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In-vitro evaluation of antinephrolithiatic effect of selected medicinal plants (Bryophyllumpinnatum, Durantaerecta, Cynodondactylon, Duchesnea indica, Taraxacum officinalis)

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ABSTRACT: There is an old and famous fact "the garden is the poor man's apothecary" and treatment of each and every disease is hidden in nature. Medicinal plants are highly esteemed all over the world as a rich source of therapeutic agents for the prevention and treatment of various diseases. Since ages, herbs are being used for treating different ailments in different parts of world by different communities. A kidney stone, also known as a renal calculus is a solid concretion or crystal aggregation formed in the kidneys from dietary minerals in the urine. Nephrolithiasis is a complex process that occurs from series of several physicochemical event including super-countries, the prevalence of upper urinary tract stones has persistently increased in the twentieth century, yet there are significant contrasts among countries and furthermore inside a similar country. Most of the people usually can have renal stones at any phase of life. The rate of prevalence of renal calculi is mostly high in males as well as in females. The basic pathophysiology for stone formation is super saturation of components of stones in urine; elements influencing solubility of these components include pH and volume of urine, and total excretion of solute. Majority of the calculi is chemically composed of calcium oxalate. Nephrolithiasis (Kidney stone) is one of the oldest known and common illnesses in the urinary tract system. Most of the people generally can have renal stones at any phase of life. Still, there is no drug that can be reasonably used in the treatment of nephrolithiasis. Data collected from in vitro and clinical trials suggest that medicinal plants could be used as an alternative therapeutic approach in the management of nephrolithiasis. The results as presented in this review demonstrate the promising role of medicinal plants (Bryophyllumpinnatum, Durantaerecta, Cynodondactylon, Duchesnea indica, Taraxacum officinalis) in the prevention and management of kidney stones. Additional investigations are required to approve the safety and efficacy of these compounds. This study focusses on the activity shown by various plant extracts on the calcium oxalate stones and their effectiveness by dissolution methods. It was observed that the selected plants have appreciable beneficial effects in preventing kidney stones and their treatment.

KEYWORDS: Medicinal plants, Nephrolithiasis, plant extracts,calcium oxalatecrystals

I.INTRODUCTION

A kidney stone is a solid object comprised of urine-derived minerals. Stones can be single or many, with varying sizes and forms, and they can occur in the pelvis, ureter, or bladder, among other places. The prevalence of kidney stones, a frequent clinical condition, is affected by changes in geography, racial and ethnic composition, lifestyle choices, and other variables. The calcium type of kidney stones is made of calcium oxalate, calcium phosphate, or a compound of oxalate and phosphate. The third most frequent issue with the urinary system is kidney and urinary stones [1]. The incidence and prevalence of kidney stones are both very high and are common clinical diseases. According to estimates, traditional medicine is used to cure illnesses in 80% of the world's population. Worldwide, medicinal plants are considered to be safer than synthetic medications due to their lengthy history of use. For finding new drugs, they are a trustworthy source. The development of drugs from medicinal plants is the current area of research [2]. Nephrolithiasis affects 12% of the world's population now, with men more likely to experience it again (70-80%) than women (47-60%). If a patient develops a kidney stone, he is more likely to experience a recurrence; the recurrence rate of stones is roughly 10% within one year, 35% within five years, and 50% within ten years. Crystals of oxalate make up around 75% of kidney stones. This is improved by specific unique dietary varieties, such as spinach, almonds and other foods high in oxalates, where calcium oxalate stones are created when it mixes with calcium

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[3].Nephrolithiasis is thought to be related to systemic diseases such diabetes mellitus (Type II), dyslipidaemia, obesity, and hypertension. Environmental and behavioural factors both have a role in the formation of calculus. Colic and renal discomfort are frequent presentations, so treatment should not be postponed [4]. Many treatments, such as thiazide diuretics and alkali-citrate, are employed in an effort to avoid recurrence, although there is insufficient scientific data to support their effectiveness. Urinary calculi are the third most common condition affecting the urinary system, and they can lead to blockage, hydronephrosis, infection, and bleeding. To remove the calculi, surgical treatments, lithotripsy, and local calculus disruption are frequently utilised; however, these procedures are costly, and recurrence is frequent [5].

Throughout the past few years, both their incidence and onset age have increased. Moreover, after 10 years, there is a significant recurrence rate of more than 50%. Normal people frequently experience crystalluria since urine is typically a supersaturated solution, but if crystals are left apart, urine flow will wash them away. Yet, under specific conditions, they are bound together as a result of forces that are both chemical and electrical that start the aggregation process. Once attached to the epithelium, the crystals or aggregates might continue to grow and eventually form the stone. In addition, the main component of human urinary calculi, calcium oxalate (CaOx) crystals, may adhere in a particular way to the plasma membrane of epithelial cells. This process is followed by endocytosis of the crystals, which causes cell damage or death. Injured cells show a proliferation response, which boosts the production of fibro-genic chemicals and provides more stimulus for crystal formation [6]. The intense colic pain experienced by those who have kidney stones is frequently not fully alleviated by over-the-counter analgesics, necessitating the administration of opioid painkillers. Infection and severe urinary tract bleeding can happen in kidney stone patients in addition to discomfort, severe urinary tract blockage, and hydronephrosis. One of the procedures for treating big kidney stones that is currently available is kidney stone open surgery [7]. Renal calculi fall roughly into two categories: tissue-connected and tissue-unattached. Calcium oxalate monohydrate (COM) renal calculi with a discernible connection site to the renal papilla, essentially as well as a middle positioned close to the connection website (concave area), and particularly striated concentrically laminated peripheral layers are the most common connected calculi. Unattached calculi, which are formed in renal cavities with limited or reduced urodynamic efficacy and may exhibit a variety of compositions and morphologies, lack a discernible point of connection to the papilla. In most cases, medical experts just treat kidney stones pain before they are expelled from the body. A vegetarian diet that emphasises herbal foods and beverages may be beneficial for kidney stone treatment and prevention. Hence, consuming plenty of water and following a vegetarian diet high in magnesium are the best ways to prevent kidney stones. The standard medications used to treat urolithiasis are not effective in all patients, and many of them have side effects that prevent their continued use [8].

Urolithiasis is a widespread and frequent human ailment characterised by a high recurrence rate, complex pathophysiological grounds and multiple aetiology. Mineral-rich urine has a strong propensity to form stones, but in healthy people, this tendency is naturally suppressed by less crystal aggregation. Calcium oxalate (CaOx) crystals are produced by the most typical urolithiasis. The process of forming a stone involves numerous and intricate physicochemical processes, starting with crystal nucleation and development and continuing through crystal aggregation, attachment to renal tubular cells, and internalisation into renal epithelial cells. All of these activities take place in a complicated environment that contains both promoters and inhibitors, and when crystals form, develop, and are maintained inside the kidney, they cause damage to the renal epithelial cells that eventually result in stones [9]. Randall's plaques (RPs), a foundation of CaP that starts at the basement membranes of the narrow limbs of the loop of Henle on the renal papillary surface, is where kidney stones develop [10]. Because urinary risk factors for stone development differ depending on the type of stone, a reliable stone diagnosis is a crucial prerequisite for particular treatment regimens. Moreover, a comprehensive metabolic evaluation of the stone patient is necessary, including a complete review of their medical history, dietary evaluation, and blood and urine tests. To find common metabolic disturbances such hypercalciuria, hypocitraturia, hyperoxaluria, and hyperuricosuria as well as to find dietary risk factors for kidney stone development, it is advised to collect two consecutive 24-hour urine samples. It has been shown that specific nutritional therapy, based on dietary assessment and metabolic evaluation, is more successful than generic

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nutritional therapy [11]. The precipitation and aggregation of crystals are accelerated by the urine's supersaturation. Crystal growth is prevented by urine's inherent ability to suppress the production of stones. These stone inhibiting agents; such as, citrate and magnesium are masked in individuals with higher risk of developing stones [12].In this context, it has been demonstrated that numerous plants that have been historically used to treat kidney stones are useful [13]. The most significant issue with this disease is its high rate of recurrence following stone removal and the steadily rising number of new patients [14].

The use of extracts from traditional herbal remedies alone, in combination with other herbs, or as isolated phytochemical substances offers numerous prospects for the development of prospective medicinal medications [15]. For the treatment of kidney stones as well as a number of other ailments, the Indian school of medicine suggests the use of medicinal herbs [16]. In many historic traditional medical systems, medicinal plants were a key component. For the majority of people on earth, plants continue to be a low-cost source of medicine that is regarded to be fairly safe and has few to no negative effects [17]. Since a few decades ago, scientists and researchers have developed a newfound interest in the therapeutic benefits that plants can impart. They have long-term benefits without any negative side effects and when put into practice, contribute positively to one's healthy lifestyle. Plants are the source of a wide range of different chemicals that are physiologically active and have therapeutic characteristics [18]. The smooth, defenceless shrub Durantaerecta, sometimes referred to as "golden dewdrops," has drooping, straggly branches and is a rich source of plant compounds. Alkaloids, glycosides, and saponin have been found in this plant. A kidney stone can be removed from the body using Durantaerecta's diuretic capabilities, which have also been described [19]. Natural resource extraction is thought to have more potential. The Crassulaceae family includes the Bryophyllumpinnatum Lam. plant, which is frequently used in traditional medicine. The plant has been used to cure a wide range of illnesses in tropical America, India, China, Australia, and Africa, including rheumatism, bodily pain, arthritis, heartburn, skin ulcers, peptic ulcers, diabetes mellitus, nephrolithiasis, microbiological infections, and hypertension. This plant is known to contain a variety of active phytochemicals, including alkaloids, triterpenes, bufadienolides, lipids, and organic acids. The plant's numerous pharmacological properties have been attributed to these chemicals [20]. Urolithiasis can be effectively managed with Taraxacumofficinale L. These plants have bioactive substances like flavonoids, phytosterols, saponins, furanochromones, alkaloids, and terpenoids that can reduce urinary saturation by inhibiting crystallisation, nucleation, and crystal aggregation. These substances also have antimicrobial, litholytic, antispasmodic, diuretic, antioxidant, and anti-inflammatory properties. They can therefore have a positive impact on urolithiasis [21].

II.RATIONALE OF THE STUDY

There is currently no medication that is completely effective to treat renal stones or stop their recurrence. In order to effectively treat urolithiasis with novel medications, more study is needed to understand the pathophysiology of kidney stone formation. This can be achieved by collection of current knowledge on the pathogenesis, etiology, and prevention of kidney stones [22].Since ancient times, medicinal plants like Cynodondactylon, Bryophyllumpinnatum, Duchesneaindica, Durantaerecta and Taraxacumofficinale and others have been used because they are safer, more effective, and acceptable to different ethnic groups than synthetic medicines. The current study focuses on strategies to use for the medicinal plants' ability to dissolve stones [8]. Nephrolithiatic drugs are derived from medicinal plants. In the last few decades, herbal remedies have improved their ability to treat stone illness. Medicinal plants lessen kidney stone discomfort and stop lithogenesis. medicinal herbs help in the expulsion of kidney stones. Calcium oxalate, uric acid, struvite, and cysteine kidney stones are frequently treated with medicinal herbs. The evolution of modern society includes the use of herbal medicine. Urolithiasis is treated with a variety of medicinal herbs [23].

In addition to the above mentioned possible therapeutic benefits for lithiatic patients, we have been examining how these plants affect various stages of stone formation. Our group have conducted experimental studies that have yielded intriguing and reassuring results regarding the possible therapeutic use of Cynodondactylon, Bryophyllumpinnatum, Duchesneaindica, Durantaerecta and Taraxacumofficinale to treat nephrolithiasis [6]. The purpose of this research was to find out how these plants are used in traditional

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medicine and affects the in vitro dissolution of calcium oxalate stones [24].

III.MATERIAL AND METHODOLOGY

Serial no.	Chemicals to be used	Plants to be used
1.	Methanol	Cynodondactylon
2.	Sodium carbonate	Bryophyllumpinnatum
3.	Ethanol	Duchesnea indica
4.	Sulphuric acid	Durantaerecta
5.	Calcium chloride dihydrate	Taraxacum officinale
6.	Disodium hydrogen phosphate	
7.	Ammonia	
8.	Sodium oxalate	
9.	Bromocresol	
10.	Gallic acid	
11.	Folin's solution	
12.	Petroleum ether	
13.	Ethyl acetate	
14.	Tris-aminomethane (tris) buffer	
15.	Phosphate buffer	

Table 1.1

Plants and materials:

The plants used in the study (Cynodondactylon, Bryophyllumpinnatum, Duchesneaindica, Durantaerecta and Taraxacumofficinale) were collected from nearby localities and their authentication was done by a botanist. The leaves of the plants were washed thoroughly, cut into small pieces, shade dried and then coarsely powdered.

Extraction: The method adopted for the process of extraction was "Soxhlet extraction", also known as hot continuous extraction. Around 50g of powdered plant material was extracted using methanol. The process was allowed to run for a period of 16-20 hours. After completion of the process, the extract was allowed to cool at room temperature, followed by distillation to separate out methanol from it.

Phytochemical screening: For the plants under investigation, preliminary phytochemical screening was done. Finding phytoconstituents in these plants was the goal of this qualitative analytical procedure. The appropriate test techniques were followed in the preparation of the extracts for the identification of the main classes of phytoconstituents, including proteins, reducing sugars, flavonoids, tannins, saponins, anthracene glycosides, polyphenol compounds, cyanogenic glycosides, and alkaloids [25].

Preparation of purified laboratory calcium oxalate stones

Procedure:

- 250 ml of both calcium chloride solution and sodium oxalate solution was added to a beaker.
- 10 ml of 2N sulphuric acid was added to the above mixture of solutions to create a calcium oxalate sediment, which was then used to create the stones.
- Sufficient amount of ammonia was added to neutralise the sediment.
- The sediment was then cleaned with distilled water at 60°C for 4 hours.

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Figure 1.1

Preparation of semi permeable membrane from eggs

Eggs were punctured at the apex to remove all the content, and the empty shells were properly washed with distilled water before being put in a beaker with 4 ml of concentrated HCl in 200 ml of distilled water. The semipermeable membrane was carefully removed the following day, properly rinsed with distilled water, and placed in an ammonia solution to neutralise any remaining acid traces after being left overnight to complete the decalcification process. The semi-permeable membrane was then utilised for the calcium oxalate and calcium phosphate dissolution procedures after being cleaned with distilled water and kept in the refrigerator.

Figure 1.2

Various methods employed for in vitro dissolution of calcium oxalate stones are:

1. Titrimetry method
2. Turbidity method

Estimation of calcium oxalate by titrimetric method

Titrimetric analysis use $KMnO_4$ to assess calcium oxalate that has not yet dissipated. 1mg of calcium oxalate was weighed, and 10mg each of methanolic extract, standard Neeri, and control

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were sutured into a semi-permeable membrane separately. They were permitted to suspend in a conical flask with 100 ml of 0.1 M TRIS buffer.About 1 mg of calcium oxalate was present in one group, which functioned as the negative control group. The conical flasks of all groups were placed in an incubator and preheated to 37°C for two hours, lasting for roughly 7-8 hours. Each group's semi-permeable membrane's contents were taken out and placed in test tubes. This was then treated with 2 ml of 1N sulfuric acid and 1N KMnO4 until a light pink colour was observed as end point. The entire amount of calcium oxalate utilised in the experiment was subtracted from the amount of calcium oxalate that was dissolved. This demonstrates how much calcium oxalate the test drug can actually dissolve.

Turbidity method

The Turbidimetric approach assesses turbidity in terms of calcium oxalate production in synthetic urine using a spectrophotometer at 620 nm and crystallisation inhibition evaluated by turbidity decrease. Without any inhibitors, stone nuclei were produced in vitro.In a test tube, 2ml of Tris-buffer (pH 7.4) and 1.0 ml of 0.025M $CaCl_2$ were added. Then 1.0 ml of sodium oxalate solution 0.025 M was added. As soon as the chemicals are mixed, turbidity begins to occur. Following this, the turbidity was measured (in terms of absorption at 620 nm in a UV/Vis spectrophotometer) immediately up to 10 minutes (600 seconds) later. Six replications of this control experiment were performed at 60, 120, and 240,360, 480, and 600 seconds.Absorptions were noted down and data obtained were used as the un-controlled growth of the stone nucleus. For the comparison of growth in the presence of the standard drug and plant extracts, it was taken at the concentration of 1mg/ml each and were added to the above chemicals and the turbidity formed were measured [26].

IV.RESULTS

Phytochemical Screening

Sr No	Chemical constituents	Name of Test	Bryophyllumpinnatum	Cyanodondactylon	Duchesnea indica	Taraxacum officinalis	Durant aerecta
1.	Alkaloids	Hager's Test	+	+	-	+	+
		Dragendorff's test	+	+	-	+	+
2.	Carbohydrates	Molisch's Test	+	+	+	-	-
		Fehling Test	+	+	+	-	-
3.	Amino acids	Ninhydrin Test	+	+	-	-	-
		Ferric chloride Test	+	+	-	-	-
		Gelatin Test	+	+	-	-	-
4.	Flavonoids	Shinoda Test	+	+	-	+	+
5.	Tannins	Ferric chloride Test	+	+	+	-	+
		Lead acetate Test	+	+	+	-	+
6.	Saponins	Foam Test	+	-	+	-	+
7.	Anthraquinone glycosides	Bromine Test	+	+	-	+	+
8.	Steroids	Salkowski's Test	+	+	-	-	-

Table 2.1

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1. Result of in vitro dissolution study of calcium oxalate by Titrimetry method

Figure 2.1

Result of dissolution studies of Calcium oxalate by Bryophyllumpinnatum

Group	Volume of $KMnO_4$(ml)	Weight of CaOx estimated(mg)	Weight of calcium reduced(mg)	Percentage of dissolution
Standard (Neeri)	0.5	0.40	0.28	70%
Negative	0.9	0.20	0	0
Methanolic extract	0.6	0.31	0.17	55%

Table 2.2

- In dissolution study, the negative control shows zero dissolution.
- The standard group (Neeri) showed dissolution of 70%.
- Methanolic extract group showed dissolution of 55%.

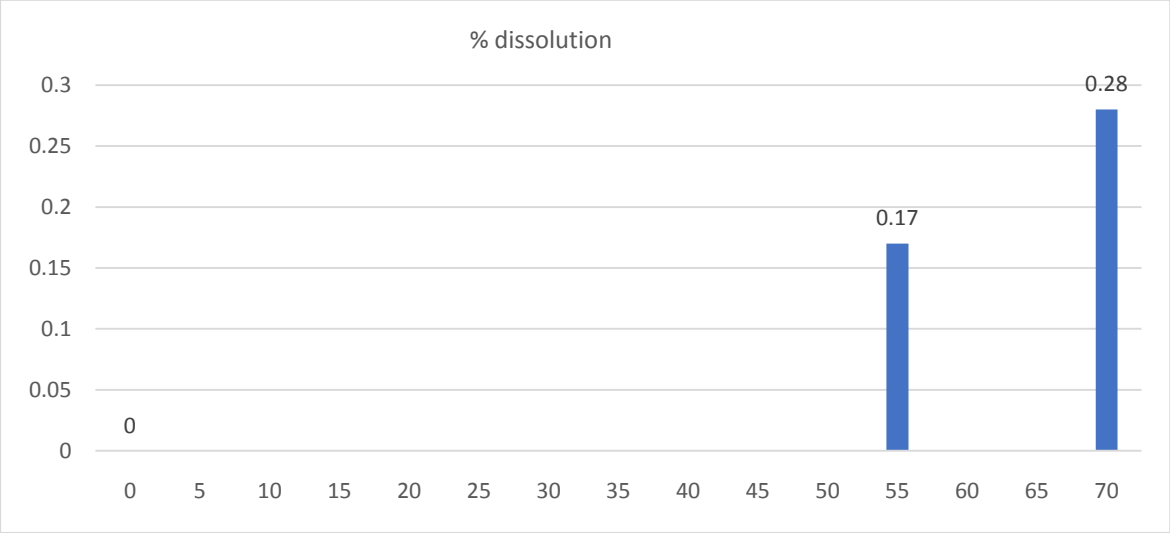

Figure 2.2

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Result of dissolution studies of calcium oxalate by Duchesnea indica

Group	Volume of $KMnO_4$(ml)	Weight of CaOx estimated (mg)	Weight of calcium reduced(mg)	Percentage of dissolution
Standard (Neeri)	0.5	0.40	0.28	70%
Negative	0.8	0.25	0	0
Methanolic extract	0.5	0.32	0.16	50%

Table 2.3

- In dissolution study, the negative control shows zero dissolution.
- The standard group (Neeri) showed dissolution of 70%.
- Methanolic extract group showed dissolution of 50%.

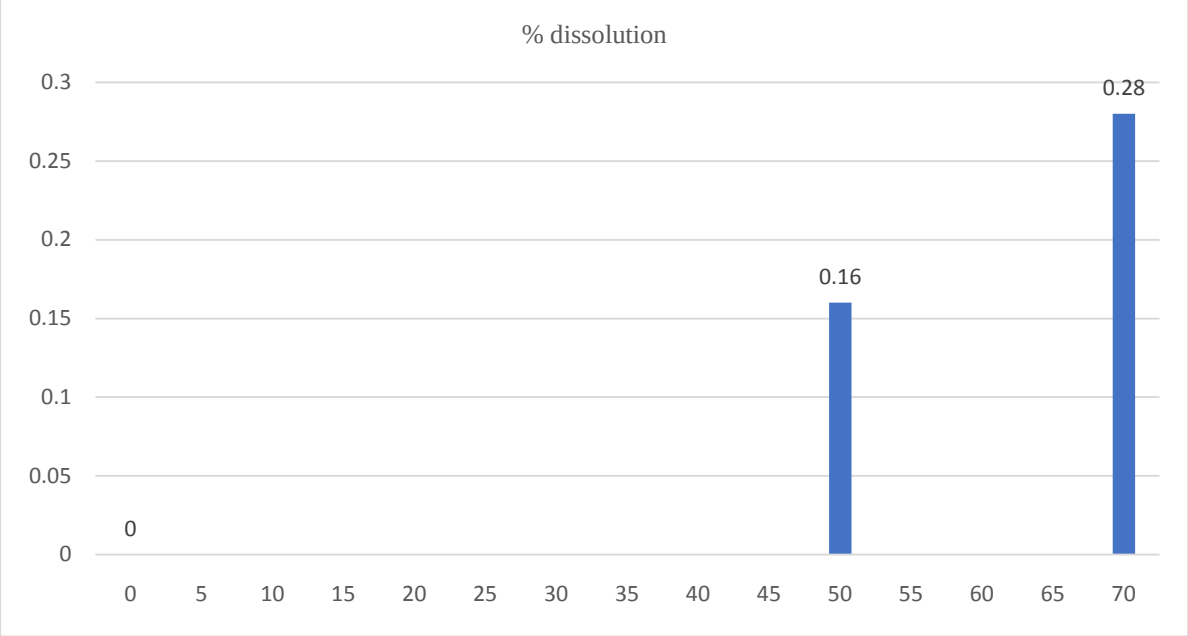

Figure 2.3

Result of dissolution studies of calcium oxalate by Cyanodondactylon

Group	Volume of $KMnO_4$(ml)	Weight of CaOx estimated (mg)	Weight of calcium reduced(mg)	Percentage of dissolution
Standard (Neeri)	0.5	0.40	0.28	70%
Negative	0.8	0.30	0	0
Methanolic extract	0.6	0.35	0.16	45%

Table 2.4

- In dissolution study, the negative control shows zero dissolution.
- The standard group (Neeri) showed dissolution of 70%.
- Methanolic extract group showed dissolution of 45%.

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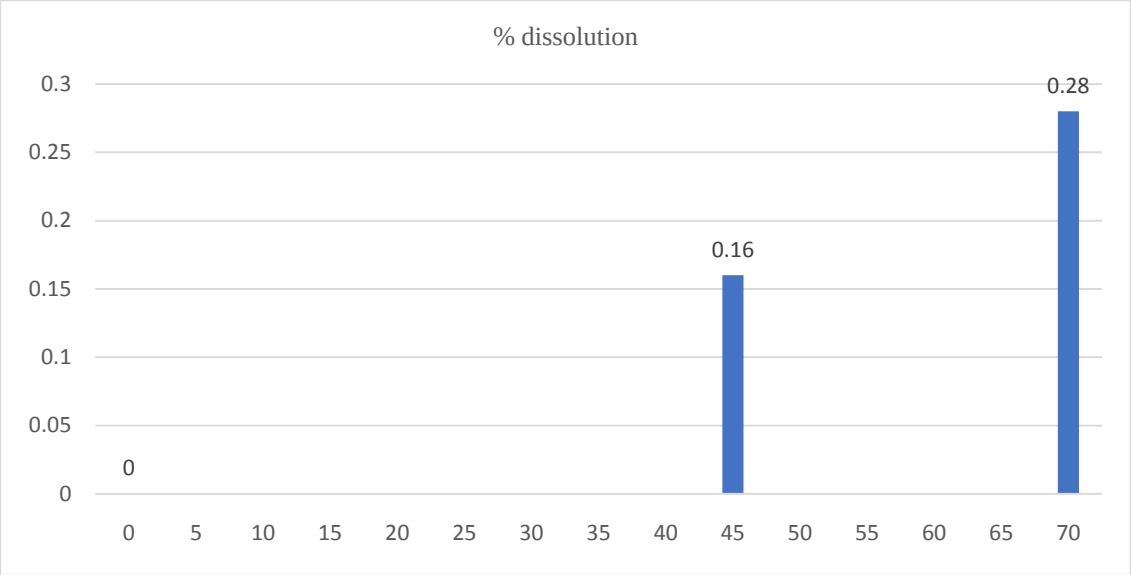

Figure 2.4

Result of dissolution studies of calcium oxalate by Durantaerecta

Group	Volume of $KMnO_4$(ml)	Weight of CaOx estimated (gm)	Weight of calcium reduced(mg)	Percentage of dissolution
Standard (Neeri)	0.5	0.40	0.28	70%
Negative	0.9	0.32	0	0
Methanolic extract	0.7	0.35	0.14	40%

Table 2.5

- In dissolution study, the negative control shows zero dissolution.
- The standard group (Neeri) showed dissolution of 70%.
- Methanolic extract group showed dissolution of 40%.

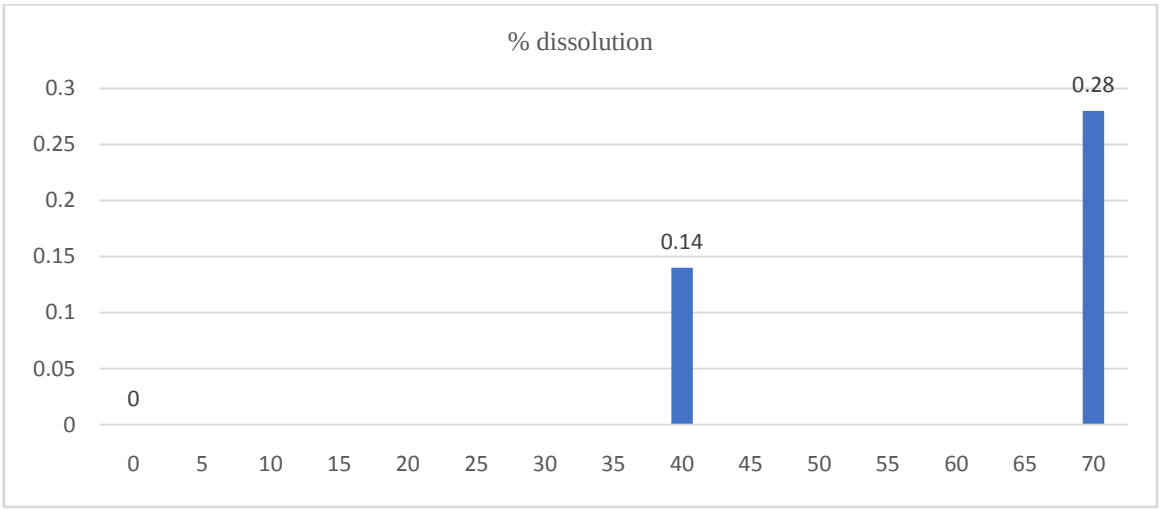

Figure 2.5

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Result of dissolution studies of calcium oxalate by Taraxacum officinalis

Group	Volume of $KMnO_4$(ml)	Weight of CaOx estimated (mg)	Weight of calcium reduced(mg)	Percentage of dissolution
Standard (Neeri)	0.5	0.40	0.28	70%
Negative	0.7	0.31	0	0
Methanolic extract	0.9	0.34	0.12	35%

Table 2.6

- In dissolution study, the negative control shows zero dissolution.
- The standard group (Neeri) showed dissolution of 70%.
- Methanolic extract group showed dissolution of 35%.

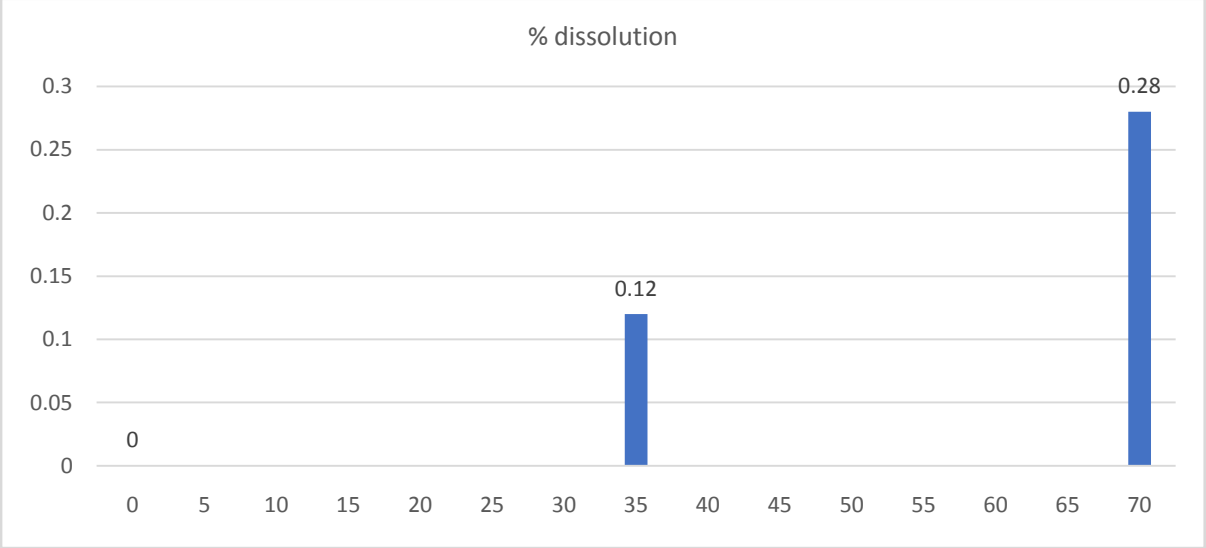

Figure 2.6

2. Result of in vitro dissolution study of calcium oxalate by turbidity method-

Time (Sec)	Control	Turbidity (methanolic extract)	Turbidity (Neeri)
0	0.401	0.090	0.086
60	0.402	0.093	0.088
120	0.408	0.094	0.089
240	0.411	0.095	0.092
360	0.416	0.095	0.093
480	0.417	0.096	0.091
600	0.419	0.098	0.094

Table 2.7 Results of turbidity method by plant extract Bryophyllumpinnatum

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Time (Sec)	Control	Turbidity (methanolic extract)	Turbidity (Neeri)
0	0.99	0.061	0.041
60	0.111	0.063	0.043
120	0.112	0.065	0.047
240	0.112	0.067	0.048
360	0.114	0.069	0.051
480	0.116	0.073	0.053
600	0.118	0.074	0.056

Table 2.8Results of turbidity method by plant extract Durantaerecta

Time (Sec)	Control	Turbidity (methanolic extract)	Turbidity (Neeri)
0	0.111	0.034	0.09
60	0.111	0.035	0.012
120	0.113	0.036	0.013
240	0.113	0.038	0.015
360	0.115	0.040	0.017
480	0.117	0.041	0.018
600	0.119	0.041	0.019

Table 2.9Results of turbidity method by plant extract Duchesnea indica

Time (Sec)	Control	Turbidity (methanolic extract)	Turbidity (Neeri)
0	0.312	0.022	0.08
60	0.314	0.023	0.09
120	0.316	0.023	0.010
240	0.317	0.024	0.010
360	0.315	0.027	0.011
480	0.314	0.030	0.013
600	0.312	0.031	0.016

Table 2.10Results of turbidity method by plant extract Cynodondactylon

Time (sec)	Control	Turbidity (methanolic extract)	Turbidity (Neeri)
0	0.312	0.012	0.07
60	0.314	0.015	0.09
120	0.309	0.017	0.012
240	0.309	0.020	0.08
360	0.307	0.018	0.012
480	0.311	0.023	0.014
600	0.312	0.025	0.08

Table 2.11Results of turbidity method by plant extract Taraxacum officinalis

- The results of this method estimated that the turbidity shown by the methanolic extract of Bryophyllumpinnatum was highly significant compared to the standard.
- The turbidity showed by Durantaerecta was comparable to the standard, the turbidity shown by Duchesnea indica, Cynodondactylon and Taraxacum officinalis was less compared to the standard (Neeri).

V.DISCUSSION

Drug therapy has developed in response to population health care needs. Among the promising areas of research and development of medicines

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from the vast highly potential plant resources. Plants are also attractive sources for the development of novel and very effective and safe therapeutic agents against kidney procumbence. Herbal medicines are also in great demand in the developed world for primary health care because of their efficacy, safety and lesser side effects. The plants selected for in vitro dissolution study of calcium oxalate kidney stones were Bryophyllumpinnatum, Durantaerecta, Duchesnea indica, Cynodondactylon and taraxacum officinalis. A comparative study was done using the above mention plant extracts and their effects on dissolution were noted. using the Titrimetry method, it was observed that the percent dissolution of Bryophyllumpinnatum was highly significant as compared to the other plant extracts, followed by Duchesnea indica, Cynodondactylon, Durantaerecta and the least activity was shown by Taraxacum officinalis. The second method used to measure dissolution was turbidity method. The results of this method estimated that the turbidity shown by the methanolic extract of Bryophyllumpinnatum was highly significant compared to the standard. The turbidity showed by Durantaerecta was comparable to the standard, the turbidity shown by Duchesnea indica, Cynodondactylon and Taraxacum officinalis was less compared to the standard (Neeri).

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