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PHYTOCHEMICAL EVALUATION, *IN-VITRO* ANTIOXIDANT POTENTIAL AND *IN-VITRO* ANTIUROLITHIATIC ACTIVITY OF *NIGELLA SATIVA* SEEDS

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ABSTRACT

Objective: The current line of investigation was focused at perusing the *in-vitro* anti-oxidant potential and anti –urolithiatic activities of aqueous extract of *Nigella sativa* seeds using various methods

Materials and Methods: The dried seed powder was subjected to maceration method to result crude aqueous extract. Phytoconstituents present in aqueous extract was examined by performing preliminary phytochemical screening. Total Phenolic Content (TPC), Total Flavonoid Content (TFC), Total alkalodal content (TAC) and Antioxidant potential for crude extracts were studied by DPPH, FRAP and Total anti-oxidant assay method methods. Anti-urolithiatic activity was screened using nucleation and aggregation assays *in-vitro.*

Results: The presence of phytochemical constituents, investigation of total phenolic, flavonoid content and total alkaloid content of aqueous extract of *Nigella sativa* seeds using various in-vitro assays were studied. The total phenolic content and flavonoid content of aqueous extract of plant was found to be 95.23 ±0.629 mg and 29.46 ±1.170 mg of GAE and Quercetin equivalents respectively. Total alkaloidal content was found to be 153mg/g of extract. The aqueous extract exhibited potent antioxidant activity as determined by 2,2-diphenyl-1-picrylhydrazyl(DPPH), ferric reducing antioxidant power assays (FRAP) and Total antioxidant assay methods. Anti urolithiatic activity was studied using nucleation and aggregation assays.

Conclusion: From the findings of current study, it was revealed that the selected medicinal plant *Nigella sativa* has good potential to treat urolithiasis.

Keywords: *Nigella sativa*, Antioxidant activity, Aggregation assay, Nucleation assay

INTRODUCTION:

Antioxidants are used to protect the body from damage caused by oxidation. Oxidation is a chemical reaction that involves the transfer of electrons from a substance to an oxidizing agent. Oxidation leads to production of free radicals **[1]**, leads to cell damage by means of lipid peroxidation or by oxidizing DNA or protein **[2]**. Oxidative stress has been insinuated in several diseases including diabetes, rheumatoid arthritis, cardiovascular diseases, atherosclerosis, neurodegenerative diseases, cancer, and aging **[3-5]**. Hence naturally derived antioxidants protect the cell damage by inhibiting the release of reactive oxygen species. Plant derived antioxidants such as phenolic acids and Flavonoid compounds may offer resistance against the oxidative stress by free radical scavenging and by other mechanisms **[6, 7]**. This has been opened a window for the researchers to emphasize focus on plant derived substances rich in good antioxidant potential.

Plants are indispensable fount of new drugs. There is an enormous historical legacy regarding the use of traditional plant medications in the field of folkforic medicine **[8]**. Efforts of researchers on plants used in ethnomedicine opened a window for the discovery of many valuable drugs like taxol, camptothecin, vincristine, etc. *Nigella sativa* L. (Ranunculaceae family) seeds, commonly known as blackseed or black cumin, have been employed for thousands of years as a spice and food preservative, as well as a protective and therapeutic remedy for various disorders **[9,10]**. In this study, we made an attempt to evaluate the antioxidant potential

and anti-urolithiatic property of aqueous extract of *Nigella sativa* seeds.

MATERIALS AND METHODS:

Collection of Plant material and extraction:

The seeds of *Nigella sativa*were purchased and powdered mechanically. The dried seed powder was allowed for method of maceration and resulted in aqueous extract, stored in refrigerator.

Phytochemical Evaluation:

Phytochemical investigation of carbohydrates, alkaloids, steroids, glycosides, flavonoids, phenols, saponins and tannins. were carried out for aqueous extract of seeds using standard protocols.

Total phenol content Estimation:

The total phenolic content (TPC) for the aqueous extract of *Nigella sativa* seeds was determined by using Foline- Ciocalteau phenol reagent method described by Singleton *et al*. **[11]**. Briefly, 1.0 mL of the extract at various concentrations was mixed 15 with 2.5 mL of 10% Foline- Ciocalteau reagent and 2.5 mL of 7.5% sodium carbonate. The contents were thoroughly mixed and allowed to stand for about half an hour (30 minutes). The absorbance was read at 750 nm in a spectrophotometer. The total phenol content was expressed as gallic acid equivalents in milligram per gram of the extract.

Total Flavonoid content estimation:

The flavonoid content (TFC) aqueous extract of *Nigella sativa* seeds was screened using aluminium chloride colorimetric method described by Chang *et al*. **[12]**. According to him, TFC is determined by the following procedure. 0.5 mL of the extract at various concentrations was mixed with 3 mL of 95% methanol, 0.1 mL of 10% (weight/volume) aluminium chloride, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water. Let the reaction mixture to stand at room temperature for about 30 minutes and absorbance was read at 415 nm against the blank sample. A calibration curve was generated using quercetin in methanol. The flavonoid content was expressed as quercetin equivalents in milligram per gram of the extract.

Total Alkaloid Content:

Total alkaloidal content was estimated by dissolving 1mg of *Nigella sativa* seeds aqueous extract in 1ml of 2N HCl. To this, 1ml of Dimethyl sulfoxide was added and filtered. 1 ml of above solution was transferred into separating funnel. To this a solution of bromo cresol green was added followed by 5 ml of phosphate buffer. The mixture was shaken with 1ml, 2ml, 3ml, 4ml of chloroform. Collected and pooled the chloroform layer after every shaking and then made up the volume up to 10ml with chloroform in 10 ml volumetric flask. The

absorbance at 470 nm was measured. Atropine sulphate was used as standard.

IN-VITRO ANTIOXIDANT ACTIVITY:

DPPH antiradical capacity:

The free radical potential of aqueous extract of *Nigella sativa* seeds was determined spectrophotometrically as described by Ilahi *et al*. **[13]**. Five different concentrations of aqueous extract of *Nigella sativa* seeds (100, 200, 400, and 800 and 1000 µg/ml) were mixed with 100 µL of DPPH radical solution in a 96-well microplate and incubated for 20 min at room temperature. The resultant mixture was read spectrophotometrically at 517 nm against a methanol blank and the following equation was used to calculate the % inhibition of each extract:

$$\% \text{ inhibition} = (Ao - As)/(Ao) \times 100$$

Where, A_o is the absorbance of the control, and *As* is the absorbance of the test sample. The IC_{50} represented the concentration of the extract that inhibited 50% of radical.

Reducing power Determination:

The reducing ability of a compound generally depends on the presence of reductants which exhibits antioxidant potential by breaking the free radical chain, donating a hydrogen atom. The Fe^{2+} reducing power of aqueous extract of *Nigella sativa* seeds was determined by the method of Oyaizu **[14]** with slight modification. Various concentrations of plant extract(0.75mL) was mixed with 0.75 ml of phosphate buffer (0.2 mole, pH 6.6) and 0.75 mL of potassium ferricyanide $K_3Fe(CN)_6$ (1%w/v), followed by incubating at 50°C for 20 mins. The reaction was stopped by adding 2.5mL of 10% (w/v) trichloroaceticacid followed by centrifugation at 3000 rpm for 10min. Finally, 1.5mL of the upper layer was mixed with 1.5mL of distilled water and 0.5mL of $FeCl_3$(0.1%) and the absorbance was measured at 700 nm. Higher the absorbance of reaction mixture indicated greater the reducing power. Ascorbic acid is used as reference compound.

Total antioxidant activity:

Total antioxidant activity was estimated by phosphomolybdenum assay **[15, 16]**.

Preparation of Molybdate Reagent Solution: 1ml each of 0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate were added in 20 ml of distilled water and made up volume to 50 ml by adding distilled water.

Procedure: Aqueous extract of *Nigella sativa* seeds in different concentration ranging from 100 µl to 800 µl were added to each test tube individually containing 3 ml of distilled water and 1 ml of Molybdate reagent solution. These tubes were kept incubated at 95 °C for 90 min. After incubation, these tubes were normalized to room temperature for 20-30 min and the

absorbance of the reaction mixture was measured at 695 nm. Mean values from three independent samples were calculated for each extract. Ascorbic acid was used as positive reference standard.

Anti-Urolithiatic activity of aqueous extract of *Nigella sativa* seeds:

Nucleation assay:

Concentrations of 5mmol/l of calcium chloride ($CaCl_2$) and 7.5 mmol/l of sodium oxalate ($Na_2C_2O_4$) solution were prepared in Tris-Hcl(0.05 mol/l) and NaCl (0.15 mol/l) buffer (pH- 6.5).Dilutions of seed extract ranging from 100-1000µg/ml were prepared in distilled water. One milliliter of each plant extract concentration was mixed with 3ml $CaCl_2$ solution followed by the addition of 3 ml $Na_2C_2O_4$ solution. Final mixtures were incubated for 30 min at 37ºC. The optical density of the mixture was then measured at 620nm wavelength.

Aggregation assay:

$CaCl_2$ and $Na_2C_2O_4$ solutions (50 mmol/l each) were mixed together, heated to 60 ºC in a water bath for 1hr and then incubated overnight at 37ºC 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 of NaCl buffer (pH 6.5). One milliliter of aliquots (100-1000 µg/ml) of seed extract were added to 3 ml CaOx solution, vortexed and then incubated at 37ºC for 30 min. The optical density of the mixture was then measured at 620nm wavelength.

RESULTS:

Phytochemical Constituents:

Phytochemical evaluation was done by performing fundamental tests for aqueous extract of *Nigella sativa* seeds. Results were shown in **(Table 1)**. '+' indicate the presence and '-' indicates the absence of phytochemical constituents.

Total phenol content Estimation:

In the present study, Total phenol content (TPC) for the aqueous extract of *Nigella sativa* seeds was measured in Gallic acid Equivalents in mg / gm extract and was found to be 95.23 ±0.629 mg. The results are described as Gallic acid equivalents (GAE)

Total Flavonoid estimation:

The flavonoid content (TFC) for the aqueous extract of *Nigella sativa* seeds was determined using aluminum chloride colorimetric method. The flavonoid content was expressed as quercetin equivalents in milligram per gram of the extract and was 29.46 ±0.170 mg.

Total alkaloid content:

The determination of total alkaloids using a visible spectrophotometric method with Bromocresol Green (BCG) is a simple and sensitive technique that requires no special equipment. BCG can react with certain

alkaloids, i.e., the ones that have nitrogen inside their structure, but not with amine and amide alkaloids. The reaction of alkaloids with BCG forms a yellow-coloured product. Total alkaloidal content of aqueous extract of *Nigella sativa* seeds was calculated in terms of Atropine equivalents in mg per gm of the extract. Total alkaloid content was found to be 154micrograms.

DPPH Free radical scavenging activity:

DPPH is a relatively stable free radical and the assay determines the ability of aqueous extract of *Nigella sativa* seeds to reduce DPPH free radicals to the corresponding hydrazine by converting the unpaired electrons to paired ones. Antioxidant can act by converting the unpaired electron to paired one. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized which can be quantitatively measured from the changes in absorbance at 517nm and also for visible deep purple colour **(Figure 1)**. and the IC50 value was calculated as 187.09 µg/mL using GraphPad Prism 8.3.1.

6.5.2. Fe Reducing power assay:

Reducing power experiment is a good reflector of antioxidant activity of the plant. The plant having high reducing power generally reported to carry high antioxidant potential too. In this experiment, Ferric ions are reduced to ferrous ions, identified by colour change from yellow to bluish green. The results for ferric reducing power activity of aqueous extract of *Nigella sativa* seeds in comparison with ascorbic acid are reported in **(Figure 2 and Table 2)**. Reducing power potential of extracts increase with the dose, however the extract exhibited low reducing power than that of ascorbic acid.

6.5.3. Total Antioxidant Activity:

The seed extract at various concentrations were assayed for their antioxidant potency by the formation of green phosphomolybdenum complex. The total anti-oxidant capacity was measured by taking ascorbic acid as standard. IC_{50} was calculated by using GraphPad Prism 8.3.1 and was 160.98µg/ml **(Figure 3)**.

Anti-Urolithiatic Activity:

Nucleation Assay:

Urine supersaturation attributes to calcium oxalate particles crystallization within the urinary tract. This is nucleation process where stone forming salts begins, united into clusters with addition of new constituents. Aqueous extract of *Nigella sativa* seeds exhibited stronger inhibition activity which is almost equipotent like the standard cystone formulation in the nucleation of calcium oxalate salts and represented in **Figure 4 and Figure 5** respectively. As in vitro crystallization study was performed, since nucleation is an important first step for the

initiation of crystals, which then grow and form aggregates. Extract of*Nigella sativa* inhibited the crystallization by inhibiting nucleation of calcium oxalate through disintegrating into smaller particles with increasing concentrations of the fraction. From the results of the nucleation assay, it was confirmed that the extract contained nucleation-preventing agents.

Aggregation Assay:

Calcium oxalate crystals begin grow; aggregate with other crystals and retained in the kidney. This is aggregation process which causes renal injury. The aqueous extract of *Nigella sativa* seeds demonstrated potency as close as nearer when compared to Cystone standard solution to inhibit the crystal aggregation and was represented in **Figure 6**.

Table 1: Phytochemical Constituents in aqueous extract of *Nigella sativa* seeds

Phytochemicals	AE
Carbohydrates	+
Proteins	-
Alkaloids	+
Steroids	+
Glycosides	+
Flavonoids	+
Fixed oils	_
Saponins	+
Phenols	+
Tannins	+

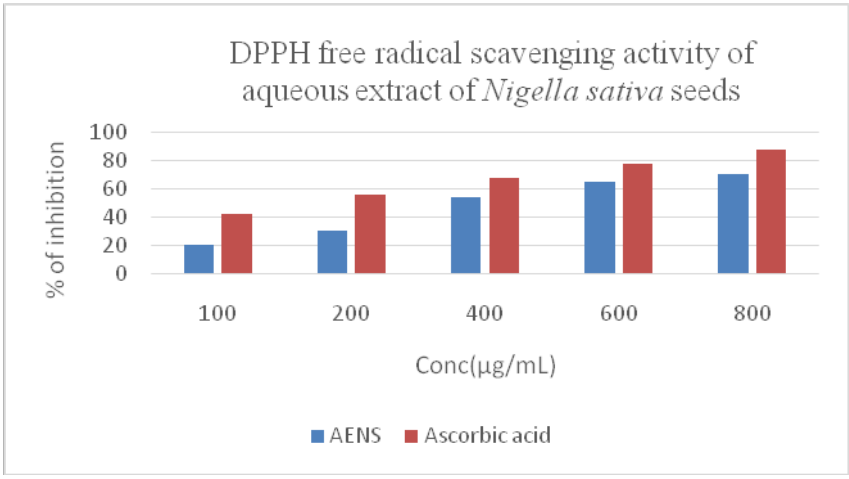


Figure 1: DPPH Free radical scavenging activity of aqueous extract of *Nigella sativa* seeds

Table 2: Ferric reducing power activity of aqueous extract of *Nigella sativa* seeds

CONCENTRATIONS	ABSORBANCE at 700nm
100µg	1.051
200µg	1.262
400µg	1.880
600µg	2.217
800µg	4.000

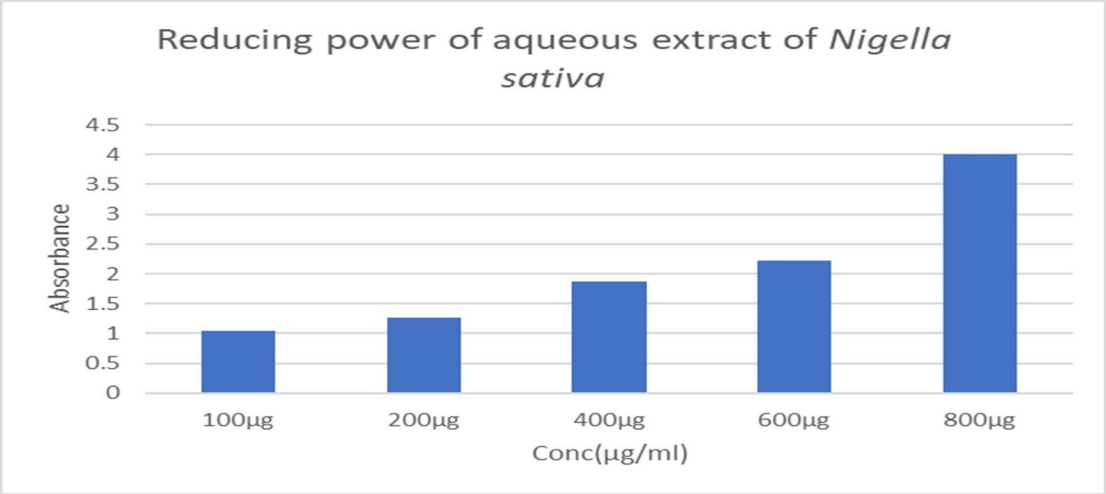


Figure 2: Ferric reducing power activity of aqueous extract of *Nigella sativa* seeds

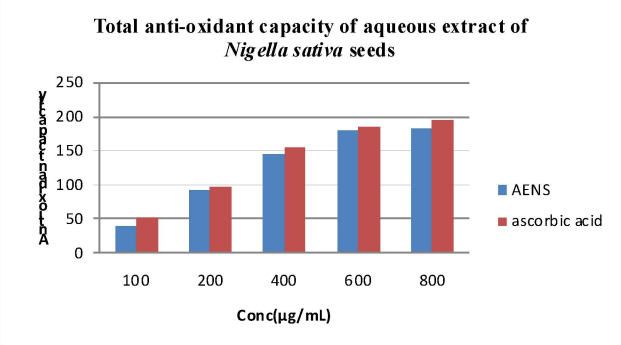


Figure 3: Total anti-oxidant assay of aqueous extract of *Nigella sativa* seeds

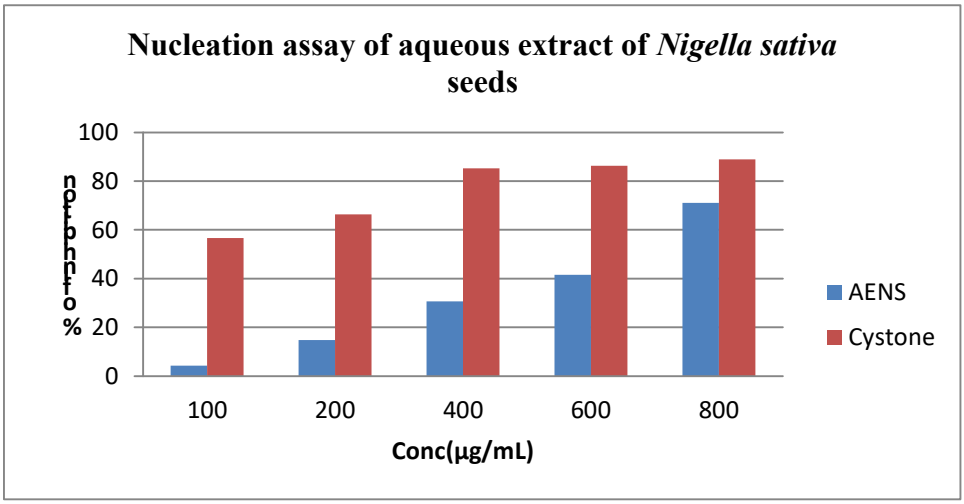
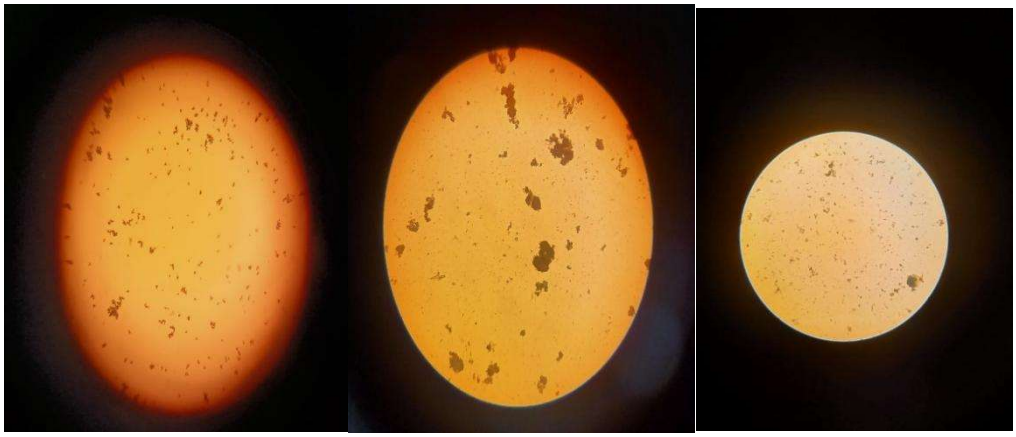
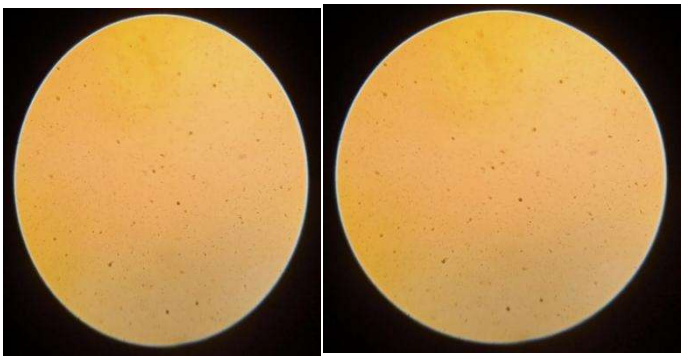


Figure 4: Effect of aqueous extract of *Nigella sativa* seeds on inhibition of calcium oxalate by nucleation assay



i) (100µg) **ii) (200µg)** **iii) (400µg)**



iv) (600µg) **v) (800µg)**

Figure 5: 100X magnification on effect of aqueous extract of *Nigella sativa* seeds on inhibition of calciumoxalate by nucleation assay

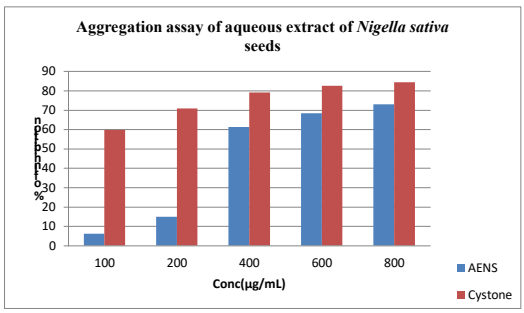


Figure 6: Effect of aqueous extract of *Nigella sativa* seeds on inhibition of calcium oxalate by aggregation assay

DISCUSSION:

From the phytochemical investigation, it was revealed that the aqueous extract of *Nigella sativa* seeds contained the presence of carbohydrates, alkaloids, steroids, glycosides, flavonoids, phenols saponins and tannins. Among all, the presence of saponins, flavonoids and phenols were of utmost significant for inhibiting urinary stone formation. Saponins possess antilithic properties and were known to disintegrate mucoproteins that are crucial components of stone matrix. Tannins and polyphenols inhibit CaOx crystal formation as well as dissolve the preformed CaOx crystals by aiding calcium complexation. Flavonoids also possess CaOx crystal dissolution potency. Phenolics and flavonoids exhibit antioxidant activity. Therefore, crystal growth defying activity of aqueous extract of *Nigella sativa* seeds would have been an outcome of these phytoconstituents present in *Nigella sativa.*

Polyphenolic compounds are the key phytochemicals with high free radical scavenging activity. It has generated a great interest among the scientists for the development of natural antioxidant compounds from plants. In the current work, phenolic and flavonid contents of the aqueous extracts of *Nigella sativa* seeds. were measured and represented in term of gallic acid and quercetin equivalents respectively.

The DPPH assay method is based on the reduction of DPPH, a stable free radical (purple colour) to the non radical form DPPH (yellow colour) in the presence of hydrogen donating antioxidants.

The degree of decolorization (yellow colour) depends on the number of electrons captured. The decolorization of the DPPH therefore reflects the radical scavenging activity of the extracts, which can be quantitatively measured at absorbance at 517nm. DPPH radical scavenging activity of aqueous extracts of *Nigella sativa* seeds was compared with ascorbic acid and was reported.

Reducing power experiment is a good reflector of antioxidant activity of the plant. The plant having high reducing power generally reported to carry high antioxidant potential too. In this experiment, Ferric ions were reduced to ferrous ions, identified by colour change from yellow to bluish green. The results for ferric reducing power activity of aqueous extract of *Nigella sativa* seeds in comparison with ascorbic acid were reported.

Urolithiasis due to CaOx is the most prevalent type of all urinary stone diseases. Significant events involved in its pathological biomineralization which includes crystal nucleation, growth and aggregation. Present

study was designed to address these key events involved in CaOx stone formation as a means to investigate the potential of aqueous extract of *Nigella sativa* seeds as an antiurolithiatic.

Nucleation is a prerequisite in the pathogenesis of CaOx urolithiasis. Nucleation basically marks a thermodynamically driven event of phase change wherein dissolved substances in a supersaturated solution spontaneously results in crystallization. Significant inhibition in the nucleation of CaOx crystals was observed in the presence of aqueous extract of *Nigella sativa*, which was as compared with that of Cystone, revealed that the aqueous extract was exhibited inhibitory property as close to cystone and suggested the anticrystallization activity of aqueous extract of *Nigella sativa* seeds against CaOx crystallization. One possible mechanism of anticrystallization activity of *Nigella sativa* seeds could be its ability to complex with free calcium and oxalate ions, thus preventing the formation of CaOx complexes.

Aggregation begins with the process where in numerous crystals in the solution come closer together and adhere, results in large crystal agglomerates. Aggregation is a key determinant of crystal retention as large crystal agglomerates are the ones that produce renal tubular obstruction thereby promoting stone formation. Aqueous extract of *Nigella sativa* seeds exhibited significant inhibitory effect on CaOx crystal aggregation.

CONCLUSION:

From the study, it was concluded that aqueous extract of *Nigella sativa* seeds was rich in phytoconstituents. Total Phenolic Content, Total Flavonoid Content and Total Alkaloid Content were found to be 95.233 mg/g , 29.46 mg/g ,153mg/g. Based on above observation it is revealed that the plant is rich in secondary metabolites and the other phytoconstituents that may have significant medicinal property to produce biological activity. The aqueous extract of *Nigella sativa* seeds proved to be the best choice for screening various pharmacological activities in order to explore medicinal profiles.

From the study, it was revealed that it has good Anti-Urolithiatic activity, which was analysed from in-vitro nucleation and aggregation assay, respectively.

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CONFLICT OF INTERESTS:

The authors declare that there is no conflict of interests regarding the publication of this paper.

REFERENCES:

[1] Valko M, LeibfritzD,Moncol J, CroninM, and Mazur M, *et al*. Free radicals and antioxidants in normal physiological functions and human disease. The International Journal of Biochemistry & Cell Biology. 2007, 39, 44-84.

[2] Sies H. Oxidative stress: Oxidants and antioxidants. Experimental Physiology. 1997, 82, 291-295.

[3] HybertsonBM, Gao B, Bose SK, and McCord JM. Oxidative stress in health and disease: the therapeutic potentialofNrf2 activation.Molecular Aspects ofMedicine. 2011, 32(4), 234–246.

[4] LiangD, Zhou Q, Gong W, Wang Y, Nie Z, He H, *et al*. Studies on the antioxidantand hepatoprotective activities of polysaccharides from*Talinumtriangulare*. Journal of Ethnopharmacology. 2011, 136(2), 316–321.

[5] Rains JL and Jain SK.Oxidativestress, insulin signaling, and diabetes.Free Radical Biology and Medicine. 2011, 50(5), 567–575.

[6] MasellaR, Di Benedetto R, Vari R, Filesi C and GiovanniniC.Novel mechanisms of natural antioxidant compounds inbiological systems: involvement of glutathione and glutathionerelated enzymes. Journal of Nutritional Biochemistry2005, 16(10), 577–586.

[7] Dai J and MumperRJ. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules. 2010, 15(10), 7313–7352.

[8] Suffness M, Douros J. Methods in drugs development. De Vita Jr and Busch H (Editors), Drugs of plant origin. New York: Academic Press; 1982.

[9] Chopra RN, Nayar SL, Chopra IC. Glossary of Indian medicinal plants. New Delhi: Council of Scientific and Industrial Research. 1956.

[10] Nadkarni K. Crocus sativus, *Nigella sativa*. In: Nadkarni KM (Editor), Indian materiamedica. Bombay: Popular Prakashan. 1976, 386- 411.

[11] Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Foline-Ciocalteu reagent. Methods of Enzymology. 1999, 299, 152-78.

[12] Chang C, Yang M, Wen H, Chern JC. Estimation of total flavonoid content

in propolis by two complementary colorimetric methods. Journal of Food DrugAnalysis 2002, 10,178-82.

[13] Ilahi I, Samar S, Khan I, Ahmad I. In vitro antioxidant activities of four medicinalplants on the basis of DPPH free radical scavenging. Pakistan journal of Pharmaceutical sciences2013, 26, 949-952.

[14] OyaizuM. Studies on products of browning reactions: Antioxidant activity of products of browning reaction prepared from glucosamine. Japan Journal of Nutrition 1986, 44, 307-315.

[15] Prieto P, Pineda M, Aguilar M. Spectrophotometric Quantitation of Antioxidant Capacity through the Formation of a Phosphomolybdenum Complex: Specific Application to the Determination of Vitamin E. Anal Biochem 1999, 269,337-341.

[16] Nayak BS, Anderson M, Pinto LM. Evaluation of wound-healing potential of *Catharanthus roseus* leaf extract in rats. Fitoterapia 2007, 78, 540-544.