1N NaOH (alcoholic)	Greenish brown	Whitish- Pink	Yellowish brown	Quinic acid
Pulp-Powder + 1N HCl	Greenish yellow	Light Pink	Light yellow	Flavonoids
Pulp-Powder + ammonia	Light brown	Light Pink	Dark brown	Linoleic acid and amino acids
Pulp-Powder + 5% iodine	Dark brown	Light Pink	Black	Mucilage and starch
Pulp-Powder + 5% $FeCl_3$	Light green	Light Pink	Greenish brown	Phenolic compounds
Pulp powder + acetic acid	Light green	Light Pink	Light brown	Anthocyanins and flavonoids
Pulp-Powder + 1N H_2SO_4	Black	Yellowish Pink	Black	Procyanidin B1, dimeric compounds such as epigallocatechin-3-O-gallate (EGCG)
Pulp-Powder + 1N HNO_3	Blackish- yellow	Blackish green	Yellowish brown	Cyanadine and rutinoside

Physicochemical analysis

The ash readings indicate the amount of inorganic material in the crude medication. Total ash, on the other hand, denotes both physiological and non-physiological ash; acid-insoluble ash denotes the herb's silica content; and water-soluble ash denotes the water-soluble component. A high ash content indicates a high concentration of phosphates, silicates, carbonates, and silica. For the pulp part of *Prunus persica* fruit, acid-insoluble ash, total ash, and water-soluble ash were 7.50 0.717% w w^{-1}, 1.21 0.211% w w^{-1}, and 9.51 0.177% w w^{-1}, respectively. The examination of physicochemical parameters is critical for determining the quality of crude pharmaceuticals and removing any dangerous chemicals, adulteration, or contamination.

Determination of Total Phenolic Content and Total Flavonoid Content

The total phenolics and total flavonoids of the powdered pulp of *Prunus persica* are given in Table 2. The standard curves of gallic acid (GA) and rutin (RU) are shown in Figures 1 and 2 respectively. Total phenolics and total flavonoids were present in quantities of 117.64±1.30 mg GAE g^{-1} extract and 87.61±2.71 mg RE g^{-1} extract, respectively.

Table 2. Total Flavonoid and Total Phenolic Content Powder Pulp of *Prunus Persica.*

S. No.	Total phenolic content (mg GAE g^{-1})	Total flavonoid content (mg RE g^{-1})
1.	117.64±1.30	87.61±2.71

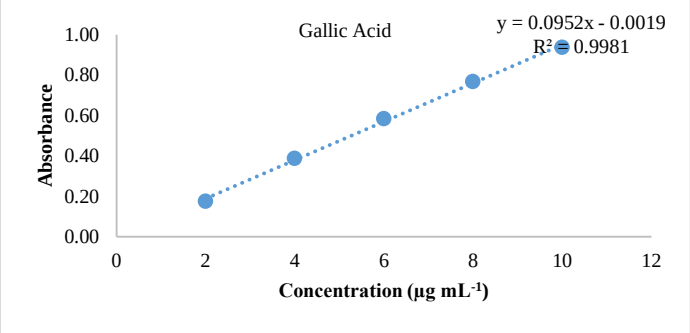

Figure 1. Standard curve of Gallic acid at 750nm using UV-visible spectrophotometer.

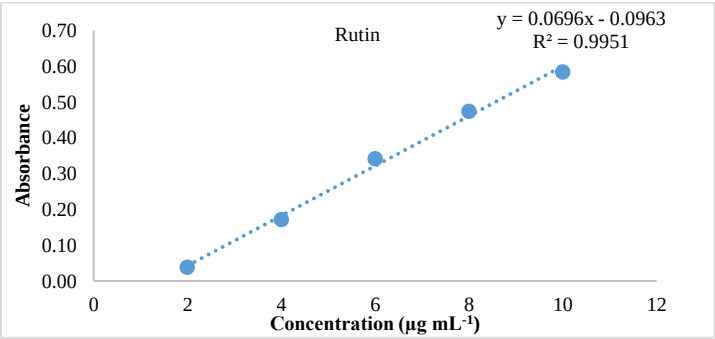

Figure 2. Standard curve of Rutin at 510nm using UV-visible spectrophotometer.

Extraction of plant material

The extraction of phytoconstituents was performed by fractionation and the results in Table 3 show the % yield of several fractions produced from hydroethanolic extracts of *Prunus persica*, indicating a high presence of numerous

phytoconstituents with variable polarity. Fraction I, which was expected to consist of non-polar phytocomponents, was found to have the highest concentration at 28.79% w w^{-1}. Fraction II was determined to be the second most abundant in yield among the other fractions, with a yield of 20.35% w w^{-1}. Fraction III, which was expected to contain phenolic compounds and flavonoid aglycones, had a value of 16.00% w w^{-1}, whereas Fraction IV had the lowest value of 15.55% w w^{-1} of all.

Table 3. Percentage Yield of Extract-Based Fractions.

Fraction	Percentage yield (% w w^{-1})
F I	28.28±1.76
F II	20.62±1.13
F III	16.41±0.87
F IV	15.42±0.91

Note. F I-F IV: Fraction I-IV.

Preliminary phytochemical screening

The results of phytochemical tests of the various extract-derived fractions are represented in Table 4 and indicate the presence of steroidal components, flavonoids, phenolic compounds, alkaloids, and glycosides. Preliminary phytochemical screening is an initial and crucial step in the identification of biologically active phytoconstituents existing in medicinal herbs, which may finally result in drug discovery and development.

Table 4. Preliminary Phytochemical Screening of various fractions of powdered pulp of *Prunus persica.*

Phytoconstituent present	Chemical test	Fractions			
		F I	**F II**	**F III**	**F IV**
Steroids and terpenoids	Sulphur powder test	+	-	-	-
	Salkowski test	+	-	-	-
Glycosides	Fehling's test	-	-	-	+
Phenols	Ferric chloride test	-	-	+	-
Alkaloids	Mayer's test	-	-	-	-
	Wagner's test	-	-	-	-
	Dragendorff's test	-	-	-	-
Flavonoids	Hager's test	-	+	-	-
	Shinoda test	-	-	-	-
	Alkaline reagent test	-	-	+	-
	Zinc-HCl test	-	-	+	-

Note. + : Presence; - : Absence; F I-IV: Fraction I-IV.

In vitro anti-urolithic studies

The *in vitro* inhibition of CaOx crystallization by Nucleation assay, Aggregation assay, and Oxalate depletion/Growth assay of various extract-based fractions were performed to test antiurolithic activity and the results are shown in Table 5-7. The graphical representation of the percentage inhibition of all assays against concentration is depicted in Figures 3-5. A significant dose-dependent increase in the response was demonstrated by all fractions as compared to their extracts as well as the standard. Fraction III of hydroethanolic extract of pulp powder expected to be composed of flavonoid aglycones and phenolic components exhibited the highest hindrance i.e. 72.01±1.12 %, 66.14±1.74% and 68.66±1.55% at 1000 µg mL^{-1} out of all other fractions to CaOx nucleation,

Aggregation and Growth assay respectively.

Table 5. Nucleation Assay powdered pulp of *Prunus persica.*

Dose (µg mL^{-1})	Percentage inhibition (%)					
	Standard (Cystone)	Extract	F I	F II	F III	F IV
200	13.81±2.02	22.11±1.41^{b}	11.12±0.12	8.20±1.15	42.04±2.01^{b,c}	6.85±1.65
400	31.53±1.85	42.31±2.79^{b}	13.04±1.29	13.20±2.12	52.64±2.03^{b,c}	31.94±2.16^{a}
600	41.90±0.52	63.11±2.58^{b}	14.60±3.26	36.47±3.5 7	63.00±0.50^{b}	35.31±0.24
800	57.34±1.54	67.12±1.51^{b}	23.14±1.05	54.34±1.26^{a}	65.09±0.26^{b}	37.11±0.57
1000	72.18±2.17	73.21±1.38^{a}	38.34±2.0	62.21±0.89	72.01±1.12	43.19±1.70

Note. Values are given as mean±SD (n=3). FI-FIV: Fraction I-IV. Statistical analysis was performed by Two-way ANOVA multiple comparison test followed by Tukey's post hoc test. [a] Non-significant difference from standard (ns); [b] Significantly different from standard ($p<<0.0001$); [c] Significantly different from extract ($p<0.0001$).

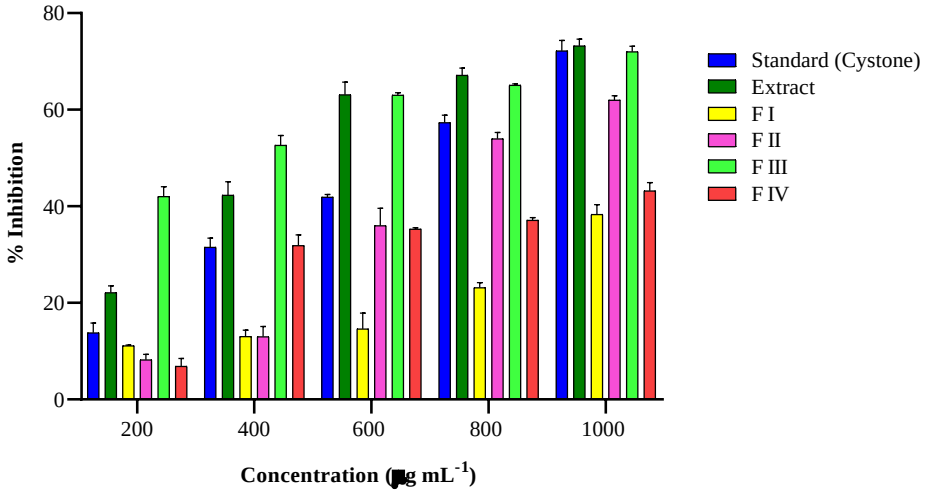

Figure 3. Graphical representation of Nucleation assay result.

Table 6. Aggregation Assay of powdered pulp of *Prunus persica.*

Dose (µg mL^{-1})	Percentage inhibition (%)					
	Standard (Cystone)	Extract	F I	F II	F III	F IV
200	22.53±0.41	32.07±0.44^{b}	24.12±0.66^{a}	17.34±1.16	26.82±3.85^{c}	17.40±0.52
400	34.31±1.14	43.13±2.86^{b}	26.81±1.21	29.54±0.62	43.17±0.89^{b,d}	24.15±1.66
600	45.64±0.24	53.89±0.87^{b}	35.50±0.73	38.27±0.72	53.90±0.42^{b,d}	32.39±0.40
800	58.41±1.24	58.95±0.12^{a}	41.54±1.27	48.92±1.23	58.70±1.7106^{a,d}	38.06±1.07
1000	71.55±1.20	69.82±3.15	43.23±0.51	59.22±0.45	66.14±1.7 4	47.51±1.44

Note. Values are given as mean±SD (n=3). FI-FIV: Fraction I-IV. Statistical analysis was performed by Two-way ANOVA multiple comparison test followed by Tukey's post hoc test. [a] Non-significant difference from standard (ns); [b] Significantly different from standard ($p<<0.0001$); [c] Significantly different from standard ($p<0.05$), [d] Non-significant difference from extract (ns).

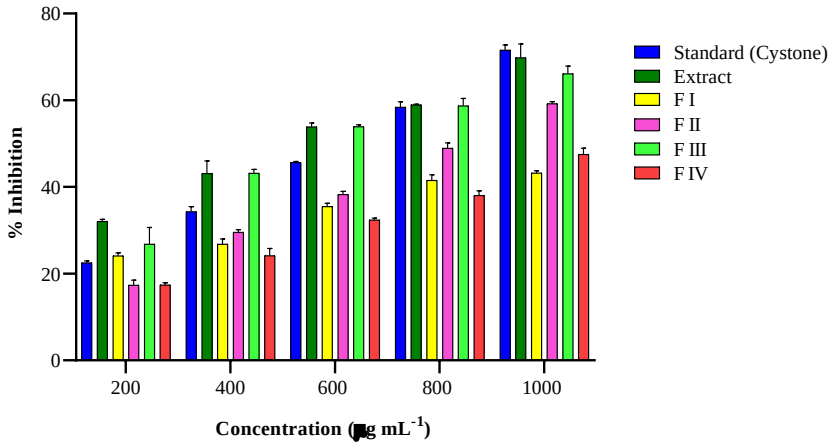

Figure 4. Graphical representation of Aggregation assay result**.**

Table 7. Growth Assay of powdered pulp of *Prunus persica.*

Dose (µg mL^{-1})	Percentage inhibition of treatment (%)					
	Standard (Cystone)	**Extract**	**F I**	**F II**	**F III**	**F IV**
200	35.17±1.65	40.04±3.43[a]	6.98±3.96	10.74±3.97	22.86±2.47	2.15±2.52
400	45.66±2.16	53.28±0.95[a]	12.01±1.78	18.90±0.71	35.27±1.04	13.06±2.40
600	57.41±1.26	63.53±1.12[a]	21.22±2.81	30.79±1.46	45.76±2.12	17.41±1.98
800	65.02±1.78	66.61±1.91[a]	28.52±3.41	43.12±1.26	57.23±0.56	23.12±1.10
1000	74.26±2.10	72.81±2.11	36.14±1.51	55.57±3.67	68.66±1.55	48.87±3.81

Note. Values are given as mean±SD (n=3). FI-FIV: Fraction I-IV. Statistical analysis was performed by Two-way ANOVA multiple comparison test followed by Tukey's post hoc test. [a] Non-significant difference from standard (ns).

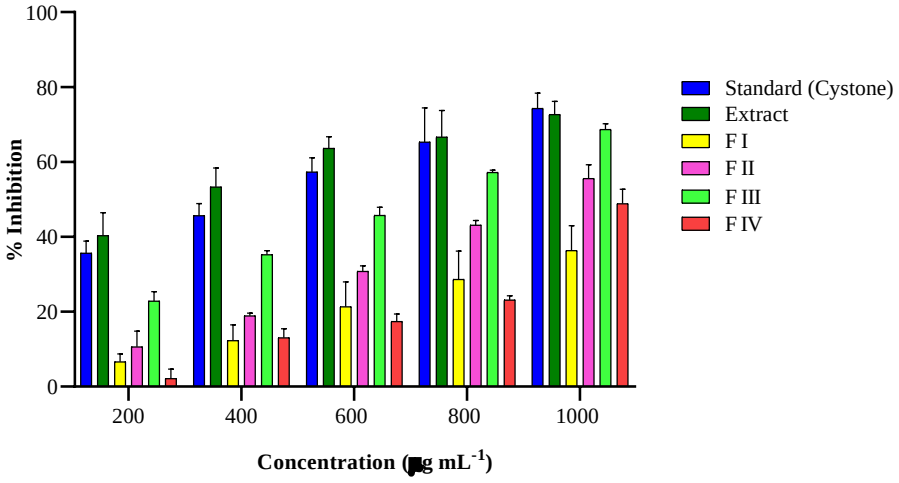

Figure 5. Graphical representation of Growth assay result.

CONCLUSIONS

In the current investigation, fraction III from a hydroethanolic extract of aerial portions comprising flavonoid aglycones and phenolic components demonstrated the greatest efficacy against CaOx nucleation, aggregation, and growth in vitro. The activity displayed was exceptional and comparable to that observed with standard Cystone, a well-known and effective polyherbal preparation. The higher efficiency of the extract

and fraction III implies the existence of stronger antiurolithic flavonoid aglycones and phenolic chemicals in the former. However, fraction I, which is expected to contain steroidal moieties as well as terpenoids, exhibited the least efficacy, while fraction IV has less antiurolithic capability of those components compared to phenolics and flavonoid aglycones and their presence in lower concentration. This medicinal herb may be a miracle source of novel antiurolithic moieties that may be extracted from hydroethanolic extracts. The current study opens the door to the isolation of novel lead compounds with antiurolithic efficacy from the best active phenolic and flavonoid aglycone-rich fraction III.

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Conflict of interest

The authors declare no conflict of interest.

REFERENCES

1. Al-Mayali Z.K., Jaffat H.S., Mohammed J.A., 2019. Effect of the indomethacin drug on kidney histology in male Albino Rats. Int J pharm Quality Assurance. 10(3), 162-167.

2. Lind M., Pivodic A., Svensson A.M., Ólafsdóttir A.F., Wedel H., Ludvigsson J., 2019. HbA1c level as a risk factor for retinopathy and nephropathy in children and adults with type 1 diabetes: Swedish population-based cohort study. BMJ (Clinical research ed.). 366, l4894.

3. Wang Z., Zhang Y., Zhang J., Deng Q., Liang H., 2021. Recent Advances on the Mechanisms of Kidney Stone Formation (Review). International Journal of Molecular Medicine. 48(2), 149.

4. Zeng G., Mai Z., Xia S., Wang Z., Zhang K., Wang L., Long Y., Ma J., Li Y., Wan S.P., Wu W., Liu Y., Cui Z., Zhao Z., Qin J., Zeng T., Liu Y., Duan X., Mai X., Yang Z., Kong Z., Zhang T., Cai C., Shao Y., Yue Z., Li S., Ding J., Tang S., Ye Z., 2017. Prevalence of Kidney Stones in China: An Ultrasonography Based Cross-Sectional Study. BJU International. 120(1), 109–16.

5. Eisner B.H., Goldfarb D.S., 2014. A Nomogram for the Prediction of Kidney Stone Recurrence. Journal of the American Society of Nephrology. 25(12), 2685.

6. Alelign T., Petros B., 2018. Kidney Stone Disease: An Update on Current Concepts. Advances in Urology. 2018, 3068365.

7. Barbas C., Garcıa A., Saavedra L., Muros M., 2002. Urinary Analysis of Nephrolithiasis Markers. Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences. 781(1–2), 433–55.

8. Ekor M., 2014. The Growing Use of Herbal Medicines: Issues Relating to Adverse Reactions and Challenges in Monitoring Safety. Frontiers in Pharmacology. 4, 177.

9. Li Y., Wang L., 2020. Genetic Resources, Breeding Programs in China, and Gene Mining of Peach: A Review. Horticultural Plant Journal. 6(4), 205–15.

10. Rudke C.R.M., Zielinski A.A.F., Ferreira S.R.S., 2023. From Biorefinery to Food Product Design: Peach (*Prunus Persica*) By-Products Deserve Attention. Food and Bioprocess Technology. 16(6), 1197.

11. Nalini R., Anuradha R., 2017. Phytochemical Screening and Fluorescence Analysis of Methanolic Flower Extracts of Punica Granatum L. Scholars Academic Journal of Pharmacy. 6(9), 378–82.

12. Mushtaq A., Akbar S., Zargar M.A., Wali A.F., Malik A.H., Dar M.Y., Hamid R., Ganai B.A. 2014. Phytochemical screening, physicochemical properties, acute toxicity testing and screening of hypoglycaemic activity of extracts of Eremurus himalaicus baker in normoglycaemic Wistar strain albino rats. BioMed research international. 2014, 867547.

13. Phuyal N., Jha P.K., Raturi, P.P., Rajbhandary S., 2020. Total Phenolic, Flavonoid Contents, and Antioxidant Activities of Fruit, Seed, and Bark Extracts of Zanthoxylum Armatum DC. Scientific World Journal. 2020, 8780704.

14. Siddiqui N., Rauf A., Latif A., Mahmood Z., 2017. Spectrophotometric Determination of the Total Phenolic Content, Spectral and Fluorescence Study of the Herbal Unani Drug Gul-e-Zoofa (*Nepeta Bracteata* Benth). Journal of Taibah University Medical Sciences. 12(4), 360.

15. Biswal B., Susanta K.P., 2022. Pharmacognostical, Hepatoprotective and Anthelmintic Evaluation of *Cuscuta Reflexa* Roxb. and *Gymnema Sylvestre* (RETZ.) Rz BR. EX. SM. International Journal of Drug Delivery Technology. 12(02), 516–21.

16. Hewagama S.P., Ruwani P.H., 2022. Antiurolithiatic Potential of Three Sri Lankan Medicinal Plants by the Inhibition of Nucleation, Growth, and Aggregation of Calcium Oxalate Crystals In Vitro. The Scientific World Journal. 2022, 8657249.

17. Saha S., Ramtej J.V., 2013. Inhibition of Calcium Oxalate Crystallisation in Vitro by an Extract of *Bergenia Ciliata*. Arab Journal of Urology. 11(2), 187.

18. Mosquera D.M.G., Yannarys H.O., Pedro C.Q., Rafael S.M., Luc P., 2020. Antiurolithiatic Activity of Boldoa Purpurascens Aqueous Extract: An in Vitro and in Vivo Study. Journal of Ethnopharmacology. 253, 112691.

19. Bawari S., Archana N.S., Devesh T., 2018. Antiurolithiatic Activity of Daucus Carota: An *In vitro* Study. Pharmacognosy Journal. 10(5), 880–84.