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ISOLATION AND CHARACTERISATION OF BIOACTIVE COMPONENTS FROM PHASEOLUS COCCINEUS SEEDS FOR ANTIOXIDANT ACTIVITY

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ABSTRACT
Historically, all medicinal preparations were derived from plants, whether in the simple form of plant parts or in the more complex form of crude extracts, mixtures, etc. The present study is aimed at evaluating the antiurolithiatic activity of the ethanolic extract and aqueous extracts of the seeds of Phaseolus coccineus. The seeds of Phaseolus coccineus Linn were collected from farmers of Ooty, Tamil Nadu, India. Coarse powder of Phaseolus coccineus Linn seeds (300 grams) were extracted by cold maceration using water for 15 days. The percentage yield of ethanolic and aqueous extract of seeds of Phaseolus coccineus linn was found to be Ethanolic extract: 8.24 % w/w. Aqueous extract: 6.32 % w/w. Preliminary Phytochemical screening of Phaseolus coccineus, revealed the presence of following phytoconstituents Alkaloids, Carbohydrates, Proteins & amino acids, Steroids, Phenols, Tannins, Flavonoids, Glycosides. Both extracts showed a very good concentration-dependent inhibition of lipid peroxidation and almost equal activity. Total Flavonoid Content of EEPC (9.18 mg) was found to be more compared with the AEPC (8.81 mg). Total Phenolic Content of EEPC (42.22 mg) was found to be more compared with AEPC (30.10 mg). The results of the present investigation on the evaluation of Phytochemical screening and anti oxidant studies of aqueous and ethanolic extracts have led to acceptable findings. Flavonoids, Polyphenols and saponins present in the extracts may be responsible of the antioxidant.

KEYWORDS: Antiurolithiatic, herbal, Phaseolus coccineus, Urinary System, kidney.

INTRODUCTION

Medicinal Plants: Scope and importance

Human race is constantly being challenged by many dreadful diseases and it is an uphill task to combat them in the present scenario. With the onset of the synthetic era, pharmaceutical industries are producing a lot of synthetic drugs, that help to alleviate the chronic diseases. With the passage of time, many problems associated with frequent use of synthetic drugs become prominent, like severe side effects and resistance of microbes against these drugs.[1] On the other side, these drugs are expensive and a large population cannot afford these drugs. In recent times, research on medicinal plants has been intensified all over the world. The natural pharmaceuticals are receiving importance and popularity as safe, efficacious and cost-effective medicines with benefits due to combination. Medicinal plants have a promising future because there are about half million plants around the world and most of their medical activities have not yet been investigated that could be decisive in the treatment of present or future studies. There has been a growing interest in Ayurveda in the past few years. To initiate fruitful dialogues between Ayurveda and modern science, in-depth understandings of both the systems become an essential pre requisite.[2]

The Urinary System

The urinary system is comprised of two kidneys, two ureters, the urethra and the bladder. Urinary production is the activity of the kidneys, and the rest of the mechanism is responsible for the urinary expulsion. The kidneys are two bean-shaped organs found under the ribs near the back end. The kidneys draw excess water and waste out of the blood to turn it into urine. Narrow tubes called ureters carry urine in the lower abdomen, a triangle-shaped area from the kidneys to the bladder. The elastic walls of the bladder stretch out and expand like a balloon to store urine. Once urine is drained from the urethra to outside the body, they flatten together.[3]

Urolithiasis

Urolithiasis (from Greek oûron, "urine", + lithos, "stone", + -iasis) is the formation of urinary calculi or urinary stones, which are formed or located anywhere in the urinary system. It comprises of nephrolithiasis (the formation of kidney stones), ureterolithiasis (the

formation of stones in the ureters) and cystolithiasis (the formation of bladder stones).[4]

Kidney stones are mainly lodged in the kidney(s).[5] Mankind has been afflicted by urinary stones since centuries dating back to 4000 B.C, and it is the most common disease of the urinary tract. The prevention of renal stone recurrence remains to be a serious problem in human health.[6] The prevention of stone recurrence requires better understanding of the mechanisms involved in stone formation.[7] Kidney stones have been associated with an increased risk of chronic kidney diseases, end-stage renal failure[8], cardiovascular diseases, diabetes, and hypertension.[9] It has been suggested that kidney stone may be a systemic disorder linked to the metabolic syndrome. Nephrolithiasis is responsible for 2 to 3% of end-stage renal cases if it is associated with nephrocalcinosis.[10]

The symptoms of kidney stone are related to their location whether it is in the kidney, ureter, or urinary bladder.[11] Initially, stone formation does not cause any symptom. Later, signs and symptoms of the stone disease consist of renal colic (intense cramping pain), flank pain (pain in the back side), hematuria (bloody urine), obstructive uropathy (urinary tract disease), urinary tract infections, blockage of urine flow, and hydronephrosis (dilation of the kidney). These conditions may result in nausea and vomiting with associated suffering from the stone event.[12]

Type of kidney stones

Calcium-containing stones

The most common type of kidney stones worldwide contains calcium. In fact, calcium-containing stones represent about 80 % of all cases. They typically contain calcium oxalate either alone or in combination with calcium phosphate in the form of apatite or brushite.[5] In India, 12% of the population is expected to have urinary stones, out of which 50% may end up with loss of kidneys or renal damage.[12]

Struvite stones

About 10-15% of urinary calculi are composed of struvite (ammonium magnesium phosphate, NH4MgPO4·6H2O. Struvite stone is (also known as "infection stones", urease or triple-phosphate stones) most often in the presence of infection by urea-splitting bacteria.[13]

Uric acid stones

About 5-10% of all stones are formed from uric acid. People with certain metabolic abnormalities, including obesity may produce uric acid stones. They also may form in association with conditions that cause hyperuricosuria (an excessive amount of uric acid in the urine) with or without hyperuricemia (an excessive amount of uric acid in the serum). They may also form in association with disorders of acid/base metabolism where the urine is excessively acidic (low pH).[14]

MATERIAL AND METHOD

Plant Collection and Authentication

The seeds of *Phaseolus coccineus* Linn were collected from farmers of Ooty, Tamil Nadu, India. The identification and authentication of plant was done by Dr. Saba Naaz, Botanist, from the Department of Botany, Saifia College of Science and Bhopal. A voucher specimen number 189/Saif. /Sci./Clg/Bpl. was kept in Department of Botany, Saifia College of Science, Bhopal for future reference.

Aqueous extract

Coarse powder of *Phaseolus coccineus* Linn seeds (300 grams) were extracted by cold maceration using water for 15 days. The extract was concentrated by surface evaporation followed by vacuum drying. Dry powder was weighed and stored in air-tight containers for further Phytochemical and pharmacological studies.

Ethanolic extract

Coarse powder of *Phaseolus coccineus* Linn seeds was extracted with 250 ml of ethanol by hot percolation method using soxhlet apparatus. The extraction was carried out for 72 hours. After extraction, the solvent was distilled out to obtain a concentrated extract. Then the concentrated extract was vacuum dried and the dry extract was stored in an air tight container for further Phytochemical and pharmacological studies.

3. IDENTIFICATION OF PHYTOCONSTITUENTS OF EXTRACTS

I. Preliminary phytochemical screening

Preliminary phytoconstituents present in the ethanol extract and aqueous extract of *Phaseolus coccineus* were identified based on the following chemical tests.

1. Tests for Carbohydrates[15,16,17]

a. Molisch's test

To 2-3 ml of aqueous extract, added few drops of alpha naphthol solution in alcohol, shaken and then added concentrated sulphuric acid from sides of test tube. A brown purple ring formed at the junction of the two liquids indicates the presence of sugars.

b. Fehling's test

Mixed 1 ml Fehling's solution A and 1ml of Fehling's solution B and boiled for 1 minute, added equal volume of test solution and heated on boiling water bath for 5-10 mins. Formation of brick red precipitate confirms the presence of sugars.

c. Benedict's test

Mixed equal volume of Benedict's reagent and test solution in a test tube, heated in boiling water bath for 5 mins. Formation of brick red precipitate confirms the presence of sugars.

d. Barfoed's test

Mixed equal volume of Barfoed's reagent and test solution. Heated for 1-2 mins in boiling water bath and

cooled. An orange red precipitate confirms the presence of sugars.

2. Tests for glycosides[18]

Heated on a water bath and the hydrolysate was subjected to Legal, Keller Killiani, Borntrager's and modified Borntrager's test to detect the presence of glycosides.

a. Legal test

To the hydrolysate, 1 ml of pyridine and few drops of sodium nitroprusside solution were added and then it was made alkaline with sodium hydroxide solution. A blood red colour indicates the presence of glycosides.

b. Borntrager's test

The hydrolysate was treated with chloroform and the chloroform layer was separated. To this, equal quantity of dilute ammonia solution was added. A light pink colour at the interface between two liquids indicates the presence of glycosides.

Test for alkaloids[19,20]

a. Mayer's test

Methanolic extract was treated with drops of hydrochloric acid and filtered. The filtrate was treated with Mayer's reagent. Yellowish buff colour indicates the presence of alkaloids.

b. Dragendroffs test

Methanolic extract was treated with few drops of Dragendroff's reagent. Orange red precipitate indicates the presence of alkaloids.

c. Wagner's test

Methanolic extract was treated with drops of hydrochloric acid and filtered' The filtrate was treated with Wagner's reagent. Reddish brown colour precipitate indicates the presence of alkaloids.

Tests for flavonoids[21,22]

a. A small quantity of solvent free methanolic extract was dissolved in alcohol separately and it was extracted with 10% sulphuric acid and cooled. Then it was extracted with diethyl ether and divided in to three portions in two separate test tubes for each extract. 1ml of sodium carbonate, 1ml of sodium hydroxide and 1ml of diluted ammonia solution were added to the first and second test tubes respectively. In each test tube development of yellow colour demonstrated the presence of flavonoids.

b. Ferric chloride test

To a small quantity of the alcohol solution of extract few drops of neutral ferric chloride solution were added. Formation of blackish red colour demonstrated the presence of flavonoids.

c. Shinoda's test

To the alcoholic solution of extract a small piece of magnesium ribbon and few drops of concentrated hydrochloric acid were added and heated, a magenta colour indicates the presence of flavonoids in methanol extract.

5. Tests for proteins[23,24]

a. Biuret test

The extract was treated with equal volume of 40% of sodium hydroxide and 2 drops of 1% copper sulphate solution. Pink or purple colour indicates the presence of proteins.

b. Millon's test

To the extract, few drops of Millon's reagent was added and heated. Appearance of red colour indicates the presence of proteins and free amino acids.

c. Ninhydrin test

A small quantity of extract solution was boiled 0.2% solution of Ninhydrin. Blue colour indicates the presence of free amino acids.

6. Tests for tannins[25,26]

Small quantity of the extract was dissolved in distilled water, filtered and tested for the presence of phenolic compounds and tannins using the following reagents:

A. With dilute ferric chloride solution (5%) – development of greenish black coloration indicates the presence of tannins.

B. With 10% lead acetate solution – development of yellow colour precipitate indicates the presence of tannis.

C. With 10% aqueous potassium dichromate solution – development of yellowish-brown precipitate indicates the presence of tannis.

7. Test for saponins[27]

Foam test

To the extract, 20ml of distilled water and agitated in a graduated cylinder for15 minutes. The formation of about 1cm layer of foam indicates the presence of saponins.

8. Tests for steroids and triterpenoids[28,29]

A. Libermann - Burchard reaction

Small quantities of solvent free methanol extract were separately dissolved in 1ml chloroform and 1ml of acetic anhydride was then added followed by 2ml of concentrated sulphuric acid. A reddish violet ring at the junction of the two layers indicates the presence of triterpenoids and steroids.

b. Salkowski's test

Concentrated sulphuric acid was added to a chloroform solution of the extract (10mg of extract in 1ml of chloroform), a reddish blue colour in the chloroform layer and green fluorescence in acid layer, suggests the presence of steroids.

9. Test for sterols
When the extracts were treated with 5% potassium hydroxide solution, appearance of pink colour indicates the presence of sterols.

10. Test for phenols
When the extracts were treated with neutral ferric chloride solution, appearance of violet colour indicates the presense of phenols. When the extracts were treated with 10% sodium chloride solution, the appearance of cream colour indicates the presence of phenol.

11. Test for Terpenoids[30]
About 0.5 g of plant extract in separate test tube was taken with 2 ml of chloroform; 5 ml of concentrated sulphuric acid was carefully added to form a layer and observed for presence of reddish-brown colour interface to show positive results for the presence of terpenoids.

Ⅱ. TLC Study on the extracts[31]
The ethanolic extract of *Phaseolus coccineus* (EEPC) was dissolved in ethanol. Then the solution was applied on Merck Aluminium plate pre coated with silica gel 60 F254 of 0.2 - 0.5 mm thickness. The plate was developed in Chloroform: Methanol: Distilled water: Toluene (8:1:0.5:0.5) solvent system. After visualization Rf values were calculated.

The aqueous extract of *Phaseolus coccineus* (AEPC) was dissolved in water. Then the solution was applied on Merck Aluminium plate pre coated with silica gel 60 F254 of 0.2-0.5 mm thickness. The plate was developed in Chloroform: Methanol: Distilled water: Toluene (8:1:0.5:0.5) solvent system. After visualization Rf values were calculated.

$$\text{Rf value} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

Determination of phytoconstituents of extracts
Determination of total flavonoid content[32]
Preparation of standard
Standard solution was prepared by adding 10 mg of accurately weighed Quercetin in 10 ml of distilled water.

Preparation of sample
10 mg of the accurately weighed AEPC and EEPC extracts were separately dissolved in 10 ml water and used for the estimation.

Procedure
The total flavonoid content of the AEPC and EEPC was determined by using aluminium chloride colorimetric method. To an aliquot of 1 ml of extract (1mg /ml) or standard solutions of Quercetin (10,20,40,60,80.100, µg/ml) methanol was added separately to make up the solution upto 2ml. The resulting mixture was treated with 0.1ml of potassium acetate and 2.8 ml of distilled water. Shaken well and incubated at room temperature for 30 minutes. The absorbance was measured at 415nm against blank, where a solution of 2ml ethanol 0.1ml potassium aetate, 2.8ml distilled water and 0.1ml of aluminium chloride serve as blank solution. The total flavanoid content was determined from the standard quercetin calibration curve. And it was expressed as milligrams of Quercetin equivalents (QE) per gram of extract

Determination of total phenolic content[33]
Preparation of standard
Standard solution was preapared by adding 10mg of accurately weighed Gallic acid in 10 ml of distilled water

Preparation of sample
10mg of the accurately weighed AEPC and EEPC extracts were separately dissolved in 10ml ethanol and used for the estimation.

Procedure
The total phenolic content of the AEPC and EEPC was determined by Folin Ciocalteau assay method. To an aliquot 100µg of AEPC or standard solution of Gallic acid (10, 20, 40, 60, 80,100µg/ml) added 0.5ml of Folin Ciocalteau reagent and made into 2ml with distilled water and the mixture is incubated for 5min at room temperature. 0.1ml of 20% Sodium Carbonate and 0.9ml of distilled water were added to make the final solution to 3ml. It was incubated for 30 ins in dark to complete the reaction. After that absorbance of the mixture was measured at 725nm against blank. Distilled water was used as reagent blank. The tests were performed in triplicate to get mean the values. The total phenolic content was found out from the calibration curve of Gallic acid. And it was expressed as milligrams of Gallic acid equivalents (GAE) Per gram of extract.

IN VITRO ANTIOXIDANT STUDIES ON THE EXTRACTS
Determination of Nitric Oxide Scavenging Assay[34,35]
The activity was measured according to the modified method of Sreejayan and Rao, to 4ml of the extract having different concentrations (100-500 µg/ml), 1 ml of sodium nitroprusside (SNP) solution (5mM) was added and incubated for 2hr at 27˚C.An aliquot (2ml) of the incubation solution was removed and diluted with 1.2ml of Griess reagent (1% Sulfanilamide in 5% H3PO4 and 0.1% naphthylethylene diamine dihydrochloride). The absorbance of the chromophore was read at 550nm and compared with standard, Ascarbic Acid.
Nitric oxide scavenging activity (%) = ($Abs_{control}$ - Abs_{sample}) / (Abs control) x100
Where, Abs (control): Absorbance of the control and Abs (sample): Absorbance of the extracts/standard.

Determination of Reducing Power Assay[36]
Various concentrations of the plant extracts in corresponding solvents were mixed with phosphate buffer (2.5ml) and potassium ferricyanide (2.5ml). This mixture was kept at 50˚C in water bath for 20 minutes. After cooling, 2.5ml of 10% trichloro acetic acid was added and centrifuged at 3000 rpm for 10mins whenever

necessary. The upper layer solution (2.5ml) was mixed with distilled water (2.5ml) and a freshly prepared ferric chloride solution (0.5ml). The absorbance was measured at700 nm. Control was prepared in similar manner excluding samples. Ascorbic acid at various concentrations was used as standard. Increased absorbance of the reaction mixture indicates increase in reducing power.

Determination of Lipid Peroxidation Assay[37]

Egg homogenate (10% in 1.15% potassium chloride, v/v) 0.1 ml of extract/standard (100-500µg) were mixed in a test tube and the volume was made up to 2ml, by adding distilled water. Finally, 0.5 ml FeSO4 (0.07M) was added to the above mixture and incubated for 30 minutes, to induce lipid peroxidation. Thereafter, 0.5ml of 20% acetic acid (pH 3.5) and 0.5ml of 0.8% TBA (w/v) (prepared in 1.1% sodium dodecyl sulphate) and 0.5ml 20% TCA were added, vortexed, and then heated in a boiling water bath for 60mins. After cooling, 5.0ml of 1-butanol was added to each tube and centrifuged at 3000 rpm for 10mins. The absorbance of the organic upper layer was measured at 532nm. For the blank 1.0ml of distilled water was used in place of the extract.

AI= (1- T/C) x 100

Where T = absorbance of Test, C = absorbance of fully oxidized control.

RESULT AND DISCUSSION

Extractive yield of *Phaseolus coccineus* linn

The percentage yield of ethanolic and aqueous extract of seeds of *Phaseolus coccineus* linn was found to be

Ethanolic extract: 8.24 % w/w. Aqueous extract: 6.32 % w/w.

1. Preliminary Phytochemical screening of *Phaseolus coccineus*

Preliminary Phytochemical screening of *Phaseolus coccineus*, revealed the presence of following phytoconstituents.

Table No: 1: Results of preliminary Phytochemical screening of *Phaseolus coccineus.*

S No	Constituents	Observation	
		Ethanol	Aqueous
1	Alkaloids	+	+
2	Carbohydrates	+	+
3	Proteins & amino acids	+	+
4	Steroids	+	+
5	Phenols	+	+
6	Tannins	+	+
7	Flavonoids	+	+
8	Glycosides	+	+
9	Saponins	_	+
10	Terpenoids	+	+

(-) indicates the absence of compound (+) indicates the presence of compound

ＩＩ. Thin Layer Chromatography of *Phaseolus coccineus*

TLC study was carried out for the separation and identification of phytoconstituents in *Phaseolus coccineus*, after development visualization was done with Iodine. Rf values were calculated.

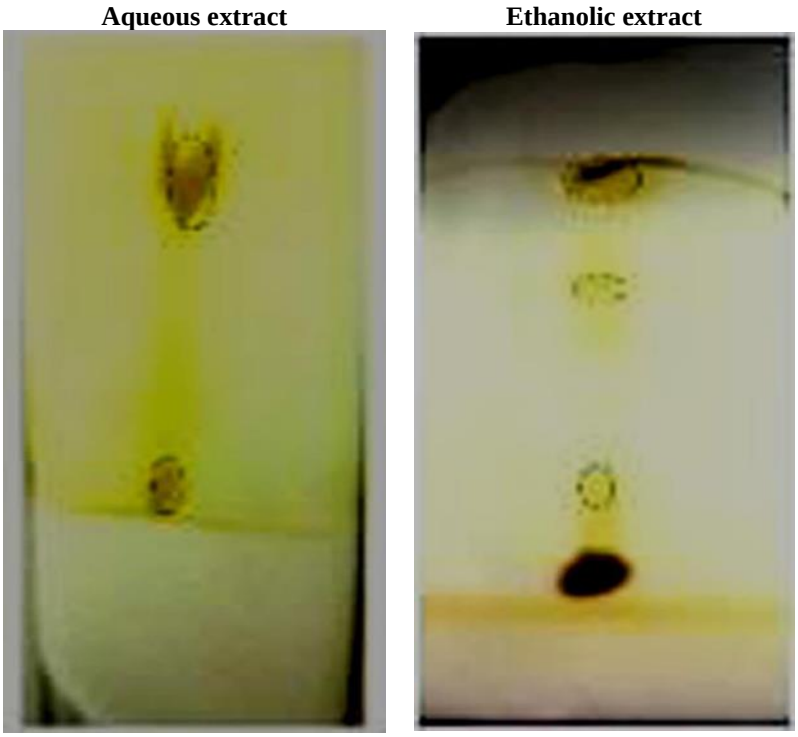

Figure No. 6: TLC Identification of phytoconstituents in AEPC and EEPC.

Table No. 2: Results of TLC screening of Phaseolus *coccineus.*

Aqueous extract		Ethanolic extract	
No of Spot	R_f Value	No of Spot	R_f Value
1	0.38	1	0.16
2	0.83	2	0.41
		3	0.55
		4	0.88

3. DETERMINATION OF PHYTOCONSTITUENTS OF EXTRACTS

1. Determination of Total Flavonoid content

Table 3: Determination of Total Flavonoid content.

Sample	Concentration µg/ml	OD value
Standard (Quercetin) 1mg/ml	10	0.03
	20	0.09
	40	0.26
	60	0.5
	80	0.78
	100	1.05
AEPC 1 mg/ml	100	0.04
	100	0.05
	100	0.04
EEPC 1 mg/ml	100	0.06
	100	0.04
	100	0.04

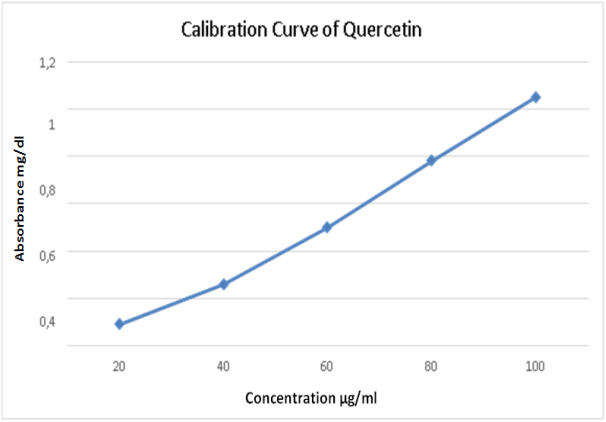

Figure No: 8 Determination of Total Flavonoid content.

Total Flavonoid Content of AEPC was found to be 8.81 mg Quercetin Equivalents /g plant Extract.

Total Flavonoid Content of EEPC was found to be 9.18 mg Quercetin Equivalents /g plant Extract.

Ⅱ. Determination of Total Phenolic content

Table No. 4: Determination of Total Phenolic content.

Sample	Concentration µg/ml	OD value
Standard (Gallic Acid) 1mg/ml	10	0.09
	20	0.12
	40	0.21
	60	0.34
	80	0.40
	100	0.50
AEPC 1 mg/ml	100	0.13
	100	0.13
	100	0.11
EEPC 1 mg/ml	100	0.15
	100	0.19
	100	0.13

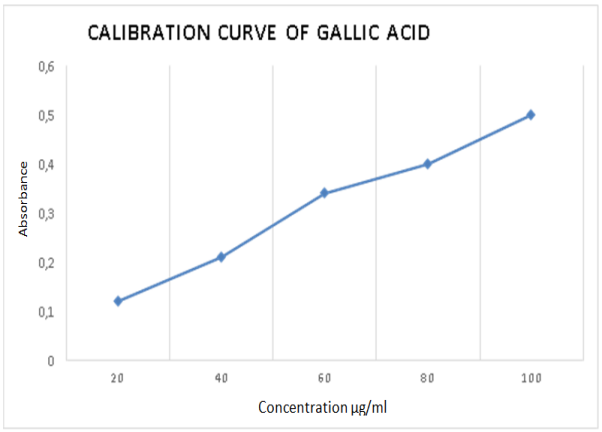

Figure No. 9: Determination of Total Phenolic content.

Total Phenolic Content of AEPC was found to be 30.10 mg Gallic acid Equivalents / g plant Extract.

Total Phenolic Content of EEPC was found to be 42.22 mg Gallic acid Equivalents / g plant Extract.

4. *IN VITRO* ANTI OXIDANT STUDIES ON THE EXTRACT

1. Determination of Nitric Oxide Scavenging Assay: EEPC: Ethanolic extract of *Phaseolus coccineus* AEPC: Aqueous extract of *Phaseolus coccineus*

Table 5: Determination of Nitric Oxide Scavenging Assay.

S. no	Concentration µg/ml	% Of Inhibition			IC_{50} Value (µg/ml)		
		AscorbicAcid	EEPC	AEPC	Ascorbic Acid	EEPC	AEPC
1	200	86.38±1.32	23.25±1.77	59.04±2.34	98.26	651	195
2	400	90.33±1.56	40.69±1.02	63.33±1.09			
3	600	91.41±1.52	47.28±1.55	84.76±0.62			
4	800	92.93±1.76	64.72±1.02	87.37±0.23			
5	1000	93.94±1.49	72.85±1.404	91.41±0.41			

Values are mean ± SEM of 3 replicates

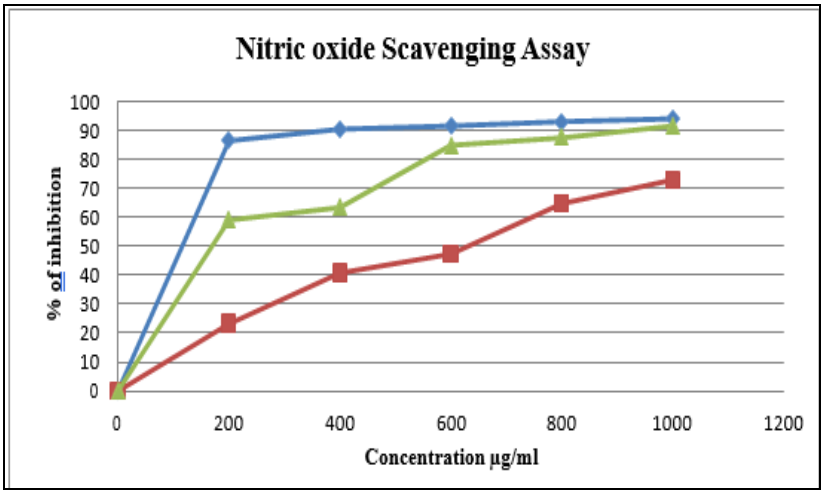

Figure No. 9: Determination of Nitric Oxide Scavenging Assay.

ⅠⅠ. DETERMINATION OF REDUCING POWER ASSAY

Table No. 6: Determination of Reducing Power Assay.

S. No	Concentrationµg/ml	OD value		
		AscorbicAcid	EEPC	AEPC
1	200	1.23 ± 0.06	0.18 ± 0.008	0.47 ± 0.01
2	400	1.61 ± 0.04	0.21 ±0.01	0.60 ± 0.02
3	600	1.68 ±0.04	0.41 ± 0.01	0.94 ± 0.03
4	800	1.77 ± 0.07	0.64 ±0.02	1.19 ±0.04
5	1000	1.80 ± 0.08	1.04 ±0.04	1.41 ± 0.01

Values are mean ± SEM of 3 replicates

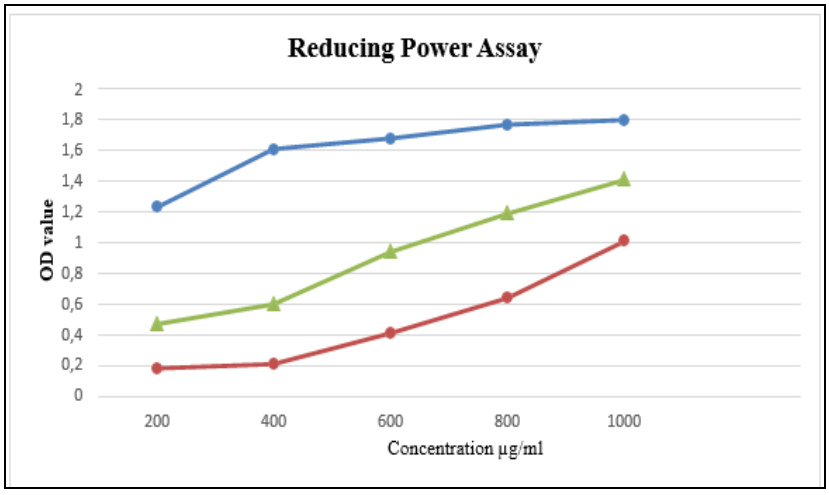

Figure No. 10: Determination of Reducing Power Assay.

III. DETERMINATION OF LIPID PEROXIDATION ASSAY

Table No: 7. Determination of Lipid Peroxidation Assay:

S. no	Concentrationµg/ml	% Of Inhibition			IC 50 Value (µg/ml)		
		Ascorbic Acid	EEPC	AEPC	AscorbicAcid	EEPC	AEPC
1	200	70.5±1.32	25.99±1.77	21.71±2.9	171.66	700.66	732.22
2	400	77.31±1.62	38.09±1.17	31.81±0.87			
3	600	82.35±1.21	46.02±0.81	43.93±1.57			
4	800	86.55±1.45	55.55±1.58	54.28±2.20			
5	1000	90.76±1.68	60.29±1.20	60.09±1.10			

Values are mean ± SEM of 3 replicates

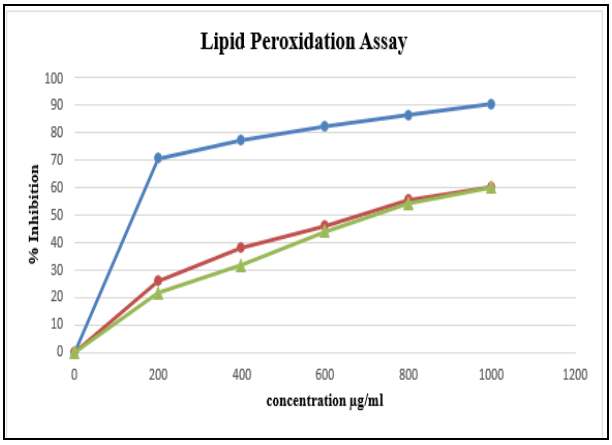

Figure No. 11: Determination of Lipid Peroxidation Assay.

CONCLUSION

Formation of kidney stones is a complex process and involves a series of biological events that are most likely triggered by genetic susceptibility together with dietary factors and lifestyle changes. The percentage yield of ethanolic and aqueous extract of Phaseolus coccineus linn was found to be 8.24 % w/w and 6.32 % w/w respectively. The ethanolic extract (EEPC) was dark brown in colour with a thick viscous consistency. The aqueous extract (AEPC) also has a thick viscous consistency and pale yellow in colour. Preliminary Phytochemical screening of EEPC and EEPC revealed the presence of Alkaloids, Carbohydrates, Proteins & amino acids, Steroids, Phenols, Tannins, Flavonoids, Terpenoids and Glycosides while the aqueous extract alone possesses saponins. Plant flavonoids are reported to possess antiurolithiatic activity through its antioxidant property. Several reports suggest that saponins are having antiurolithiatic activity through its diuretic and disaggregating the suspension of mucoproteins. The results of TLC separation of the extracts with the mobile phase Chloroform: Methanol: Distilled water: Toluene - 8:1:0.5:0.5 followed by spraying with Iodine showed 2 spots for aqueous extracts and 4 spots for ethanolic extract. The Rf value one of the compounds (0.86) separated with methanolic extract of Phaseolus coccineus seeds using the same mobile phase almost coincides with one of the spots of our aqueous extract (0.83) and one spot in ethanolic extract (0.88). The other spots in the ethanolic extracts have Rf values almost similar to that of methanolic extracts of Phaseolus lunatus methanolic extracts. Therefore the spots visualised in the extracts indicate the presence of either saponins or terpenes in aqueous extract and only terpenoids in ethanolic extract. The anti-oxidant activity of AEPC and EEPC was assessed by three methods namely nitric oxide scavenging assay, Reducing power assay and in vitro lipid peroxidation assay.

The polyphenols, tannins, content of the extracts have been reported to have metal chelating and hydroxyl radical scavenging properties. Further the AEPC and EEPC can be assessed for Anti urolithiatic activity on different animal models.

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Conflict of interest

The Authors declare no conflict of interest

REFERENCE

1. Mani, shankar. Importance and uses of medicinal plants – an overview. International journal of preclinical & pharmaceutical research, 2016; 7: 67.
2. Bushra sultana, Effect of drying techniques on the total phenolic contents and antioxidant activity of selected fruits. Journal of medicinal plants research, 2012; 6(1). Doi:10.5897/jmpr11.916
3. Boron, walter f.; boulpaep, emile l. Medical physiology. Elsevier health sciences, 2016; 738.
4. "kidney stones in adults". February 2013. Archived from the original on 11 may 2015.
5. Giannossi l., summa v. A review of pathological biomineral analysis techniques and classification schemes. In: aydinalp c., editor. An introduction to the study of mineralogy. Intech, imaa-cnr, italy: intechopen, 2012.
6. Mikawlrawng k., kumar s., vandana r. Current scenario of urolithiasis and the use of medicinal plants as antiurolithiatic agents in manipur (north east india): a review. International journal of herbal medicine, 2014; 2(1): 1–12.
7. Sigurjonsdottir v. K., l.runolfsdottir h., indridason o. S., et al. Impact of nephrolithiasis on kidney function. Bmc nephrology, 2015; 16(1): 149.
8. El-zoghby z. M., lieske j. C., foley r. N., et al. Urolithiasis and the risk of esrd. Clinical journal of the american society of nephrology, 2012; 7(9): 1409–1415. Doi: 10.2215/cjn.03210312.
9. Taylor e. N., stampfer m. J., curhan g. C. Obesity, weight gain and the risk of kidney stones. Journal of the american medical association, 2005; 293(4): 455–462. Doi: 10.1001/jama.293.4.455.
10. Courbebaisse m., prot-bertoye c., bertocchio j., et al. Nephrolithiasis of adult: from mechanisms to preventive medical treatment. Revue medicale international, 2017; 38(1): 44–52. Doi: 10.1016/j.revmed.2016.05.013.
11. Kumar s. B. N., kumar k. G., srinivasa v., bilal s. A review on urolithiasis. International journal of universal pharmacy and life sciences, 2012; 2(2): 269–280.
12. Miller nl, lingeman je. "management of kidney stones". Bmj., march 2007; 334(7591): 468–72.
13. Johri n, cooper b, robertson w, choong s, rickards d, unwin r. "an update and practical guide to renal stone management". Nephron clinical practice,

2010; 116(3).
14. Liebman m, al-wahsh ia. "probiotics and other key determinants of dietary oxalate absorption" (pdf). Advances in nutrition, may 2011; 2(3): 254–60.
15. John h. Foulger. "the user of the molisch (α-naphthol) reactions in the study of sugars in biological fluids" (pdf). J. Biol. Chem., 1931; 92: 345–35.
16. Robert d. Simoni; robert l. Hill; martha vaughan. "benedict's solution, a reagent for measuring reducing sugars: the clinical chemistry of stanley r. Benedict". J. Biol. Chem., 2002; 277(16): 10–11. Doi:10.1016/s0021-9258(19)61050-1.
17. C. Barfoed. Uber die nachweisung des traubenzuckers neben dextrin und verwandten körpern". Fresenius' zeitschrift für analytische chemie, 1873; 12(1): 27. Doi:10.1007/bf01462957. S2cid 95749674.
18. Brito-arias, marco (2007). Synthesis and characterization of glycosides. Springer. Isbn 978-0-387-26251-2.
19. Ghani a. Medicinal plants of bangladesh (1st ed.). Dhaka, bangladesh: the asiatic society of Bangladesh, 1998; 505.
20. "mayer′s reagent". Sigma aldrich. Retrieved 2022-02-08. K2hgi4
21. Yisa, jonathan. "phytochemical analysis and antimicrobial activity of scoparia dulcis and nymphaea lotus". Australian journal of basic and applied sciences, 2009; 3(4): 3975–3979.
22. Delcour ja. "a new colourimetric assay for flavanoids in pilsner beers". Journal of the institute of brewing, 1985; 91: 37–40. Doi:10.1002/j.2050-0416.1985.tb04303.x.
23. Fenk, c. J.; kaufman, n.; and gerbig, d. G. J. Chem. Educ., 2007; 84: 1676-1678.
24. Walsh, edward o. An introduction to biochemistry. London: the english universities press; hodder & Stoughton, 1961; 406–407. Oclc 421450365. Archived from the original on 2022-02-08.
25. Plant polyphenols: synthesis, properties, significance. Richard w. Hemingway, peter e. Laks, susan j. Branham, 263.
26. Antibacterial activity of leave extracts of nymphaea lotus (nymphaeaceae) on methicillin resistant staphylococcus aureus (mrsa) and vancomycin resistant staphylococcus aureus (vrsa) isolated from clinical samples". Akinjogunla o. J., yah c. S., eghafona n. O. And ogbemudia f. O., annals of biological research, 2010; 1(2): 174–184
27. El aziz mma, ashour as, melad asg. A review on saponins from medicinal plants: chemistry, isolation, and determination. J nanomed Res., 2019; 8(1): 282-288.
28. Rahman, atiqur; sitepu, irnayuli r.; tang, sui-yan; hashidoko, yasuyuki. "salkowski's reagent test as a primary screening index for functionalities of rhizobacteria isolated from wild dipterocarp saplings growing naturally on medium-strongly acidic tropical peat soil". Bioscience, biotechnology, and biochemistry, 2010; 74(11): 2202–2208. Issn 1347-6947.
29. Campbell, mary k. & shawn o. Farrell. Biochemistry. (4th ed.). Singapore: thomson asia pte ltd., 2005.
30. Ayoola ga. "phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in southwestern nigeria". Tropical journal of pharmaceutical research, 2008; 7(3): 1019–1024.
31. Asante ik, owusu e, essilfie mk, kwarteng m and amuzuah o. Phytochemical investigation and thin layer chromatography of methanolic extracts of some selected grain legumes. J pharmacogn phytochem, 2016; 5(3): 240-244.
32. Ling-jia zhao, wei liu, su-hui xiong, jie tang, zhao-huan lou, ming-xia xie, bo-hou xia, li-mei lin, duan-fang liao, "determination of total flavonoids contents and antioxidant activity of ginkgo biloba leaf by near-infrared reflectance method", international journal of analytical chemistry, article id 8195784, 2018; 7: 2018.
33. Kupina, s., fields, c., roman, m. C., & brunelle, s. L. Determination of total phenolic content using the folin-c assay: single-laboratory validation, first action 2017.13. Journal of aoac international, 2018; 101(5): 1466–1472.
34. Nitric oxide scavenging assay : (garrat , 1964.), 2018.
35. Sreejayan, & rao, m. N. Nitric oxide scavenging by curcuminoids. The journal of pharmacy and pharmacology, 1997; 49(1): 105–107.
36. Ponnusamy, jayanthi & p ramadas, lalitha. Determination of the in vitro reducing power of the aqueous extract of eichhornia crassipes (mart.) Solms. Journal of pharmacy research, 2011; 4: 4003-4005.
37. Devasagayam, t. P., boloor, k. K., & ramasarma, t. Methods for estimating lipid peroxidation: an analysis of merits and demerits. Indian journal of biochemistry & biophysics, 2003; 40(5): 300–308.