

In Vitro Evaluation of Combined Dosage of *Apium graveolens* Dulce and *Bryophyllum pinnatum* Kurz for Antigout Activity

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Abstract: The present study aimed at the In Vitro Evaluation of Xanthine Oxidase Inhibitory Activity of *Apium graveolens* dulce and *Bryophyllum pinnatum* kurz. The activity was performed using ethanolic extract of leaves and stalk of *Apium graveolens* dulce and fresh juice of leaves of *Bryophyllum pinnatum* kurz at various concentrations (100µg, 200µg, 300µg, 400µg, 500µg, 600µg, 700 µg, 800µg). In this study Allopurinol was used as a standard drug. The results were noted in terms of highest percentage inhibition of Xanthine oxidase. The combination of ethanolic extract of leaves and stalk of *Apium graveolens* dulce and fresh juice of leaves of *Bryophyllum pinnatum* kurz shows significant effect at highest concentration such as 800µg in combination group.

Keywords: Allopurinol, *Apium graveolens* dulce, *Bryophyllum pinnatum* kurz, Potassium oxonate.

INTRODUCTION

Gout is usually characterized by repeated attacks of inflammatory arthritis described as red, tender, hot, and swollen joint. Pain usually comes rapidly within twelve hours. In the most of cases the joint at the base of the big toe is affected. It may also result in nodule like formation also known as tophi, urolithiasis, or urate nephropathy.

The main reason of causing gout is the combination of diet and genetic factors. It occurs more commonly in those who eat a lot of meat, drink a lot of beer, or are obese. Elevated levels of uric acid in the blood are the important mechanism. Gout occurs at increased levels of uric acid causing the uric acid to crystallize and deposit in joints, tendons and surrounding tissues[1].

The most important approach in the treatment of gout is the use of xanthine oxidase (XO) inhibitors, which are effective in reducing blood serum and urinary uric acid levels and as well as the development of tophaceous deposits[2]. So, food components which consist of xanthine oxidase inhibitor can reduce the formation of uric acid and reduce the inflammation [3].

Treatment with nonsteroidal anti-inflammatory drugs (NSAIDs), steroids or colchicine improves symptoms. Once the acute attack reduces, levels of uric acid can be lowered by changing the way of living and in those with frequent attacks, allopurinol or febuxostat provides prevention for a longer time. Incidence of gout in India is not given very clearly[1].

The prevalence is 0.12% as per International League of Nations Against Rheumatism, Community Oriented Program for Control of Rheumatic Diseases (ILAR COPCORD) study in Bhigwan village of India[5].

Apium graveolens Dulce (family - Apiaceae) Celery is a marshland plant variety, it has been cultivated as a vegetable. Its stems, leaves, and seeds are used in the preparation of food. Celery seed is used as a spice and its extracts are used for medicinal purpose. Investigation of plant showed the use of all parts of celery plant[4].

The seed of the plant can be used as antispasmodic, antirheumatic, antibronchitis, and antiasthma. The root can be used to treat urolithiasis, and the constituent flavonoid present in plant can be used to treat diseases associated to uric acid because of its xanthine oxidase inhibitory activity, it can also be used as a diuretic. The Leaves and stalk are used for arthritis, rheumatism, gout, urinary tract inflammation, and especially for rheumatoid arthritis[6].

Bryophyllum pinnatum kurz (family – crassulenscent) commonly known as panfuti, life plant is an herb of about one meter in height, with opposite, glabrous leaves (with 3-5 deeply crenulated, fleshy leaflet). They widely grow in hot and humid areas,

around the dwelling places, along road sides and in abandoned farm and fields. It grows widely and used as folk medicine in tropical Africa, India, China, Australia and tropical America Madagascar, Asia and Hawaii. Need of Investigation Incidence of gout in India is not given very clearly.

The occurrence is 0.12% as per International League of Nations against Rheumatism, Community Oriented Program for Control of Rheumatic Diseases (ILAR COPCORD) study in Bhigwan village of India. The cause of Gout is due to elevated levels of uric acid, often related to relatively high levels in the blood.

This can occur because diet, genetic predisposition, or under excretion of uric acid. Allopurinol is the most frequently used XO inhibitor prescribed for the treatment of gout. Allopurinol can cause the side effects, such as nephrolithiasis. Thus, the development of new anti-gout agents with greater efficacy and a broader safety profile is greatly needed. Focus on plant research has increased all over the world lately, and a large number of evidence has collected to show immense potential of medicinal plants used in various traditional systems [7].

Hence the present study is undertaken to investigate the anti hyperuricemic effect of the plant *Apium graveolens* dulce (celery) and *Bryophyllum pinnatum* kurz (Panfutti) which are traditionally used as antigout agent

PLANT COLLECTION AND AUTHENTICATION

The plant *Apium graveolens* dulce (celery) was collected in month of October from local region of Sangli, Maharashtra state, India. The plant was identified and authenticated by Dr. S.S Sathe (Asso. Professor Department of Botany) in Rajeramrao College, Jath, Dist: Sangli.

The plant *Bryophyllum pinnatum* kruz (panfutti) was collected in the month of December from local region of Sangli, Maharashtra state, India. The plant was identified and authenticated by Dr. U.S. Yadav (Asso. Professor and HOD of Botany) in Willingdon College of Sangli

MATERIALS USED

In the present study Allopurinol, Xanthine, xanthine oxidase enzyme were used in antigout activity. All the material was used in laboratory grade.

PREPARATION OF PLANT EXTRACT

Preparation of Aqueous extract -Fresh juice was collected from the plant *Bryophyllum pinnatum* kurz by putting the plant leaves in the grinder and extracting the juice with the help of muslin cloth. The extract obtained was used for further use.

Preparation of Alcoholic extract-Fresh leaves and stalks of *Apium graveolens* dulce were washed and air dried for about 6-7 days, and powdered. That powder was extracted with 95% ethanol by Soxhlet apparatus for 48 hrs and was used for further oral administration.

PREPARATION OF CONCENTRATIONS

Into each extracts ranging from 100 to 800 µg a pH 7.5 buffer of 50 mM of potassium phosphate was added until the volume reached 1.9 mL, One mL of 2.1 mM xanthine and 0.1 mL of 0.1 unit/mL of XO were added. The solutions were incubated for 45 min at 20 °C, and then 1 mL of HCl 0.58 M was added to terminate the reaction [8].

THE INHIBITORY ASSAY TO XANTHINE OXIDASE (XO)

The optimum condition of the assay referred to a previous work [8] with modification. We tried to measure the inhibitory effect of the crude extract in various concentrations, ranging from 100 to 800 µg/ml. into each extracts; a pH 7.5 buffer of 50 mM of potassium phosphate was added until the volume reached 1.9 M. One mL of 2.1 mM xanthine and 0.1 mL of 0.1 unit/mL of XO were added. The solutions were incubated for 45 min at 20 °C, and then 1 mL of HCl 0.58 M was added to terminate the reaction. The absorbance of the mixed solution was measured at 262 nm and further results were determined using statistical analysis [6].

RESULTS AND DISCUSSION

Qualitative phytochemical investigation:

The plant of *Apium graveolens* dulce contained carbohydrates, flavonoids, alkaloids, steroids, glycosides, phenols, furocoumarins, volatile oils, sesquiterpene alcohols, fatty acids as reported by Ali Esmail Al-Snafi 2014 [9].

The plant *Bryophyllum pinnatum* kurz contains alkaloids, triterpenes, lipids, flavonoids, glycosides, bufadienolides, phenols and organic acids as reported by Firoz Anwar 2012.

The inhibitory assay toward XO was done on all extracts with concentration variant, ranging from 100 to 800 µg. Moreover, the observation on the activity of the enzyme without the addition of extract was completed to see the inhibition effect of the extract against the activity of the enzyme. The results of inhibitory assay showed that all extract tested had lower activity than that of control. This lower activity indicated that celery extract potentially inhibit the activity of xanthine oxidase as reported by Dyah Ishwantini *et al.* [6] Overall, the inhibition power of the enzyme activity increased with the increasing of the extract concentration. All results showed that combination of extract had the inhibition effect more

than that of extract from individual herb. On the average the inhibition effects of the combination of extract of *Apium graveolens dulce* (AGD) and *Bryophyllum pinnatum kurz*(BPK) were high, with the

highest percentage of inhibition of 58% obtained from the applied concentration of 800 µg/ml compared to other extract groups as shown in Table no. 1

Table-1: In vitro xanthine oxidase inhibitory activity

| Groups | Control | Allopurinol | <i>Apium graveolensdulce</i> (ethanolic extract) | <i>Bryophyllum pinnatum kurz</i> (aqueous extract) |
|---------------|---------|-------------|--|--|
| Concentration | | | | |
| 100µg/ml | 1.2344 | 0.0177 | 0.4655 | 0.1203 |
| 200µg/ml | 1.3455 | 0.0356 | 0.4995 | 0.1221 |
| 300µg/ml | 1.4643 | 0.0988 | 0.5550 | 0.2473 |
| 400µg/ml | 1.5674 | 0.1529 | 0.7998 | 0.3635 |
| 500µg/ml | 1.7644 | 0.1679 | 0.8234 | 0.3645 |
| 600µg/ml | 1.7857 | 0.2355 | 0.8834 | 0.4734 |
| 700µg/ml | 1.8966 | 0.3452 | 0.9123 | 0.4876 |
| 800µg/ml | 1.9065 | 0.4355 | 0.9345 | 0.8978 |
| % inhibition | — | 77.1% | 50% | 52% |

CONCLUSION

Celery extract are inhibitory against the activity of xanthine oxidase enzyme to change xanthine into uric acid. The combination of extract *Apium graveolense dulce* and *Bryophyllum pinnatum kurz* of herbal at 800 µg had the greatest (highest) inhibition effect with percent inhibition value at 58%.

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