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Comparative Study On The Phytochemical Analysis And Antioxidant Assessment Of Medicinal Plants Used To Treat Urolithiasis

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Abstract:

The growth of solid crystalline forms, or stones, in the urinary tract is referred to as urolithiasis, also known by the name's kidney stones and urinary calculi. Several plants that are believed to have medicinal properties have been used in traditional medicine to treat various conditions. Many cultures have employed these medicines because of their ability to prevent, treat, or decrease kidney stone symptoms. When plants are used to treat urolithiasis, they often cure multiple aspects of the condition, including diuresis (increased urine production), prevention of crystal formation, and assistance in dissolving or removing existing stones. While anecdotal evidence and conventional wisdom may provide support to their use, thorough scientific investigation is necessary to validate them and ascertain their safety and effectiveness. Based on the results of the ethnobotanical survey, *Abutilon indicum* (L.) Sweet, *Ouret*lanata (L.) Kuntze, and *Tribulus terrestris* L. were selected for the current study with this objective in mind. Phenols were detected in all samples after a phytochemical screening of the leaves of *Tribulus terrestris* L., *Ouret*lanata (L.) Kuntze, and *Abutilon indicum* (L.). Methanolic extracts showed the highest concentration of phenols. Naturally occurring antioxidants such tannin, total phenols, flavanoids, vitamin C, and vitamin E were also investigated. *Tribulus terre*stris had higher antioxidant levels than *Ouret*lanata and *Abutilon indicum*. These plants had substantially higher levels of flavonoids than other antioxidants because they have a better capacity to catalyse urolithiasis.

Keywords: Urolithiasis, Flavonoid, phytochemical, calculi, antioxidant

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Introduction:

Calculi production or deposition in the urinary tract is known as urolithiasis, and it is the third most frequent condition in the world, affecting approximately 12% of the population(Sharma *et al*., 2016; Khan, 2013).Kidney stone production, or urolithiasis, is a widespread condition and the third most prevalent disease among urinary disorders(Bahmani *et al*., 2016), with no guarantee of effective treatment and a high recurrence rate of 1%–5% in Asia (İlhan *et al*., 2014; Premgamone *et al.*, 2009). Urine volume, pH, elevated calcium or sodium oxalate, and urates are among the several biological activities that aid in the physiochemical event that leads to crystal nucleation, aggregation, and development that results in calcified kidney stone formation (Ratkalkar and Kleinman, 2011; Khan *et al.*, 2016). Reports state that 10%–12% of individuals in affluent nations—3% of women and 10% of men—will experience a urinary stone at some point in their lives. Genetics, nutrition, and inactivity are some of the multiple aetiologies of this condition (Gindi *et al*., 2013). Kidney stones are mostly caused by the crystallisation, nucleation, and development of insoluble particles such as calcium, magnesium ammonium phosphates, uric acid, and cystine, while the exact mechanisms underlying their formation are unknown(Sun *et al*., 2015; Ratkalkar and Kleinman, 2011).

This disease has a complex aetiology, however dietary lifestyle choices and behaviours have a strong correlation with it (Taylor *et al*., 2005). While some studies have shown that medicinal interventions can improve the removal of calculi in the distal ureters (Dellabella *et al.*, 2005), no effective antiurolithiatic agent has been identified for this purpose, particularly in terms of preventing the recurrence of stones. Numerous plants have been traditionally used to treat kidney calculi, and their efficacy has been demonstrated. Numerous plants are utilised in Ayurveda and traditional medicine to treat urolithiasis. Numerous scholars worldwide are investigating the possibility of using herbs to cure this illness (Patil *et al*., 2010).

With this goal in mind, *Abutilon indicum* (L.)*Ouret lanata* (L.) Kuntze, and *Tribulus terrestris* (L.) were chosen for the current study based on the ethnobotanical survey. To perform qualitative analysis of phytochemical in various extracts of selected plants.To evaluate the antioxidant components of leaf samples of selected plats being used in treating kidney stones.

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Materials and Methods

The fresh plants of *Abutilon indicum*,*Ouret lanata* and *Tribulus terrestris* were collected from the Korampallam village, Thoothukudi district.The leaves were washed with water to remove contaminants and shade dried. The coarse powder (100g) will be extracted successively with petroleum ether, chloroform, methanol, ethanol and water each 250 ml in a closed flask for 24 hrs. All the extracts were filtered through Whatman No. 41 filter paper. All the extracts (petroleum ether, chloroform, methanol, ethanol and water) were stored for further testing.

Phytochemical screening:

Test for alkaloids- Mayer's test

To 1 ml of extract, 2 ml of Mayer's reagent was added; a dull white precipitate reveals the presence of alkaloids.

Test for Terpenoids- Noller's test

To 1 ml extract with tin (one bid) and thionyl chloride (1ml) were added. Appearance of pink colour indicates the presence of terpenoids.

Test for steroids- Limbermann Burchard's test

The powder was dissolved in two ml of chloroform in a dry test tube. Ten drops of acetic anhydride and two drops of concentrated sulphuric acid were added. The solution became red, then blue and finally bluish green, indicates the presence of steroid.

Test for Coumarin

To 1 ml of extract, 1 ml of 10% sodium hydroxide as added. The presence of coumarin is indicated by the formation of yellow colour.

Test for Tannin

The test solution was mixed with basic lead acetate solution. Formation of a white precipitate indicates the presence of tannins.

Test for Saponin

The test solution was shaken with water. Copious lather formation indicates the presence of saponin.

Test for Flavones (Shinadow's test)

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To a few mg of the powder, magnesium turnings and 1-2 drops of concentrated hydrochloric acid were added. Formation of red colour shows the presence of flavonoids.

Test for Anthraquinones (Borntrager's test)

The powder/extract was macerated with ether and after filtration; aqueous ammonia or caustic soda was added. Pink red or violet in the aqueous layer after shaking indicates the presence of anthraquinone.

Test for Phenols

To 1 ml of the extract, 2 ml of distilled water was added followed by few drops of 10% aqueous ferric chloride. Appearance of blue or green colour indicates the presence of phenols.

Test for Quinone

To 1 ml of extract, 1ml of con.H_2SO_4 was added. Appearance of red indicates the presence of quinone.

Quantitative analysis of antioxidant: -

Total phenolic content: (Singleton*et al.*, 1974).

The amount of total phenolics was determined using Folin – Ciocalteau reagent (Singleton et al., 1974). To 0.5 ml of the sample, 0.5 ml H_20, 2ml Folin – ciocalteau reagent (1.5 H_2O) was added, after 3 min. 10 ml of 10% (w/v) Na_2Co_3 and the contents were mixed and allowed to stand for 30 min. Absorbance at 725nm was measured in a UV – via Spectrophotometer. The amount of total phenolics was calculated as gallic acid equivalent (GAE) in mg per g of fresh weight (FW).

Total flavonoid content: (Chang*et al.*, 2002).

The total flavanoid content (TFC) of each extract was ainvestigated using the aluminium chloride colorimetry method described by chang et al., (2002) with slight modifications. In brief, the extract sample was diluted with methanol until 100µg/ml. The calibration curve was prepared by diluting quercetin (2.0 ml) was mixed with 0.1 ml of 10% (w/v) aluminium chloride solution and 0.1 ml of 0.1 mm potassium acetate solution. The mixture was kept at room temperature for 30 minutes. Then the maximum absorbance of the mixture was measured at 15nm using a UV-

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Vis Spectrophotometer. TFC was expressed as milligram quercetin equivalent per gram (mg QCE/g DFLA).

Vitamin C [Ascorbic acid] (Hickey, 2005):

100 mg of plant material was homogenized with 10 ml of 5% Trichloro acetic acid (TCA). The homogenate was centrifuged. To 2ml of indophenols reagent and 0.5 ml of DT reagent was added and incubated at 10^0Cfor 1 hour and then cooled in ice bath and 2.5ml of 85% sulphuric acid was added and shaken well for 30 minutes (until) red colour appeared. The absorbance was measured at 540 nm. Ascorbic acid was used as standard and the results were expressed as mg /g Fw.

Estimation of Tannin (Sun *et al*., 1998)

Condensed tannis (Proanthocyanidins) were determined according to the method of Sun et al.(1998). To 50 ml of diluted sample, 3ml 0f 4% vanillin solution in methanol and 1.5 ml of concentrated HCL were added. The mixture was allowed to stand for 15 min and absorption was measured at 500 nm against methanol as a blank. The amount of total condensed tannis is expressed as mg $(+)^-$ catechin /g DW. All samples were analysed in triplicane.

Vitamin E (Tocopherol) (Burton *et al*., 1983)

The plant sample (2.5 g) was homogenized in 50 ml of 0.1 N sulphuric acid and allowed to stand overnight the content in the flask was shaken vigorously and filtered through what manNo:1 filter paper. Aliquot of the filterate was used for estimation.In Stoppard centrifuge tubes 3ml of extract and 3ml of water were pipette out separately. To both the tubes, 3ml of ethanol and 3 ml of xylene were added, mixed well and centrifuged. Xylene (2.0ml) layer was transferred into another Stoppard tube. To each tube, 2.0 ml of dipyridyl reagent was added and mixed well, the mixture (3ml) was pipette out into a cuvette and the extinction was read at 460 nm. Fercic chloride solution (0.66ml) was added to all the tubes and mixed well. The red colour developed was read exactly after 15 minutes at 520nm. Tocopherol was used as standard.

Tocopherol (ug) = $\frac{\text{Sample A520} - \text{A460}*0.29*0.15}{\text{Standard A520}}$

Result and Discussion

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The plants include a variety of secondary metabolites, including tannins, steroids, terpenoids, coumarins, alkaloids, flavones, anthroquinones, phenols, saponins, and quinones, according to preliminary results of a phytochemical screening that are shown in Table 1. Specifically, phenols were found in all samples and were particularly noticeable in the methanolic extracts.

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It was also noted that all of the plant extracts used in this investigation included tannins. Tiwari *et al.* 2011 reported that this particular collection of metabolites exhibited antibacterial, antidiarrheal, and anthehelmintic effects. Remarkably, quinone proved negative for every plant extract examined. One possible explanation is that the solvent did not work well to extract the quinone molecule. In support of this was Leksawasdi *et al.* 2008.

Table 1: Preliminary Phytochemical screening of selected plant samples

Bioactive compounds	*Abutilon indcium*					*Aerva lanata*					*Tribulus terrestris*				
	Petroleum Ether	Water	Ethanol	Methanol	Chloroform	Petroleum Ether	Water	Ethanol	Methanol	Chloroform	Petroleum Ether	Water	Ethanol	Methanol	Chloroform
Tannins	–	–	+	+	+	+	+	+	+	–	–	–	+	+	+
Steroids	+	+	–	–	–	–	+	+	–	+	+	+	–	–	–
Terpenoids	+	+	+	–	–	+	+	+	+	+	+	+	+	–	–
Coumarins	+	+	–	–	+	–	+	+	–	–	+	+	–	–	+
Alkaloids	–	–	–	–	+	–	+	+	+	–	–	–	–	–	+
Flavones	–	–	–	+	+	–	+	+	+	+	–	–	–	+	+

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Anthroquinones	+	+	+	+	–	+	–	–	–	–	+	+	+	+	–
Phenols	–	–	+	+	+	+	–	–	–	+	–	–	+	+	–
Saponins	–	–	–	–	–	–	+	+	+	–	–	–	–	–	–
Quinones	–	–	–	–	+	–	–	–	–	–	–	–	–	–	+

(+) indicates presence and (-) indicates absence

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Total Phenol content:

Table 2 displays the total phenol concentration of three distinct plant extracts made using five different solvents. The results indicate that out of the five different solvents, the methanol solvent has the highest phenol content (1.87 ± 7.26^{c}, 2.31 ± 0.028^{e}, and 2.42 ± 0.028^{e}, respectively) for *Abutilon indicum*, *Ouret lanata*, and *Tribuls terrestris*. The chloroform extracts of the same extract come in second with 1.75 ± 3.27c, 1.88 ± 0.032d, and 1.95 ± 0.028d. *Tribulus terrestris* has a higher phenol content out of the three plants that were used.

Phenolic compound isolated from ethyl acetate fraction of the leaves, demonstrated higher dissolution of stones. It was more effective in calcium phosphate stones (67.74%) than oxalate stone (Vivek V, Byahatti *et al*., 2010). Phenolics compounds are known to be a powerful chain breaking antioxidant, they possess scavenging ability due to their hydroxyl groups (Hatano *et al*., 1989).

Table 2: Total Phenol content of different solvents of selected plants

Different extracts	*Abutilon indicum*	*Ouret lanata*	*Tribulus terrestris*
Ethanol	1.21 ± 6.25c	1.28 ± 0.018^{c}	1.3 ± 0.032^{c}
Methanol	1.87 ± 7.26c	2.31 ± 0.028^{e}	2.42 ± 0.028^{e}
Chloroform	1.75 ± 3.27c	1.88 ± 0.032^{d}	1.95 ± 0.028^{d}
Petroleum	0.32 ± 3.47a	0.96 ± 0.014^{a}	1.03 ± 0.018^{a}
Water	1.10 ± 3.45a	1.04 ± 0.028^{b}	1.10 ± 0.018^{b}

Values are expressed as the mean ± SD; statistical significance (p) calculated by one way ANOVA followed by Duncan's Range test

Total Flavanoid content:

Table 3 displays the total flavonoid content of three distinct plant extracts made using five different solvents. Out of the five distinct solvents, the methanol solvent has the highest flavonoid content, measuring 167.4 ± 2.53e, 2.207 ± 2.53e, and 209.7 ± 2.53e for *Abutilon indicum*, *Ouret lanata*, and *Tribuls terrestris*, respectively. The chloroform extracts of the same

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extracts come in second and third, with 126.0 ± 1.46d, 134.3 ± 1.46d, and 149.8 ± 1.46d, respectively. The plant Tribulus terrestris has a higher flavonoid concentration than the other two.

Flavonoid is one of the main groups of phenolic compounds and widely distributed flavonoid, flavones and flavonols. Many flavonoids and related compounds are reported to possess strong antioxidative characteristics (Dziedzic and Hudson 1983).

Table 3: Total Flavanoid content of different solvents of selected plants

Different extracts	*Abutilon indicum*	*Aerva lanata*	*Tribulus terrestris*
Ethanol	107.4± 1.46^{c}	112.6 ± 1.46^{c}	118.8 ± 1.46^{c}
Methanol	167.4± 2.53^{e}	207.7 ± 2.53^{e}	209.7 ± 2.53^{e}
Chloroform	126.0± 1.46^{d}	134.3 ± 1.46^{d}	149.8 ± 1.46^{d}
Petroleum	57.8± 1.46^{a}	66.1 ± 1.46^{a}	73.3 ± 1.46^{a}
Water	68.2± 2.53^{b}	97.1 ± 1.46^{b}	100.2 ± 1.46^{b}

Values are expressed as the mean ± SD; statistical significance (p) calculated by one way ANOVA followed by Duncan's Range test

Total tannin content:

Table 4 displays the total tannin content of three separate plant extracts made using five different solvents. Out of the five distinct solvents, the methanol solvent has the highest tannin content, measuring 48.4 ± 0.42e, 48.1±0.73e and 50.8 ± 0.77e for *Abutilon indicum*, *Ouret lanata*, and *Tribuls terrestris*, respectively. The chloroform extracts of the same specimens come in second and third, with 34.8 ± 0.42d, 33.9 ± 0.42d, and 37.5 ± 0.42d, respectively. *Tribulus terrestris* has a higher tannin concentration than the other two plants that were employed.

Tannins are astringer, bitter plant polyphenols that either bind and precipitate or shrink proteins and various other organic compounds including amino acids and alkaloids. Tannins have shown potential antiviral, antibacterial and antiparasitic effects (Kolodziej and Kiderlen 2005).Tannin belong to the phenolic class. All phenolics compounds are in one way or another formed via shikimic acid pathway also known as phenyl propanoid pathway. Previous studies

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revealed that tannin phytochemicals in blueberries may help to prevent the development of a particular type of kidney stone known as struvite stone. Research has linked struvite stones to chronic infection of then urinary tract, and tannin have been shown to protect against urinary treat infections(Whole Health md, 2014).

Table 4: Total tannin content of different solvents of selected plants

Different extracts	*Abutilon indicum*	*Aerva lanata*	*Tribulus terrestris*
Ethanol	25.1± 0.42^{c}	24.5 ± 0.73^{c}	27.5 ± 0.42^{c}
Methanol	48.4± 0.42^{e}	48.1 ± 0.73^{e}	50.8 ± 0.77^{e}
Chloroform	34.8± 0.42^{d}	33.9 ± 0.42^{d}	37.5 ± 0.42^{d}
Petroleum	16.6± 0.42^{a}	16.9 ± 0.42^{a}	18.4 ± 0.42^{a}
Water	23± 0.42^{b}	23 ± 0.42^{b}	24.5 ± 0.73^{b}

Values are expressed as the mean ± SD; statistical significance (p) calculated by one way ANOVA followed by Duncan's Range test

Total vitamin C content:

Table 5 displays the total vitamin C content of three distinct plant extracts made using five different solvents. Out of the five distinct solvents, the methanol solvent has the highest vitamin C content (14.6 ± 0.09e, 14.6 ± 0.09e, and 15.4 ± 0.09e, respectively) for *Abutilon indicum*, *Ouret lanata*, and *Tribuls terrestris*. The chloroform extracts of the same solvent have the next highest vitamin C content (10.2 ± 0.09e, 10.2 ± 0.09e, and 10.7 ± 0.09e). *Tribulus terrestris* is the plant chosen that has the highest vitamin C content out of the three.

Vitamin C also known as L-ascorbic acid is water – soluble vitamin, Human, unlike most animals, are unable to synthesize vitamin C endogenously. Vitamin C is a co factor in enzymatic reaction and antioxidant against oxidative stress. However high-dose of vitamin C is linked to kidney stones in men. Research also established that ascorbate in low or high doses generally do not cause significant increase in urinary oxalate. Ascorbate tends to prevent formation of calcium oxalate kidney stones (Hickey, 2008).

Table 5: Total Vitamin C content of different solvents of selected plants

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Different extracts	*Abutilon indicum*	*Aerva lanata*	*Tribulus terrestris*
Ethanol	7.5± 0.09^{c}	7.5 ± 0.09^{c}	8.1 ± 0.09^{c}
Methanol	14.6± 0.09^{e}	14.6 ± 0.09^{e}	15.4 ± 0.09^{d}
Chloroform	10.2± 0.09^{d}	10.2 ± 0.09^{d}	10.7 ± 0.09^{e}
Petroleum	4.2± 0.09^{a}	4.2 ± 0.09^{a}	4.7 ± 0.09^{a}
Water	6.5± 0.09^{b}	6.5 ± 0.09^{b}	7.1 ± 0.09^{b}

Values are expressed as the mean ± SD; statistical significance (p) calculated by one way ANOVA followed by Duncan's Range test

Total vitamin E content

The total Vitamin E content of three different plants extracts obtains from 5 different solvents is shown in Table 6. Among the 5 different solvents the Vitamin E content is observed to be more in the methanol solvent with 0.009, 0.009 and 0.01 respectively for *Abutilon indicum, ouret lanata and Tribuls terrestris* which is then followed by the chloroform extracts of the same with0.005, 0.007 and 0.005. Among the three plants used *Tribulus terrestris* have more vitamin E content.

Vitamin E is a potent chain-breaking antioxidant that inhibits the production of reactive oxygen species molecules when fat undergoes oxidation and during the propagation of free radical reactions (Burton *et al*., 1983).It has numerous important roles with in the body because of its antioxidant activity. Oxidation has been linked to numerous possible conditions and diseases, including cancer, ageing, arthritis and cataracts; vitamin E has been shown to be effective against these (Saliha Rizvi *et al*., 2013).

Table 6: Total vitamin E content of different solvents of selected plants

Different extracts	*Abutilon indicum*	*Aerva lanata*	*Tribulus terrestris*
Ethanol	0.005± 0^{a}	0.005 ± 0	0.001± 0.0004^{a}

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Methanol	0.009± 0.0004[a]	0.009 ± 0	0.01 ± 0[e]
Chloroform	0.005± 0[a]	0.007 ± 0	0.005 ± 0[d]
Petroleum	0.002± 0[a]	0.001 ± 0	0.002 ± 0[b]
Water	0.004± 0[a]	0.005 ± 0	0.004 ± 0.0004[c]

Conclusion

The presence of phenols, flavones, and tannin contents was revealed by the qualitative examination of the samples, particularly in the polar solvents. The methanol solvent showed higher antioxidant content than the other four solvent extracts out of the five. *Tribulus terrestris* showed higher antioxidant levels than the other plants. We can conclude that the antioxidant content of plants aids in their antimicrobial properties and may be used to treat urolithiasis.

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Conflict of interest

None

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