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Original article

Comparative evaluation of xanthine oxidase inhibitory activity of *Allium ceba* L., *Azadirachta indica* A.Juss. and *Piper betle* L. extracts alone and in combination

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Abstract

The present study was conducted to assess the xanthine oxidase inhibitory activity of *Allium ceba* L., *Azadirachta indica* A.Juss. and *Piper betle* L. alone and in combination, using double beam visible spectrophotometer. *A. ceba* (bulb), *A. indica* (leaf) and *P. betle* (leaf) were selected as per the existing indigenous practice among poultry farmers in Namakkal district. All the plants were collected locally and they were processed to obtain fresh extracts. The phytochemical analysis of these herbs was conducted to identify the active constituents. The xanthine oxidase inhibitory activity of fresh extracts of all three herbs were assessed alone and in combination at the dose rate of 100 µg/ml in double beam UV/visible spectrophotometer in comparison with standard antigout drug allopurinol. The results revealed that among the herb extracts, the *P. betle* extracts were produced potential xanthine oxidase inhibitory activity (72.39 ± 0.60 per cent) in comparison with allopurinol (89.30 ± 0.77 per cent). Hence, it was concluded that the xanthine oxidase inhibitory property of *P. betle* leaf extracts will be utilized for the development of potential antigout remedy for poultry.

Key words: Xanthine oxidase, allopurinol, *Allium ceba* L., *Azadirachta indica* A.Juss., *Piper betle* L.

1. Introduction

Xanthine oxidase (XO) catalyses the metabolism of hypoxanthine to xanthine, and xanthine into uric acid, which is responsible for the medical condition leading to painful inflammation called gout (Chiang *et al.*, 1994). Xanthine oxidase also serves as an important biological source of oxygen derived free radicals that contribute to oxidative damage to living tissues involved in many pathological processes such as inflammation, atherosclerosis, cancer and ageing (Rohman *et al*., 2010). *In vitro* bioassays are used to examine test material for xanthine oxidase enzyme inhibition, as inhibitors of xanthine oxidase may be potentially useful for the treatment of gout or other xanthine oxidase enzyme induced diseases.

Gout is a common metabolic disorder that results in abnormal accumulation of urates in domestic birds. It is a clinical manifestation of severe renal function disorder associated with hyperuricemia, resulting in the precipitation of monosodium urate monohydrate crystals on the visceral surface(s) and also the articular surfaces of the joints. Incidence of gout has been reported in India by several workers (Rahamathulla and Mohmuddeen, 1973; Paresh and Swain, 2005). In broiler flocks, symptoms of gout was reported at about 7- 8 days of age. Similarly, higher incidences of gout were observed in broilers less than 3 weeks of age (Uma *et al*., 1996).

Visceral gout is considered to be the acute form of disease, causing huge mortality characterized by the urate deposits on serosal surfaces, most often in the liver, kidney, pericardium, heart and air sacs. Visceral gout is among the most commonly diagnosed causes of mortality in poultry. The economic loss to farmers due to the gout has been reported regularly. Narsapur (2011) reported that gout affected broiler birds were shown mortality rate of above 15% and feed conversion ratio were drastically increased (>2), which in turn results in heavy economic loss to farmers.

Xanthine oxidase inhibitors are employed as a significant mediator by suppressing the uric acid generation in the treatment of gout. Allopurinol used in the therapeutic treatment for hyperuricemia by inhibiting the biosynthesis of uric acid from purine (Khanna *et al*., 2012). Because of adverse effects like superoxide generation (Berry and Hare, 2004), hepatitis produced by the allopurinol, the focus is now shifted towards the use of natural products which are devoid of such disadvantages. Thus, we focus on the medicinal plants and their active chemical constituents currently used and those with the potential to be developed as antigout medications in the future.

Xanthine oxidase is one of the most important enzyme that are inhibited by some flavonoids (Van Hoom *et al*., 2002). The plants like *A.ceba* and *A.indica* have rich flavonoids components (Finnegan *et al.*, 1992) like quercetin which may show xanthine oxidase inhibitory activity. Piperaceous plants are known to contain various

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catecholic compounds which exhibit antioxidant activity, also piperine obtained from *P. betle* leaves has shown anti-inflammatory activity (Sabina *et al.,* 2011). *A. ceba* (Haidari *et al.,* 2008), *A. indica* (Kumar and Kumar, 2014) and *P. betle herbs* (Murata *et al*., 2009) were possess to have xanthine oxidase inhibitory activity. These research backgrounds have prompted us to investigate their xanthine oxidase inhibitory activity. These plants are commonly available in Indian conditions and can easily be recognized by the farmers. In order to explore the scientific efficacy of these plants in poultry as antigout agents, this preliminary study was carried out.

2. Materials and Methods

Allium ceba L. (Onion - small size) was purchased from local market and the species identity was confirmed with the help of botanist. The fresh extract of *A. ceba* was prepared by removing the outer dry skins and inedible portions and the remaining edible portion bulb was cut into small pieces. Then they were grinded and the fresh extract was obtained through filtration. Whereas *Azadirachta indica* A.Juss. (Neem) leaves were collected locally and by the addition of small quantum of water, they were grinded in a mixer and filtered for fresh extract preparation. Also, the local variety (Karpooram or Karpoori) of *Piper betle* L. leaves were purchased from market and the fresh extracts was prepared by crushing the leaves in small portions and grinding in a mixer and then filtered for extract preparation.

2.1 Phytochemical analysis

Qualitative phytochemical analysis of *A.ceba, A.indica* and *P.betle* extracts were carried out to detect the presence of alkaloids, flavonoids, terpenoids, saponin, tannin, amino acids and proteins, volatile oil, phylobatannin, carbohydrates and glycosides (Kokate *et al.,* 1990).

2.2 *In vitro* spectrophotometric assay

Double beam visible spectrophotometer (Systronics, UV - VIS double beam spectrophotometer 2201) was used for the *in vitro* xanthine oxidase enzyme inhibitory activity assessment as per the method described by Umamaheswari *et al.* (2007). The dose of all herbs equivalent to that of allopurinol drug dose were fixed as 100 µg/ml (Kumar and Azmi, 2014; Umamaheswari *et al.,* 2007). The experimental design is as follows:

Group	Drug/plant extract
T 1	Allopurinol drug
T 2	*A. ceba* bulb aqueous extract
T 3	*A. indica* leaves aqueous extract
T 4	*P. betle* leaves aqueous extract
T 5	*A. ceba* aqueous extract + *A. indica* aqueous extract
T 6	*A. ceba* aqueous extract + *P. betle* aqueous extract
T 7	*A. indica* aqueous extract + *P. betle* aqueous extract
T 8	*A. ceba* + *A. indica* + *P. betle* aqueous extracts

The xanthine oxidase activity was assayed in double beam visible spectrophotometer under aerobic conditions for all the three plant extracts as per the experimental design mentioned above. The assay mixture consists of 1 ml extract of each plant (100 µg/ml), 2.9 ml of phosphate buffer (pH 7.5) and 0.1 ml of enzyme solution (xanthine oxidase-0.01 units/ml in phosphate buffer, pH 7.5) which was prepared immediately before use. After preincubation at 25°C for 15 min, the reaction was initiated by the addition of 2 ml of substrate solution (150 mM xanthine in the phosphate buffer). The assay mixture was incubated at 25°C for 30 min. The reaction was then stopped by the addition of 1ml of 1N hydrochloric acid and the absorbance was measured at 290 nm in double beam visible spectrophotometer. The assay for each group was carried out in triplicate. One unit of xanthine oxidase activity is defined as the amount of enzyme required to produce 1 mmol of uric acid per min at 25°C.

Xanthine oxidase activity was expressed as the percentage inhibition of xanthine oxidase in the above assay system, calculated as Percentage of inhibition = $(A - B) - (C - D) / (A - B) \times 100$.

Where A is the activity of the enzyme without test extract, B is the control of A without test extract and enzyme, C and D are the activities of the test extract with and without xanthine oxidase. Allopurinol, a known inhibitor of xanthine oxidase was used as a positive control. Statistical analysis was carriedout by one-way ANOVA using SPSS statistical package, version 17.0. The values were represented as mean ± SE. The significant difference in results between various groups was determined by Duncan Multiple Range Test (DMRT) which were represented in the Table.

3. Results and Discussion

3.1 Phytochemical analysis

The results of qualitative phytochemical analysis of *A. ceba* bulb powder extract, *A. indica* leaves extract and *P. betle* leaves extract were given in Table 1. The presence of saponin, alkaloids, flavonoids, terpenoids and tannins were noticed in *A. ceba* bulb powder extract, *A. indica* leaves extract and *P. betle* leaves extracts. Whereas the presence of carbohydrates, phenol and volatile oil was noticed only in *P. betle* extracts.

Table 1: Qualitative phytochemical analysis results of *A. ceba*, *A. indica* and *P. betle extracts*

Phytochemical	*A. ceba*	*A.indica*	*P. betle*
Saponin	Present	Present	Present
Tannin	Present	Present	Present
Phenol	Absent	Absent	Present
Alkaloids	Present	Present	Present
Terpenoids	Present	Present	Present
Flavonoids	Present	Present	Present
Amino acids and proteins	Absent	Absent	Absent
Carbohydrates	Absent	Absent	Present
Volatile oil	Absent	Absent	Present
Phylobatannin	Present	Absent	Absent
Glycosides	Absent	Absent	Absent
Vitamin C	Absent	Present	Absent

Ling and Bochu (2014) stated that flavonoids, alkaloids, tannins and phenolic compounds which were present in certain herbs showed antigout effects by their xanthine oxidase inhibitory action. The presence of alkaloids, flavonoids and tannin compounds in *A. ceba* bulb powder extract, *A. indica* leaves extract and *P. betle*

leaves extract might results in their xanthine oxidase inhibition. Whereas the absence of phenolic compounds and volatile oils in *A. ceba* and *A. indica* might results in their reduced xanthine oxidase inhibitory activity in comparison with *P. betle*. Previous studies have shown that flavonoids interact with xanthine oxidase by competitively inhibiting its action (Jiao *et al.*, 2006). However, tannins act through non-selective binding of the enzyme (Owens and Johns, 1999). It is possible that compounds belonging to these classes are responsible for the observed bioactivity. Literature search revealed no terpenoids has been previously identified with xanthine oxidase inhibitory action.

3.2 *In vitro* spectrophotometric assay

The results of xanthine oxidase inhibition of *A. ceba* bulb powder extract, *A. indica* leaves extract and *P. betle* leaves extract were given in Table 2. The absorbance of all groups was measured at 290 nm in double beam visible spectrophotometer.

Table 2: *In vitro* xanthine oxidase enzyme inhibitory activity of *A.ceba, A.indica* and *P.betle* extracts

Herbal extract(s) group	Xanthine oxidase inhibition (Percentage)
Allopurinol drug	$89.30^{a} \pm 0.77$
A. ceba extract	$37.54^{ef} \pm 0.57$
A. indica extract	$32.54^{ef} \pm 0.89$
P. betle extract	$72.39^{ab} \pm 0.60$
A. ceba extract + *A. indica* extract	$27.12^{f} \pm 0.74$
A. ceba extract + *P. betle* extract	$56.11^{cd} \pm 1.45$
A. indica extract + P. *betle* extract	$42.04^{de} \pm 0.65$
A. ceba + *A. indica* + *P. betle* extract	$61.20^{bc} \pm 0.83$

Overall means bearing different superscripts within column differ significantly ($p < 0.05$)

The standard drug allopurinol has shown highest rate of (89.30 per cent) xanthine oxidase enzyme inhibition. The results are in agreement with Umamaheswari *et al.* (2007) where the same drug at 100 µg/ml level has exhibited 93.2 per cent inhibition of xanthine oxidase enzyme.

Among the three herbs extracts, the *P. betle* leaves extract has given at the dose rate of 100 µg/ml were shown higher rate of inhibition (72.39 ± 0.60) which was comparable to that of allopurinol. Also *P.betle* extract has shown higher xanthine oxidase enzyme inhibitory activity than the different combinations of all three plant extracts. Combination of *A.ceba , P.betle* (T6) and *A. ceba, A. indica* and *P. betle* (T8) extracts showed moderate inhibition of xanthine oxidase enzyme. Other group results are insignificant.

Inhibition of xanthine oxidase enzyme has been recognized as one of the promising targets or the treatment of hyperuricemia which is observed in poultry gout cases. Based on the literature, *A. ceba* (Haidari *et al.*, 2008), *A. indica* (Kumar and Kumar, 2014) *and P. betle herbs* (Murata *et al.*, 2009) were selected and their potency to inhibit the xanthine oxidase was studied in UV/Visible spectrophotometer at 290 nm. Among the individual and different combination of *A. ceba, A. indica and P.betle extracts*, the highest inhibition of xanthine oxidase enzyme was noticed in *P. betle* extracts. Therefore, *P.betle* leaves extract was proved to show higher xanthine oxidase inhibition property than the other plants. In Tamil Nadu, three varieties of *P. betle* leaves (Sirugamani, Karpoori and Vellaikodi) are accessible mostly (Bhattacharya *et al.*, 2007). They have considerable potency to act as an anti-inflammatory, antioxidant, antibacterial (Dwidedi and Tripathi 2014) agents. These properties might be useful in their xanthine oxidase enzyme inhibition activity. Also, the *A. indica* leaf and its constituents have shown anti-inflammatory, antihyperglycaemic, antioxidant, antimutagenic and anticarcinogenic properties (Williamson, 2002) which might results in their partial xanthine oxidase inhibitory activity. The present result of *A.ceba* herbs on xanthine oxidase inhibition was in par with the same plant study in rats (Haidari *et al.*, 2008). The hypouricemic property of *A.ceba* juice observed in this study could be explained by the inhibitory effects of its flavonoids on xanthine oxidase enzyme.

Combined extracts (T8) showed moderate (61.20 ± 0.83 per cent) xanthine oxidase inhibitory activity. This moderate inhibition of xanthine oxidase enzyme rather than higher activity might be due to the absence of phenolic and volatile oil compounds in *A. ceba* and *A. indica* extracts, which ultimately affect the results of combined extract. Also, *A. ceba* cannot able to exert high inhibitory activity on the normal xanthine oxidase enzyme than the inducible one (Haidari *et al.*, 2008).

4. Conclusion

In the present study, based on the indigenous practice of the farmers and scientific backgrounds, the qualitative phytochemical analysis of *A. ceba* bulb, *A. indica* leaf and *P.betle* leaf were conducted to detect the active principles. The analysis revealed the presence of saponin, tannin, alkaloids, terpenoids and flavonoids in all three extracts, whereas the presence of volatile oil and carbohydrates were noticed only in *P. betle* leaf extracts. In order to evaluate their possible antigout effects, an *in vitro* xanthine oxidase inhibition assay was performed for all three plants individually and also in combination, using allopurinol as standard drug. The results revealed that the potency to inhibit the xanthine oxidase enzyme was high in *P. betle* extract than the other plant groups. Since the synthetic drug allopurinol might produce the unwanted side effects, the natural plant, *P. betle* could be used as an alternative xanthine oxidase inhibitor. Therefore, the outcome of this study indicated that *P. betle* could be used as a potential antigout agent as a replacement for allopurinol. Further, detailed *in vivo* trial of these plants on the gout affected live birds may give detailed insight about their use as possible antigout agents in poultry.

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Conflict of interest

We declare that we have no conflict of interest.

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