Investigation on antiurolithiatic activity of aqueous extract of Ananas fruit (in-vitro)

N F A Rahim1,2, N Muhammad1,2* and N Abdullah1,2

1Department of Technology and Natural Resources, Faculty of Applied Sciences and Technology, Universiti Tun Hussein Onn Malaysia, Pagoh Educational Hub, 84600 Pagoh, Muar, Johor, Malaysia.
2Centre of Research for Sustainable Uses of Natural Resources (CoR-SUNR), Universiti Tun Hussein Onn Malaysia (UTHM), Parit Raja, 86400 Batu Pahat, Johor, Malaysia.

*Corresponding author: norhayatim@uthm.edu.my

Abstract. Urolithiasis or kidney stone disease is a urologic ailment that has a high prevalence rate worldwide and medicinal plants have been widely used for alternative therapy. In this present study, the antiurolithiatic potential in Ananas nanus (dwarf pineapple) and Ananas comosus (L.) Merr (pineapple) fruit extracts were investigated through in-vitro assays. The fruit is extracted with aqueous distilled water via the decoction method. The antiurolithiatic properties were evaluated by titrimetric and turbidity assay. The results showed that A. nanus 2.5 fold higher (p<0.05) than A. comosus but no significant difference with standard drug, cystone for titrimetric assay. For turbidity assay, A. nanus has significant antiurolithiatic properties (66.44±3.30%) as compared to A. comosus (15.07±0.59%) but lower than cystone (90.75±6.42%). This might be influenced by the phytochemical contents found in the A. nanus, for instance, phenols, flavonoids, alkaloids, saponins, and terpenoids. This finding indicates the potential of A. nanus to be developed into nutraceutical products for urolithiasis in the future.

1. Introduction

Urolithiasis or kidney stone problem is a multi-factorial ailment that has a high prevalence and reoccurrence rate globally including Malaysia that might be due to lifestyle habits and climate environment [1][2]. Kidney stone refers to solid non-metallic minerals from supersaturated solution in the urinary tract that involves phytochemical events such as crystal nucleation, aggregation and retention within the urinary tract [3][4]. There are four categories of urinary stones which are struvite, uric acid, cysteine, and the most common are calcium oxalate stones that representing up to 80% of the analyzed stones [5].

Nowadays, recent mechanisms to manage urolithiasis are through surgical procedures such as Extracorporeal Shock Wave Lithotripsy (ESWL), ureteroscopy (URS), and percutaneous nephrolithotomy (PNL) or by consuming synthetic drugs. However, those mechanisms have side effects and quite costly [6]. Medicinal plants have been used traditionally to treat kidney stone even before inventing all those modern treatments as it is proven to be effective and naturally safe remedies [7][8]. Thus, treatment with the medicinal plant is recommended as it contains phytochemical contents that may be associated with antiurolithiatic properties.

Malaysia is renowned to have a wide variety of medicinal plants such that Ananas species which have been used as remedies for urolithiasis. Ananas nanus (dwarf pineapple) and Ananas comosus (pineapple) are both belonging to the family Bromeliaceae. A. nanus is also locally called as “nenas
“batu” and looked likely to a common form of pineapple as in Figure 1 (a). It has been documented with the ability to dissolve kidney stone and have diuretic properties by herbal healers where the fruit is boiled with three cups of water for 10 minutes and drink it as juice [9][10]. Meanwhile, *A. comosus* or locally known as “*nanas*” as in Figure 1 (b) is a fruit grown in the tropical and sub-tropical regions. In folk remedies, the extract of ripe pineapple fruit is mixed with extract of *Averrhoa carambola* (star fruit) in equal proportions with common salt. Then, it was kept overnight before consuming it to prescribed against kidney stone troubles [11].

![Figure 1(a). Ananas nanus (dwarf pineapple)](image1)

![Figure 1 (b). Ananas comosus (pineapple)](image2)

Therefore, this study aims to investigate the antiurolithiatic properties of aqueous fruit extract of *A. nanus* and *A. comosus* through titrimetric and turbidity assay (*in-vitro*). This research was focusing on experimental calcium oxalate (CaOx) that was prepared in the laboratory as it is a common type of kidney stones that occur in our urinary tract or kidney. Besides that, their phytochemical contents were also screened to validate their antiurolithiatic potential.

### 2. Materials and Methods

#### 2.1. Plant material

The fruit of *Ananas nanus* and *Ananas comosus* were collected in the month of February 2019 from Pusat Ujian Varieti Baru Tumbuhan, Serdang, Selangor, and the local market in Batu Pahat, Johor, Malaysia respectively. The fruit plants were authenticated by Dr. Ab Rasip Ab Ghani, Director of Forest Plantation Programme in Biotechnology Division at Forest Research Institute Malaysia (FRIM), Malaysia and the voucher specimen were deposited in the herbarium for future reference. The fruit was cut into smaller pieces and dried in the drying oven (Memmert, Germany) at 40°C for a week or until the moisture content below 10% to avoid fungally and mold contamination [12]. The dried fruit is powdered using a heavy-duty professional blender (TM767, Warriors, Malaysia).

#### 2.2. Preparation of fruit extract

The crude aqueous fruit extracts for both *Ananas sp.* were prepared using the decoction method as described by Rathod et al., (2013) with slight modifications [13]. Approximately 100 g of powdered fruit was extracted with 1000 mL of boiled distilled water for about 20 minutes. Then, the extracts were filtered using filter paper (Whatman no.1) and then filtrates were collected. The aqueous extracts were then freeze-dried using a freeze dryer (FreeZone Freeze Dryers, Labconco, Kansas City) for 48 hours. The dried extracts were placed in a universal bottle, stopped, and then stored at -20°C for further analysis of antiurolithiatic activity.
2.3. Chemical used
The chemicals and reagents used were Cystone tablet (The Himalaya, India), potassium citrate (QRec, Malaysia), calcium chloride dihydrate (CaCl$_2$) (HmbG, Malaysia), sodium oxalate (Na$_2$C$_2$O$_4$) (QRec, Malaysia), Tris-buffer (Sigma-Aldrich, USA), ammonia solution (QRec, Malaysia), sulphuric acid (H$_2$SO$_4$) (QRec, Malaysia), potassium permanganate (KMnO$_4$) (Vchem, Thailand), 37% hydrochloric acid (HCl) (Qrec, Malaysia), iron (III) chloride (FeCl$_3$) (R&M, UK), chloroform (R&M, UK), Mayer’s reagent (Bendosen, Malaysia) and sodium hydroxide pellets (NaOH) (QRec, Malaysia). All items were analytical grade and purchased from procured suppliers.

2.4. Antiurolithiatic assay

2.4.1. Titrimetric (calcium oxalate dissolution) assay. The titrimetric or calcium oxalate dissolution assay was conducted according to Atodariya et al., (2013) with minor modifications [14]. The experimental kidney stones of calcium oxalate (CaOx) were prepared by the homogenous precipitation method. An equal concentration of calcium chloride dihydrate in distilled water and sodium oxalate in 10 mL of 2N H$_2$SO$_4$ was allowed to react in sufficient distilled water in a beaker. The precipitate was freed from traces of sulphuric acid by ammonia solution, washed with distilled water, and dried at 60°C for 4 hours. Meanwhile, semi-permeable membranes were prepared from farm eggs by decalcifying the eggs in 2M HCl for overnight until the complete removal of eggshell as shown in Figure 2(a) and 2(b). The contents were squeezed out completely from decalcified eggs and then they were washed thoroughly with distilled water, placed in an ammonia solution for a while, and rinsed again with distilled water. The dissolution percentage of calcium oxalate was evaluated by weighing exactly 1 mg of experimental calcium oxalate and 10 mg of the fruit extract, packed it together in the semi-permeable membrane. This was allowed to suspend in a conical flask containing 100 ml of 0.1 M Tris buffer as shown in Figure 2(c). The first group was served as blank containing only 1 mg of calcium oxalate. The second and third groups served as a positive control containing 1 mg of calcium oxalate and along with the 10 mg standard drugs which are cystone and potassium citrate. The fourth and fifth groups along with 1 mg of calcium oxalate were contained aqueous extracts for both Ananas sp. The conical flasks of all groups were kept in an incubator preheated to 37 °C for 2 hours. The contents of semi-permeable membranes from each group were removed into separate test tubes. Approximate 2 mL of 1N sulphuric acid was added to each test tube and titrated with 0.9494 N KMnO$_4$ till a light pink color end-point was obtained. The amount of remaining calcium oxalate that is not dissolved was subtracted from the total quantity used in the experiment, in the beginning, to determine the total quantity of dissolved calcium oxalate by Ananas sp. fruit extracts.

Figure 2 (a). Decalcification process of eggshell in 2M HCl.

Figure 2 (b). Decalcified eggs.

Figure 2 (c). Egg membrane along with the contents suspended into Tris buffer.
2.4.2. Turbidity (nucleation) assay. Turbidity or nucleation assay was done by using a similar method as described by Khare et al., (2014) with a slight modification [15]. The initial step was conducted to represent the absence of an inhibitor where 1 mL of 0.025 M CaCl₂ and 2 mL of Tris-buffer (pH 7.4) was added in a test tube. Then, proceed with the addition of 1 mL of 0.025 M sodium oxalate. The measurement of the turbidity that occurred was taken by using UV-Vis spectrophotometer (U-3900H, Hitachi, Japan) at 620 nm immediately after mixing the above chemicals up to a period of 5 min (300 seconds). This control experiment was done in three replications. The study was continued on the effect of standard drugs, cystone and potassium citrate as well as Ananas sp. For this experiment, four sets of test tubes with 1 mL of 0.025 M CaCl₂, 2 mL Tris-buffer and 1 mL (10 mg/mL solution) of samples were taken. The induction time in the presence of the samples was compared with that of control to calculate the nucleation rate. The results were expressed in percentage. The measurement of change in turbidity of the solution was conducted up to 5 min post mixing. Inhibition in stone nucleus formation was calculated by the graphical method using the following mathematical formula:

\[
\text{Inhibition} \% = \{1 \text{-} \frac{\text{Si}}{\text{Sc}}\} \times 100
\]

Where;
Si: the slope of the graph in the presence of inhibitor (standard drugs/extracts).
Sc: the slope of the graph without inhibitor (control)

2.5. Phytochemicals screening
Preliminary phytochemical evaluation of Ananas sp. were carried out for qualitative estimation of phenols, tannins, alkaloids, saponins, flavonoids, and terpenoids by using the following methods:

a) **Phenols and tannin**: It was done by using Ferric chloride test where 2 mL of 5% solution of FeCl₃ were added to 1 ml crude extracts. A black or blue-green color indicated the presence of tannins and phenols [16].

b) **Alkaloids**: It was done using Mayer's test where 2 ml of concentrated hydrochloric acid was added to 2 ml of extract. Then few drops of Mayer's reagent were added. The presence of green color or white precipitate indicates the presence of alkaloids [17].

c) **Saponin**: It was determined by a foam test where a few drops of the crude extract sample are placed in the test tube. About 5 ml of distilled water was added and shaken vigorously. The formation of a stable bubble froth indicates the presence of saponin [18].

d) **Flavonoids**: Flavonoid content was conducted using an alkaline reagent test. The amount of 3 mL of plant extract was treated with 1 mL of 10% sodium hydroxide solution. The formation of intense yellow color is an indication of the presence of flavonoids [16].

e) **Terpenoid**: It was conducted by Salkowski test where a few drops of the crude extract sample were added to 2 ml of chloroform. Then, concentrated sulfuric acid (3 ml) was carefully added to form a layer. A reddish-brown coloration indicates the presence of terpenoids [18].

3. Result and discussion
In general, urolithiasis is a process of the removal or formation of stones in any part of the urinary system such that the kidney, the ureters, or the urinary bladder [19]. Basically, urine supersaturation initiates crystallization when their concentrations are higher than the thermodynamic solubility for those stone materials [20]. This situation leads to the nucleation process where stone-forming salts in supersaturated solution associate with microscopic particles [21]. The stone formation can also be influenced by the irregular urinary composition that proposed the unevenness between promoters and inhibitors in the kidneys [22].

Many previous and recent studies have mentioned that there are many various types of herbal remedies among the local community to treat urolithiasis diseases in different traditional systems of medicine such as Ayurveda, Traditional Chinese medicine (TCM), and others [23][24]. This includes A. nanus and A. comosus that have been used in folk remedies for kidney stones therapy since ages ago [11][9]. Traditional medicinal plants have great potential as therapeutic drugs either single extract or in
combination with other herbs or the form of phytochemical compounds isolated from them with minimal side effects [25].

Based on the result obtained in Table 1, *A. nanus* has shown a significant difference of antiurolithiatic properties with *p*<0.05 as compared to *A. comosus* for both assays tested but demonstrated no significant difference (*p*>0.05) with standard drug, cystone for titrimetric assay. In comparison for both *Ananas sp.*, *A. nanus* was presented with 2.5 times higher than *A. comosus* for titrimetric antiurolithiatic activity while turbidity assay was indicated with an estimation of 4.4 times better than *A. comosus*. In general, cystone is a marketed herbal formulation commercialized as it has anti-calculifying properties proven to expel the stones from the urinary tract within 55 days of treatment [26][27]. Meanwhile, potassium citrate is a clinically used drug that is a low molecular weight inhibitor for crystallization [28].

Table 1. Antiurolithiatic activity of *A. nanus* and *A. comosus* fruit extracts.

| Antiurolithiatic assay | Standard drugs (%) | Plant samples (%) |
|-----------------------|--------------------|-------------------|
|                       | Cystone            | Potassium citrate | *Ananas nanus* | *Ananas comosus* |
| Titrimetric           | 89.54 ± 3.00<sup>a</sup> | 79.74 ± 4.08<sup>a</sup> | 88.89 ± 4.93<sup>a</sup> | 35.29 ± 3.92<sup>b</sup> |
| Turbidity             | 90.75 ± 6.42<sup>a</sup> | 80.14 ± 8.24<sup>a</sup> | 66.44 ± 3.30<sup>b</sup> | 15.07 ± 0.59<sup>c</sup> |

<sup>a</sup>-<sup>c</sup>Different letter at each line indicates significant differences (*p* < 0.05)

Theoretically, the titrimetric’s purpose is to dissolve the already formed stone in the kidney or urinary tract while the turbidity method aims to inhibit the pre-formed crystal [15]. In turbidity assay, the turbidity slope was rising linearly up to 5 minutes which indicates the nucleation process occurred and then decline linearly up to 15 minutes showing the aggregation process [29]. *A. comosus* had low antiurolithiatic potential might be because it was tested individually which does not follow the traditional practice recorded. It is reported that, the traditional practitioners used it with star fruit to enhance the dissolution activity of stones [11].

The antiurolithiatic properties of *A. nanus* could be contributed by the phytochemicals present in the fruit extract. These findings have been proven in the previous study where reported that different types of phytochemicals might have some positive contribution to the antiurolithiatic effect against CaOx crystals either in terms of dissolution or inhibition properties [30][29]. Aqueous extract of *A. nanus* fruit was strongly present with terpenoid content while *A. comosus* only has traces of terpenoids as summarized in Table 2. Previous studies have reported that extracts with high terpenoid content exhibited potent antiurolithiatic properties that prevent CaOx formation [31].

Besides, the presence of saponin was proven to have good properties of stone inhibition as reported on the extract of *Daucus carota*, *Kalanchoe pinnata*, and *Bergenia ciliata* [32][33][25]. This is due to saponin’s ability to exhibit anti-crystallization properties by disintegrating the mucoprotein suspensions which are promoter components to the crystallization process [33]. Besides that, phenols and flavonoids may also influence the effectiveness of antiurolithiatic activity of *A. nanus* extracts. They have potential in antioxidant activity to upgrade reactive oxygen species (ROS) that meditated oxidative stress which could be helpful in the management of urolithiasis [25]. This is confirmed by the previous study on *Lepidagathis prostrata* which showed a reduction on stone formation [34]. Traces of alkaloid contents in *A. nanus* also helps in antispasmodic that help in smooth muscle relaxation specific to the urinary tract which could facilitate the stone expulsion [35].
Table 2. Phytochemical screening of *A. nanus* and *A. comosus* fruit extracts

| Sample   | Phenols | Tannin | Alkaloids | Saponin | Flavonoids | Terpenoid |
|----------|---------|--------|-----------|---------|------------|-----------|
| *A. nanus* | ++      | -      | +         | ++      | +          | +++       |
| *A. comosus* | ++      | -      | -         | +       | +          | +         |

‘*’ indicates traces, ‘**’ indicates moderately present, ‘***’ indicates strongly present, ‘*’ indicates absent

4. Conclusion

The aqueous extract of *Ananas* fruit extract was obtained by decoction method that has been established in folk remedies for urolithiasis. In conclusion, *A. nanus* has higher antiurolithiatic potential compared to *A. comosus*. It is also comparable with the standard drug, cystone. This might be because of the strongly presence of saponin, phenols, and terpenoids in *A. nanus* extract due to the dissolution and inhibition activity of CaOx stones. The study provides a platform for further analysis of antiurolithiatic properties on *Ananas* fruit extract through *in-vivo* study. Since *A. nanus* have a good potential for antiurolithiatic properties, it can be developed into nutraceutical products for urolithiasis in the future.

Acknowledgments

This research is funded by the Ministry of Higher Education Malaysia under Fundamental Research Grant Scheme (FRGS/1/2017/WAB11/UTHM/03/1) Vot No. 1646 and partially funded by Universiti Tun Hussein Onn Malaysia under Postgraduate Research Grant Scheme (GPPS) Code H290.

References

[1] Baharudin N N, Shahar S, and Md. Zainuddin Z 2017 Dietary and lifestyle factors and its risk to kidney stone disease: a case control study at UKM Medical Centre *J. Sains Kesihat. Malaysia* 15 113–130

[2] Liu Y, Chen Y, Liao B, Luo D, Wang K, Li H and Zeng G 2018 Epidemiology of urolithiasis in Asia *Asian J. Urol.* 5 205–214.

[3] Gilhotra U K, Mohan G and Christina A J M 2013 Antilithiatic activity of poly-herbal formulation tablets by in-vitro method *J. Appl. Pharm. Sci.* 3 43–48

[4] Rani M, Sridivyagoud K, Himabindhu J and Ramanjaneyulu K 2018 Evaluation of in-vitro antiurolithiatic activity of *Aloe vera* *World J. Pharm. Pharm. Sci.* 15 1232–29

[5] Aggarwal A, Tandon S, Singla S K and Tandon C 2010 Reduction of oxalate-induced renal tubular epithelial (NRK-52E) cell injury and inhibition of calcium oxalate crystallisation in vitro by aqueous extract of *Achyranthes aspera* *Int. J. Green Pharm.* 4 159–164

[6] Tiwari A, Soni V, Londhe V, Bhandarkar A, Bandawane D and Nipate S 2012 An overview on potent indigenous herbs for urinary tract infirmity: Urolithiasis *Int. J. Green Pharm.* 5 7–12

[7] Butterweck V and Khan S R 2009 Herbal medicines in the management of urolithiasis: Alternative or complementary? *Planta Med.* 75 1095–1103

[8] Kumar S P, Latheef K A and Remashree A B 2014 Ethnobotanical survey of diuretic and Antilithiatic medicinal plants used by the traditional practitioners of Palakkad District *Int. J. Herb. Med.* 2 52–56

[9] Ramli M R, Milow P and Chooi O H 2015 Traditional knowledge of a practitioner in medicinal plants of Masjid Ijok village, Perak, Malaysia *Stud. Ethnomedicine* 9 59–66

[10] Syed Ibrahim S M 2015 *Misteri keajaiban alam ciptaan tuhan: tumbuhan penyembuh penyakit*. Kuala Lumpur, Malaysia: Green Dome Publication

[11] Lokendraj N, Swapana N, Singh C D and Singh C B 2011 Herbal folk medicines used for urinary and calculi/stone cases complaints in Manipur *NeBIO* 2 1–5
[12] Pauzi A N, Muhammad N, Sairi N H, Tuan Putra T N M, Gul M T, Rahim N F A, Marzuki, N A S, Abu Bakar M F, Talip B A and Abdullah N 2019 The effect of different solvent extraction towards antiurolithiatic properties of Euphorbia hirta and Orthosiphon stamineus IOP Conf. Ser. Earth Environ. Sci. 269 1–7

[13] Rathod V D, Fitwe P, Sarnaik D and Kshirsagar S N 2013 In-vitro anti-urolithiatic activity of corn silk of Zea mays Int. J. Pharm. Sci. Rev. Res. 21 16–19

[14] Atodariya U, Barad R, Upadhyyay S and Upadhyyay U 2013 Anti-urolithiatic activity of Dolichos biflorus seeds J. Pharmacogn. Phytochem. 2 209–213

[15] Khare P, Mishra V K, Arun K., Bais N and Singh R 2014 Study on in-vitro antilithiatic activity of Phyllanthus niruri Linn. leaves by homogenous precipitation and turbiditoy method Int. J. Pharm. Pharm. Sci. 6 124–127

[16] Madike L N, Takaidza S and Pillay M 2017 Preliminary phytochemical screening of crude extracts from the leaves, stems, and roots of Tulbaghia violacea Int. J. Pharmacogn. Phytochem. Res. 9 1300–08

[17] Roghini R and Vijayalakshmi K 2018 Phytochemical screening, quantitative analysis of flavonoids and minerals in ethanolic extract of Citrus paradisi Int. J. Pharm. Sci. Res. 9 4859–64

[18] Maskam N A, Mohamad Ravi N H, Mohd. Hassan H H and Mohamad Nor M 2017 Phytochemicals screening and antioxidant activity of three different solvent extracts of Euodia redlevi leaves Mod. Agric. Sci. Technol. 3 18–21

[19] Mamillapalli V, Khamtamneni P L, Mohammad Z, Mathangi A, Nandigam N, Namburi S and Katta V 2016 Phytochemical & in vitro antilithiatic studies on the leaf extracts of Bauhinia variegata Linn Int. J. Pharm. Sci. Res. 7 4074–84

[20] Worcester E M and Cee F L 2008 Nephrolithiasis Prim. Care 35 369–391

[21] Ratkalkar V N and Kleinman J G Mechanisms of stone formation Clin. Rev. Bone Miner. Metab. 9 187–197

[22] Chinnala K M, Shanigarm S and Elsani M M 2013 Antiurolithiatic activity of the plant extracts of Solanum virginianum on ethylene glycol induced urolithiasis in rats Int. J. Pharm. Biol. Sci. 3 328–334

[23] Kasote D M, Jagtap S D, Thapa D, Khyade M S and Russell W R 2017 Herbal remedies for urinary stones used in India and China: A review J. Ethnopharmacol. 203 55–68

[24] Ghatapanadi S R, Johnson N and Rajasab A H 2011 Documentation of folk knowledge on medicinal plants of Gulbarga district, Karnataka Indian J. Tradit. Knowl. 10 349–353

[25] Saha S and Verma R J 2013 Inhibition of calcium oxalate crystallisation in-vitro by an extract of Bergenia ciliata Arab J. Urol. 11 187–192

[26] Bijnarnia R K, Kaur T, Singla S K and Tandon 2010 C Non-surgical management therapies for kidney stones J. Pharm. Educ. Res. 1 21–25

[27] Radha S R and Vijayakumari B 2013 Herbal plants used in the treatment of urolithiasis: a review Int. J. Pharm. Res. Dev. 5 66–70

[28] Sharifa A A, Jamaludin J, Kiong L S, Chia L A and Osman K 2012 Anti-urolithiatic terpenoid compound from Plantago major Linn. (ekor anjing) Sains Malaysiana 41 33–39

[29] Sharma D, Dey Y N, Sikarwari, Sijoria R, Wanjarai M M and JadHAV A D 2016 In-vitro study of aqueous leaf extract of Chenopodium album for inhibition of calcium oxalate and brushite crystallization Egypt. J. Basic Appl. Sci. 3 164–171

[30] Gul M T, Dheyab A S, Shaker E K, Muhammad N and Pauzi A N 2020 In vitro evaluation of anti-urolithiatic properties of Strobilanthes crispus extracted using different solvents Res. J. Chem. Environ. 24 117–121

[31] Velu V, Fuloria N, Fuloria S, Panda J, Panda B P and Malipeddi H 2018 In-vitro and in-vivo anti-urolithiatic activity of terpenoid-rich ethyl acetate extract of rhizomes of Curcuma zedoaria Stud. Ethno Med. 12 31–39

[32] Bawari S, Negi Sah A and Tewari D 2018 Antiurolithiatic activity of Daucus carota: an in
vitro study *Pharmacogn. J.* **10** 880–884

[33] Phatak R S and Hendre A S 2015 In-vitro antiurolithiatic activity of *Kalanchoe pinnata* extract,” *Int. J. Pharmacogn. Phytochem. Res.* **7** 275–279

[34] Devkar R A, Chaudhary S, Adep S, Xavier S K, Chandrashekar K S and Setty M M 2016 Evaluation of antiurolithiatic and antioxidant potential of *Lepidagathis prostrata*: A Pashanbhed plant *Pharm. Biol.* **54** 1237–45

[35] Eweka A O and Enogieru A 2011 Effects of oral administration of *Phyllanthus amarus* leaf extract on the kidneys of adult wistar rats-a histological study *African J. Tradit. Complement. Altern. Med.* **8** 307–311