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In vitro XANTHINE OXIDASE INHIBITORY ACTIVITY OF SELECTED MEDICINAL PLANTS

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Abstract: Gout is a painful disease and its prevalence has increased in recent years. This metabolic disorder arises when uric acid is deposited in the capillary vessels of the joints, which leads to inflammation and sharp pain. The enzyme xanthine oxidase (XO) plays a very important role which catalyses the metabolism of hypoxanthine to xanthine and then xanthine to uric acid. The inhibitory effect of the extracts of leaves and seeds of some medicinal plants on XO was measured spectrophotometrically. The plant parts used were leaves of *Azadirachta indica, Catharanthus roseus, Citrus limon, Cymbopogon citratus, Nyctanthus arbour-tristis, Psidium guajava, Tinospora cordifolia* and seeds of *Apium graveolens*, *Salvia hispanica, Trigonella foenum-graecum.* Allopurinol, a well known drug to treat gout has been used as positive control. The drug prevents the formation of uric acid by inhibiting XO. The medicinal plants are the source of traditional life saving drugs with minimal side effects. Results of the study indicated that two potential medicinal plants *Apium graveolens* and *Citrus limon* may be useful for the treatment of gout.
Key words: Allopurinol, gout, medicinal plants, xanthine oxidase

Introduction

Gout is an inflammatory arthritis associated with hyperuricemia. The disease is triggered by the crystallization of uric acid within the joints. Hyperuricemia is a pathological state that arises from the overproduction or under excretion of uric acid. This state is characterized as increased serum urate level of more than 7.0 mg/dL for men and more than 5.7 mg/dL for women (Zhu et al., 2014). As a result, the insoluble uric acid forms microscopic crystals in the capillary vessels of the joints. These crystals cause inflammation and sharp pain, which is termed as acute gouty arthritis or acute gout. Gout is a painful disease which can lead to decrease the quality of life and its prevalence has increased in recent years (Kuo et al., 2015)

Enzyme xanthine oxidase (XO) is known to be associated with the development of gout where uric acid is produced as the end product of purine metabolism (Wong et al., 2014). XO catalyses the conversion of hypoxanthine to xanthine, and then xanthine to uric acid (Johnson et al., 2001). Abnormalities associated with this pathway of purine metabolism leads to increased concentration of uric acid. Compounds that would inhibit XO may be potentially

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useful for the treatment of gout or other XO enzyme induced diseases (Chakravarthi and Selvaraju, 2017). Allopurinol is one such drug that is being widely used to treat hyperuricemia and gout (Singer and Wallace, 1986; Emmerson, 1996). It works by preventing the formation of uric acid through the inhibition of the enzyme XO. Allopurinol is the only clinically used XO inhibitor (XOI). Using allopurinol has many side effects including skin rashes, headaches, feeling drowsy or dizzy, feeling or being sick and can change the sense of taste. It also induces serious side effects such as renal failure, impaired liver function and allergic reactions. Hence, there is a need to look for new xanthine oxidase inhibitors. (Argulla and Chichioco-Hernandez, 2014). Thus, alternative treatment of the gout with therapeutics of lesser side effects derived from medicinal plants has drawn the attention of researchers. Several medicinal plants growing in India, Philippine, Vietnam and North-Eastern America are known to possess XO inhibition activity (Owen and Johnson, 199; Umamaheswari et al., 2007).

Today, there is a renewal interest in traditional medicines and an increasing demand for more drugs from plant sources. Using green medicine is safe and cost effective as compared to synthetic drugs, many of which have adverse side effects (Sharma and Patel, 2013). These disadvantages of synthetic drug consumption have drawn the attention of researchers to explore for new alternatives to lower the uric acid with minimal side effects. Thus, the present study has been undertaken to evaluate *in vitro* XO inhibitory activity potential of ten different medicinal plant extracts.

2. Material and methods

2.1 Collection of medicinal plants

Full grown fresh leaves of *Azadirachta indica* (Neem), *Cymbopogon citrates* (Lemongrass), *Citrus limon* (Lemon), *Catharanthus roseus* (Periwinkle), *Nyctanthus arbour-tristis* (Night jasmine), *Psidium guajava* (Guava), *Tinospora cordifolia* (Giloy) were collected from local areas and seeds of *Apium graveolens* (Celery), *Salvia hispanica* (Chia), *Trigonella foenum graecum* (Fenugreek) were purchased from the local market of Chhattisgarh.

2.2 Preparation of plant extracts

The leaves were washed properly with tap water initially and then twice with double distilled water to remove the traces of dirt. The washed leaves weighed were ground in a blender with addition of water. The slurry so obtained was filtered. The filtrate was boiled for 10 min and then cooled. The extract was again filtered using Whatman number 1 filter paper to remove particulate matters. The filtrate was concentrated in a hot air oven. The seeds of the plants

were finely powdered using a grinder. The powdered seeds weighed and were mixed with absolute methanol and stirred for one hour using a shaker at room temperature. The extract was then subjected to filtration using Whatman number 1 filter paper and was concentrated in a hot air oven.

2.3 *In vitro* xanthine oxidase inhibition assay

The inhibitory effect of leaf and seed extracts on XO was measured spectrophotometrically at 295 nm following the method reported by Uno et al., 2004. A well-known XO inhibitor, allopurinol (100 µg/mL) was used as positive control for the inhibition test. The plant extracts were dissolved in 1% dimethylsulfoxide (DMSO) and made into dilution to obtain final concentrations of 100 µg/mL. The reaction mixture consisted of 300 µL of 50 mM sodium phosphate buffer (pH 7.5), 100 µL of sample solution diluted in DMSO, 100 µL of freshly prepared enzyme solution (0.2 unit/mL of XO in phosphate buffer) and 100 µL of distilled water. The assay mixture was pre-incubated at 37 ^{o}C for 15 min. Then, 200 µL of substrate solution (0.15 mM of xanthine) was added into the mixture. The mixture was incubated at 37 ^{o}C for 30 min. The reaction was then stopped with the addition of 200 µL of 0.5 M HCl. The absorbance of the reaction mixture was measured at 295 nm using a UV-Visible spectrophotometer (ELICO, SL20). The measurement was taken against a blank prepared in the same way but the enzyme solution being replaced with the phosphate buffer. Another reaction mixture was prepared (control) having 100 µL of DMSO instead of test compounds in order to have maximum uric acid formation (Ummaheshwari et al., 2007). The degree of XO inhibitory activity was evaluated using the formula: I % = [$\boldsymbol{A}_{\boldsymbol{control}} - \boldsymbol{A}_{\boldsymbol{sample}}$]/$\boldsymbol{A}_{\boldsymbol{control}}$*100. Where, I % = inhibition percentage, $\boldsymbol{A}_{\boldsymbol{control}}$ = absorbance of control and $\boldsymbol{A}_{\boldsymbol{sample}}$ = absorbance of sample at 295 nm.

2.4 Statistical analysis

All the data presented in this study are the arithmetic mean of at least three independent experiments along with the standard deviation (±SD). Mean and standard deviations have been calculated using MS office 2007.

3. Results

The distilled water and methanol extracts of leaves and seeds of the above-mentioned plants were evaluated for their XO inhibitory activity. The extracts of *Apium graveolens* seeds exhibited 63.6% inhibition, the highest inhibition as compared to the rest of the plant extracts studied in the present work. The leaf extract of *Citrus limon* and *Cymbopogon citrates* showed 54.2% and 44.3% inhibition activity respectively. Of all the extracts, the seed

extracts of *Trigonella foenum graecum* was found to possess least inhibition of XO activity (2.7%). The inhibition activity was found to be absent with seed extracts of *Salvia hispanica*. Allopurinol, the standard drug that is being widely used for treatment of gout has shown about 83.9% XO enzyme inhibition activity. The percentage inhibition of XO by leaf and seed extracts of different medicinal plants has been shown in Table 3.1. The inhibition percentage of different extracts compared to allopurinol is shown in Fig. 3.1.

Table 3.1: Inhibition of XO by leaf and seed extracts of different medicinal plants. Concentration of XO used for the assay was 100 µg/mL.

Name of plant	**Common name**	**Plant parts used**	**Solvent preferred**	**% inhibition of XO (Mean ± SD.)**
Apium graveolens	Celery	Seeds	Methanol	63.6±0.4
Azadirachta indica	Neem	Leaves	Water	10.1±0.8
Catharanthus roseus	Periwinkle,	Leaves	Water	30.6±0.2
Citrus limon	Lemon	Leaves	Water	54.2±0.4
Cymbopogon citratus	Lemongrass	Leaves	Water	44.3±1.6
Nyctanthus arbour-tristis	Night jasmine	Leaves	Water	34.6±0.1
Psidium guajava	Guava	Leaves	Water	18.5±0.3
Salvia hispanica	Chia	Seeds	Methanol	---
Tinospora cordifolia	Giloy	Leaves	Water	24.7±0.8
Trigonella foenum graecum	Fenugreek	Seeds	Methanol	2.7±0.2
Allopurinol	Allopurinol	---	Water	83.9±0.2

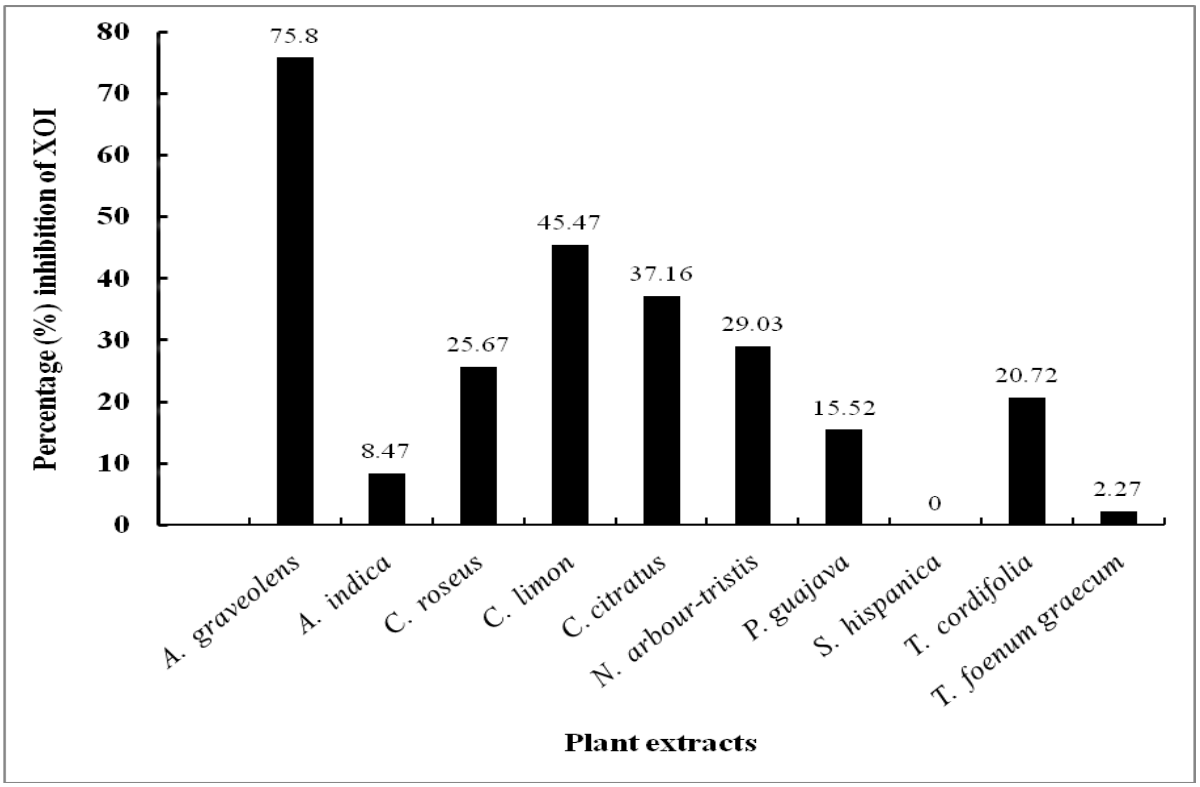

Fig. 3.1 The inhibition percentage of different plant extracts compared to allopurinol. The percentage inhibition of XO by allopurinol (100 µg/mL) was considered as 100%.

Discussion

The present study was carried out using different plant extracts namely *Apium graveolens, Azadirachta indica, Catharanthus roseus, Citrus limon, Cymbopogon citratus, Nyctanthus arbour-tristis, Psidium guajava, Salvia hispanica, Tinospora cordifolia* and *Trigonella foenum-graecum*, extracted in two different solvents: water and methanol. The plant parts used are leaves and seeds. The study was undertaken with an objective to examine the potential of these plants for their inhibitory activity against the enzyme called XO.

Out of the ten plant extracts examined for XO inhibition assay, nine extracts demonstrated XOI activity at 100 µg/mL. Two of these extracts showed an inhibition greater than 50%. The result was compared with a positive control drug allopurinol which showed the highest activity. Allopurinol showed 83.9% of inhibition in the present study. Ummamaheshwari et al, 2007 have reported 82% of inhibition by allopurinol at 50 µg/mL concentration, whereas, at 100 µg/mL, the study showed 93.2% of inhibition. Maximum number of findings establish the fact that allopurinol shows inhibition of less than 85% for the treatment of gout. Among the plant extracts used in the study, the seed extract of *Apium graveolens* and leave extract of *Citrus limon* showed XO inhibition of 63.6% and 54.2% respectively. Rahman et al, (2015)

have observed 73.8% inhibition by ethanol extract of *A. graveolens*. 43.22%. Inhibition of XO by leaves of *Citrus limon* at 50 µg/mL have been noticed by Muthiah (2012).

Apart from the *Apium graveolens* and *Citrus limon* plant extracts which showed more than 50% XO inhibition, the remaining plant extracts showed inhibition of less than 50%. The seed extracts of *Salvia hispanica* showed no XO inhibition activity. *Azadirachta indica* leaf extract showed 10.15% inhibition. In the present study, the leaves of *Catharanthus roseus* showed 30.6% of inhibition. Rini et al, 2016 have reported 50.27% of XO inhibition at 100 µg/mL. The leaves of *Cymbopogon citratus* showed 44.3% of inhibition in the present study whereas previous studies have suggested more than 50% inhibition by the plant (Mirghani et al., 2012). The leaves of *Nyctanthus arbour-tristis* showed 34.6% of inhibition. Valentina et al, (2016) have reported 45.6% inhibition in the same plant at 50 µg/mL concentration. *Psidium guajava* plant extract showed 18.5% inhibition because of abundance of polyphenols present in the plant extract. The leaves of *Tinospora cordifolia* showed 24.7% inhibition whereas previous findings have suggested 25-30% inhibition of XOI. The seeds of *Trigonella foenum-graecum* showed very less inhibition of 2.7%, giving insignificant result.

The variation XO inhibition of the same plant extracts in different studies might be due to the fact that inhibition of different plant extracts might be affected by the environmental condition, locality and physiological factors influencing the growth of the plants which in turn collectively affects the phytochemical properties of the plant extracts both in positive and negative way. However, an extensive study pertaining to XO inhibition by different plant extracts is needed to establish the findings.

In conclusion, the study indicates two potential medicinal plants, *Apium graveolens* and *Citrus limon* may be useful for the treatment of hyperuricemia and gout. This provides the basis for further investigation on these plants to isolate active compounds and drug development.

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