Original Research Article

PRELIMINARY PHYTOCHEMICAL ANALYSIS OF ANTI HYPERLIPIDIMIC AND XANTHINE OXIDASE INHIBITORY ACTIVITIES OF *Malus domestica*

Running title: Xanthine oxidase inhibitory activities of *malus Domestica*

ABSTRACT:

Aim: The aim of this study is to analyse phytochemical constituents and to evaluate antihyperlipidemic and xanthine oxidase inhibitory activities of *Malus domestica* aqueous extract.

Introduction: Malus Domestica is a well known plant commonly known as apple belonging to the family *Rosaceae.* The fruit is rich in flavonoids and many other phytochemicals. *The* fruit is also reported to have many therapeutic properties.

Materials And method: The phytochemical screening, and assessment of in vitro anti cholesterol and xanthine oxidase inhibitory activity were done in aqueous extract of Malus domestica using standard procedures.

Results and discussion: Many phytochemical elements, such as alkaloids, proteins, amino acids, terpenoids, flavonoids, carbohydrates, saponins, and steroids, were found in Malus domesticus extract. The extract also has anticholesterol effect in vitro, according to the findings. The extract is also efficient in inhibiting the xanthine oxidase inhibitory activity in a concentration dependent manner.The results obtained in the study show that *Malus domestica* has significant anti cholesterol and antioxidant activities.

Conclusion: The present study established the potent in vitro anti cholesterol and xanthine oxidase inhibitory potential of *Malus domestica.*

KEY WORDS: Innovative technique, Cholesterol,xanthine oxidase,Antioxidant activities,Malus domestica, novel method

INTRODUCTION:

Malus domestica, commonly known as apples, are cultivated worldwide and are the most widely grown species in genus *Malus*. Apple is rich in vitamin A, B complex and it is rich in antioxidants that help to maintain healthy and glowing skin. *Malus Domestica* is a well known antioxidant and rich in vitamins. The apple is the most important temperate fruit crop, having been grown in Asia and Europe since antiquity (1). According to most experts, the genus Malus encompasses 25-30 species and subspecies of the so-called crabapple. Interspecific hybridization is thought to have produced the cultivated apple. The scientific nomenclature Malus x domestica has been widely considered as the most appropriate (2). Malus sieversii, which grows wild in the heavenly mountains, is thought to be the main parent of the domestic apple (3). Dietary fibre, carbohydrates, vitamins, and phenolic compounds are just a few of the health and sensory components found in apples (4). Apple's antioxidant potential is largely due to phenolic components like flavonoids and phenolic acids.

Xanthine oxidase is well known for conversion of purines from proteins rich in food such as organ meat and fish. Gout is an inflammatory condition that affects the joints and is caused by an abnormal buildup of uric acid in the blood. Gout is treated with xanthine oxidase inhibitors, which work by preventing purines from being converted to uric acid (5). Allopurinol is a well-known xanthine oxidase inhibitor that is commonly used to treat gout and hyperuricemia in both clinical and therapeutic settings (5,6). Hyperlipidemia is one of the key risk factors which contribute to the prevalence of coronary heart diseases (7). Hyperlipidemia is associated with many lipid disorders which are considered a causative agent for atherosclerotic cardiovascular disease. Higher lipid levels are caused by increased intestinal absorption or increased endogenous production. Treatment of hyperlipidemia is one of the main strategies used by therapists to slow down the atherogenic process (8). Our team has extensive knowledge and research experience that has translate into high quality publications (9-28).

Hence the aim of the present study is to analyse the phytochemical constituents, anti cholesterol and xanthine oxidase inhibitory activities of *Malus domestica*.

MATERIALS AND METHODS

1. 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. H_2SO_4 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 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.

2. In vitro xanthine oxidase inhibitory activity of *Malus domestica*

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

3. In vitro anticholesterol activity of *Malus domestica*

The anti-cholesterol assay was performed according to the kit's instructions (Spinreact, S.A.U-Ctra Santa Coloma, Girona, Spain). At a concentration of 2.5 mg mL/ml, cholesterol was dissolved in chloroform. A ten microliter sample of the extract was pipetted into a microtiter plate, followed by 2000 litres of R1 reagent and ten microliters of cholesterol. As a blank, 20 microliters of distilled water and 2000 litres of R1 reagent were utilised. The negative control was 20 mL cholesterol and 2 mL R1, while the standard was 20 mL simvastatin and 2000 mL R1 reagent. The contents were incubated between 0-30 min at room temperature and the absorbance was read at 500 nm in a UV-Vis spectrophotometer against reagent blank. Anti-cholesterol assay of the extract was calculated using the following equation:

$$\text{Inhibition (\%)} = \frac{\text{Negative control-Sample}}{\text{Negative control}} \times 100$$

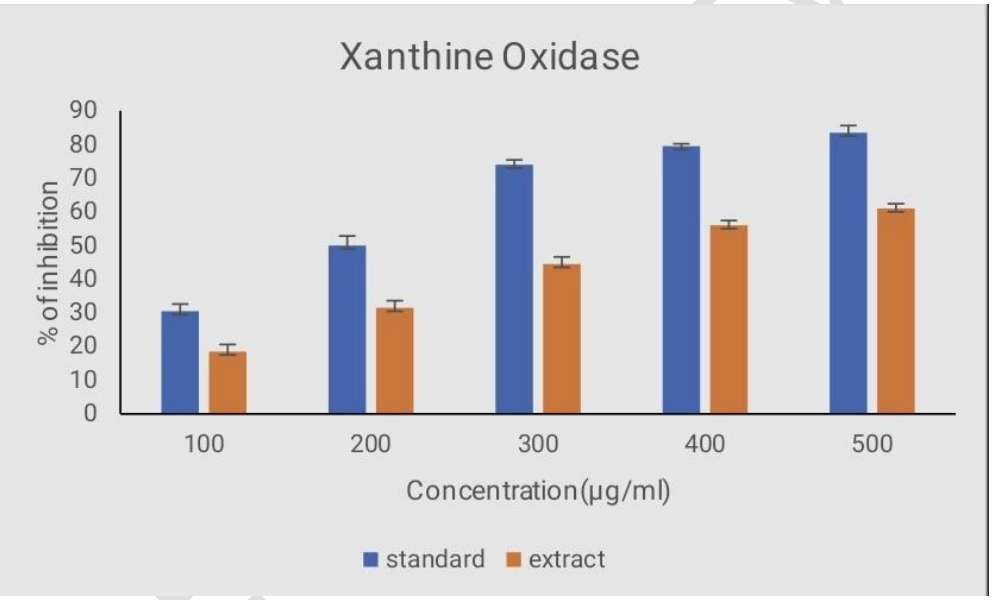
RESULTS AND DISCUSSION

Table 1: Phytochemical analysis of Malus domestica

PHYTOCHEMICAL	MALUS DOMESTICA
Proteins	(+)
Amino acid	(-)
Terpenoids	(+)
Flavonoids	(+)

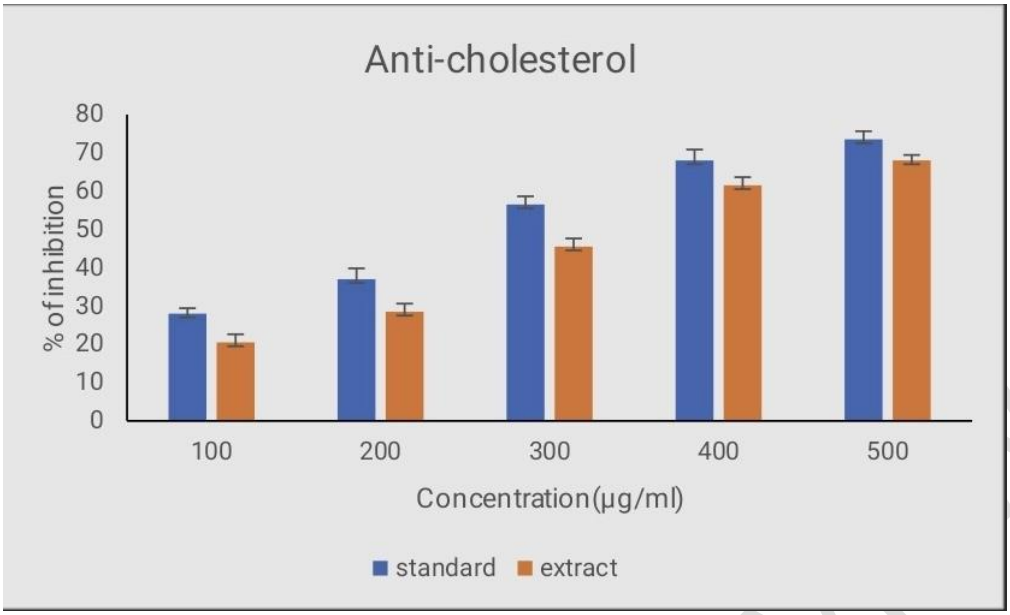
Alkaloids	(+)
Carbohydrates	(+)(+)
Saponins	(+)
Steroids	(+)

Figure 1: In vitro xanthine oxidase inhibitory potential of Malus domestica



Each bar represents the mean ±SD of three independent observations. Significance at the levels of $p<0.05$.

Figure 2: In vitro xanthine oxidase inhibitory activity of *Malus domestica*



Each bar represents the mean ±SD of three independent observations. Significance at the levels of $p<0.05$.

The phytochemical screening analysis revealed the presence of many phytochemicals in the aqueous extract of Malus domestica such as terpenoids, flavonoids, alkaloids, proteins, carbohydrates, saponins and steroids. Phytoconstituents are the bioactive compounds in plants. They work with nutrients and fibers to form an integral part of the defense system against various diseases and stress conditions (29). Phytochemicals contain a broad spectrum of chemical structures which are capable of preventing and treating many diseases. Hence, conducting preliminary phytochemical screening of plants is an important tool in determining the discovery and development of novel therapeutic agents in plant materials (30). The rich existence of the phytochemicals might be the underlying reason for the beneficial activities of *Malus domestica* extract.

The gold standard for determining anti-gout potential is the xanthine oxidase inhibition assay. Phytochemical found in plants that inhibits xanthine oxidase (5). In addition, our extract inhibited xanthine oxidase enzyme activity in a concentration-dependent manner. The extract's activity was compared to that of the conventional medication allopurinol. Allopurinol is the standard drug used for treatment. However, the use of this drug is associated with many side effects such as hepatitis, nephropathy, and allergic reactions (31). Since our extract being a natural product, which exhibits xanthine oxidase inhibitory activity, can avoid the side effects caused by the synthetic drugs, if we can formulate a drug against gout.

The findings also indicated the extract's significant in vitro anticholesterol action, which was concentration dependant. When compared to the usual medicine simvastatin, the activity is slightly lower. Simvastatin is the most commonly prescribed medication for

hypercholesterolemia. HMG CoA reductase, an enzyme involved in cholesterol production, is inhibited by the statin family of medicines. Many side effects of statin medicines have been described, including cognitive decline, neuropathy, pancreatic and hepatic malfunction, and sexual dysfunction. (32). Hence the need for alternative natural products may be required for the treatment of hypercholesterolemia. Our extract can be such an alternative medicine in this way.

Limitation:
Xanthine oxidase inhibition assay is considered as the gold standard to study anti- gout potential. The activity of the extract was compared with the standard drug allopurinol.

Future scope:
Other medicinal effects of Malus domestica extract on other diseases can be studied.

CONCLUSION

The present study can be concluded as, the Malus domestica extract is showing good in vitro anticholesterol and anti gout properties.

COMPETING INTERESTS DISCLAIMER:

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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