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Research Article

PREPARATION OF NIOSOMES USING ETHANOLIC EXTRACT OF CAESALPINIA BONDUCELLA LEAVES AND INVITRO EVALUATION OF ANTIUROLITHIATIC ACTIVITY

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ABSTRACT

Objective: Caesalpinia bonducella (Fabaceae), has traditionally been used for various renal diseases including urolithiasis. Considering the therapeutic and nutritional values, the present study was designed to investigate the antiurolithiatic potential of C.bonducella leaves as niosomes through in-vitro approaches. Methods: Ethanolic aqueous extract of C.bonducella leaves was prepared and screened for phytoconstituents through GC-MS analysis, while anti urolithiatic activity was determined by nucleation assay. Whereas, Presence of alkaloids, phenolic compounds, glycosides, proteins and flavonoids was confirmed . Results: C.bonducella niosomal extract significantly inhibited the ammonium oxalate crystal nucleation, parameter. Conclusions: Thus, C.bonducella niosomal extract demonstrated marked stone inhibiting potential which can be due to its antioxidant, lowering of urinary concentration of stone forming constituents and anti-crystallization effects.

Keywords: Caesalpinia bonducella leaves, niosomal extract, invitro anti-urolithiasis activity.

INTRODUCTION

Physiologically speaking, urolithiasis refers to the formation of stones in any part of the urinary system, such as the kidney, bladder, or ureter [1]. During the process of urinary stone formation, urine is supersaturated, crystals are nucleated, grow, accumulate, and translocate to the surface of the renal epithelium [2]. Renal calculi may be caused by an imbalance between stone-promoting factors (albumin, oxalate, and uric acid) and stone-inhibiting factors (citrate, magnesium, nephrocalcin, and urinary prothrombin fragment I). Stones of the kidney are classified according to their composition, such as calcium oxalate, calcium phosphate, uric acid, struvite, and cystine. CaOx crystals are divided into two types: calcium oxalate monohydrate (COM) and calcium oxalate dihydrate (COD). The renal epithelium is damaged by dendritic COM crystals with sharp edges caused by hyperoxia [3]. The defense against the retention of crystals that spontaneously form in the urine has been proposed to include the formation of COD crystals in the urine that inhibit crystal attachment to the renal tubular epithelium and reduce inflammation. Several factors contribute to the etiology of this condition, including lack of physical activity, diet, and genetics. Additionally, the interaction between crystals and epithelial cells of the renal tubule plays a significant role in the development of renal stones as well as the physicochemical mechanism (precipitation, growth, and aggregation) [4]. As well as crystallization in urine, supersaturation is also responsible for nucleation of crystals. Symptoms of urinary supersaturation, hypercalciuria, and hyperoxaluria may result in oxidative stress and necrosis of cells. Due to the resultant cell injury and damage to the cell membrane, crystal-binding molecules (such as osteopontin) are upregulated and

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enhanced, facilitating the binding of crystals to the cell membrane. Upon binding, crystals translocate into the interstitium, causing inflammation, which results in the release of monocytes, which are responsible for adhesion and retention of crystals [5]. Even though various treatment options have been developed over the years for the mitigation of urolithiasis, there are variations in their clinical indications and effectiveness [6]. Currently, treatment for urolithiasis is aimed at reducing stone recurrence rather than addressing the underlying causes. In the past, plant-based remedies have been used successfully to cure renal stones disease [7].

The use of medicinal plants as a remedy for kidney stones is therefore valuable. Caesalpinia bonducella is a rapidly growing plant that belongs to the Fabaceae family [8,9,10]. C.bonducella is native to India, Sri Lanka, Bangladesh, Burma, Myanmar, China, and Vietnam. Additionally, it can be found in the Andaman and Nicobar Islands, as well as the tropical regions of India. There have also been reports of different plants modulating and inhibiting the crystallization process and crystal morphology of calcium oxalate monohydrate [16,17].

While C.bonducella niosomal leaves extract has traditionally been used for treating renal disorders and has been reported to have antiurolithiatic properties in vitro, there is no information available on its effectiveness against kidney stones. Consequently, the ethanolic aqueous extract of C.bonducella leaves as niosomal extract, an edible component, was investigated for its effect on urolithiasis by using various in-vitro crystallization (nucleation assay) techniques [11-14].

MATERIAL AND METHODS

Plant material

The leaves of Caesalpinia bonducella was collected from the rural side of the Villupuram district and it was authenticated by Dr. V. Nandagopalan, vice principal, Associate professor, Department of botany, National college, Tiruchirappalli. Voucher numbers (NCT/BOT/PI/24/200076) was issued for future reference. he fresh Caesalpinia bonducella was properly cleaned and shade dried and powdered.

Preparation of crude extract

The Caesalpinia bonducella leaves was extracted by Maceration process. The dry powder of Caesalpinia bonducella leaves are taken in a round bottom flask with ethanol in the ratio of 1:10 for the maceration process. The process is done for 96 hours with occasional shaking. This flask was kept in the room temperature in a room. After filtration, the filtrate was subjected to evaporation to obtain a semi-solid paste. The prepared C.bonducella was weighed, labelled and stored in freezer for future use[15,18].

Chemicals

Analytical grade chemicals such as cystone (Himalaya, India), ammonium oxalate (Nice chemicals, Tamilnadu), calcium chloride (pure chemicals, Tamilnadu), sodium chloride, sodium dihydrogen ortho-phosphate (Nice chemicals, Tamilnadu), chloroform (merck lifescience, Karnataka), Span 60 (Srl chemicals, Mumbai), Tween 60 (D2C Chemicals, Mumbai), ethanol (zenthol, Tamilnadu), cholesterol (matrix lifescience, Maharastra) was used in the study.

Phytochemical Screening

Phytochemical analysis was performed to confirm the presence of secondary metabolites like alkaloids, amino acids, carbohydrates, flavonoids, phenolic compounds, glycosides, saponins, tannins, sterols and terepenoids in C. bonducella ethanolic extract [19-21].

Preparation of niosomal extract

A thin film hydration method was used to prepare niosomes. A total of 10 ml of chloroform was dissolved in 2:2:1 with Span 60, Tween 60 and cholesterol to prepare 250 mg and 500 mg niosomes loaded with drug extract. After stirring with a magnetic stirrer, the solvent was evaporated with a heating mantle at 600C until a thin film formed. At room temperature, a specific volume of water was added to the resulting thin film for hydration. After the niosome suspension was formed, it was subjected to ultrasonication in order to reduce its size. The sample was then centrifuged for 30 minutes at 12000 rpm. Separate supernatants and sediments were collected after centrifugation. We followed the same procedure for the blank niosomes devoid of drug extract.

Evaluation of niosomal extract

UV visible spectroscopy

Niosomal drug solutions were diluted in buffer solution and buffer solution served as a blank. Niosomal solutions and the control were simultaneously scanned from 190-600nm using a UV- vis spectrometer.

Scanning Electron Microscopy

It is based on electron scanning principle. It is used to determine shape, morphology, and dispersion of nanoparticles in the bulk or matrix. Sample Preparation 1. The niosomal extract was placed on carbon tape. 2.The images were performed at certain voltage and pressure at different magnification.

GC-MS Analysis

The Clarus 680 GC was used in the analysis employed a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250μm df) and the components were separated using Helium as carrier gas at a constant flow of 1 ml/min. When

running the chromatographic experiment, the injector temperature was set at 260°C. The 1µL of extract sample injected into the instrument the oven temperature was as follows: 60 °C (2 min); followed by 300 °C at the rate of 10 °C min−1; and 300 °C, where it was held for 6 min. There were three conditions for the mass detector: 230 °C transfer line temperature, 230 °C ion source temperature, and 70 eV electron impact mode, with a scan time of 0.2 seconds and a scan interval of 0.1 seconds. Fragments ranging in size from 40 to 600 Da. A comparison was conducted between the spectrums of the components and the database of spectra of known components in the NIST GC-MS.

Invitro Anti Uroliathiatic Activity

Nucleation Assay

Principle: Nucleation is a process of formation of crystals. In this process, there occurs a phase change of dissolved salts into a solid. Invitro crystallization systems are widely used to process of crystal nucleation. Inhibitory activity of test compound against the calcium oxalate nucleation was measured using the nucleation assay. Crystallization can be triggered by adding calcium, oxalates, phosphates to the reaction medium. Growth of the crystals was expected due to the following reactions. CaCl2 + Na2C2O4 CaCa2o4 + 2NaCl

Procedure: Calcium chloride (5 mmol/l) and sodium oxalate (7.5 mmol/l) solutions were prepared in the presence of phosphoric acid buffer (0.05 mol/l) and sodium chloride (0.15 mol/l) at pH 6.5 in a buffer containing sodium chloride and phosphate buffer at pH 6.5. Different concentrations of the extract and standard drug (cystone) was prepared in distilled water (10,20,30 and 40µg/ml). A solution of calcium chloride and sodium oxalate solution was mixed with a solution of 1 ml of each concentration of the extract and Cystone. 370C was used as an incubation temperature for 30 minutes to incubate the mixtures. The mixture should be cooled down to room temperature after 30 minutes. In order to measure the optical density of the mixtures, a UV spectrophotometer was used to measure the optical density at 620nm. Based on the formula below, we are able to calculate the percentage inhibition of nucleation for the extract and Cystone [22-24].

$$\% \text{ Inhibition} = [\, 1 - (OD_{test} / OD_{control})] \times 100$$

RESULT

Phytochemical screening of C.bonducella leaves extract:

Phytochemical screening revealed the presence of carbohydrate, alkaloids, flavonoids, proteins, phenolic compounds and tannins in ethanolic aqueous extract of C.bonducella.

GC-MS analysis

The major functional groups of C.bonducella were identified and categorized by GC-MS results based on peak values and RT values. The compounds like Phytol (RT 17.309), Alpha.-D Mannofuranoside, 1 Nonyl (RT 19.215), Hexadecanoic Acid, 1 (Hydroxymethyl)-1,2 Ethanediyl ester [28] (RT 19.525), Undecanoic acid (RT 20.375), Octadecanoic acid (RT 20.816), Sulfurous acid, Octadecyl 2-Propyl ester (RT 21.026), 11-Eicosenoic acid, Trimethylsilyl ester (RT 21.331), 2,6,10,14,18,22 Tetracosahexaene, 2,6,10,15,19,23 Hexamethyl-, (All-E) (RT 23.747), Pregna-1,4-Diene-3,20 Dione, 11,22-Diacetoxy 16,17-Propylidenedioxy(RT26.893),3-(1-Acetyl-2-OxoPropylsulfanyl)-3,3Difluoro-2 Trifluoromethyl-Propionic acid Methyl ester (RT 27.068), 4,4,6A,6B,8A,11,11,14B Octamethyl 1,4,4A,5,6,6A,6B,7,8,8A,9 ,10,11,12,12A,14,14A,14B-Octadecahydro-2 (RT 27.348),URS-12-EN-3-OL,Acetate,(3.BETA.)-(RT27.588),2,4,4-Trimethyl-3 Hydroxymethyl-5A-(3-Methyl-But-2-Enyl) Cyclohexene (RT 30.054) as shown in Figure 1 and Table 1.

UV-Visible Spectroscopy

This is the primary charecterization of synthesized niosomal extract. The appearance of specific absorbance peak at 190nm in the UV-visible region of the spectrum confirmed the presence of niosomes as shown in Figure 4 and 5.

Scanning Electron Microscopy Sem

is a technique which is basically used to determine the particle size, particle size distribution surface morphology, a shape of the niosomal extract. In this niosomal extract were circular shape, the particle size ranged from 2-10µm as shown in Figure 2 and 3.

In-Vitro Antiurolithiatic Activity

The anti-urolithiatic effects of ethanolic extract of Caesalpinia bonducella was evaluated by invitro nucleation, aggregation and growth assay methods. The nucleation, aggregation and growth are the basic mechanisms by which stones form within the urinary tract. An increase in the solute concentration in the urine leads to supersaturation it and results in formation of clusters of urinary crystals, the process called nucleation. Then crystal growth and aggregation will takes place. The present study evaluated the effects of HECB on different stages of crystallization by in vitro nucleation, growth and aggregation assay

Table 1: Phytocomponents identified in ethanolic extract of leaves of Caesalpinia bonducella

S.No	RT	Compound Name	Mol. Formula	Mol. Wt	%Peak Area
1.	17.309	Phytol	$C_{20}H_{40}O$	296	2.178
2	Alpha.-D-Mannofuranoside, 1-Nonyl--	C15H30 O6	306	13.995	19.215
3.	19.525	Hexadecanoic Acid, 1-(Hydroxymethyl)-1,2-Ethanediyl ester [28]	$C_{35}H_{68}O_5$	568	15.403
4.	20.375	Undecanoic acid	$C_{11}H_{22}O_2$	186	1.113
5.	20.816	Octadecanoic acid	$C_{18}H_{36}O_2$	284	3.252
6.	21.026	Sulfurous acid, Octadecyl 2-Propyl ester	$C_{21}H_{44}O_3S$	376	2.162
7.	21.331	11-Eicosenoic acid, Trimethylsilyl ester	$C_{23}H_{46}O_2Si$	382	1.357
8.	23.747	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-Hexamethyl-, (All-E)-	$C_{30}H_{50}$	410	3.894
9.	26.893	Pregna-1,4-Diene-3,20-Dione, 11,22-Diacetoxy-16,17-Propylidenedioxy-	$C_{28}H_{36}O_8$	500	1.223
10.	27.068	3-(1-Acetyl-2-Oxo-Propylsulfanyl)-3,3-Difluoro-2-Trifluoromethyl-Propionic acid Methyl ester	$C_{10}H_{11}O_4F_5S$	322	4.035
11.	27.348	4,4,6A,6B,8A,11,11,14B-Octamethyl-1,4,4A,5,6,6A,6B,7,8,8A,9,10,11,12,12A,14,14A,14B-Octadecahydro-2	$C_{30}H_{48}O$	424	2.220
12.	27.588	URS-12-EN-3-OL, Acetate, (3.BETA.)-	$C_{32}H_{52}O_2$	468	1.918
13.	30.054	2,4,4-Trimethyl-3-Hydroxymethyl-5A-(3-Methyl-But-2-Enyl)-Cyclohexene	$C_{15}H_{26}O_2$	222	0.817

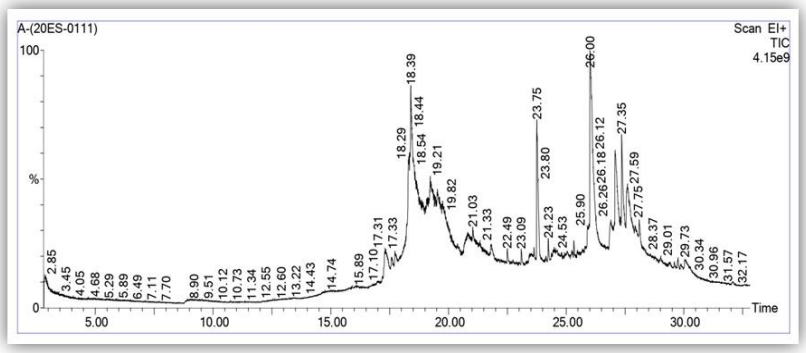

Figure 1: GC-MS of ethanolic extract of *Caesalpinia bonducella*

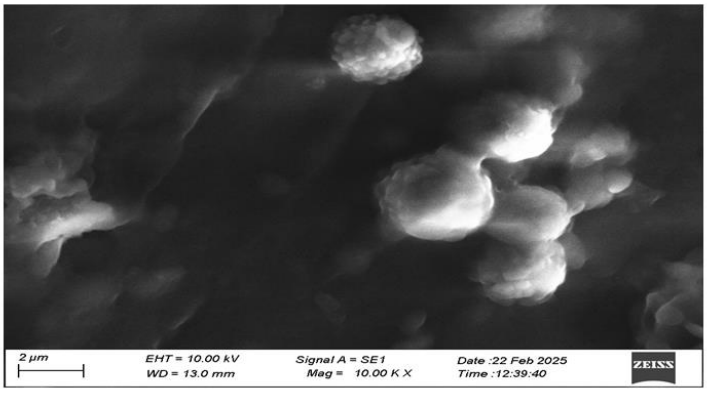

Figure 2: Scanning Electron Microscopy of 250mg

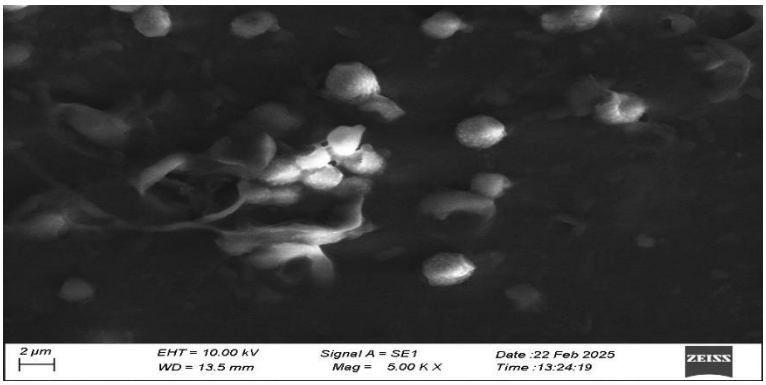

Figure3: Scanning Electron Microscopy of 500mg

Entrapment Efficiency

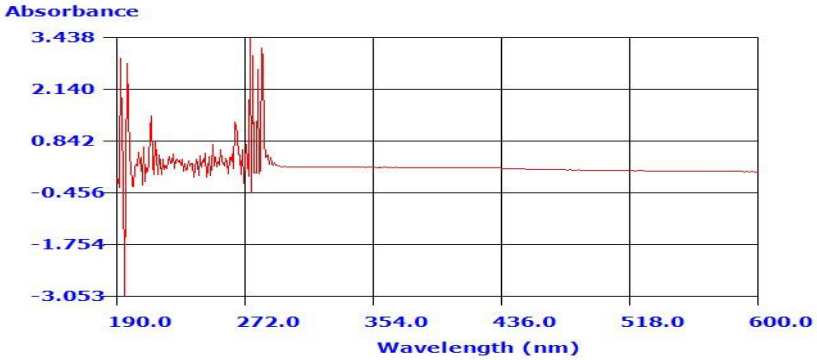

Figure 4: base peak of 500 mg drug extract niosomes *250mg*

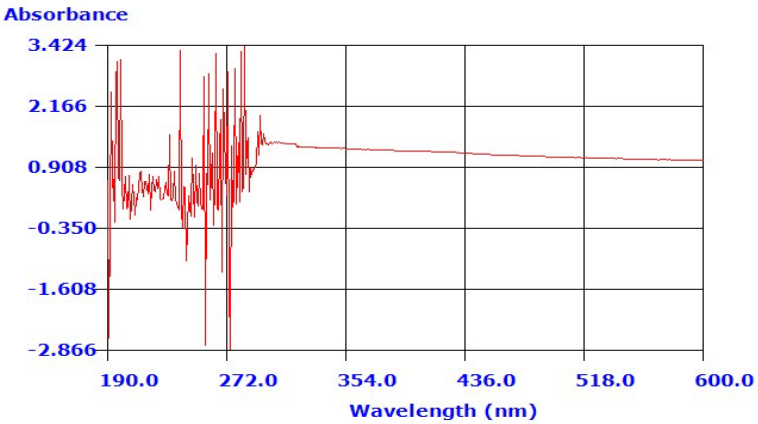

Figure 5: Base peak of 250 mg drug extract niosomes

Table 2: different concentrations and its absorbance of 250 mg and 500 mg drug extract niosomes at 190nm

S. No	Concentration(µg/ml)	250 mg drug extract niosomes (absorbance) at 190nm	500mg drug extract niosomes (absorbance) at 190nm
1	10	2.334	-0.077
2	20	0.486	-0.015
3	30	-3.394	-0.113
4	40	-0.947	-0.433

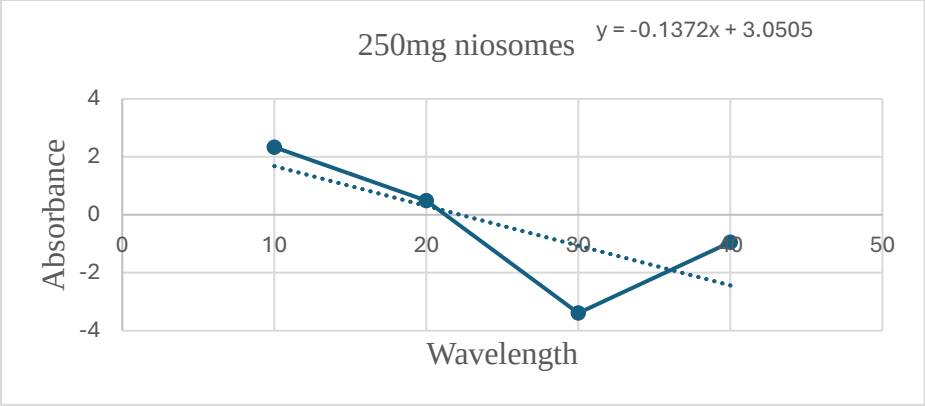

Figure 6: 250 mg drug extract niosomes and its absorbance at different concentrations

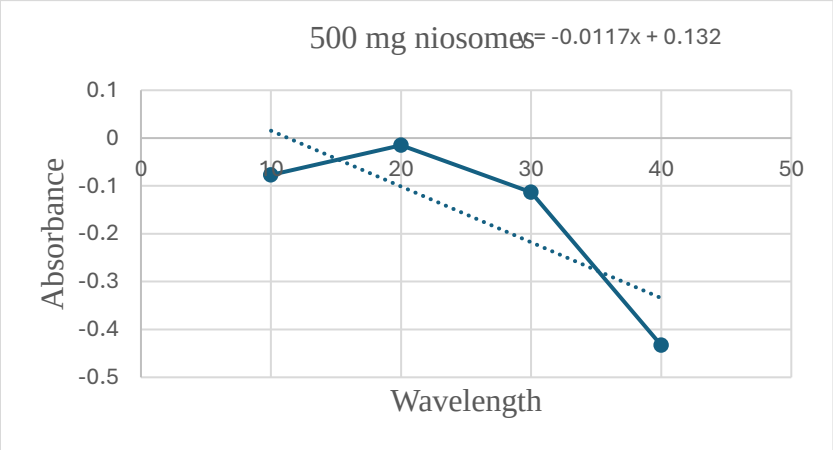

Figure 7: 500mg drug extract niosomes and its absorbance at different concentrations

S.No	Concentration (µg/ml)	Entrapment efficiency (%) of 250 mg drug extract Niosomes	Entrapment efficiency (%) of 500 mg drug extract Niosomes
1	10	97.91%	96.42%
2	20	92.52%	90.34%
3	30	81.21%	95.81%
4	40	88.34%	97.48%

Table 3: entrapment efficiency (%) of 250 and 500 mg drug extract niosomes

SAMPLE	CONCENTRATION	ABSORBANCE	%INHIBITON
Control	-	1.279	-
Standard (cystone)	10	2.281	78.2
	20	1.891	47.8
	30	1.300	1.6
	40	1.157	90.64
250 mg Niosomes with drug extract	10	2.239	1.841
	20	2.472	30.72
	30	2.318	78.3
	40	2.158	86.51
500 mg Niosomes with drug extract	10	2.454	7.58
	20	2.474	30.83
	30	2.439	87.61
	40	2.313	99.91

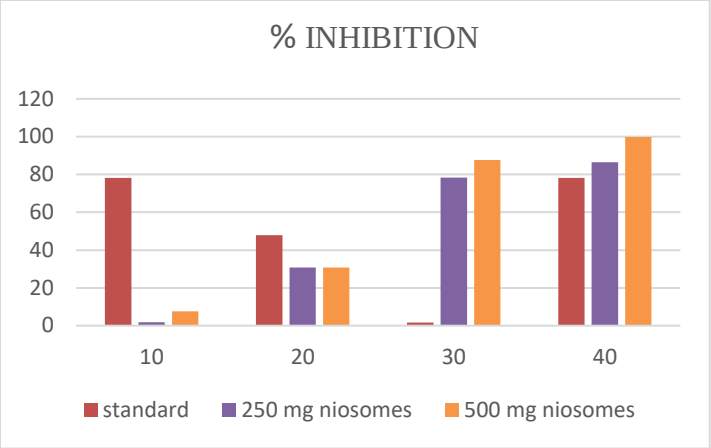

Figure 8: % inhibition of standard, 250 and 500 mg drug extract niosomes

DISCUSSION

Studies have shown that calcium oxalate (CaOx) is the most common component of most stones. The purpose of this study was to verify the ethnomedicinal use of Caesalpinia bonducella leaves in treating kidney stones. A niosome formulated from Caesalpinia bonducella extract had a beneficial effect on reducing the incidence of ammonium oxalate "induced urolithiasis" in an invitro study. Caesalpinia bonducella has a unique anti-urolithiatic action due to its oral administration. The in vitro method was used to assess ammonium oxalate's role in the development of urolithiasis. A preliminary phytochemical analysis of Caesalpinia bonducella extract showed the presence of alkaloids, glycosides, flavonoids, tannins, and phenolic compounds.

Caesalpinia bonducella was analysed using GC-MS. Caesalpinia bonducella ethanolic extract was developed into niosomes using span-60, tween-60, cholesterol. Niosomes were confirmed using SEM analysis. Absorbance was noted at 190 nm at various dilutions and entrapment efficiency was calculated.

Caesalpinia bonducella extract had previously been shown to have an anti-crystallisation effect in vivo, which supports the current findings. Phenolic compounds are said to prevent stones from forming. According to previous research, antioxidant properties play an important role in the antilithiasis properties of these plants. Caesalpinia bonducella niosomal extracts contain phytochemicals, including flavonoids and polyphenols, which may contribute to the anti-urolithiatic activity. There is, however, a need for more research to determine the specific phytochemical ingredients and the mechanism of action of Caesalpinia bonducella niosomal extract in preventing urolithiasis.

CONCLUSION

This review comprehensively addressed synthesis and applications of Caesalpinia bonducella niosomal extract with special emphasis on in vitro anti urolthiatic activity. This study also suggests that Caesalpinia bonducella niosomal extract possess potent anti-urolithiatic activity due to presence of phenolic compounds rich in level of Caesalpinia bonducella. The development of niosomes as anti-urolithiatic is one of the most interesting approaches, it can overcome poor delivery.

The conclusion of the research was to study in vitro anti-urolithiatic activity of Caesalpinia bonducella niosomal extract. The developed niosomes was characterized by uv-visible spectroscopy, SEM. The in-vitro aanti urolithiatic activity of niosomes was evaluated by nucleation assay that shows better result

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