EVALUATION OF ANTIOXIDANT AND XANTHINE OXIDASE INHIBITORY POTENTIAL OF METHANOLIC ROOT EXTRACT OF *ACORUS CALAMUS*- AN *IN VITRO* STUDY

Running title: Antioxidant and Xanthine Oxidase inhibitory potential of methanolic root extract of *Acorus calamus*

Abstract:

Background: Gout is an inflammatory joint disease that elevates the uric acid levels in blood that triggers the formation of urate crystals in the joint, especially since the past 50 years. Xanthine oxidase catalyses oxidative hydroxylation of hypoxanthine to Xanthine to uric acid. show it's higher in men aged above 50 years. *Acorus calamus* is a mid term, perennial, fragrant herb. Therefore, the main approach for the treatment of gout is reducing uric acid.

Aim: To analyze the antioxidant and Xanthine Oxidase inhibitory potential of methanolic root extract of *Acorus calamus.*

Materials and method: Preparation of methanolic root extract of *Acorus calamus* was done by hot percolation method. Phytochemical screening test was done. The antioxidant activity was carried out by DPPH radical scavenging assay. Anti gout potential of the herbal extract was analysed by the evaluation of Xanthine oxidase inhibitory potential. The data were analyzed statistically by a one-way analysis of variance (ANOVA) followed by Duncan's multiple range test to see the statistical significance among the groups. The results with $p<0.05$ level were considered to be statistically significant.

Result: Methanolic root extract of *Acorus calamus* shows a strong presence of phytochemicals such as alkaloids, terpenoids, steroids, saponins and flavonoids. IC_{50} of antioxidant potential of Methanolic root extract of *Acorus calamus* was found to be 210 µg/ml. Ic 50 of xanthine oxidase inhibitory potential of methanolic root extract of *Acorus calamus* was found to be 310 µg/ml.

Conclusion: Methanolic root extract of *Acorus calamus* exhibited significant antioxidant and anti gout potential. Further research on the natural Xanthine oxidase inhibitors especially *in vivo* studies and investigation of active compounds and its pharmacokinetics to be elucidated.

Keywords: Gout, Xanthine oxidase, *Acorus calamus*, pharmacokinetics, DPPH, Innovative technology, Novel method

Introduction:

Acorus calamus also known as sweet flag is a mid-term, perennial, fragrant herb. The plant's rhizomes are brown in colour, twisted, cylindrical, curved and shortly nodded, with radiant green leaves, a sword-like structure, which has curvy margins and is thick in the middle (1,2). It is a tall wetland monocot from the family Acoraceae and species Acorus(3)

Gout is a chronic disorder or an inflammatory joint disease that elevates the uric acid levels in blood that triggers the formation of monosodium urate crystals in the joint. It can also cause tophi, joint deformities and kidney stones (4). Its occurrence has increased since the past 50 years, especially in developing countries (5) only 5% of the individuals who had hyperuricemia above 9 mg/dL developed gout. Accordingly, it is proved that the incidence of gout is shared by factors such as genetic predisposition . In India, approximately 0.3% of the population is affected by gout, and the statistics prove that it's higher in men aged above 50 years (6).

Xanthine oxidase is an enzyme that generates reactive oxygen species that catalyses oxidative hydroxylation of hypoxanthine and xanthine to uric acid in further oxidation ,leading to inflammation at joints that causes severe pain,redness and soreness.(7)However, it forms uricase which is not a functional human enzyme,as a result people can develop hyperuricemia (8). Hyperuricemia is the key predictor for development of gout (1), it occurs when serum uric acid levels are more than 0.42 mmol/L and the main cause is due to the unbalanced excretion and production of uric acids, therefore the main approach for treating gout is by reducing the uric acid production (1).

Recently it has been reported that treating gout using medicinal plants is gaining new interest (9), due to its less side effects and lower cost (3). The synthetic drugs used in the treatment of gout

are reported to have various side effects and cannot be used for a longer period of time(10). Our team has extensive knowledge and research experience that has translate into high quality publications (11),(12),(13),(14),(15),(16),(17),(18),(19),(20),(21),(22),(23),(24),(25), (26),(27), (28), (29),(30)The aim of this current research is to validate the *in vitro* antioxidant and anti gout potential of methanolic root extract of *Acorus calamus.*

Materials and methods

Preparation of methanolic root extract of *Acorus calamus:*

Acorus calamus was purchased from a herbal health care centre. Air dried, crushed and made into powder form. Methanol was added to it. 80% of methanolic extract was obtained. The extract was then prepared by a hot percolation method. Later it was dried and used to analyze the antioxidant and anti-inflammatory potential(31)..

DPPH radical scavenging activity

Scavenging of 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radicals was assessed by the method of Hatano et al (1989). DPPH solution (1.0 ml) was added to 1.0 ml of extract at different concentrations (0.1 to 0. 5mg/ml). The mixture was kept at room temperature for 50 minutes and the activity was measured at 517nm. Same concentration of ascorbic acid was used as standard. The capability to scavenge the DPPH radical was calculated and expressed in percentage (%) using following formula:

$$\text{DPPH radical scavenging (\%)} = \frac{\text{Control OD - Sample OD X 100}}{\text{Control OD}}$$

***In vitro* anti-inflammatory activity of methanolic root extract of *Acorus calamus* by Xanthine oxidase inhibitory activity**

The anti-inflammatory activity of methanolic root extract of *Acorus calamus* was studied by using inhibition of albumin denaturation technique which was studied according to the method of Leela Prakash and Mohan Dass, (2010). The reaction mixture consisted of 1% aqueous solution of bovine albumin fraction and test extracts, the reaction mixture's pH was adjusted using a little amount of 1N HCl. The sample extracts were incubated at 37 ºC for 20 min and then heated to

51°C for 20 min, after cooling the samples the turbidity was measured at 660nm. (UV Visible Spectrophotometer Model 371, Elico India Ltd). The experiment was performed in triplicate. The Percentage inhibition of protein denaturation was calculated as follows:

Percentage inhibition(%) = (Abs Control – Abs Sample) X 100 / Abs control

Statistical analysis

The triplicate analysis results of the experiments performed on control and experimented *Acorus calamus* were expressed as mean ± standard deviation. Results were analyzed statistically by a two-way analysis of variance (ANOVA) and significant differences between the mean values were measured using Tukey's multiple range test using Graph Pad Prism version 5. The results with the $p<0.05$ level were considered to be statistically significant.

Results and discussion:

Serial no.	Phytochemicals	Presence
1	Amino acids	+ +
2	Protein	+
3	Alkaloids	-
4	Terpenoids	+
5	Steroids	+
6	Carbohydrates	-
7	Sapanoids	+
8	Flavonoids	+ +

Table 1: Phytochemical screening of methanolic root extract of ***Acorus calamus***

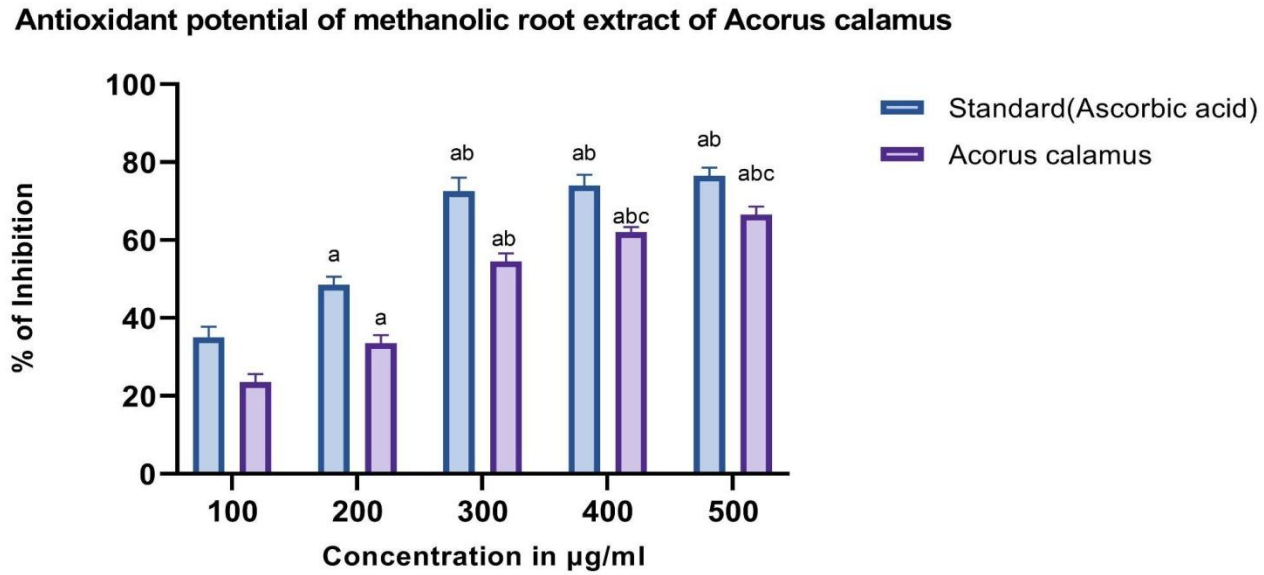

Figure 1: Represents antioxidant potential of methanolic root extract of *Acorus calamus* by DPPH Assay against the standard Ascorbic acid.. X-axis represents the concentration in µg/ml while Y-axis represents the inhibitory potential of the extracts.Blue bar represents the standard – Ascorbic acid, purple bar represents methanolic root extract of *Acorus calamus.*Each bar represents Mean ± SEM of 3 independent observations. Significance at $p < 0.05$.

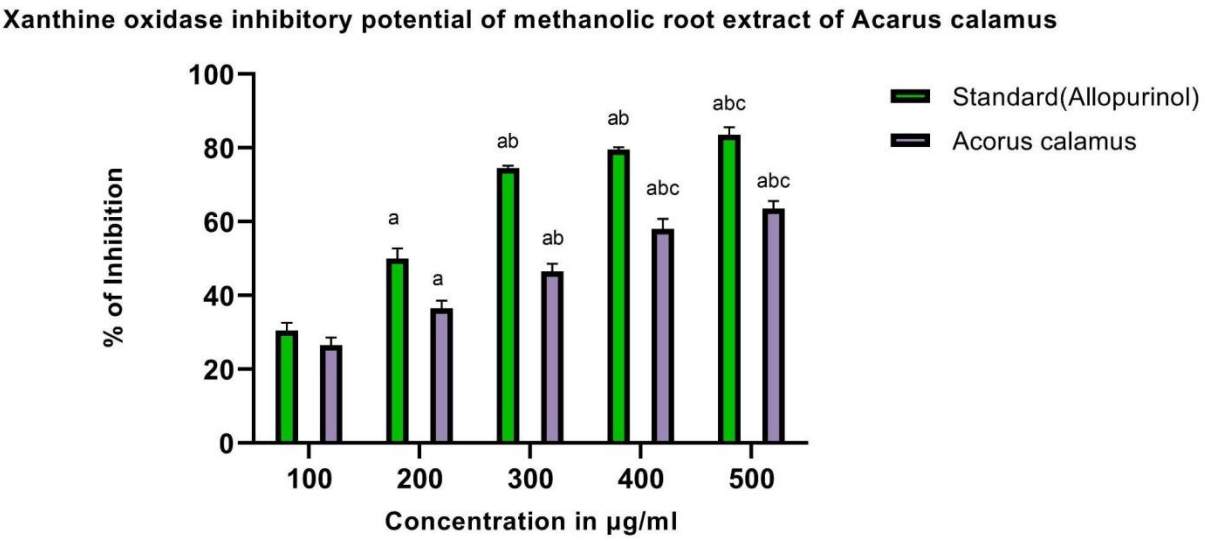

Figure 2: Represents anti-inflammatory activity - Xanthine oxidase inhibitory potential of *Acorus calamus* root ethanolic extract compared with the standard - Allopurinol. X-axis represents the concentration in µg/ml and Y-axis represents the inhibitory potential of the extracts.Green bar represents the standard -Allopurinol, purple bar represents methanolic root extract of *Acorus calamus.* Each bar represents Mean ± SEM of 3 independent observations. Significance at $p < 0.05$.

From the above study the methanolic root extract of *Acorus calamus* shows a presence of phytochemicals such as alkaloids, terpenoids, steroids, saponins and flavonoids (Table 1). Phytochemicals are secondary metabolites which are exclusively present in plants. The presence of these phytochemicals are responsible for the medicinal property of these plant extracts. (32). The antioxidant activity of *Acorus Calamus* was determined by performing the DPPH radical scavenging assay. They are molecules possessing unpaired electrons emerging from phenolic compounds. Plant extract with phenolic phytochemicals can scavenge the free radicals and thus the antioxidant potential can be estimated (6). Methanolic root extract of *Acorus calamus* exhibited a significant antioxidant potential and increased in a dose dependent manner as compared to the standard. IC_{50} of Methanolic root extract of *Acorus calamus* was found to be 210 µg/ml (Figure 1).

A dose dependent Xanthine oxidase inhibitory activity (anti-gout) was observed for the extract in the present study, the standard drug allopurinol showed greater percent of inhibition of

Xanthine oxidase than the extract of the compounds with some concentration (33). IC_{50} of xanthine oxidase inhibitory potential of methanolic root extract of Acorus calamus was found to be 310 µg/ml (Figure 2) Gout is a disorder whose prevalence has increased in the last 50 years. Reasons for the disorder can be genetic, highliving and excess alcohol consumption. The current treatment for gout predominantly depends on synthetic drugs with serious side effects (34). Prolonged usage of synthetic drugs can lead to adverse side effects and now research is focussed to explore the rich phytochemicals of herbal extracts which are a part of indegenous medicine. (35). More research and awareness has to be spread to cure the disorder instead of just managing one. Rawlani et al (2014) has stated that conventional radiography is an effective diagnostic method to treat chronic tophaceous gout and ultrasonography has been used recently to assist in both diagnosing and monitoring the disease (36). Rübenthaler et al (2016) has stated that utilizing computed tomography is associated with ionizing radiation, with a little added benefit over ultrasonography and computed tomography, Reiser and Clevert et al (2016) stated that magnetic resonance imaging is a great modality to image bones and soft tissues as it has less radiation and better quality (37).The current common clinical treatments for gout are mainly self medication and anti-inflammatory medications (NSAIDs) by (38).

Conclusion:

The bioactive compounds found in medicinal plants remain as an important component of research for the development of new drugs with potential to fight against several diseases and disorders. Methanolic root extract of *Acorus calamus* shows significant antioxidant and anti-gout properties as compared to the standards. Hence it can be used as an antioxidant and anti gout drug for the management of various health ailments. Further studies on the *in vitro* cell line and *in vivo* experimental models need to be carried out in order to ascertain its potential mechanisms of action towards the development of clinical utility.

- **COMPETING INTERESTS DISCLAIMER:**
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- Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.
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- **NOTE:**

- The study highlights the efficacy of "herbal" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

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