EVALUATION OF POLYHERBAL FORMULATION FOR UROLITHIATIC TREATMENT

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ABSTRACT

In comparison to allopathic medications, which are not only more expensive in terms of "leads" but also have a variety of adverse effects, polyherbal medications are more practical. The *Abutilon indicum, Tribulus terrestris*, and *Phyllanthus niruri* exhibit strong anti-urolithiatic activity, according to ethnopharmacological usage. A formulation containing the plant materials was created after a thorough analysis of the powdered ethanolic extract of the leaves of *Abutilon indicum, Tribulus terrestris,* and *Phyllanthus niruri*. The formulation was assessed and standardised in accordance with pharmacopoeia requirements. Pre-formulation studies findings showed that every value was within permissible bounds. Significant hardness characteristics (4.35 kg/cm2) were present in the formulation, which speeds up disintegration. The formulation's low friability (0.8%) suggested that the tablets were mechanically stable. The permissible weight variation range is 7% because the average tablet weight was 505 mg. As a result, the complete tablet formulation passed the weight variation test. Formulations took longer than a minute to disintegrate. Thus, the old Indian medical systems' assertions that this herb might be used to treat kidney stones were proven true. The final inference made from the facts stated above is that since there are no significant adverse effects compared to synthetic drugs, it may be possible to employ these affordable, relatively safe, and natural treatments of plant origin.

Keywords: *Abutilon indicum*, *Tribulus terrestris*, *Phyllanthus niruri*, Pre-formulation

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1. INTRODUCTION

Plants used directly or indirectly to extract medications for the treatment of diseases are known as medicinal plants. Pharmacogenomics researchers are working to uncover the past benefits of therapeutic plants for the world's suffering people. In the modern world, 30% of pharmaceutical

preparations are produced from plants. It has been discovered that people employ a variety of plants as a source of medication. People employ a variety of plant components for medical purposes, including roots, stems, barks, gums, leaves, berries, seeds, and flowers, but the way that they are used can vary depending on local customs.[1,2] It is well known that some plants are used in numerous ways. Tribal drug trafficking has long been thought to be necessary due to the growing demand for drugs and opiates. The collection, classification, processing, and utilisation of native plant material for medical reasons are desired. A preliminary investigation of the taxonomic, pharmacognostic, and phytochemical characteristics of plants is the logical first step towards the ultimate goal of natural medicine creation. Typically, it involves bioactive therapeutic elements that have been extracted from natural areas. They have endured the test of time because to their protection, effectiveness, cultural acceptability, and less adverse effects.[3,4] They are considered to be more similar to the human body because the chemical components are a part of the biological processes of living flora. There is a wealth of literature that recommends age-related natural therapies for ailments including memory loss, osteoporosis, diabetic wounds, and liver problems. However, it is clear from the existing medical system that there is no effective treatment that can totally heal the aforementioned diseases. The existence of man on this planet is only conceivable because to the vital role that the plant kingdom plays in this. Nature is always a shining example of how extraordinary synergy can occur.[5,6]

1.1 Urolithiasis (kidney stone)

A hard mass known as urolithiasis forms when crystals in the urinary tract separate from urine. Oxalate and calcium are frequent components of the human diet and play important roles in maintaining the health of the muscles and bones. Urine commonly contains calcium and phosphate, which are excreted through the urinary system. When some substances that naturally prevent the production of calcium oxalate and calcium phosphate crystals do not function effectively in a particular person, crystal formation may take place.[7,8] Urinary infection is one of the major factors that contribute to stone development. The tiny crystal particle can pass through the renal tract undetected and exit the body through the urine. A struvite or infection stone is the name given to this type of stone. Uric acid stones, another type of stone, are a little less typical. There have been attempts at specific phytotherapy treatments, and interest in effective, secure, and socially

acceptable medicinal plants has recently grown again. However, there aren't many studies that have been done specifically to evaluate their effectiveness in treating urolithiasis.[9,10]

2. MATERIAL AND METHODS

2.1 Physicochemical parameter

Various ash values, including total ash (TA), water soluble ash (WSA), and acid insoluble ash (AIA), were calculated in accordance with protocol. It demonstrated the identification, cleanliness, and purity of herbal medications. All types of ash values for AI, TT, and PN were discovered to be within pharmacopeial limits. It demonstrated the purity and cleanliness of the crude medications chosen for the current investigation. The level of active chemicals in a volume of plant material after being extracted with a solvent is measured by extractive value estimation.

2.2 Qualitative chemical evaluation

2.2.1 Petroleum ether extract of AI, TT and PN: -

❖ The presence of fixed oil and lipids is demonstrated by the petroleum ether extracts of AI, TT, and PN.

2.2.2 Extract from Chloroform of AI, TT and PN: -

❖ The presence of tannins and phenol compound was revealed in the chloroform extract of AI, TT, and PN.

2.2.3 Water extract of AI, TT and PN: -

❖ Water extract of AI showed the presence of Carbohydrates.

2.2.4 Ethanolic extract of AI, TT and PN: -

Ethanol extract shows the existence of carbohydrates, glycosides, tannins and flavonoid and phenolic compounds. On analysis and comparison to data recorded from phytochemical studies, the tannins, glycosides, phenolic and carbohydrates from Ethanolic AI, TT and PN extract involved various pharmacological behaviors on the urinary system. The presence of glycosides, flavonoids and phenolic compounds in the extracts that cause anti-urolithic activity. We can conclude from the results that the ethanol extract of *Abutilon indicum, Tribulus terrestris and Phyllanthus niruri* leave has anti-urolithic activity against urolithiasis caused by EG in rats. *Abutilon indicum* leave ethanolic extract was found to be more potent in showing anti-urolithic activity. Results shows that *Abutilon indicum, Tribulus terrestris and Phyllanthus niruri* leave should be used to treat urological disorders as a necessity of modern health science.

2.3 Fluorescence studies: -

Studies of Fluorescence help to identify difficult to distinguish of drugs Fluorescence experiment in combination of drugs of two or more species, helps recognize a specific drug by the using fluorescence intensity estimates. A sample of known identity should be used to compare the unknown. Fluorescence is an important phenomenon shown in plant materials by different phytoconstituents. Some display fluorescence in daylight in the visible range. In many natural products ultraviolet light generates fluorescence that is not clearly Fluorescence in daylight. Some of the substances can often be transformed into fluorescent derivatives using distinct chemical reagents and chemicals although they are not fluorescent, so we can often use fluorescence to evaluate some crude drugs qualitatively as it is the most significant pharmacognostical parameter.

2.4 Polyherbal Formulation

2.4.1 Powdering of extracts: - Colloidal silicon dioxide (Aerosil 200) was added to the extract in a percentage of 2% of adjuvant to dry residue.

2.4.2. Pre-formulation studies

2.4.2.1 Organoleptic studies: In these studies, the organoleptic features like colour, odour and physical appearance were observed and recorded.

2.4.2.2 Solubility testing

The solubility were checked in water pH (7.0), 0.1 N hydrochloric acid solution, absolute alcohol, ethyl acetate, and hexane.

2.4.2.3 Angle of repose

Angle of repose is an important parameter to study the Flow property analysis of any powdered formulation with respect to their frictional forces. It was measured by taking reading of height of pile and radius of pile. Mathematically it was calculated by level of the pile (H) divided by radius of the pile(R).

Angle of repose (Tan θ) = height of the pile (H)/ radius of the pile(R)

S.no.	Angle of repose	Flow property
1.	Less than 25	Excellent
2.	Between 20 to 30	Good
3.	Between 30 to40	Passable
4.	More than 40	Very poor

Table 1. Relationship between Angle of repose and powder Flow property

2.4.2.4 Loss on drying

Weighing bottle was dried a in an oven at 105°C.and weight (w_1) was taken. 3 g of the drug was to be found in it. It was dried at temperature of 100-105^{0}C in oven approximately for 3 hours. Drug was then allowed to cool in desiccators. And weigh it again (w_2).

$$\% \text{ Loss on drying (LOD)} = \{(w1- w_2) / w_1\} \text{ x100}$$

2.4.2.5 Bulk density

Bulk density was determined by measuring the amount of sample required to fill 3/4th volume of a 10ml. capacity graduated measuring cylinder via a funnel and measuring the volume occupied and weighed.

The following formula was used to determine bulk density.

Bulk density (Db) = mass of powder (M) / bulk volume (Vb) (Aulton ,1988)

2.4.2.6. Tapped density

The following formula was used to determine tapped density. Tapped density (Dt) = mass of powder (M) /tapped volume (Vt)

Tapped density was determined by tapping the graduated 10ml. measuring cylinder 100 times from a height of about 1.5 inch.

2.4.2.7. % Compressibility

% Compressibility determined by formula given below

Tapped density – Bulk density/ tapped density

Then mulplied value obtanned by 100

2.4.2.8. Hausner ratio

Hausner ratiocan be obtained by formula given below.

Tapped density / Bulk density

2.4.2.9. pH

pH was determined by pH meter.

2.4.2.10 Particle size

These studies were carried out by sieve analysis. Sieve no. 60, 80 and 120 were used. Accurately weighed sample was placed (2 g) on topmost /coarsest sieve. Sieves were arranged in the ascending order from top. Agitated the nest of sieves for 5 minutes. Stopped the sieve shaker. Then carefully removed each sieve from the nest, without any loss of material. Reweighed each sieve and determined the weight of material on each one Determined the mass of material in the collecting pan. Reassembled the nest of sieves and agitated for another 5 min. This was done repeatedly for

three times. After three times of agitation the end point criterion was achieved (when the change in mass of any of the test sieve is not more or less than 5% of the previous mass on that sieve).

2.5 Extract excipients interaction study

Powdered extracts excipients interaction study was performed by determination of total flavonoid in mixture of powder of *Abutilon indicum*, *Tribulus terrestris*, *Phyllanthus niruri* and excipients at initial, 15 and after 30 days.

2.6 Formulation of anti-urolithiatic Tablets

The extract powder was taken that was properly dried. Ethanolic extract of *Abutilon indicum*, *Tribulus terrestris* and *Phyllanthus niruri* were implemented by wet granulation in tablet form. Five formulations of were prepared by taking individual extract of *Abutilon indicum* seeds, *Tribulus terrestris* and *Phyllanthus niruri*. Then five formulations were prepared by taking combination of above extracts. Combination formulation given as PHF (Poly herbal formulation). Formulations have the following composition.

2.7 Evaluation of Tablets

The various physical parameters were used to evaluate the tablets.

2.7.1. Organoleptic properties

Size (thickness), shape, color, taste was determined.

2.7.2. Weight Variation Test

For determination of weight variation take 20 tablets. Weight these 20 tablets and their average was determined. Compare the average of tablet weight with single tablet.

Tablet average weight (mg)	Total deviation percentage allowed
130 mg or less	10 Percentage
130 mg to 324 mg	7.5 Percentage
More than 324 mg	5.0 Percentage

2.7.3. Tablet strength

The tablet strength was represented as the capacity of the tensile (Kg/cm^2). The tablet crush load, in which the pressure force needed to break a tablet in half. A tablet hardness tester (Monsanto hardness tester) was used to calculate this.

2.7.4. Friability

Friability testing was carried out to evaluate friction and shocks effect. This can often lead to chiping, caping, and breaking of tablets. For this reason, Roche friabilator was used. This machine puts many tablets under the cumulative impact of abrasion. The tablet was shaken in a plastic chamber which rotates with speed of 25 RPM. The tablets were dropped from height of 6 inch per rotation. The tablets were again weight after dusting. The loss of weight of tablets should not more than 1 %.

2.7.5. DT

One tablet was mounted in each of the six DT apparatus. DT was performed at 37 ±2$_0$C Disintegration time defined as time required disintegrating and passing all fragments through the sieve (# 10)**.**

3. RESULTS

3.1 Physicochemical parameter

Parameters	Values Obtained		
	AI	**TT**	**PN**
Total ash (TA) %w/w	7.25	8.29	7.26
Ash which is soluble in Water (WSA) %w/w	3.50	6.20	4.19
Acid insoluble ash (AIA) %w/w	1.13	3.19	3.10
Moisture content (MC) %w/w	1.29	2.09	1.72
Swelling index (SI) %w/w	3.16	1.18	1.28
Foreign organic matters (FOM) %w/w	1.89	1.12	1.02
Water soluble extractive value %w/w	19.86	18.25	16.27
Extract that was soluble in alcohol, value %w/w	12.27	13.92	10.28

Table 2. Physicochemical parameter of selected herbs

3.2 Qualitative chemical evaluation

S. No.	Chemical constituents	AI			
		Petroleum Ether	**Chloroform**	**Water**	**Ethanol**
1	**Fixed oils& fats**	+	-	-	-
2	**Alkaloid**	-	-	-	-
3	**Carbohydrate**	-	-	+	+
4	**Glycoside**	-	-	-	+
5	**Tannins and phenolic**	-	+	-	+

6	**Flavonoid**	**-**	**-**	**-**	+
7	**Saponin**	**-**	**-**	**-**	**-**
8	**Steroid**	**-**	**-**	**-**	**-**

Figure 2. Qualititative Evaluation of AI

S. No.	**Chemical constituents**	**TT**			
		Petroleum Ether	**Chloroform**	**Water**	**Ethanol**
1	**Fixed oils& fats**	+	-	-	-
2	**Alkaloid**	-	-	+	+
3	**Carbohydrate**	-	+	+	+
4	**Glycoside**	-	-	-	+
5	**Tannins and phenolic**	-	+	-	+
6	**Flavonoid**	-	-	-	+
7	**Saponin**	-	+	+	-
8	**Steroid**	-	-	-	-

Table 3. Qualititative Evaluation of TT

S. No.	**Chemical constituents**	**PN**			
		Petroleum Ether	**Chloroform**	**Water**	**Ethanol**
1	**Fixed oils& fats**	+	-	-	-
2	**Alkaloid**	-	-	-	+
3	**Carbohydrate**	-	-	+	+
4	**Glycoside**	-	-	-	-
5	**Tannins and phenolic**	-	+	-	+
6	**Flavonoid**	-	+	-	+
7	**Saponin**	-	-	+	-
8	**Steroid**	-	-	-	-

Table 4. Qualititative Evaluation of PN

3.3 Fluorescence studies: -

S. No	**Treatment**	**Under ordinary light**	**Ultra violet light (254 nm)**	**Ultra violet light (365 nm)**
1	Leave powder	Brown	No Fluorescence	No Fluorescence
2	Leave powder + 50% Sulphuric acid	Dark brown	Greenish black	Dark green
3	Leave powder + 1N NaOH	Yellowish green	Dark greenish	Dark green

4	Leave powder + Ammonia	Orange	Light yellow	Light yellow
5	Leave powder + Acetic acid	Dark orange	Yellowish brown	Yellowish brown
6	Leave powder + 5% KOH	Dark greenish	Greenish black	Yellowish green
7	Leave powder + 5% FeCl3	Dark green	Light green	Light green
8	Leave powder + 50% HNO3	No florescence	Light yellow	Light yellow
9	Leave powder + 5% Iodine sol.	Golden yellow	Dark green	Dark green

Table 5. Fluorescence studies of Leave powder of AI

S. No	Treatment	Under ordinary light	Ultra violet light (254 nm)	Ultra violet light (365 nm)
1	Leave powder	Yellowish green	Dark greenish	Dark green
2	Leave powder + 50% Sulphuric acid	Orange	Light yellow	Light yellow
3	Leave powder + 1N NaOH	Golden yellow	Light green	Dark green
4	Leave powder + Ammonia	Dark green	Dark green	Light green
5	Leave powder + Acetic acid	No florescence	Light yellow	Light yellow
6	Leave powder + 5% KOH	Dark green	Light green	Light green
7	Leave powder + 5% FeCl3	Dark greenish	Greenish black	Yellowish green
8	Leave powder + 50% HNO3	Golden yellow	Dark green	Light yellow
9	Leave powder + 5% Iodine sol.	Light yellow	Light yellow	Dark green

Table 6. Fluorescence studies of leave powders of TT

S. No	Treatment	Under ordinary light	Ultra violet light (254 nm)	Ultra violet light (365 nm)
1	Leave powder	Dark greenish	Yellowish green	Dark green
2	Leave powder + 50% Sulphuric acid	Golden yellow	Light yellow	Light yellow

3	Leave powder + 1N NaOH	Orange	Dark green	Dark green
4	Leave powder + Ammonia	Light green	Dark green	Light green
5	Leave powder + Acetic acid	No florescence	Light yellow	Light yellow
6	Leave powder + 5% KOH	Dark greenish	Light green	Light green
7	Leave powder + 5% FeCl3	Dark green	Greenish black	Yellowish green
8	Leave powder + 50% HNO3	Golden yellow	Dark green	Light yellow
9	Leave powder + 5% Iodine sol.	Light yellow	Light yellow	Dark green

Table 7. Fluorescence studies of leave powders of PN

3.4 Pre-formulation study of powders of extracts

Pre-formulation study of powder shows that % compressibility of were found poor so they are unable to process as directly compressible formulation to give sufficient compressibility to the powder they should process through wet granulation. Angle of repose concluded that powders having poor flow so sufficient lubricant should be added, to improve flow of powder.

S.no	Parameter	EEAI	EETT	EEPN
1	Description	Light Brown Powder with characteristic odour	Light Brown Powder with characteristic odour	Dark green Powder with characteristic odour
2	Solubility			
	Alcohol	Soluble	Soluble	Soluble
	Water (pH 7)	Slightly soluble	Slightly Soluble	Slightly Soluble
	0.1 N HCl solution	Soluble	Soluble	Insoluble
	Hexane	Insoluble	Insoluble	Insoluble

Table 8. Pre-formulation study of powders

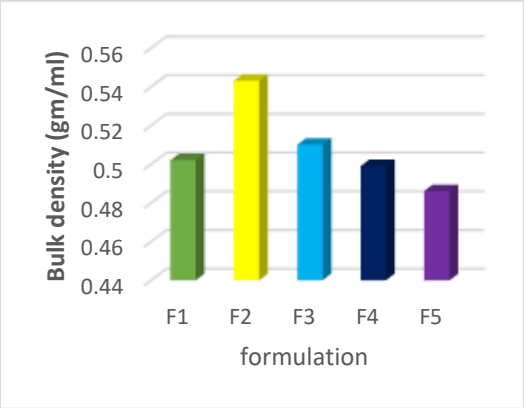
3.5 Pre-compression parameters of Extracts

S. No	Parameter	EEAI	EETT	EEPN
1.	Angle of repose	32	31	31
2.	Loss of drying	12.4%	9.8%	11.2%
3.	Bulk density	0.66g/ml	0.67g/ml	0.65g/ml
4.	Tapped density	0.88g/ml	0.91g/ml	0.87g/ml
5.	% compressibility	17.93%	17.88%	18.22%

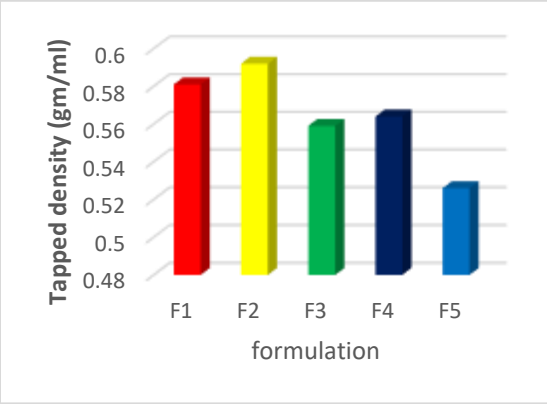
6.	Hausner ratio	1.19	1.17	1.23
7.	pH	6.3	6.4	6.5
8.	Particle size	50-150 µ	50-150 µ	50-150 µ

Table 9. Pre-compression parameters of Extracts

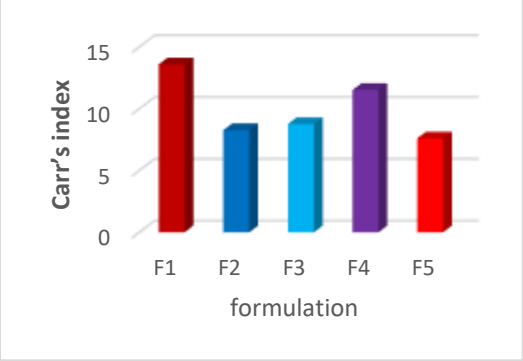
3.5.1 Pre-compression parameters of Formulation containing EEAI



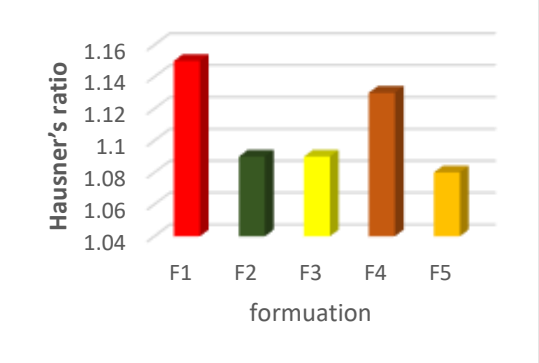
Bulk density



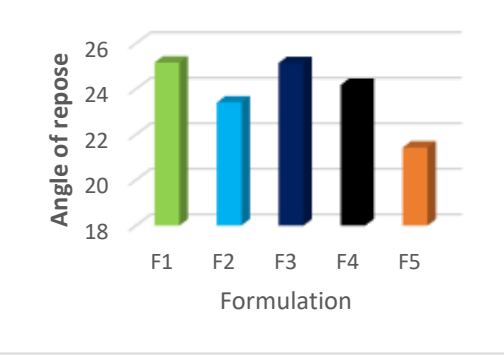
Tapped density



Carr's index

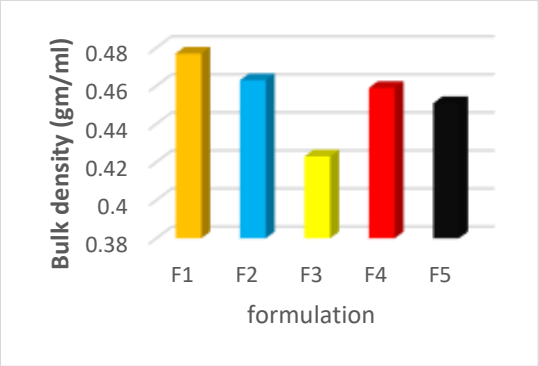


Hausner's ratio

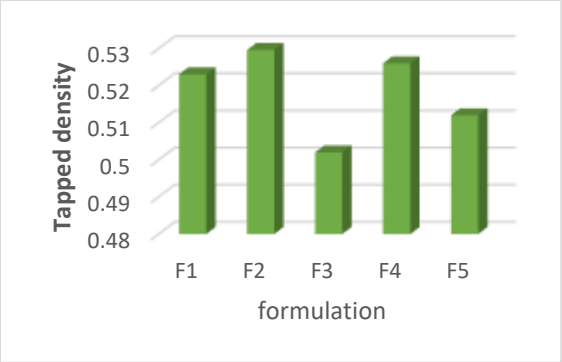


Angle of repose

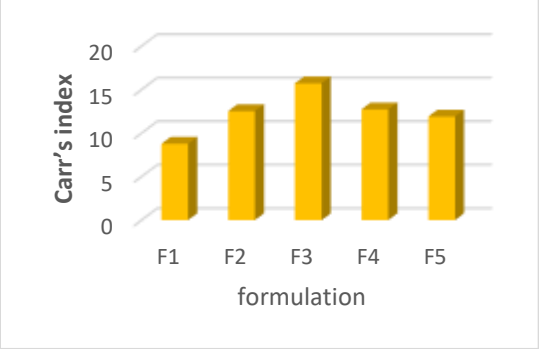
3.5.2 Pre-compression parameters of Formulation containing EETT



Bulk density



Tapped density



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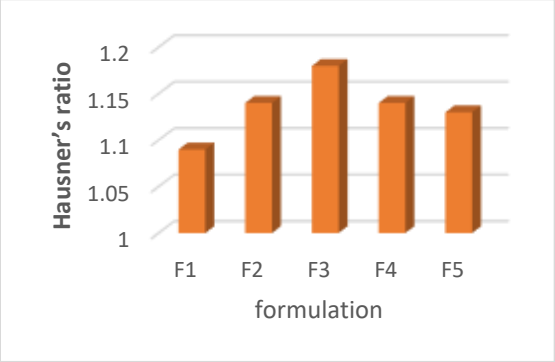
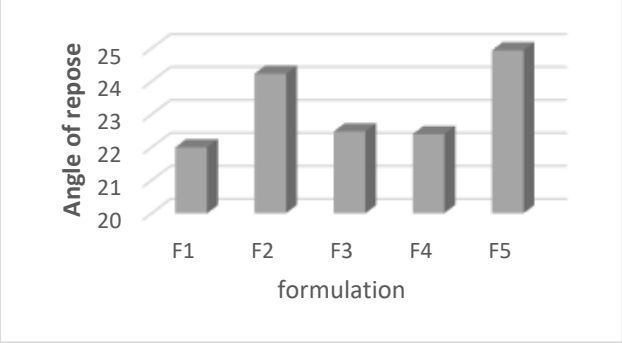
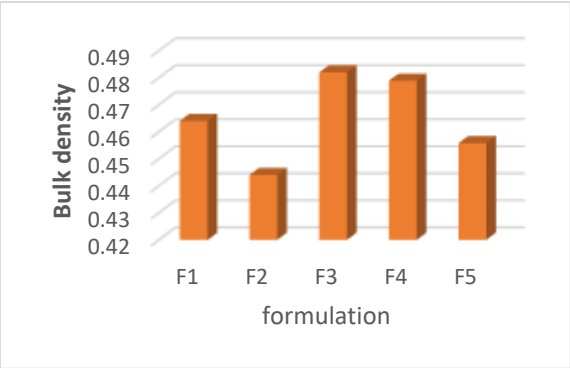


Hausner's ratio

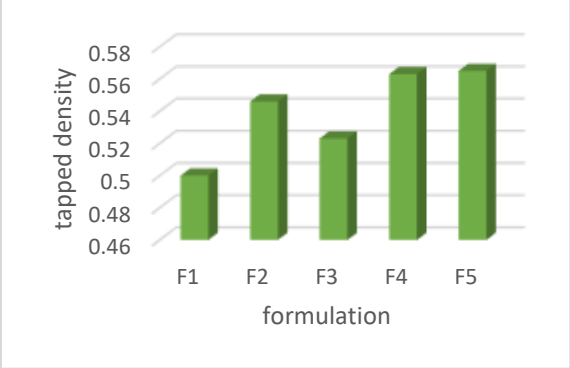


Angle of repose

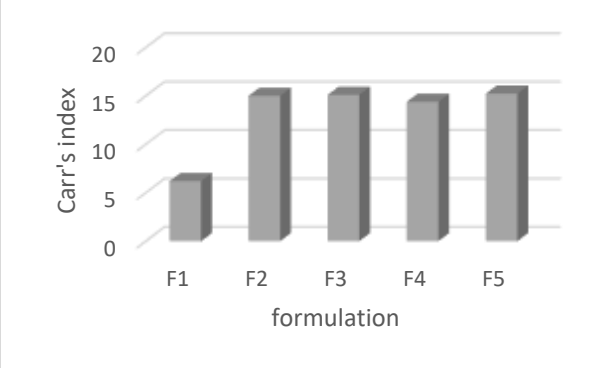
3.5.3 Pre-compression parameters of Formulation containing EEPN



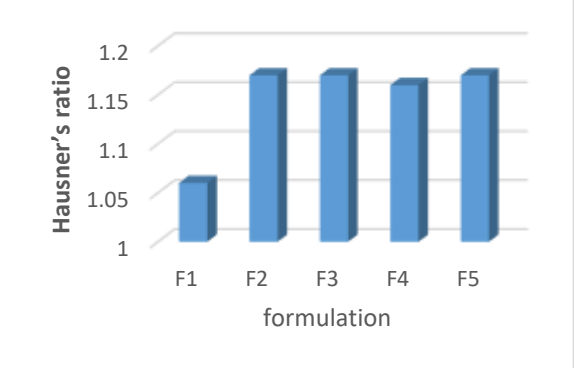
Bulk density



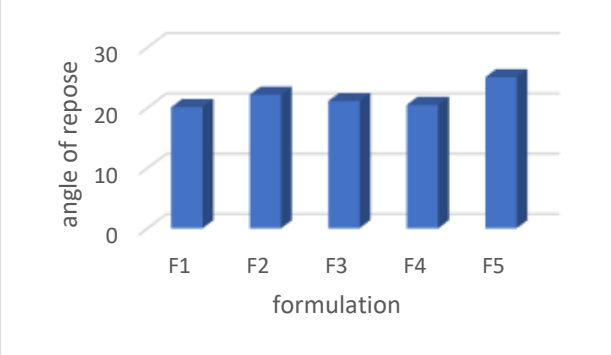
Tapped density



Carr's index



Hausner's ratio



Angle of repose

3.6 Drug - excipient interaction

S. No.	Day	Determination of total flavonoid content (QE mg/100mg)
1	Initial	0.755
2	After 15 days	0.755

3	After 30 days	0.755

Table 10. Drug - excipient interaction

3.7 Evaluation of formulation (Tablets)

3.7.1 Organoleptic properties of Formulations containing EEAI

Parameter	EEAI				
FC	**F1**	**F2**	**F3**	**F4**	**F5**
Color	Brown	Brown	Brown	Brown	Brown
Odor	characteristics	characteristics	characteristics	characteristics	characteristics
Taste	Good	Good	Good	Good	Good

3.7.2 Organoleptic properties of Formulations containing EETT

Parameter	EEAI				
FC	**F1**	**F2**	**F3**	**F4**	**F5**
Color	Brown	Brown	Brown	Brown	Brown
Odor	characteristics	characteristics	characteristics	characteristics	characteristics
Taste	Good	Good	Good	Good	Good

3.7.3 Organoleptic properties of Formulations containing EEPN

Parameter	EEAI				
FC	**F1**	**F2**	**F3**	**F4**	**F5**
Color	Green	Green	Green	Green	Green
Odor	characteristics	characteristics	characteristics	characteristics	characteristics
Taste	Good	Good	Good	Good	Good

3.8 Organoleptic properties of PHF (Poly Herbal Formulation)

Parameter	PHF				
FC	**F1**	**F2**	**F3**	**F4**	**F5**
Color	Dark brown	Dark brown	Dark brown	Dark brown	Dark brown
Odor	characteristics	characteristics	characteristics	characteristics	characteristics
Taste	Good	Good	Good	Good	Good

3.9 Evaluation Parameters of Formulation containing EEAI

Parameter	EEAI				
FC	**F1**	**F2**	**F3**	**F4**	**F5**

Hardness (kg/cm^2)	5.1±0.57	5.2±0.47	4.8±0.35	4.9±0.30	4.3±0.32
Friability	0.65±0.03	0.72±0.04	0.57±0.05	0.63±0.03	0.51±0.06
Weight variation	±5.01	±4.89	±4.90	±4.51	±4.23
DT	20.15	30.30	32.10	40.10	10.10
DC	94.23	95.91	96.49	97.01	98.89

3.10 Evaluation Parameters of Formulation containing EETT

Parameter	**EETT**				
FC	**F1**	**F2**	**F3**	**F4**	**F5**
Hardness (kg/cm_2)	4.1±0.34	4.6±0.37	5.8±0.45	4.8±0.55	4.9±0.32
Friability	0.49±0.03	0.65±0.06	0.58±0.05	0.23±0.04	0.51±0.03
Weight variation	±3.01	±4.23	±4.48	±4.59	±5.28
DT	10.15	18.36	32.18	23.10	17.13
DC	98.32	96.23	96.10	95.05	96.12

3.11 Evaluation Parameters of Formulation containing EEPN

Parameter	**EEPN**				
FC	**F1**	**F2**	**F3**	**F4**	**F5**
Hardness (kg/cm_2)	4.8±0.37	4.5±0.42	4.3±0.51	4.9±0.61	4.9±0.33
Friability	0.59±0.07	0.55±0.05	0.28±0.03	0.23±0.06	0.61±0.07
Weight variation	±4.01	±4.29	±4.26	±4.57	±5.19
DT	19.45	17.36	12.10	13.12	27.03
DC	96.02	97.13	98.03	94.92	96.29

3.12 Evaluation Parameters of Formulation containing PHF

Parameter	**PHF**				
FC	**F1**	**F2**	**F3**	**F4**	**F5**
Hardness (kg/cm_2)	5.8±0.42	4.9±0.57	4.8±0.55	4.5±0.35	3.9±0.27
Friability	0.50±0.03	0.61±0.02	0.78±0.05	0.33±0.07	0.19±0.06
Weight variation	±5.16	±5.09	±4.06	±4.12	±3.09
DT	22.91	18.27	13.92	15.78	10.11
DC	93.09	95.89	94.69	97.01	98.99

4. SUMMARY

From these studies it is concluded that tablet, which is more acceptable dosage forms, able to solve the various complications which are associated with kidney stone. From the overall study and the physicochemical parameters, pre-formulation and evaluation we concluded that the prepared dosage form proved to be effective medicament in the management of urolithiasis.

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