

EXPLORING THE INHIBITORY EFFECT OF MEDICINAL PLANT EXTRACTS ON XO: A PROMISING SOURCE OF LIFE-SAVING DRUGS

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Abstract: Gout is a metabolic disorder that results from the deposition of uric acid in joint vessels, causing sharp pain and inflammation. The enzyme xanthine oxidase (XO) plays a key role in this process. This study aimed to evaluate the inhibitory effect of extracts from leaves and seeds of ten selected medicinal plants on XO, using spectrophotometry to measure the percentage of inhibition. The medicinal plants were *Azadirachta indica*, *Catharanthus roseus*, *Citrus limon*, *Cymbopogon citratus*, *Nyctanthus arbour-tristis*, *Psidium guajava*, *Tinospora cordifolia*, and seeds of *Apium graveolens*, *Salvia hispanica*, and *Trigonella foenum-graecum*. The results showed that *Apium graveolens* and *Citrus limon* have potential as alternative treatments for gout. Allopurinol, a drug used to treat gout, was used as the positive control. This study provides evidence for plant extracts from traditional medicines as a potential source of life-saving drugs with minimal side effects.

Keywords: Gout, medicinal plants, xanthine oxidase, *Apium graveolens*, *Citrus limon*.

Introduction

There has been an increasing demand for drugs from plant sources due to the adverse side effects of synthetic drugs. This has resulted in a renewed interest in traditional medicines, which are often safe and cost-effective. The present study aimed to evaluate the potential of medicinal plants as alternative treatments for gout, a painful metabolic disorder resulting from the deposition of uric acid in joint vessels. The enzyme xanthine oxidase plays a crucial role in the process and catalyses the metabolism of hypoxanthine to xanthine and then to uric acid. Allopurinol is a well-known drug used to treat gout by inhibiting XO and preventing the formation of uric acid. However, its adverse side effects have highlighted the need for alternative treatments with minimal side effects. Ten medicinal plants were selected for the study, and their extracts were evaluated for their inhibitory effect on XO using spectrophotometry to determine the percentage of inhibition. This study provides evidence for the potential of plant extracts from traditional medicines as a source of life-saving drugs with minimal side effects, which could help reduce the burden of gout worldwide.

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 °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 °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: $I \% = [A_{control} - A_{sample}] / A_{control} * 100$. Where, I % = inhibition percentage, $A_{control}$ = absorbance of control and A_{sample} = absorbance of sample at 295 nm.

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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.)
<i>Apium graveolens</i>	Celery	Seeds	Methanol	63.6±0.4
<i>Azadirachta indica</i>	Neem	Leaves	Water	10.1±0.8
<i>Catharanthus roseus</i>	Periwinkle,	Leaves	Water	30.6±0.2
<i>Citrus limon</i>	Lemon	Leaves	Water	54.2±0.4
<i>Cymbopogon citratus</i>	Lemongrass	Leaves	Water	44.3±1.6
<i>Nyctanthus arbour-tristis</i>	Night jasmine	Leaves	Water	34.6±0.1
<i>Psidium guajava</i>	Guava	Leaves	Water	18.5±0.3
<i>Salvia hispanica</i>	Chia	Seeds	Methanol	---
<i>Tinospora cordifolia</i>	Giloy	Leaves	Water	24.7±0.8
<i>Trigonella foenum graecum</i>	Fenugreek	Seeds	Methanol	2.7±0.2
Allopurinol	Allopurinol	---	Water	83.9±0.2

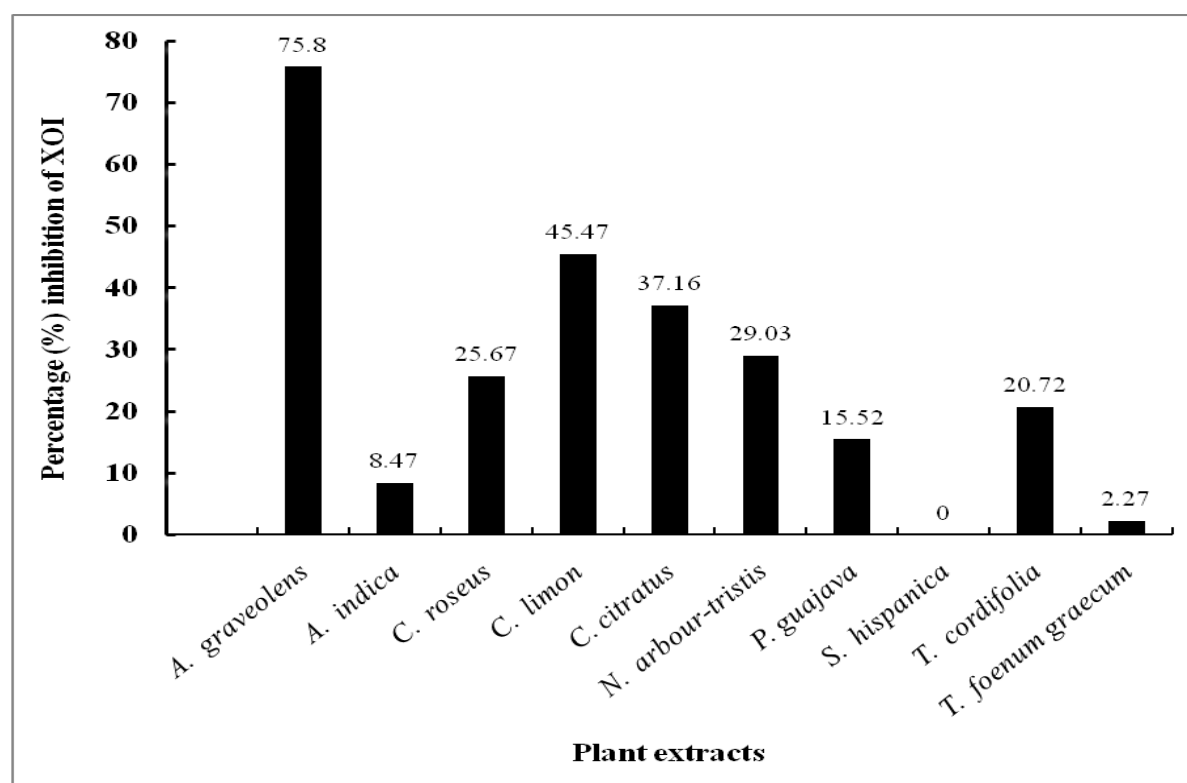


Fig. 3.1 The inhibition percentage of different plant extracts compared to allopurinol. The percentage inhibition of XO by allopurinol (100 µg/mL) was considered as 100%.

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 XO inhibition 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 XO. 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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References

- Argulla, L., and Chichioco-Hernandez, C. L., 2014. Xanthine oxidase inhibitory activity of some leguminosae plants. *Asian Pacific Journal of Tropical Disease*. 4 (6): 438-441.
- Chakravarthi, V. P., and Selvaraju, M., 2017. Comparative evaluation of xanthine oxidase inhibitory activity of *Allium cepa* L., *Azadirachta indica* A. Juss. and *Piper betle* L. extracts alone and in combination, *Annals of Phytomedicine*, 6 (1): 132-135.
- Emmerson, B. T., 1996. The management of gout, *New England Journal of Medicine*, 334 (7): 445-451.
- Johnson, R. J., Sautin, Y. Y., Oliver, W. J., Roncal, C., Mu, W., Lozada, G. S., Iturbe, B. R., Nakagawa, T., and Benner, S. A., 2001. Lessons from comparative physiology: could uric acid represent a physiologic alarm signal gone awry in western society?, *Journal of Comparative Physiology B*, 179 (1): 67-76.
- Kuo, C. F., Grainge. M. J., Zhang. W., and Doherty M., 2015. Global epidemiology of gout: prevalence, incidence and risk factors, *Nature Review Rheumatology*, 11(11): 649-662. [6] Mirghani, M. E. S., Liyana, Y., and Parveen, J., 2012. Bioactivity analysis of lemongrass (*Cymbopogon citratus*) essential oil, *International Food Research Journal* 19(2): 569-575. [7] Muthiah, P. L., 2012. *In vitro* xanthine oxidase inhibitory activity of leaves, fruits and peel extracts of *Citrus aurantium*, *Citrus limetta* and *Citrus limon*. *International Journal of Phytopharmacy*. 2(3): 77-80.
- Owen, P. L., and Johns T., 1999. Xanthine oxidase inhibitory activity of northeastern North American plant remedies used for gout, *Journal of Ethnopharmacology*, 64 (2): 149-160.
- Rahman-El, H. S. M., and ElHak, N. A. M., 2015. Xanthine Oxidase inhibitory activity and antigout of celery leek parsley and Molokhia. *Advances in Biochemistry*. 3(4): 40-50. [10] Rini, H., Elin, Y. S., Kusnandar, A., and Sukrasno., 2016. *In vitro* evaluation of xanthine oxidase inhibitory activity of selected medicinal plants, *International Journal of Pharmaceutical and Clinical Research*, 8(4): 235-238.
- Sharma, V. J., and Patel, P. M., 2013. Evaluation of antibacterial activity of methanolic extract of plant *Rivea ornate*, *International research Journal of Pharmacy*, 4(1): 233-234. [12] Singer, J. Z., and Wallace, S. L., 1986. The allopurinol hypersensitivity syndrome, *Arthritis Rheumatology*, 29(1): 82-87.
- Umamaheswari, M., Kumar, K.A., Somasundaram, A., Sivashanmugam, T., Subhadradevi V., and Ravi T. K., 2007. Xanthine oxidase inhibitory activity of some Indian medical plants, *Journal of Ethnopharmacology*, 109(3): 547-551.
- Unno, T., Sugimoto, A., and Kakuda, T., 2004. Xanthine oxidase inhibitors from the leaves of *Lagerstroemia speciosa* (L.), *Journal of Ethnopharmacology* 93 (2-3): 391-395.

- Valentina, P., Maheshwaran, A., Monisha, M., Mythili, R., Shiba, V. N., and Nallasivam, A., 2016. *In silico* and *in vitro* xanthine oxidase inhibitory activity of *Nyctanthes arbor-tristis* leave extract, International Journal of Research in Pharmacology and Pharmacotherapeutics, (1): 72-76.
- Wong, Y. P., Ng, R. C., Chuah S. P., Koh, R. Y., and Ling, A. P. K., 2014. Antioxidant and Xanthine oxidase inhibitory activities of *Swietenia Macrophylla* and *Punica Granatum*, International Conference on Biological, Environment and Food Engineering August 4-5, Bali (Indonesia) 53-58.
- Zhu, Y., Pandya, B., and Choi, H., 2011. Prevalence of gout and hyperuricemia in the US general population, Arthritis and Rheumatism, 63(10): 3136–3141.