

In vitro antiurolithiatic activity of green and black tea extract of *Camellia sinensis* (L)

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Abstract

Kidney stones are one of the most established known and regular infections in the Urinary tract system. Urolithiasis is a multifaceted cycle that happens from the arrangement of a few physicochemical occasions including nucleation and aggregation. Natural items are more valuable for the body since they characteristically help the fixed component. Tea is delivered from the plant *Camellia sinensis* (L). It has been esteemed for its therapeutic properties. Green and Black tea remove have been depicted to have antiurolithiatic property. *In vitro* study was led to evaluate the antiurolithiatic impact of green and Black tea separates with standard medication specifically, Cystone. Nucleation and Aggregation strategies were polished to get to the hindrance of stone arrangement and disintegration of stone gems individually. The tiny investigation was accomplished for the relative appraisal of precious stone thickness and size in every strategy. Green tea removal demonstrated its potential result equivalent to the standard medication. The current examination was attempted to assess the *in vitro* antiurolithiatic movement of green and Black tea extract of *Camellia sinensis* (L).

Keywords: kidney stone, nucleation, and aggregation, microscopic evaluation

Introduction

Urolithiasis is derived from the Greek words ouron means urine and lithos means stone. Urolithiasis is characterized by the formation of the stone in the kidneys or urinary tract in a large number of people. Urolithiasis is one of the most common diseases of the urinary tract which has been afflicting human kidneys. Nearly 10-30% of the population is currently suffering from kidney stones. World Health Organization (WHO) estimated that about 12% of men and 55% of women have at least one episode of kidney stone during their lifetime (Narendra *et al.*, 2013) [12]. The cause of Urolithiasis is still unknown but probably positive family history, overweight, obesity or increased body mass index (BMI) (Mellisa, and Cadnapaphoranchai 2008) [10]. Epidemiological studies revealed that urolithiasis is more common in men than women but rare in children (Smith, 1978) [17] and are more prevalent between the ages of 20-40 in both sexes.

Urolithiasis is associated with calculus formation at any level in the urinary collecting system, but calculus often arises in the kidney. Recurrent stone formation is probably the most important problem in aftercare patients who have undergone operations for renal and ureteric calculi. Urolith formation is a multifactorial process that may relate to diet, urinary tract infection, altered urinary solutes, and colloids decreased urinary drainage and urinary stasis, prolonged immobilization, macrolides, etc., (Fowler *et al.*, 1995) [4].

The worldwide incidence of urolithiasis is quite high, and more than 80% of urinary calculi are calcium oxalate stones alone or calcium oxalate mixed with calcium phosphate. Kidney stone formation is a complex process which is the outcome of several physio-chemical events such as nucleation, growth, aggregation, and retention (Khan, 1997) [8].

Despite substantial progress in the pathophysiology and treatment of urolithiasis, there is no satisfactory drug being

used in clinical therapy. Endoscopic stone removal and extracorporeal shock wave lithotripsy are prohibitively costly and recurrence is quite common with these procedures (Hadjzadeh *et al.*, 2007) [5]. The surgical operation, local calculus disruption using high power laser are widely used to remove the calculi. Many remedies have been employed for ages to treat renal stones and most of them were from plants and proved to be useful. In Ayurveda and folklore medicine many herbs are used in the management of urolithiasis (Baumann, 1998) [3]. Medicinal plants have played a significant role in various ancient traditional systems of medicine. Even today, plants provide a cheap source of drugs for the majority of the world's population. Several pharmacological investigations on the medicinal plants used in traditional antiurolithic therapy have revealed their therapeutic potential in the *in vitro* models (Leonetti, 1996) [9].

Plants provide food, raw materials for medicine, and various other requirements for the very existence of life from the origin of human beings (Suhail Asghar *et al.*, 2011) [19]. The majority of the global population utilizes medicinal plants for their health care. Even the current conventional medicine is using a lot of plant-derived chemicals as therapeutic agents. The overuse of synthetic drugs results in a higher incidence of adverse drug reactions has motivated humans to return to nature for safe remedies. Herbs and herbal drugs have created interest among the people by its clinically proven effects (Sumayya sikandari and Prathima Mathad, 2015) [20].

Tea is one of the most popular drinks in the world. Tea is produced from the plant *Camellia sinensis* (L) grows best in tropical and subtropical regions. It has been valued for its medicinal properties. Moreover, it has received more attention with regards to health benefits *Camellia sinensis* (L) belongs to Theaceae and commonly called as the Tea plant. Green tea is made from unfermented leaves, Black tea

from fully fermented leaves (Sanjay kumar Gupta *et al.*, 2012) [15]. Therefore, the current investigation was carried out for the screening of the antilithic potential of green and black tea against calcium oxalate crystallization in an *in vitro* setting.

Materials and Methods

Plant collection

The fresh leaves of *camellia sinensis* (L) were collected from the dense tea state garden, Ooty, Tamilnadu, South India. The plants were identified based on the morphological plant characteristics. Commercial black tea powder was purchased from the local market at Ooty. The leaves were identified and authenticated by Dr. S. John Britto, the Director Rabinat herbarium and center for molecular systematics, St. Joseph's college Trichy. A voucher specimen (T4001) has been deposited at the Rabinat Herbarium, St. Joseph's College, Trichy.

Processing and extraction

The fresh tea plant leaf sample was shade dried at room temperature for three days, made into small pieces. Both dried form of fresh leaf and black tea powder grind into powder by using mortar and pestle. Extract from the fresh leaf of the tea plant was done by following the standard method (12). For water extraction about 10 g of powder fresh green tea leaves and black tea, the powder was weighed and soaked in 100 ml of distilled water separately and kept for overnight in a shaker (130 r/min at 37 °C). The extracts were filtered by using Whatman No. 1 filter paper to remove the residues. The filtrate was then concentrated by using a rotary evaporator and stored in bottles and refrigerated at 4 °C until use.

Preliminary phytochemical screening

Preliminary phytochemical evaluation of Green and Black tea powder was carried out for the qualitative estimation of Phytoconstituents. (Harborne, 1973) [6].

In vitro Antiuro lithiatic Activity

Sample preparation

The leaf extract of green and black tea was prepared as 100µg/ml -1000µg/ml for antiuro lithiatic activity

Nucleation assay (Patel *et al.*, 2012) [13].

Effect of calcium oxalate (CaOx) crystal formation was determined using nucleation assay. Calcium chloride (CaCl₂) (5 mmol/l) and sodium oxalate (Na₂C₂O₄) solution (7.5 mmol/l) were prepared in Tris-HCl (0.05 mol/l) and NaCl (0.15 mol/l) buffer (pH 6.5). Dilutions of samples ranging from 100-1000 µg/ml were prepared in distilled water. One milliliter of each sample concentration was mixed with 3 ml CaCl₂ solution followed by the addition of 3 ml Na₂C₂O₄ solution. Final mixtures were incubated for 30 min at 37°C. The optical density (OD) of the mixtures was then measured at 620 nm wavelength. Percent inhibition of nucleation was calculated using under the mentioned formula and compared to that calculated for the standard polyherbal drug, Cystone.

$$\% \text{ of inhibition} = [1 - (\text{Test OD}/\text{Control OD})] \times 100$$

Aggregation assay (Patel *et al.*, 2012) [13]

Effect of CaOx crystal aggregation was determined through aggregation assay. CaCl₂ and Na₂C₂O₄ solutions (50 mmol/l each) were mixed together, heated to 60 °C in a water bath for 1 hour, and then incubated overnight at 37 °C to prepare seed CaOx crystals. After drying, CaOx crystal solution (0.8 mg/ml) was prepared in a 0.05 mol/l Tris-HCl and 0.15 mol/l NaCl buffer (pH 6.5). One milliliter of aliquots (100-1000 µg/ml) of the sample was added to 3 ml CaOx solution, vortexed, and then incubated at 37 °C for 30 min. OD of the final mixtures was then read at 620 nm wavelength and percent inhibition of aggregation was then calculated for the standard polyherbal drug, Cystone.

$$\% \text{ of inhibition} = [1 - (\text{Test OD}/\text{Control OD})] \times 100$$

Microscopic evaluation

The number, size, and morphology of CaOx crystals formed in the absence or presence of Green tea and Black tea powder extract were determined using a light microscope at 100X magnification.

Statistical analysis

Tests were carried out in triplicate for 3–5 separate experiments. The amount of sample needed to inhibit the stone formation by 50%, IC₅₀, was graphically estimated using a nonlinear regression algorithm

Results and Discussion

Preliminary phytochemical screening

Qualitative phytoconstituents determination in Green and Black tea powder extract showed the presence of Flavonoids, Polyphenol, Saponins, Terpenoids, Triterpenoids, and Tannin.

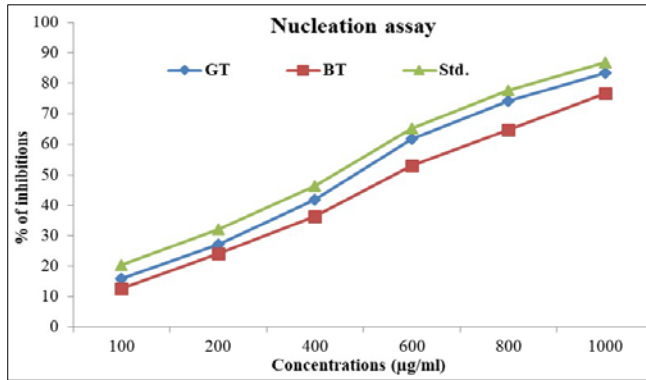
Nucleation assay

The addition of Na₂C₂O₄ solution to the reaction mixture consisting of CaCl₂ resulted in the formation of numerous CaOx crystals. The presence of Green tea extract (1000µg/ml) in the reaction mixture produced a percent reduction in the nucleation of 83.50±5.84 which was significantly higher than that produced by Black tea extract (76.61±5.36). The half inhibition concentration (IC₅₀) of Green tea extract and Black tea extract was 507.44 and 594.04 and cystone was 453.16 respectively. Green tea has high potential activity than Black tea extracts and was near to standard (Table-1, Figure 1).

Table 1: Effect of Green and Black tea extract and Cystone on CaOx crystals by Nucleation assay.

Concentration (µg/ml)	GT (Green Tea)	BT (Black Tea)	Std. (Cystone)
100	15.81 ± 1.10	12.65 ± 0.88	20.33 ± 1.42
200	27.11 ± 1.89	24.18 ± 1.69	32.09 ± 2.24
400	41.80 ± 2.92	36.38 ± 2.54	46.32 ± 3.24
600	61.80 ± 4.32	53.10 ± 3.71	65.19 ± 4.56
800	74.12 ± 5.18	64.63 ± 4.52	77.62 ± 5.43
1000	83.50 ± 5.84	76.61 ± 5.36	86.89 ± 6.08
IC ₅₀ value	507.44	594.04	453.16

Values expressed as Mean ± SD for triplicate



GT= Green tea BT= Black tea Std= Standard

Fig 1: Effect of Green and Black tea extract and Cystone on CaOx crystals by Nucleation assay.

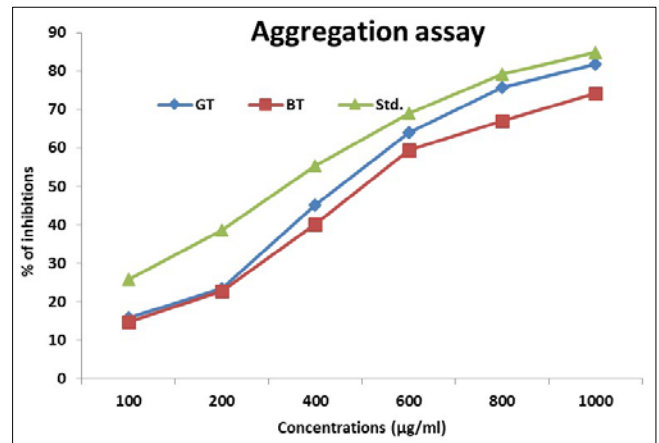
Aggregation Assay

Green and Black tea extract produced a significant reduction in the aggregation of performed CaOx crystals. The present reduction in aggregation produced by Green tea was found to be 81.72 ± 5.72 comparable to that of Black tea 74.11 ± 5.18 at 1000 µg/ml concentration. The half inhibition concentration (IC₅₀) of Green and Black tea extract was 504.85 and 569.44 and the cystone was 381.82 respectively. Green tea has high potential activity than Black tea extract and was near to standard (Table-2, Figure 2).

Table 2: Effect of Green and Black tea extract and Cystone on CaOx crystals by Aggregation assay.

Concentrations (µg/ml)	GT (Green Tea)	BT (Black Tea)	Std. (Cystone)
100	15.73 ± 1.10	14.72 ± 1.03	25.88 ± 1.81
200	23.35 ± 1.63	22.84 ± 1.59	38.57 ± 2.69
400	45.17 ± 3.16	40.10 ± 2.80	55.32 ± 3.87
600	63.95 ± 4.47	59.39 ± 4.15	69.03 ± 4.83
800	75.63 ± 5.29	67.01 ± 4.69	79.18 ± 5.54
1000	81.72 ± 5.72	74.11 ± 5.18	84.77 ± 5.93
IC ₅₀ value	504.85	569.44	381.82

Values expressed as Mean ± SD for triplicate



GT= Green tea BT= Black tea Std= Standard

Fig 2: Effect of Green and Black tea extract and Cystone on CaOx crystals by Aggregation assay.

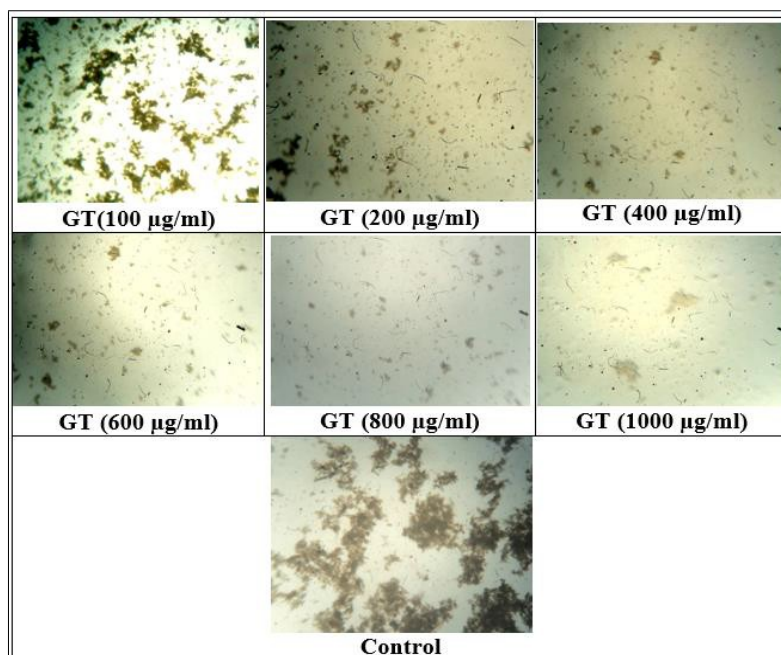
Microscopy of CaOx crystals

Various huge CaOx monohydrate (COM) precious stones of either rectangular propensity or dendrites with sharp edges were transcendent in the Control group.

Green tea at higher focus and Black tea structure lower fixations itself supported the arrangement of tetrahedral molded calcium oxalate dry out (COD) crystals with smoother morphology.

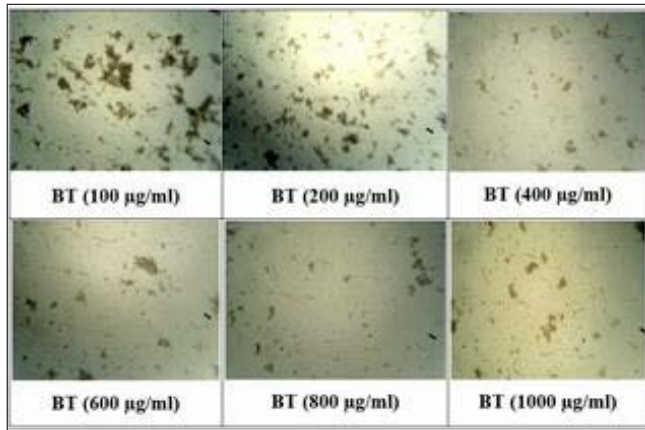
Green and Black tea extract and cystone likewise decreased the size and number of CaOx crystals. Percent decrease in the size of CaOx crystals created by Green tea was similar to that of black tea extract.

The number of CaOx crystals was reduced to a far greater extent by Green tea extract than black tea extract. Speak to photos of CaOx precious stones as seen under the light microscope instrument (X100) in the nonappearance or presence of Green and Black tea extract and cystone (Figure-3,4,5).



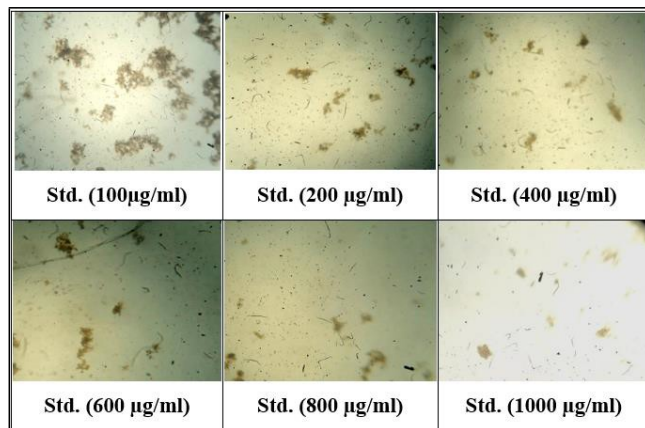
GT= Green Tea

Fig 3: Represent photographs of CaOx crystals as observed under a light microscope (X100) in the absence or presence of Green Tea extract.



BT= Black tea

Fig 4: Represent photographs of CaOX crystals as observed under the light microscope (X100) in the presence of Black Tea extract



Std= Standard

Fig 5: Represent photographs of CaOX crystals as observed under the light microscope (X100) in the presence of Standard as Cystone

Drug therapy has developed in response to population health care needs (Michael Dickson and Jean Paul Gagnon, 2004) [11]. Plants are also attractive sources for the development of novel and very effective and safe therapeutic agents against kidney procumbens. Herbal medicines are also in great demand in the developed world for primary health care because of their efficacy, safety, and lesser side effects (Kamboj, 2000) [7]. Unlike allopathic medicines which target is only one aspect of urolithiatic pathophysiology, most plant-based therapies are effective at different stages of stone pathophysiology (Archana *et al.*, 2013) [2]. About 80% of the world's populations rely on the use of traditional medicine which is predominantly based on plant materials (Subramoniam and Pushpangadan, 1999) [18]. Plant-based drug discovery programs continue to provide an important source of new drug leads (Sanjay *et al.*, 2007) [16].

In India, as in many countries, recent interest has been focused on the therapeutic potential of traditional plants in the context of controlling various diseases by using scientific methods. Lithiasis (stone formation) is an important cause for acute and chronic renal failure, includes both nephrolithiasis (stone formation in the kidney) and urolithiasis (stone formation in the 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)

[14].

CaOx urolithiasis is the most prevalent type of all urinary stone. Key events involved in its pathological biomineralization include crystal nucleation and aggregation (Aggarwal *et al.*, 2013) [1]. The present study was designed to address these key events involved in CaOx stone formation as a means to investigate the efficacy of Green and Black tea extract as an antiurolithiatic.

Conclusion

Both Green and Black tea concentrates of *Camellia sinensis* (L) indicated their most elevated disintegration of calcium oxalate. Green tea extract was discovered to be significantly more viable than Black tea extract in the disintegration of calcium oxalate. This examination has given essential proof for *Camellia sinensis* (L) as the plant which has antiurolithiatic property. This *in vitro* study has given lead information and demonstrated that Green and Black tea extracts are very encouraging for additional investigations in such manner. The current investigation found that the organization of Green tea successfully forestalled the improvement of urolithiasis. These discoveries uphold the utilization of green tea and Black tea as an elective medication to forestall urolithiasis.

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