PRELIMINARY PHYTOCHEMICAL ANALYSIS OF ANTI HYPERLIPIDIMIC AND 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: The results showed the presence of many phytochemical constituents in Malus domesticus extract such as alkaloids, proteins, amino acids, terpenoids, flavonoids, carbohydrates, saponins and steroids. The results also showed that the extract possessed in vitro anticholesterol activity. 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. Apple is the most important temperate fruit crop and has been cultivated in Asia and Europe from antiquity.(1) The genus malus has, according to most authorities, contains 25-30 species and several subspecies of so called crabapple. The cultivated apple is supposed to be the result of interspecific hybridisation. The denomination *Malus x domestica* has been generally accepted as the appropriate scientific name (2). The main progenitor of the domestic apple is considered to be *malus sieversii* which grows wild in the heavenly mountains (3). Apple fruit contains several health and sensory related constituents including dietary fiber, sugars, vitamins and phenolic compounds (4). The antioxidant capacity of apple is mostly attributed to phenolic compounds such as 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 disease which targets the joints and is caused by an abnormal buildup of uric acid in the blood and treatment of gout includes the use of therapeutic agents such as xanthine oxidase inhibitors that act by blocking the conversion of purines to uric acid (5). Allopurinol is a well known xanthine oxidase inhibitor and widely used in the therapeutic and clinical management of gout and hyperuricemia (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. Elevated lipid levels result from increased absorption through the gut or enhanced endogenous synthesis. Therapists consider the treatment of hyperlipidemia as one of the major approaches to decelerate the atherogenic process (8). Our team has extensive knowledge and research experience that has translate into high quality publications (9),(10),(11),(12),(13),(14),(15),(16),(17),(18),(19),(20),(21),(22),(23),(24),(25),(26),(27),(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. 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 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 activity 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.

\[
\text{Inhibitory activity(%) = (1 - As/Ac) \times 100}
\]

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 carried out as described as per the kit method (Spinreact, S.A.U-Ctra Santa Coloma, Girona, Spain). Cholesterol was dissolved in chloroform at a concentration of 2.5 mg mL/ml. Ten microliter of the extract was pipetted into a microtiter plate followed by the addition of 2000 μL of R1 reagent and 10 μL of cholesterol as sample. Twenty microliter of distilled water and 2000 μL of R1 reagent were used as blank. Negative control consisted of 20 μL cholesterol and 2ml R1; standard consisted of 20 μL 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:

\[
\text{Inhibition(%) = Negative control-Sample × 100}
\]

\[\text{Negative control}\]

RESULTS

Table 1: Phytochemical analysis of *Malus domestica*

<table>
<thead>
<tr>
<th>PHYTOCHEMICAL</th>
<th>MALUS DOMESTICA</th>
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<tbody>
<tr>
<td>Proteins</td>
<td>(+)</td>
</tr>
<tr>
<td>Amino acid</td>
<td>(-)</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>(+)</td>
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<tr>
<td>Flavonoids</td>
<td>(+)</td>
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<tr>
<td>Compounds</td>
<td>Activity</td>
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</tr>
<tr>
<td>Alkaloids</td>
<td>(+)</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>(+)(+)</td>
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<tr>
<td>Saponins</td>
<td>(+)</td>
</tr>
<tr>
<td>Steroids</td>
<td>(+)</td>
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</tbody>
</table>

**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*
DISCUSSION

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.

Xanthine oxidase inhibition assay is considered as the gold standard to study anti-gout potential. Phytochemical present in plants and acts as xanthine oxidase inhibitors (5). Our extract also showed potent activity in the inhibition of xanthine oxidase enzyme in a concentration dependent manner. The activity of the extract was compared with the standard drug 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 results also revealed the potent in vitro anticholesterol activity of the extract in a concentration dependent manner. The activity is somewhat less compared to the standard
drug simvastatin. Simvastatin is the standard drug for hypercholesterolemia. Statin groups of drugs are inhibitors of HMG CoA reductase, which is an enzyme associated with the cholesterol biosynthesis. The statin drugs are reported to produce many adverse effects such as cognitive loss, neuropathy, pancreatic and hepatic dysfunction, 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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