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In vitro antiurolithiatic activity of *Butea monosperma* Lam. and *Nigella sativa* Linn. seeds

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Abstract

The present study was undertaken to evaluate the *in vitro* antiurolithiatic activity of two medicinal plants, *i.e. Butea monosperma* Lam. and *Nigella sativa* Linn. It was observed in the first experimental set of *in vitro* model study that the highest calcium oxalate dissolution was observed in the aqueous extract and the lowest was recorded in hexane extract of *B. monosperma* seeds. In the second experimental set, the highest dissolution was found in the methanolic extract and the lowest was observed in the hexane extract of *N. sativa* seeds. The efficacy of various extracts of both the plants have shown that hexane extract was found less efficient to dissolve calcium oxalate aqueous extract whereas the methanolic extract was very efficient and near to the standard drug, cystone. Our results have clearly indicated that the aqueous extract of *B. monosperma* seeds and methanolic extract of *N. sativa* seeds were quite promissing for further studies in this regard.

Key words: *In vitro*, antiuroithiatic activity, urolithiasis, *Butea monosperma* Lam, *Nigella sativa* Linn.

1. Introduction

Plants provide food, raw materials for medicine and various other requirements for the very existence of life from the origin of human beings. Even the current conventional medicine is using a lot of plant derived chemicals as therapeutic agents. It should be noted that in the present day also, the majority of the global population utilizes medicinal plants for their healthcare. Therefore, there is a compelling need for detailed scientific validation of all traditional medicinal plant drugs to establish their efficacy and safety in light of modern science (Subramonium, 2014).

Stone formation in the kidney is one of the oldest and most wide spread diseases known to man. In India, people living in different states utilize different plants for curing urolithiasis (Chitme *et al*., 2010; Agarwal and Varma, 2014). Urolithiasis is derived from the Greek words “ouron” (urine) and “lithos’ (stone). It is considered as the third most common affliction of the urinary tract (Khan and Pradhan, 2012).

Urolithiasis is characterized by the formation of the stone in the kidneys or urinary tract in a large number of people. Nearly 4-15% of the human population is currently suffering from urinary stone problem all over the globe. The crystals of calcium oxalate (CaOx) are the primary constituent of more than 60% of the majority of human kidney stones. In spite of substantial progress in the pathophysiolgy and treatment of urolithiasis, there is no satisfactory drug being used in clinical therapy. Kidney dialysis, endoscopic stone removal and extra corporeal shock wave lithotripsy are prohibitively costly and reoccurrence is quite common with these procedures. Data from *in vitro* and *in vivo* clinical trials revealed that phytotherapeutic agents could be useful as alternative therapy in the management of urolithiasis. Medicinal plants and their products are more useful because they promote the repair mechanism in natural way. All over the world especially in developing countries, approximately 80% of population continues to use traditional medicines for the treatment of primary medical problems. In the past decade, research has been focussed on scientific evaluation of traditional drugs of plants origin. There is an urgent need to systematically evaluate the plants used in traditional medicines. Such research could lead to new drug discovery or advanced use of indigenous herbal medicines for treatment. This interest in plants derived drug is mainly due to the current widespread belief that green medicines were safe and more dependable than the costly synthetic drugs, many of which have adverse side effects. The two selected plants, *i.e. B. monosperma* Lam. and *N. sativa* Linn. have occupied an important place in Indian culture and folk medicines. These plants have been used in almost all the traditional systems of medicines in India such as Unani, Ayurveda, Siddha, and Homeopathy. From the ancient times the tribal and rural people of our country commonly used these plants in treating various disorders (Yadav *et al*., 2012; Shiny *et al*., 2013; Deepika *et al*., 2014).

The *B. monosperma* Lam. has been traditionally reported to possess astringent, aphrodiasic, antihelminthic, antilithiatic, antimicrobial and antiasthamatic property (Anil Kumar, 2012). The *N. sativa* Linn has a long history of folklore usage in various systems of medicines. The seeds are said to be “The cure for all the diseases except death” (Aisha Kamal, 2014).

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2. Materials and Methods

The seeds of two selected plants, *i.e. Butea monosperma* Lam. and *Nigella sativa* Linn. were commercially purchased from M/S Jajee Super Market, Kalaburgi, Karnataka State, India, a certified dealer in Ayurvedic products. The seeds of both the plants were separately shade dried and powdered. The crude plant extracts were prepared by Soxhlet extraction method. 50 g of powdered plant material was extracted with 500 ml of solvents in the increasing order of their polarity, *viz.* hexane, petroleum ether, methanol and water individually. The process of extraction was carried out up to 6 cycles, till the solvent in siphon tube of an extractor became colourless. All the extracts were filtered separately, the filtrates were placed in a beaker for evaporation at 30-40ºC. Further, the dried extracts were maintained in a refrigerator at 4ºC for further antiurolithiatic activity (Harborne, 1973).

The experimental kidney stones of calcium oxalate (CaOx) were prepared in the laboratory by taking equimolar solution of calcium chloride dehydrate in distilled water and sodium oxalate in 10 ml of 2N H_2SO_4. Both were allowed to react in sufficient quantity of distilled water in a beaker, the resulting precipitate was calcium oxalate. The precipitate was freed from traces of sulphuric acid by ammonia solution, washed with distilled water and dried at 60^{O}C. The dissolution percentage of calcium oxalate was evaluated by taking exactly 1 mg of calcium oxalate and 10 mg of the extract, packed it together in semipermeable membrane of egg as shown in the model designed given below (Figure 1 a-c). This was allowed to suspend in a conical flask containing 100 ml of 0.1M Tris buffer. First group served as blank containing only1 mg of calcium oxalate. The second group served as positive control containing 1 mg of calcium oxalate and along with the10 mg standard drug, *i.e.*cystone. The 3rd 4th 5th and 6th groups along with 1 mg of calcium oxalate contain hexane, aqueous, methanolic, pet-ether extracts. The conical flasks of all groups were kept in an incubator preheated to 37^{O}C for 2 h. Remove the contents of semipermeable membranes from each group into separate test tubes, add 2 ml of 1N sulphuric acid to each test tube and titrated with 0.9494 N $KMnO_4$ till a light pink colour end point obtained. The amount of remaining undissolved calcium oxalate is substracted from the total quantity used in the experiment in the beginning to know the total quantity of dissolved calcium oxalate by various solvent extracts (Unnati *et al*., 2013).

Figure 1: *In vitro* experimental model setup to evaluate anti-urolithiatic activity

Figure 1(a) Figure 1(b) Figure 1(c)

Figure 1(a): Decalcification of egg shell in 2M HCl overnight

Figure 1(b): Egg membrane along with the contents suspended into the 0.1 M Tris buffer

Figure 1(c): Titration with 0.949 N $KMnO_4$

3. Results and Discussion

There are many crucial areas in medicine such as liver diseases, arthritis, old age related problems, certain viral infections and cancer where the conventional medicine is devoid of satisfactory treatment. These are among the promising areas of research and development of medicines 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 problems. Development of valuable phytomedicines and healthcare products from locally available plants will result in multidimensional socio-economical progress (Subramonium and Pushpangadan, 1999; Kamboz, 2000; Beigh *et al*., 2002; Dickson and Gagnon, 2004; Jachak and Saklani, 2007; Subramonium, 2014).

Lithiasis (stone formation) is an important cause for acute and chronic renal failure, includes both nephrolithiasis (stone formation in kidney) and urolithiasis (stone formation in ureter or bladder or both). Among the various kinds of stones identified, calcium stones occur mainly in men, while phosphate stones formation is more in women (Ramachandran *et al*., 2011). A number of medicinal plants have been used in India and elsewhere which claim efficient cure of urinary stones (Mukharjee *et al*., 1984)

This study evaluates the antiurolithiatic activity of different extracts *of B. monosperma* Lam. and *N. sativa* Linn. seeds (Table 1 and Figure 2). The highest percentage, *i.e.* 58.2% of calcium oxalate (CaOx) dissolution was observed in aqueous extract and lowest percentage, *i.e.* 41.7% of calcium oxalate dissolution was observed in hexane extract of *B. monosperma* seeds. The highest percentage, *i.e.* 58.54% of calcium oxalate dissolution was observed in methanolic extract and lowest percentage, *i.e.* 38.4% of calcium oxalate dissolution was observed in hexane extract of *N. sativa* seeds, The hexane extract was found less effective in dissolution of CaOx in the seed extracts of both the plants. Whereas methanolic and aqueous extracts were found more effective (Rajput and Pal, 2011; Patil and Pawar, 2006; Gupta and Chauhan, 2012).

From this study, it was observed that aqueous extract of *B. monosperma* and methanolic extract of *N. sativa* seeds showed highest dissolution of calcium oxalate in comparison to other extracts. This study has given primary evidence for *B. monosperma* and *N. sativa* as the plants which possess lithotriptic property. This *in vitro* study has given lead data, and shown that aqueous and methanolic extracts are quite promising for further studies in this regard (Aggarwal and Singla, 2012; Eman Moussa and Jawaher, 2013).

Table 1: Shows % dissolution of calcium oxalate (CaOx) by *in vitro* antiurolithiatic activity of *B. monosperma* and *N. sativa* seeds extracts

		% of dissolution of calcium oxalate	
Sl/No.	Groups	*B. monosperma*	*N. sativa*
1	Blank	0	0
2	Positive control	56.4	60.1
3	Hexane extract	41.7	38.4
4	Aqueous extract	58.2	52.3
5	Methanolic extract	55.9	58.54
6	Petroleum ether extract	43.6	50.31

4. Conclusion

In vitro urolithiasis has been performed on two selected medicinal plants, *i.e. B. monosperma* and *N. sativa* by using the standard drug, cystone, commercially purchased from KBN Pharma Station Bazaar, Kalaburgi, Karnataka State, India. The work was performed by using *in vitro* antiurolithiatic model for calculating percentage dissolution of kidney stone. This study has given primary evidence for *B. monosperma* and *N.sativa* as the plants which possess lithotriptic property.

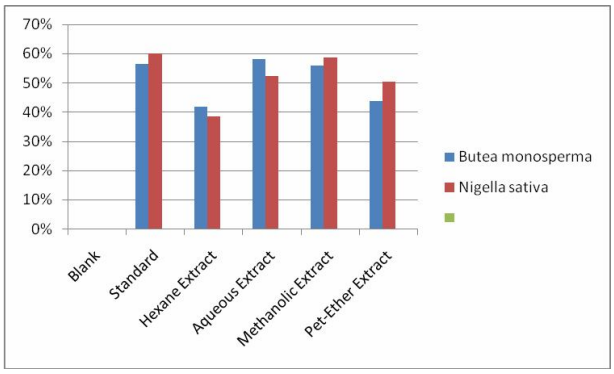

Figure 2: Graphical representation of dissolution of calcium oxalate

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Conflict of interest

We declare that we have no conflict of interest.

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