

Comparative study of hydroalcoholic extracts of *Bryophyllum pinnatum* and *Macrotyloma uniflorum* for their antioxidant, antiurolithiatic, and wound healing potential

Chetna Faujdar, Priyadarshini* 

Department of Biotechnology, JIIT, Noida, India.

ARTICLE INFO

Article history:

Received on: July 6, 2021

Accepted on: September 13, 2021

Available online: January 07, 2022

Key words:

Urolithiasis, antioxidant, injury, wound healing

ABSTRACT

The recurrence and associated side effects of modern treatment methods for urolithiasis highlight the need for a safer phytotherapy-based alternative medicine. In the present study, the seeds of *Macrotyloma uniflorum* (MUE) and leaves of *Bryophyllum pinnatum* (BPE) were evaluated for their antioxidant, antiurolithiatic, and wound healing potential. Phytochemical screening of extracts was carried out through gas chromatography–mass spectrometry analysis. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) assays were used to determine antioxidant potential of plant extracts. Percentage radical activity increased from 1.91% to 53.99% in DPPH assay and 39.26%–97.44% in ABTS assay, with an increase in concentration of BPE. Different concentrations of MUE also resulted in dose-dependent antioxidant activity from 5.70% to 45.14% in DPPH assay and 17.96%–96.04% in ABTS assay. Extracts were investigated for their effect on calcium oxalate monohydrate (COM) crystals-induced Vero cell injury. BPE resulted in the retention of 98.5% viability after COM crystal exposure to Vero cells against the injured group (57.44%). Similarly, retained cell viability was found to be in the range of 77.4%–90.74% with different MUE concentrations. Wound healing potential was examined through scratch assay. Along with the prevention of cell injury, extracts also accelerated the wound closure rate as compared to control. Treatment with EC50 and EC90 of BPE resulted in 84.48% and 74.08% wound closure, respectively, as compared to the control group (73.79%). However, EC50 and EC90 of MUE resulted in 85.66% and 91.09% wound closure, respectively. The present study concludes the effectiveness of these herbal extracts in minimizing risk factors leading to urolithiasis.

1. INTRODUCTION

Urolithiasis is a disease caused due to the precipitation and retention of crystal-forming salts within the kidneys. The disease is marked by a recurrence rate of approximately 40% in 5 years of initial treatment [1]. Currently available medical expulsive therapy is a wait-and-watch kind of approach where success rate depends upon a number of factors such as location of the stone, size of the stone, associated obstructions, etc. [2]. The high recurrence rate of urolithiasis [3] and risk factors associated with synthetic medicines [4] have necessitated the need for safer,

reliable, cost-effective, and noninvasive alternatives to manage urolithiasis. During the last few decades, tremendous research has been focused on revealing the role of medicinal plants in the management of urolithiasis. Traditional medicines have been known to result in significant outcomes and play a critical role in treating and preventing human ailments along with paving the way for discovering new drugs. Antiurolithiatic herbs have been used to treat or prevent lithiasis worldwide throughout history.

The interaction of calcium oxalate monohydrate (COM) crystals with renal tubular cells elevate the production of reactive oxygen species (ROS) and stimulate epithelial cell injury, leading to inflammation and eventually cell death [5,6]. Renal epithelial cells

*Corresponding Author

Priyadarshini, Department of Biotechnology, JIIT, Noida, India.

E-mail: priyadarshini@mail.jiit.ac.in

injury by COM crystals provide adhesion sites for crystals, which serve as fixed particles for stone formation [7,8]. Epithelial cell injury also adds oxidative stress, further increasing the chances of stone formation [9]. Considering the role of oxidative stress, treatment modality for urolithiasis should also include therapeutic agents possessing antioxidant abilities. It is a proven fact that the plants having diuretic and antioxidant activities possess inhibitory effects on crystallization, nucleation, and aggregation of crystals, thus having antiurolithiatic activity [10]. Both the plants used in this study have been explored for their diuretic, antispasmodic, antioxidant, and other medicinal properties yet not well explored for their antiurolithiatic property [11–13]. These plants possess strong historical background for being used traditionally as antiurolithiatic agents [14–16]. Therefore, in the present investigation, we have compared the effect of hydroalcoholic extracts of two traditional medicinal plants *Bryophyllum pinnatum* (BPE) and *Macrotyloma uniflorum* (MUE) on ROS, COM crystal-induced cell injury, and wound healing to evaluate their antiurolithiatic potential.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1. Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich. Fetal bovine serum (FBS), Dulbecco's modified Eagles's medium (DMEM), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H tetrazolium bromide (MTT) were purchased from HiMedia Laboratories, India. Remaining chemicals were purchased from Central Drug House (P) Ltd (CDH) fine chemicals, India.

2.1.2. Plant materials

Plants and seeds of BPE and MUE, respectively, were collected locally and identified at the Department of Botany, University of Delhi. Voucher specimens of the authenticated BPE (accession number DUH14500) and MUE (accession number DUH14479) were submitted in the herbarium unit for future reference.

2.1.3. Cell culture

Vero cells were procured from the National Centre of Cell Sciences (NCCS, Pune, India). The cells were maintained as subconfluent monolayers in DMEM supplemented with 10% FBS and 1.2% antibiotics (penicillin-streptomycin) in a humidified incubator at 37°C with 5% CO₂.

2.2. METHODS

2.2.1. Preparation of plant extracts

The leaves of BPE (50 g) were crushed to form a paste and mixed with 70% ethanol, while the powdered seeds of MUE (5 g) were mixed with 70% methanol to prepare hydroalcoholic extracts. After filtration and solvent evaporation, the obtained concentrate was lyophilized and stored in air-tight containers at 4°C for further use. The percentage yield of each extract was calculated using the following formula:

$$\% \text{Yield} = \left[\frac{W_1}{W_2} \right] \times 100$$

where W_1 is the weight of the extract after solvent evaporation and W_2 is the weight of the leaves/seeds taken.

The powdered hydroalcoholic extracts of BPE and MUE were dissolved in distilled water to get desired concentrations before each experiment.

2.2.2. Phytochemical screening of the plant extracts

Qualitative phytochemical screening of BPE and MUE was carried out to detect the presence of phytoconstituents, such as tannins, flavonoids, saponins, alkaloids, glycosides, terpenes, and steroids, using standard procedures. Afterward, total phenolic content of the extracts was estimated spectrophotometrically at 723 nm using UV-Vis spectrophotometer and expressed as Gallic acid equivalent (GAE) milligrams per gram of the extract [17].

To confirm the presence of individual phytochemicals, gas chromatography–mass spectrometry (GC–MS) analysis of plant extracts was carried out. Model QP-2010 Plus (Shimadzu, Japan) of the gas chromatograph mass spectrometer was used for the GC–MS analysis. Samples of BPE and MUE were prepared by mixing them in ethanol and methanol, respectively. The samples were dissolved properly by vortexing. Sample solutions having a concentration of 1 mg/ml were used for analysis. Out of which 1 µl of sample was injected into an Rtx-5 MS capillary column (30 m length × 0.25 mm i.d. × 0.25 µm film thickness) for analysis with injector–detector temperatures to be 250°C and 280°C respectively. The stepped temperature was programmed as held at 80°C for 3 minutes and then from 80°C to 280°C at a ramp rate of 10°C/minute. The retention time of components was compared with standard components provided by different databases such as WILEY8LIB and NIST11LIB for identification [18].

2.2.3. Determination of antioxidant activity

The free radical scavenging activity of extracts was evaluated using DPPH and ABTS assays as reported in the literature. DPPH and ABTS assays were carried out according to the method described by Jadid *et al.* [19] and Saeed *et al.* [20], respectively.

2.2.4. Effect of plant extracts on COM crystal-injured cells

COM crystals were prepared using the method described by Semangoen *et al.* [21]. Before using, purity of harvested crystals was confirmed by Fourier transform infrared (FTIR) analysis (data not shown) and crystals were exposed to UV radiation to avoid any contamination.

Vero cells were divided into control, injured, and extract-treated groups. Injury was induced by adding fresh DMEM (without serum) containing COM crystals (200 µg/ml). For the extract-treated group, injured cells were exposed to different concentrations of BPE (10–400 µg/ml) and MUE (10–400 µg/ml) extracts. Cells exposed to serum-free DMEM without COM crystals were used as control. The effect of BPE and MUE on the repair of cell injury was assessed by measuring cell viability

through MTT assay [22] against cells injured with COM crystals (injury group without treatment). Percentage cell viability was calculated using the following formula:

$$\% \text{Cell viability} = \left[\frac{At - Ab}{Ac - Ab} \right] \times 100$$

where *At* is the absorbance of test sample, *Ac* is the absorbance of control, and *Ab* is the absorbance of blank.

Finally, effective concentrations (EC₅₀ and EC₉₀) of both the extracts, i.e., BPE and MUE, were calculated using AAT bioquest EC₅₀ calculator (<https://www.aatbio.com/tools/ec50-calculator/>).

2.2.5. Wound healing assay

Extracts were evaluated for their wound healing potential through scratch assay using the method reported by Danciu *et al.* [23]. Cells were divided into test (with extract) and control (without extract) groups. For the test group, cells were exposed to serum-free DMEM containing BPE and MUE extracts at a concentration equivalent to calculated EC₅₀ and EC₉₀ values. Pictures of the scratched surface were taken at different time intervals (0, 3, 18, and 24 hours) under an inverted microscope (Olympus Life Science) at 10× magnification. Image Pro-Plus software (Media Cybernetics) was used to compare the images at different time intervals [24] and wound closure percentage was calculated [25] against control.

3. STATISTICAL ANALYSIS

Each experiment was carried out in triplicates ($n = 3$) and results were presented as mean ± SD. GraphPad prism 6.0 software (GraphPad Software, San Diego, CA) was used for data presentation. One-way ANOVA was used to compare differences among different groups. Associated probability (*p*) value of < 0.05 was considered as statistically significant.

4. RESULTS

4.1. Preparation of plant extracts

The % yield of seeds extract of MUE was 11.4% as compared to 9.2% yield of leaves extract of BPE.

4.2. Phytochemical screening of the plant extracts

Preliminary phytochemical screening of BPE and MUE confirmed the presence of different classes of bioactive phytoconstituents in the extracts. Tannins, flavonoids, alkaloids, and steroids were found to be present in both the extracts. Unlike MUE, BPE was found to possess saponins. Cardiac glycosides were present in MUE and absent in BPE. Total phenolic contents of BPE and MUE were estimated to be 74.11 ± 0.003 and 34.11 ± 0.0015 µg/mg GAE, respectively.

Finally, the extracts were analyzed using GC–MS to screen out specific phytoconstituents. GC–MS analysis of BPE led to the identification of 62 compounds. Out of these, many compounds, such as eucalyptol, 1-tridecanol, terpinyl acetate, beta caryophyllene, squalene, vitamin E, gamma sitosterol, kolavenol acetate, alpha amyryl, etc., are of medical significance (Fig. 1, Table 1).

However, GC–MS analysis of MUE confirmed the presence of medicinally important phytocompounds such as terpinyl acetate, gamma sitosterol, beta caryophyllene, mome inositol, etc. (Fig. 2, Table 2).

4.3. Determination of antioxidant activity

BPE exhibited significant radical scavenging activity against DPPH as well as ABTS free radicals. Percentage radical activity increased from 1.91% to 53.99% in DPPH assay and 39.26%–97.44% in ABTS assay with an increase in the concentration of BPE (12.5–400 µg/ml) (Fig. 3a).

Percentage radical scavenging activity of MUE was comparable with that of BPE. Different concentrations of the extract (12.5–400 µg/ml) resulted in dose-dependent antioxidant activity from 5.70% to 45.14% in DPPH assay and 17.96%–96.04% in ABTS assay (Fig. 3b).

4.4 Effect of plant extracts on COM crystal-injured cells

The effect of treatment with BPE and MUE was evaluated using cell viability assay against the injured group (without treatment). Exposure of the cells to COM crystals resulted in more than 40% decrease in cell viability against control (without COM crystal exposure). However, treatment of the injured cells with different

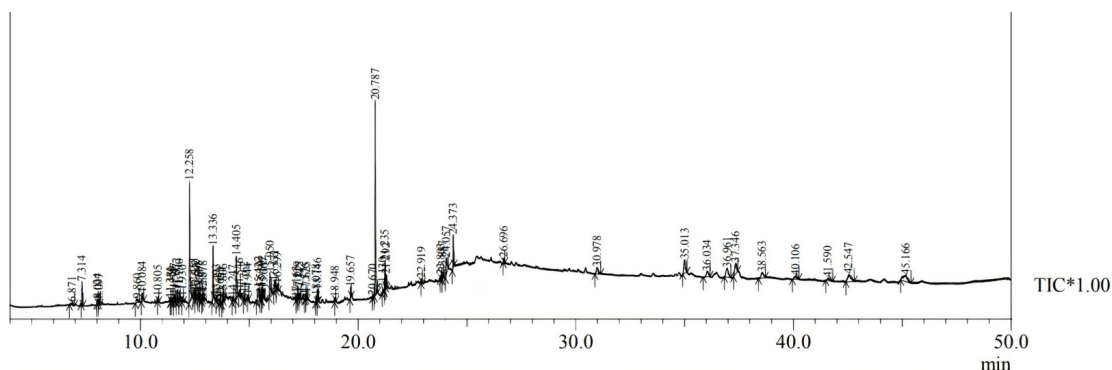


Figure 1: GC–MS chromatogram of hydroalcoholic extract of BPE leaves' extract.

Table 1: Chemical compositions of hydroalcoholic extract of BPE leaves (GC–MS).

S. No.	Name of compound	Retention time	Area%
1.	3,3,4-trimethylhexane	6.871	0.61
2.	Eucalyptol	7.314	1.65
3.	2,4-dimethyl-1-heptanol	8.024	0.64
4.	1-isopropyl-4-methyl-3-cyclohexen-1-ol	9.860	0.68
5.	4-pentenoic acid, 2-methyl-, octyl ester	10.084	1.04
6.	Linalyl acetate	10.805	0.42
7.	Isobornyl acetate	11.381	0.24
8.	Isobornyl thiocynoacetate	11.430	0.45
9.	1-tridecanol	11.547	1.73
10.	Pentadecafluorooctanoic acid, tetradecyl ester	11.672	1.01
11.	Myrtenyl acetate	11.930	0.37
12.	Terpinyl acetate	12.258	11.45
13.	14-methyl-8-hexadecyn-1-ol	12.451	0.75
14.	4-tert-butylcyclohexyl acetate	12.542	0.90
15.	2-heptadecyloxirane	12.668	0.46
16.	Tricyclo [4.4.0.0 (2,7)] dec-3-ene, 1,3-dimethyl-8-(1-methylethyl)	12.717	0.34
17.	1-acetyl-1,4-dihydropyridine	12.837	0.11
18.	Beta-elemene	12.878	0.41
19.	Beta-caryophyllene	13.336	4.26
20.	2-cyclohexylethyl methylphosphonofluoridate	13.493	0.44
21.	3-ethyl-3-methylheptane	13.653	0.28
22.	2,8,8-trimethyldecane	13.757	0.31
23.	1,4,8-cycloundecatriene, 2,6,6,9-tetramethyl	13.806	0.27
24.	10-12-pentacosadiynoic acid	14.247	0.48
25.	Phenol, 3,5-bis(1,1-dimethylethyl)	14.405	3.98
26.	1-octadecanesulphonyl chloride	14.546	0.65
27.	Nonadecyl pentafluoropropionate	14.762	0.59
28.	Bicyclo [3.1.1] hept-3-en-2-one, 4,6,6-trimethyl	14.944	0.27
29.	2,4,7,14-tetramethyl-4-vinyl-tricyclo [5.4.3.0(1,8)] tetradecan-6-ol	15.422	2.61
30.	Ethanol, 2-(3,3-dimethylbicyclo [2.2.1] hept-2-ylidene	15.519	0.62
31.	2(3 hours)-furanone, dihydro-5-pentyl	15.592	0.66
32.	3-Isopropyl-6,8a-dimethyl-2,3,4,5,8,8a-hexahydro-3a(1 hours)-azulenol	15.667	1.20
33.	5-ethyl-1,3-dioxane-5-methanol, tert-butyl dimethylsilyl ether	15.950	2.65
34.	Silane, [(1,1-dimethyl-2-propenyl)oxy]dimethyl	16.171	1.45
35.	1-(4-Isopropylphenyl)-2-methylpropyl acetate	16.299	1.11
36.	2-(1-methoxy-2-(trimethylsilyl)ethyl)-4,4-dimethylcyclopentane-1-carboxaldehyde dimethyl acetal	17.163	0.13
37.	2,4-diethyl-1-heptanol	17.229	0.38
38.	Tert-hexadecanethiol	17.576	0.32
39.	1-nonadecene	17.625	0.52
40.	Neophytadiene	18.078	0.48
41.	2-pentadecanone, 6,10,14-trimethyl	18.146	1.00
42.	7-hexadecenal, (Z)	18.948	0.30
43.	Hexadecanoic acid, ethyl ester	19.657	0.82
44.	Diglycolic acid, decyl 2-methylphenyl ester	20.670	0.21
45.	3,7,11,15-tetramethylhexadec-2-en-1-ol	20.787	18.11
46.	Trans, trans-9,12-octadecadienoic acid, propyl ester	21.235	2.04

continued

S. No.	Name of compound	Retention time	Area%
47.	Ethyl (9Z,12Z)-9,12-octadecadienoate	21.292	0.90
48.	4,8,12,16-tetramethylheptadecan-4-olide	22.919	0.34
49.	Benzedrex	23.803	0.42
50.	1,3,5-trisilacyclohexane	23.887	0.39
51.	2-ethylbutyric acid, eicosyl ester	24.057	2.46
52.	1,2-benzenedicarboxylic acid	24.373	2.62
53.	Squalene	26.696	0.40
54.	Vitamin E	30.978	1.60
55.	Gamma.-sitosterol	35.013	3.38
56.	24-noroleana-3,12-diene	36.034	1.46
57.	Kolavenol acetate	36.961	2.88
58.	Alpha-amyrin	37.346	2.81
59.	Olean-12-en-3-one	38.563	2.06
60.	Alpha-caryophyllenol	41.590	0.88
61.	Methyl ursal-2,12-dien-28-oate	42.547	3.28
62.	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, octadecyl ester	45.166	3.69

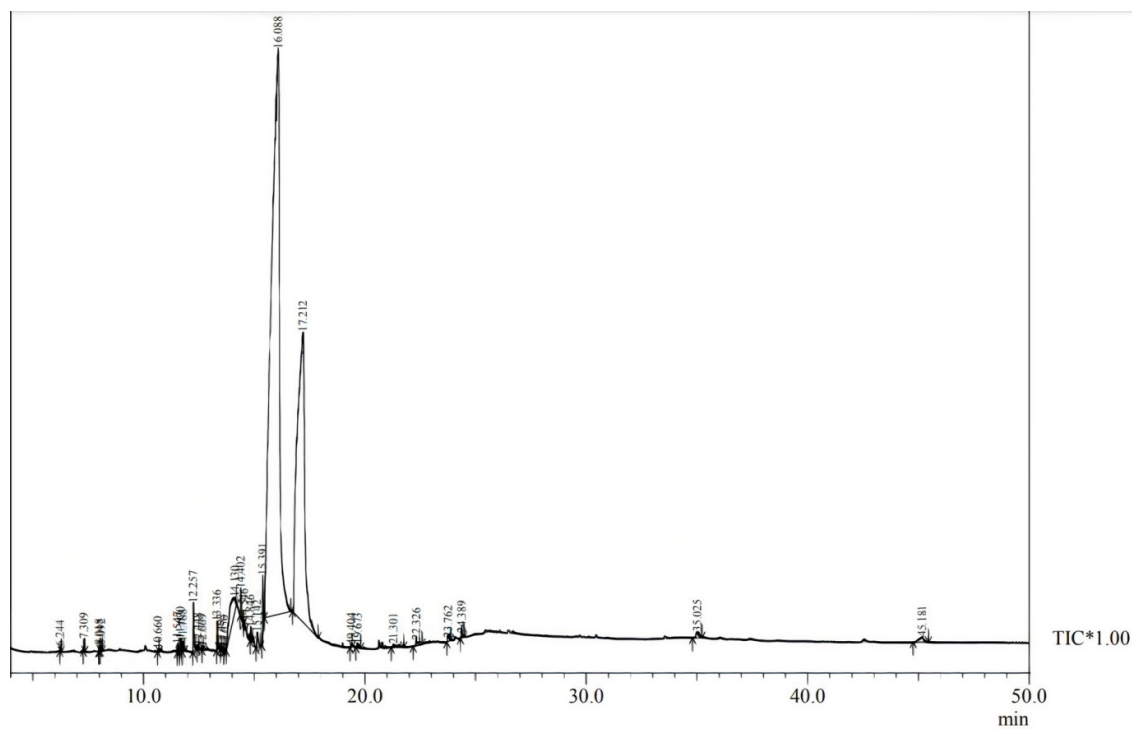


Figure 2: GC-MS chromatogram of hydroalcoholic extract of seeds of MUE.

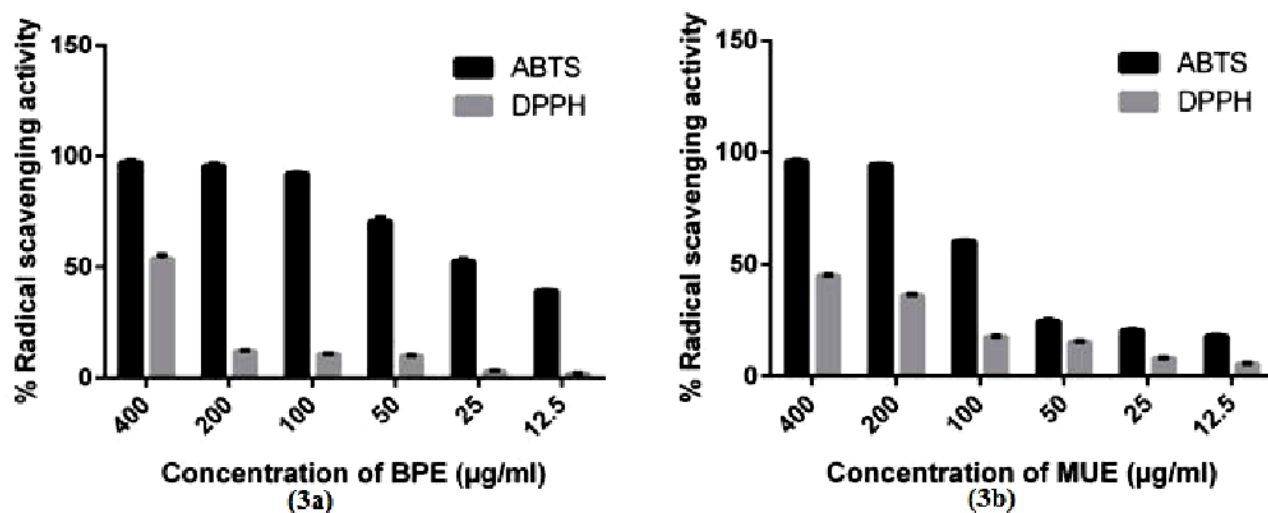
concentrations of BPE and MUE extracts resulted in significantly improved cell viability which was at a comparable difference with the control group. BPE at a concentration of 200 $\mu\text{g/ml}$ resulted in the retention of 98.5% viability after COM crystal exposure to Vero cells. The effect was found to be dose-dependent and more than 76.56% cell viability was retained at the lowest concentration of BPE (10 $\mu\text{g/ml}$).

Similarly, MUE also exhibited dose-dependent effect on cell viability against the injured group (Fig. 4b). Retained cell viability was found to be in the range of 77.4%–90.74% with different MUE concentrations. Both the extracts showed maximum activity at 200 $\mu\text{g/ml}$.

Using the data of cell viability assay, EC50 and EC90 values were calculated for both the extracts. EC50 and EC90 for BPE were

Table 2: Chemical compositions of hydroalcoholic extract of seeds of MUE (GC-MS).

S. No.	Name of compound	Retention time	Area%
1.	Tetraethyl silicate	6.244	0.03
2.	Eucalyptol	7.309	0.09
3.	Diisodecyl ether	8.018	0.05
4.	1-tridecanol	8.092	0.16
5.	Dodecane, 4,6-dimethyl-	10.660	0.04
6.	Pentadecafluorooctanoic acid, tetradecyl ester	11.547	0.07
7.	2-isopropyl-5-methyl-1-heptanol	11.788	0.08
8.	Terpinyl acetate	12.257	0.46
9.	14-methyl-8-hexadecyn-1-ol	12.448	0.04
10.	Cyclohexanol, 5-methyl-2-(1-methylethyl)-, acetate	12.669	0.02
11.	Beta-caryophyllene	13.336	0.21
12.	Cholestan-22(26)-isoepoxy	13.492	0.03
13.	1,8-nonadien-3-ol	14.130	2.06
14.	3,5-bis(1,1-dimethylethyl)phenol	14.402	0.29
15.	Oxirane, [(dodecyloxy)methyl]	14.546	0.05
16.	Cyclopropanemethanol, alpha,2-dimethyl-2-(4-methyl-3-pentenyl	14.846	0.18
17.	5,9-undecadien-2-ol, 6,10-dimethyl	15.142	0.28
18.	5-ethyl-1,3-dioxane-5-methanol, tert-butyldimethylsilyl ether	15.391	0.78
19.	Methyl. alpha.-D-galactoside	16.088	64.28
20.	Mome inositol	17.212	29.52
21.	2-aminoethanethiol hydrogen sulfate (ester)	19.404	0.04
22.	Heptadecanoic acid, ethyl ester	19.673	0.07
23.	(Z,z)-6,9-cis-3,4-epoxy-nonadecadiene	21.301	0.20
24.	3-cyclopentylpropionic acid, 2-dimethylaminoethyl ester	22.326	0.19
25.	Hexanoic acid, 2-dimethylaminoethyl ester	23.762	0.13
26.	1,2-benzenedicarboxylic acid, diisooctyle	24.389	0.12
27.	Gamma.-sitosterol	35.025	0.20
28.	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4- hydroxy-, octadecyl ester	45.181	0.34

**Figure 3:** Free radical scavenging potential of BPE (3a) and MUE (3b). Results are expressed as mean \pm SD.

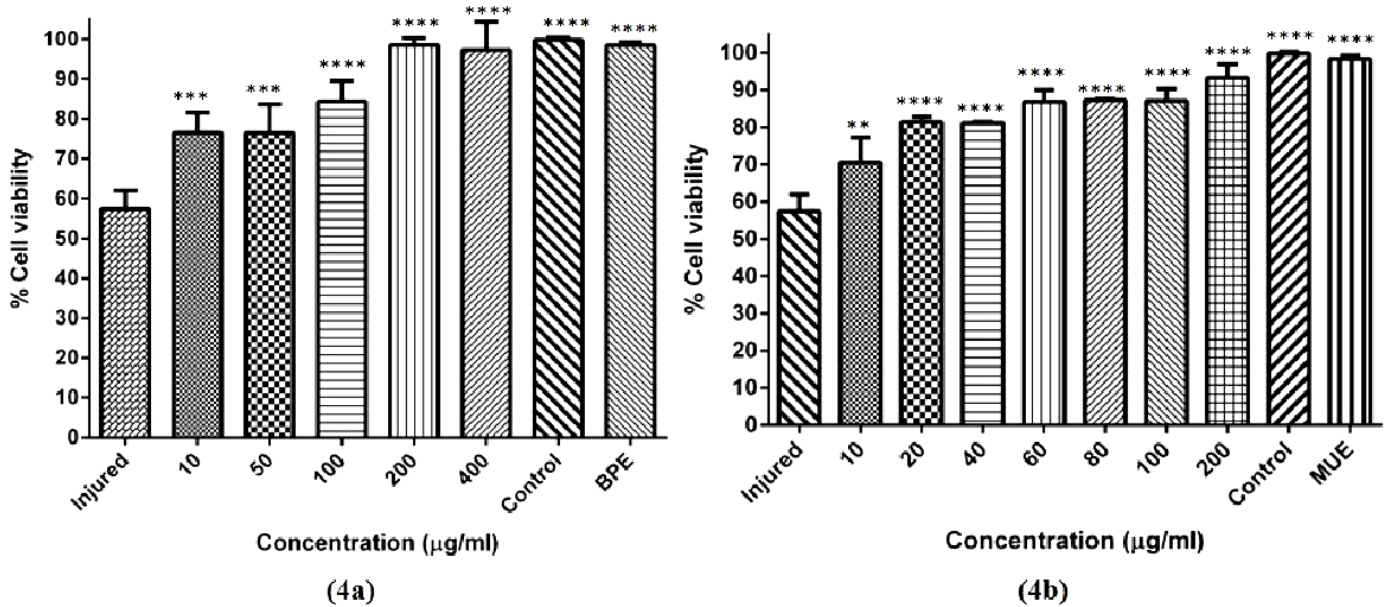


Figure 4: Treatment of COM crystal injured Vero cells with BPE (4a) and MUE (4b). Activity of both plant extracts was dose-dependent. Values are expressed as mean \pm SD.

** $p < 0.005$, *** $p < 0.0005$, and **** $p < 0.0001$ versus injured group of cells.

found to be 104.26 and 109.18 $\mu\text{g/ml}$, respectively. However, in case of MUE, EC50 and EC90 were found to be 74.64 and 246.09 $\mu\text{g/ml}$, respectively.

4.5. Wound healing assay

Wound healing potential of both the extracts was evaluated at EC50 and EC90 concentrations. Treatment with EC50 of BPE (Fig. 5a) resulted in 84.48% wound closure after 24 hours which was significantly higher than the control group (73.79%). However, treatment with EC90 of BPE led to 74.08% wound closure which was not at a significant difference with the control (Fig. 5b). EC50 and EC90 of MUE resulted in 85.66% and 91.09% wound closure which were significantly higher than the control group (Fig. 5c), respectively.

5. DISCUSSION

Urolithiasis remains a medical challenge worldwide and the problem is increasing day by day due to the modern lifestyle. Moreover, the recurrence rate of urolithiasis is very high. In the present study, we evaluated the antioxidant, wound healing assays, and anti-urolithiatic potential of hydroalcoholic extracts of BPE and MUE. Both extracts exhibited dose-dependent radical scavenging activity against DPPH as well as ABTS free radicals. In the cell viability assay, it was observed that exposure of the Vero cells to COM crystals significantly reduced the cell viability to 57.4% with respect to 100% in case of control group (without COM crystal treatment). The data clearly indicated that exposure of the cells to COM crystals resulted in cytotoxicity and the results were found to be in agreement with the findings of Mittal *et al.* [26]. Interestingly, the treatment of injured cells with the plant extracts resulted in a dose-dependent significant improvement ($p <$

0.0005) in the retained cell viability even at a lower concentration (10 $\mu\text{g/ml}$) for the BPE and MUE versus injured group. When we increased the extract concentration from 10 to 400 $\mu\text{g/ml}$, there was a significant increase in the retained cell viability. The results were found comparable to the control group at higher concentration of BPE (above 200 $\mu\text{g/ml}$). The exact mechanism responsible for this improved cytoprotective activity is not clear yet but it may be correlated with high antioxidant property and excellent wound healing potential of both the extracts.

MUE exhibited better wound healing efficacy than BPE when compared to control (untreated group). Treatment with EC50 of BPE resulted in significant improvement ($p < 0.05$) in wound closure. However, both EC50 ($p < 0.0005$) and EC90 ($p < 0.0001$) of MUE led to improved wound closure rates and were at comparable differences with the control. The results of the cell viability assay, antioxidant activity, and wound healing assay may be correlated with a number of pharmacologically active phytoconstituent identified during preliminary screening and GC-MS. Phytoconstituents such as eucalyptol, 1-tridecanol, terpinyl acetate, beta caryophyllene, 3, 7, 11, 15-tetramethylhexadec-2-en-1-ol, squalene, vitamin E, gamma sitosterol, kolavenol acetate, and alpha amyryn are capable of influencing metabolic reactions in different ways. Eucalyptol is reported to possess ulcer healing abilities involving antioxidant and cytoprotective effect [27]. A sesquiterpene, β -caryophyllene which was present in a significant concentration in BPE, has also been investigated for its cytoprotective effects and antioxidant activities [28,29]. Vitamin E along with another compound 3, 7, 11, 15-tetramethylhexadec-2-en-1-ol, which is also known as phytol, was found in significant concentrations in BPE. Phytol is a precursor of vitamin E and can be considered as a source of antioxidant [30,31]. Another polyunsaturated hydrocarbon squalene present in BPE is capable

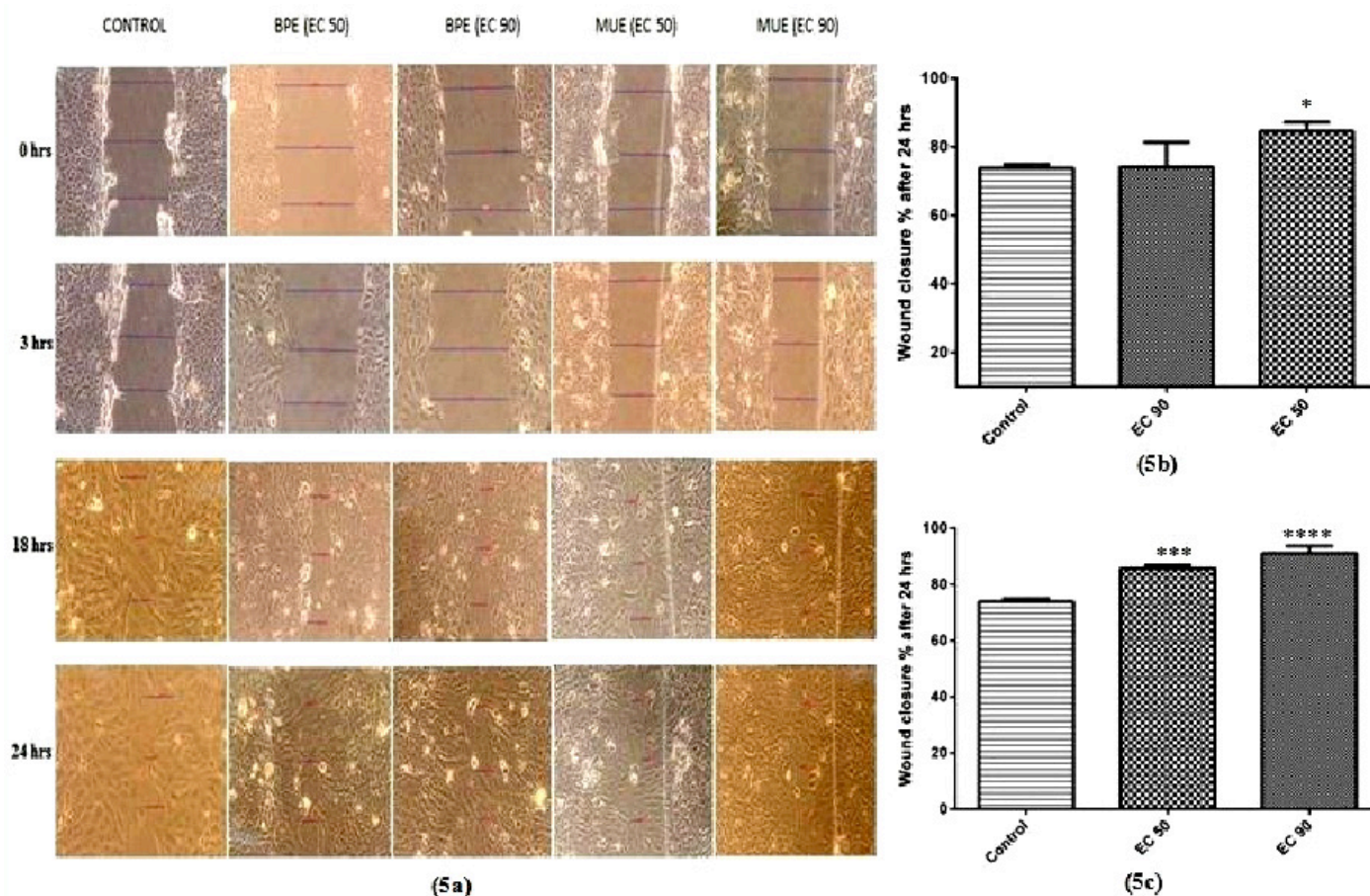


Figure 5: Image showing wound closure at different time points (5a). Percentage of wound closure in BPE (5b) and MUE (5c) treated groups. Values are expressed as mean \pm SD.

* $p < 0.05$, *** $p < 0.0005$, and **** $p < 0.0001$ versus untreated control.

of preventing oxidative damage and act as a natural antioxidant [32,33]. Beta elemene and its derivatives, present in BPE, are already reported to possess potent antioxidant activity against ROS production [34]. The presence of mome inositol as a major phytoconstituent along with terpinyl acetate, gamma sitosterol, and beta caryophyllene in smaller amounts in MUE also justifies its role in repairing the cell damage due to COM crystal exposure. The literature reveals the antioxidant activity of alpha amyryn [35]. These phytochemicals could be responsible for providing the extract its antioxidant and wound healing abilities. Many medicinal properties have already been attributed to mome inositol, such as antiallopecic, anticirrhotic, antineuropathic, cholesterolytic, lipotropic, and sweetener [36,37].

The presence of eucalyptol, β -caryophyllene, vitamin E, phytol, and squalene may be providing the antioxidative property to BPE. In MUE also, the presence of compounds like mome inositol, terpinyl acetate, β -caryophyllene, etc. could probably be the factors attributing its antioxidative potential.

In recent years, many surgical and nonsurgical treatment methods for urolithiasis have been emerged, but recurrence of the disease

remains a challenge with modern treatment modalities. According to the literature, the generation of free radicals in kidneys plays the key role in the pathogenesis of renal calculi [38]. In such conditions, using plant-derived antioxidants can be a wonderful approach to combat urolithiasis. Seeds of MUE and leaves of BPE are rich sources of antioxidants and have been used traditionally to treat kidney stones, thus serving the purpose.

6. CONCLUSION

In summary, hydroalcoholic extracts of MUE and BPE are rich in phytochemicals having antioxidative, cytoprotective, and wound healing properties. They are capable of protecting the renal cells which are injured with calcium oxalate crystals. However, specific phytochemicals of the extracts may be explored for therapeutic efficacy against urolithiasis.

7. ACKNOWLEDGMENT

The authors would like to acknowledge Jaypee Institute of Information Technology, Noida, India, for providing the facilities to conduct the research work.

8. AUTHORS' CONTRIBUTIONS

We, the authors hereby declare that both authors have made substantial contributions to the conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content and both agreed to submit to the current journal. Therefore, both the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

9. FUNDING

There is no funding to report.

10. CONFLICT OF INTEREST

The authors declare that they have no financial or any other conflicts of interest in this work.

11. ETHICAL APPROVAL

Not applicable.

12. ABBREVIATIONS

ABTS: 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt

BPE: *Bryophyllum pinnatum* leaves' extract

COM: Calcium oxalate monohydrate

DMSO: Dimethyl sulfoxide

DPPH: 2,2-Diphenyl-1-picrylhydrazyl

GC-MS: Gas chromatography-mass spectrometry

MUE: *Macrotyloma uniflorum* seeds' extract.

REFERENCES

- Daudon M, Jungers P, Bazin D, Williams JC. Recurrence rates of urinary calculi according to stone composition and morphology. *Urolithiasis* 2018;46(5):459–70.
- Shafi H, Moazzami B, Pourghasem M, Kasaeian A. An overview of treatment options for urinary stones. *Casp J Intern Med* 2016;7(1):1–6.
- Gomase PV, Pawar SP. Urolithiasis (kidney stones) current pharmacological diagnosis and management. *J Drug Deliv Ther* 2019;9(4):726–37.
- Patle A, Hatware KV, Patil K, Sharma S, Gupta G. Role of herbal medicine in the management of urolithiasis—a review for future perspectives. *J Environ Pathol Toxicol Oncol* 2019;38(2):97–118.
- Chaiyarit S, Thongboonkerd V. Changes in mitochondrial proteome of renal tubular cells induced by calcium oxalate monohydrate crystal adhesion and internalization are related to mitochondrial dysfunction. *J Proteome Res* 2012;11(6):3269–80.
- Yu L, Gan X, Liu X, An R. Calcium oxalate crystals induces tight junction disruption in distal renal tubular epithelial cells by activating ROS/Akt/p38 MAPK signaling pathway. *Ren Fail* 2017;39(1):440–51.
- Khan SR. Histological aspects of the “fixed-particle” model of stone formation: animal studies. *Urolithiasis* 2017;45(1):75–87.
- Khamchun S, Thongboonkerd V. Cell cycle shift from G0/G1 to S and G2/M phases is responsible for increased adhesion of calcium oxalate crystals on repairing renal tubular cells at injured site. *Cell Death Discov* 2018;4(1):1–2.
- Gan QZ, Sun XY, Bhadja P, Yao XQ, Ouyang JM. Reinjury risk of nano-calcium oxalate monohydrate and calcium oxalate dihydrate crystals on injured renal epithelial cells: aggravation of crystal adhesion and aggregation. *Int J Nanomed* 2016;11:2839–54.
- Nirumand MC, Hajjalyani M, Rahimi R, Farzaei MH, Zingue S, Nabavi SM, *et al.* Dietary plants for the prevention and management of kidney stones: preclinical and clinical evidence and molecular mechanisms. *Int J Mol Sci* 2018;19(3):765.
- Ozoluua RI, Eboka CJ, Durua CN, Uwayaa DO. Effects of aqueous leaf extract of *Bryophyllum pinnatum* on guinea pig tracheal ring contractility. *Niger J Physiol Sci* 2010;25(2):149–57.
- Adibe MK, Gabriel IM, Akintunde AAM, Esther AO. Chemical compositions and antioxidant activity of leaf and stem essential oils of *Bryophyllum pinnatum* (lam.) Kurz. *GSC Biol Pharm Sci* 2019;9(2):057–64.
- Upadhyay S, Jain V, Upadhyay U. Glossary of *Dolichos biflorous*-A Legume with miraculous activities. *Res J Pharmacol Pharmacodyn* 2015;7(2):103–16.
- Ahmed S, Hasan MM, Mahmood ZA. Antiuro lithiatic plants: multidimensional pharmacology. *J Pharmacogn Phytochem* 2016;5(2):4.
- Shukla AK, Shukla S, Garg A, Garg S. A review on anti-urolithiatic activity of herbal folk plants. *Asian J Biomater Res* 2017;3(2):1–1.
- Ahmed S, Hasan MM, Mahmood ZA. Antiuro lithiatic plants in different countries and cultures. *J Pharmacogn Phytochem* 2016;5(1):102.
- Bonomo MG, Cafaro C, Russo D, Calabrone L, Milella L, Saturnino C, *et al.* Antimicrobial activity, antioxidant properties and phytochemical screening of *Aesculus hippocastanum* mother tincture against food-borne bacteria. *Lett Drug Des Discov* 2020;17(1):48–56.
- Painuli S, Rai N, Kumar N. Gas chromatography and mass spectrometry analysis of a methanolic extract of leaves of *Rhododendron arboreum*. *GAS* 2016;9(1):101–4.
- Jadid N, Hidayati D, Hartanti SR, Arraniry BA, Rachman RY, Wikanta W. Antioxidant activities of different solvent extracts of *Piper retrofractum* Vahl. using DPPH assay. Paper presented at: AIP Conference Proceedings, 2017.
- Saeed N, Khan MR, Shabbir M. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L. *BMC Complement Altern Med* 2012;12(1):221.
- Semangoen T, Sinchaikul S, Chen ST, Thongboonkerd V. Proteomic analysis of altered proteins in distal renal tubular cells in response to calcium oxalate monohydrate crystal adhesion: Implications for kidney stone disease. *Proteomics Clin Appl* 2008;2(7-8):1099–109.
- Kumar P, Nagarajan A, Uchil PD. Analysis of cell viability by the MTT assay. *Cold Spring Harb Protoc* 2018;2018(6):469–471.
- Danciu C, Muntean D, Alexa E, Farcas C, Oprean C, Zupko I, *et al.* Phytochemical characterization and evaluation of the antimicrobial, antiproliferative and pro-apoptotic potential of *Ephedra alata* Decne. hydroalcoholic extract against the MCF-7 breast cancer cell line. *Molecules* 2019;24(1):13.
- Liang CC, Park AY, Guan JL. *In vitro* scratch assay: a convenient and inexpensive method for analysis of cell migration in vitro. *Nature Protoc* 2007;2(2):329–33.
- Felice F, Zambito Y, Belardinelli E, Fabiano A, Santoni T, Di Stefano R. Effect of different chitosan derivatives on *in vitro* scratch wound assay: a comparative study. *Int J Biol Macromol* 2015;76:236–41.
- Mittal A, Tandon S, Singla S, Tandon C. *In vitro* inhibition of calcium oxalate crystallization and crystal adherence to renal tubular epithelial cells by *Terminalia arjuna*. *Urolithiasis* 2016;44(2):117–25.
- Caldas GFR, da Silva Oliveira AR, Araújo AV, Lafayette SS, Albuquerque GS, Silva-Neto Jda C, *et al.* Gastroprotective mechanisms of the monoterpene 1, 8-cineole (eucalyptol). *PLoS One* 2015;10(8):e0134558.

28. Francomano F, Caruso A, Barbarossa A, Fazio A, La Torre C, Ceramella J, *et al.* β -Caryophyllene: a sesquiterpene with countless biological properties. *Appl Sci* 2019;9(24):5420.
29. Al-Tae H, Azimullah S, Meeran MN, Alaraj Almheiri MK, Al Jasmi RA, *et al.* β -caryophyllene, a dietary phytocannabinoid attenuates oxidative stress, inflammation, apoptosis and prevents structural alterations of the myocardium against doxorubicin-induced acute cardiotoxicity in rats: an *in vitro* and *in vivo* study. *Eur J Pharmacol* 2019;858:172467.
30. Byju K, Vasundhara G, Anuradha V, Nair S, Kumar N. Presence of phytol, a precursor of vitamin E in *Chaetomorpha antinnina*. *Mapana J Sci* 2013;12(2):57–65.
31. Islam MT, Ali ES, Uddin SJ, Shaw S, Islam MA, Ahmed MI, *et al.* Phytol: a review of biomedical activities. *Food Chem Toxicol* 2018;121:82–94.
32. Kabuto H, Yamanushi TT, Janjua N, Takayama F, Mankura M. Effects of squalene/squalane on dopamine levels, antioxidant enzyme activity, and fatty acid composition in the striatum of Parkinson's disease mouse model. *J Oleo Sci* 2013;62(1):21–8.
33. Micera M, Botto A, Geddo F, Antoniotti S, Berteau CM, Levi R, *et al.* Squalene: more than a step toward sterols. *Antioxidants* 2020;9(8):688.
34. Chen J, Duan W, Bai R, Yao H, Shang J, Xu J. Design, synthesis and antioxidant activity evaluation of novel β -elemene derivatives. *Bioorg Med Chem Lett* 2014;24(15):3407–11.
35. Santos FA, Frota JT, Arruda BR, de Melo TS, da Silva AA, Brito GA, *et al.* Antihyperglycemic and hypolipidemic effects of α , β -amyrin, a triterpenoid mixture from *Protium heptaphyllum* in mice. *Lipids Health Dis* 2012;11(1):1–8.
36. Das S, Vasudeva N, Sharma S. Chemical composition of ethanol extract of *Macrotyloma uniflorum* (Lam.) Verdc. using GC-MS spectroscopy. *Org Med Chem Lett* 2014;4(1):13.
37. Kumar NR, Reddy JS, Gopikrishna G, Solomon KA. GC-MS determination of bioactive constituents of *Cycas beddomei* cones. *Int J Pharm Bio Sci* 2012;3(3):344–50.
38. Ceban E, Banov P, Galescu A, Botnari V. Oxidative stress and antioxidant status in patients with complicated urolithiasis. *J Med Life* 2016;9(3):259.

How to cite this article:

Priyadarshini, Faujdar C. Comparative study of hydroalcoholic extracts of *Bryophyllum pinnatum* and *Macrotyloma uniflorum* for their antioxidant, antiurolithiatic, and wound healing potential. *J Appl Biol Biotech* 2022; 10(01):196–205.