PRELIMINARY PHYTOCHEMICAL ANALYSIS AND INVITRO EVALUATION OF XANTHINE OXIDASE INHIBITORY ACTIVITY OF Fragaria ananassa (strawberry) EXTRACT

Running title : Phytochemical analysis and invitro evaluation of xanthine oxidase inhibitory activity of Fragaria ananassa extract.

Type of study: Original research

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ABSTRACT :
Introduction : Xanthine oxidase is a superoxide producing enzyme which synthesis uric acid from hypoxanthine. Gout is an inflammatory disease which targets the joints and is caused by an abnormal buildup of uric acid in the blood. Allopurinol is a well known xanthine oxidase inhibitor and widely used in the therapeutic and clinical management of gout and used as a first line treatment for hyperuricemia and gout. Fragaria ananassa is a well known antioxidant and rich in Vitamin C.
Aim : The aim of this study is to analyse phytochemical constituents and evaluate xanthine oxidase inhibitory activity of Fragaria ananassa (strawberry) extract.
Methods : The extract was tested for its phytochemical constituents and analysed for its anti gout potential by estimating the xanthine oxidase inhibitory potential.
Results : The results of qualitative phytochemical analysis of strawberry extract are given in table 1. The results revealed the presence of Terpenoids, Flavonoids, Carbohydrates and Steroids in strawberry extract.The extract also showed a significant presence of anti gout potential.

Conclusion : Aqueous extract of strawberry exhibits xanthine oxidase inhibitory activity. Further in vitro studies and detailed molecular mechanisms may be required for developing these extracts into commercially available drugs for treatment.

Key words : Novel method, Xanthine oxidase; strawberry extract; Phytochemical analysis; Gout; Hyperuricemia; Antioxidant, Innovative technique

INTRODUCTION :

Medicinal plants have been used all over the world as an alternative medicine. Nowadays, the use of herbal medicines has increased due to their ability to perform against the disease, availability and safety claims. Medicinal plants are rich in important phytochemicals i,e., active compounds and many are secondary metabolites, such as flavonoids, terpenoids, alkaloids and many others. These active compounds contain antioxidant properties which are important for drug development and also used as therapeutic agents. Antioxidants play an important role in preventing the oxidation of molecules inside the body and they have potential to destroy the free radicals present inside the body(1).

Fragaria ananassa (strawberry) is one of the dominant medicinal plants widely grown all over the world. It belongs to the family of Rosaceae. It is bright red in colour and contains a good aroma, texture and sweetness. Strawberries are a worthy source of natural antioxidants especially, the existence of phenolic compounds which are known as polyphenolic, protect the human body from diseases and infections like damage caused by oxidative stress(2). Previous study depicted that fruits, pericarps, pulps, and seeds are well known to contain many phenolic components, which are capable of protecting the human body from oxidative damage through an anti-inflammatory mechanism(3). When compared to other fruit plants of the Rosaceae family including raspberries, blackberries, peaches, pears and apples, strawberry plant is rich in secondary metabolites compositions and contains hundreds of volatile and non-volatile compounds(4).

Xanthine oxidase (XO) is a form of xanthine oxidoreductase which catalyzes the oxidation of hypoxanthine and further catalyzes the oxidation of xanthine to uric acid. This enzyme helps in maintaining normal uric acid metabolism. Xanthine oxidase inhibitory activity (XO) is an enzyme assay which is used in determining the anti-gout activity of the plant extracts. XO enzyme is known for causing the accumulation of uric acid in the blood which eventually leads to gout(5). When compared to anti-inflammatory agents, xanthine oxidase inhibitors have less side effects. Excessive production or excretion of uric acid results in hyperuricemia(6).

Gout is an inflammatory disease which targets the joints and is caused by an abnormal buildup of uric acid in the blood. Xanthine oxidase is well known for conversion of xanthine to uric acid. It is linked to multiple human diseases and development of various cardiovascular and inflammatory diseases. Treatment of gout includes the use of therapeutic agents such as xanthine oxidase inhibitors that act by blocking the conversion of purine and uric acid(7).

Allopurinol is a well known xanthine oxidase inhibitor which is used as a first line treatment for hyperuricemia and gout.a molecular target involved in nucleic acid metabolism and the enzymes also responsible for the development of gout disease. It is known to cause hypersensitivity reactions. Allopurinol reduces superoxide anion production because XO is one of the main producers of superoxide anions(8). Gout is the most common form of inflammatory arthritis and caused by the chronic elevation of serum uric acid levels leading to monosodium urate crystal formation(9)**.** Our team has extensive knowledge and research experience that has translate into high quality publications (10),(11),(12),(13),(14),(15),(16),(17),(18),(19),(20),(21),(22),(23),(24),(25),(26),(27),(28),(29)

The aim of this study is to analyse phytochemical constituents and evaluate xanthine oxidase inhibitory activity of Fragaria ananassa.

MATERIALS AND METHODS :

Phytochemical Screening test :

Test for phlobatannin :

1ml of the extract was treated with 1ml of 1% HCl and boiled for 10 mins. The formation of red color precipitate indicates the presence of phlobatannin.

Test for Carbohydrates :

Three to five drops of Molisch reagent was added with 1 mL of the extract and then 1 mL of concentrated sulphuric acid was added carefully through the side of the test tube. The mixture was then allowed to stand for two

minutes and diluted with 5 mL of distilled water. The development of a red or dull violet ring at the junction of the liquids showed the presence of carbohydrates.

Test for Flavonoids :

Few drops of 1% liquid ammonia were taken in a test tube and along with it 1ml of the extract was added resulting in the formation of yellow color thereby indicating the presence of flavonoids.

Test for Alkaloids :

2ml of sample was mixed with 2ml of HCl. Then 6 drops of HCN was added and further 2 drops of picric acid was added that resulted in a creamish pale yellow ppt indicating the presence of alkaloids.

Test for Terpenoids :

2 ml of sample along with 2ml of chloroform and 3ml of con. H2SO4 was added. Red color ppt obtained indicates the presence of terpenoids.

Test for proteins :

One milliliter of ninhydrin was dissolved in 1 mL of acetone and then a small amount of extract was added with ninhydrin. The formation of purple colour revealed the presence of protein.

Detection of saponins :

Foam test: A fraction of the extract was vigorously shaken with water and observed for persistent foam.

Test for steroids :

One milliliter of chloroform was mixed with 1 mL of extract and then ten drops of acetic anhydride and five drops of concentrated sulphuric acid were added and mixed. The formation of dark red colour or dark pink colour indicates the presence of steroids.

In Vitro Xanthine Oxidase Inhibitory Activity of strawberry extract :

In vitro Xanthine oxidase inhibitory of the extract was assessed as per the method of (Nguyen et al, 2004; Umamaheswari et al., 2007). Briefly, the assay mixture consisted of 1 ml of the fraction (0.1 to 0.5g/ml), 2.9 ml of phosphate buffer (pH 7.5) and 0.1 ml of xanthine oxidase enzyme solution (0.1 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 the substrate solution (150 M xanthine in the same buffer). The assay mixture was incubated at 25◦C for 30 min. The reaction was then stopped by the addition of 1 ml of 1N hydrochloric acid and the absorbance was measured at 290 nm using a UV spectrophotometer. Allopurinol (0.1 to 0.5mg/ml), a known inhibitor of XO, was used as the positive control. One unit of XO is defined as the amount of enzyme required to produce 1 mmol of uric acid/min at 25◦C. XOI activity was expressed as the percentage inhibition of XO in the above assay system calculated as percentage of inhibition as follows.

Inhibitory activity (%) = (1 - As/Ac) x100 Where,

As – absorbance in presence of test substance, Ac – absorbance of control.

STATISTICAL ANALYSIS :

The data were subjected to statistical analysis using one - way analysis of variance (ANOVA) and Duncan's multiple range test to assess the significance of individual variations between the groups. In Duncan's test, significance was considered at the level of $p<0.05$.

RESULTS AND DISCUSSIONS :

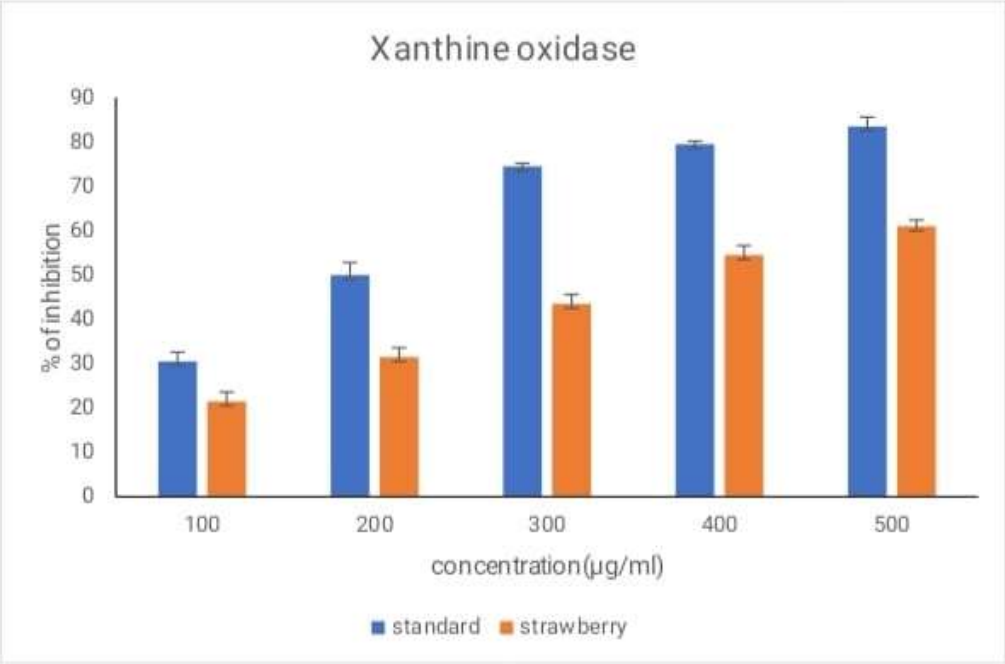
Phytochemical analysis : Phytochemical constituents such as Alkaloids, Proteins, Amino acids, Terpenoids, Flavonoids, Carbohydrates, Saponins and Steroids were analysed.

The results obtained was as follows,

Table 1: Qualitative phytochemical analysis of strawberry extract

Phytochemical	**Strawberry Extract**
Proteins	(-)
Amino acids	(-)
Terpenoids	(+)
Flavonoids	(+)

Alkaloids	(-)
Carbohydrates	(+)
Saponins	(-)
Steroids	(+)



Graph 1 represents the xanthine oxidase inhibitory potential of strawberry extract . Each bar represents the mean±SD of three independent observations. Significance at the levels of $p<0.05$

Phytochemical analysis exhibited the presence of phytoconstituents such as terpenoids, flavonoids, steroids etc in the strawberry extract(Table1). The xanthine oxidase inhibition assay is considered as the gold standard to study the anti gout potential of strawberry extract. Phytochemicals present in plants can act as xanthine oxidase inhibitors. These compounds are also safe if an appropriate amount is taken and have few side effects. Previous studies have shown that different parts of the same plants can contribute differently to affect uric acid levels(30). Allopurinol is the standard drug used in the treatment of gout. The standard synthetic drugs when taken for a longer period of time exhibit various side effects. Moreover synthetic medicines can only help in the management not a complete cure for disorders like gout, diabetes etc(31). In this study, the plant extracts are compared with standard drugs and found to have efficient xanthine oxidase inhibitory potential, with an Ic 50=380µg/ml.(Graph 1).

From previous studies, the role of herbal extracts and its phytoconstituents are well understood(32). The ability of herbal medicine in curing disorders lies especially in its phytoconstituents. The disorders like gout need long term drug ingestion to have a better management of the disorder. Prolonged ingestion of synthetic drugs to manage gout has led to various serious side effects. (33). Thus more research has been made to explore the phytochemicals and its health benefits(34).

From the above study, it is evident that the anti gout potential of the plant extract is significantly less than the standard drug Allopurinol. Though the potential is less, it can be used as a safe drug in the long run.

Strawberries, as a fruit, are easily available and cost effective. More research is required to evaluate the pharmacotherapy role of the extract , so that it can be made into a drug formulation in future.

CONCLUSION :

From this we may conclude that aqueous extract of strawberry exhibits xanthine oxidase inhibitory activity. Fragaria ananassa is also a good source of potent antioxidative molecules along with antibacterial activity. Further in vitro studies and detailed molecular mechanisms may be required for developing these extracts into commercially available drugs for treatment.

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AUTHORS CONTRIBUTION :

Sujitha S : Literature search, data collection, analysis, manuscript drafting.

Dr.V.Vishnu Priya, Dr.R.Gayathri, Dr.S.Kavitha : Study designing, data verification, manuscript correcting.

CONFLICTS OF INTEREST :

The authors declare that there are no conflicts of interest in the present study.

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