

## 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:** 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. H<sub>2</sub>SO<sub>4</sub> 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. XO activity was expressed as the percentage inhibition of XO in the above assay system calculated as percentage of inhibition as follows.

$$\text{Inhibitory activity (\%)} = (1 - A_s/A_c) \times 100$$

Where,  $A_s$  – absorbance in presence of test substance,  $A_c$  – 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  $\mu$ L of R1 reagent and 10  $\mu$ L of cholesterol as sample. Twenty microliter of distilled water and 2000  $\mu$ L of R1 reagent were used as blank. Negative control consisted of 20  $\mu$ L cholesterol and 2ml R1; standard consisted of 20  $\mu$ L 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:

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

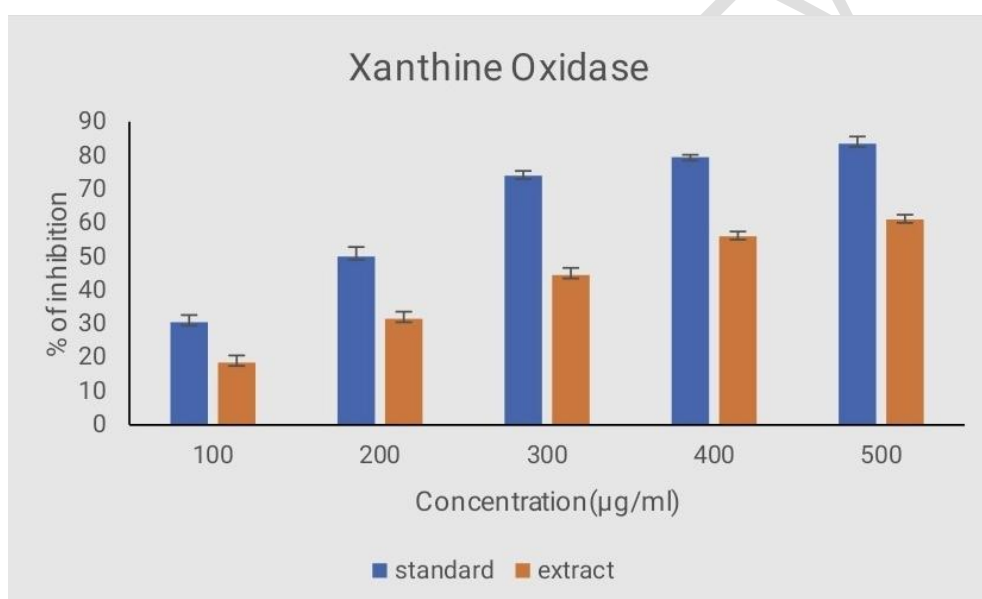
## RESULTS

**Table 1: Phytochemical analysis of *Malus domestica***

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

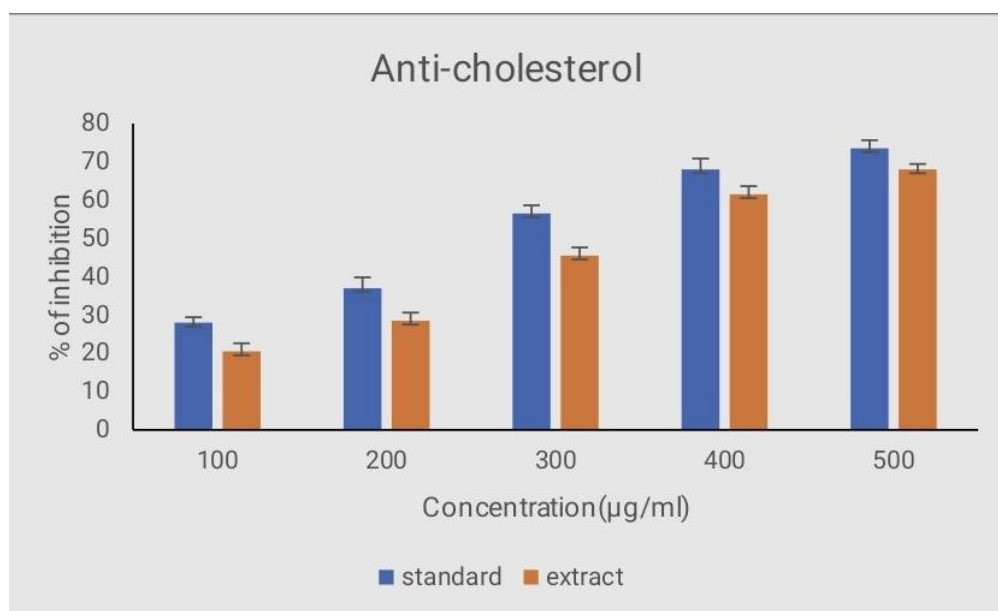
Alkaloids	(+)
Carbohydrates	(+)(+)
Saponins	(+)
Steroids	(+)

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



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

## 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.

#### **REFERENCES**

1. Weisshaar B. Faculty Opinions recommendation of The genome of the domesticated apple (*Malus × domestica* Borkh.) [Internet]. Faculty Opinions – Post-Publication Peer Review of the Biomedical Literature. 2010. Available from: <http://dx.doi.org/10.3410/f.5107956.5043054>
2. Smyth D. Faculty Opinions recommendation of The genome of the domesticated apple (*Malus × domestica* Borkh.) [Internet]. Faculty Opinions – Post-Publication Peer Review of the Biomedical Literature. 2010. Available from: <http://dx.doi.org/10.3410/f.5107956.5067054>
3. Mühlbauer W, Müller J. Apple (*Malus domestica* Borkh.) [Internet]. Drying Atlas. 2020. p. 259–68. Available from: <http://dx.doi.org/10.1016/b978-0-12-818162-1.00029-8>
4. Hagen SF, Borge GIA, Bengtsson GB, Bilger W, Berge A, Haffner K, et al. Phenolic contents and other health and sensory related properties of apple fruit (*Malus domestica* Borkh., cv. Aroma): Effect of postharvest UV-B irradiation [Internet]. Vol. 45, Postharvest Biology and Technology. 2007. p. 1–10. Available from:

<http://dx.doi.org/10.1016/j.postharvbio.2007.02.002>

5. Sreejith M, Kannappan N, Santhiagu A, Marathakam A, Ajith PM, Jasemine S. In vitro xanthine oxidase inhibitory and antioxidant activities of aerial parts of *Flacourtia sepiaria* Roxb [Internet]. Vol. 13, *Oriental Pharmacy and Experimental Medicine*. 2013. p. 113–20. Available from: <http://dx.doi.org/10.1007/s13596-012-0100-4>
6. Sahgal G, Ramanathan S, Sasidharan S, Mordi MN, Ismail S, Mansor SM. In Vitro Antioxidant and Xanthine Oxidase Inhibitory Activities of Methanolic *Swietenia mahagoni* Seed Extracts [Internet]. Vol. 14, *Molecules*. 2009. p. 4476–85. Available from: <http://dx.doi.org/10.3390/molecules14114476>
7. El-Tantawy WH, Temraz A, Hozaien HE, El-Gindi OD, Taha KF. Anti-hyperlipidemic activity of an extract from roots and rhizomes of *Panicum repens* L. on high cholesterol diet-induced hyperlipidemia in rats [Internet]. Vol. 70, *Zeitschrift für Naturforschung C*. 2015. p. 139–44. Available from: <http://dx.doi.org/10.1515/znc-2014-4147>
8. Nomura H, Kimura Y, Okamoto O, Shiraishi G. Effects of antihyperlipidemic drugs and diet plus exercise therapy in the treatment of patients with moderate hypercholesterolemia. *Clin Ther*. 1996 May;18(3):477–82.
9. Wu F, Zhu J, Li G, Wang J, Veeraraghavan VP, Krishna Mohan S, et al. Biologically synthesized green gold nanoparticles from Siberian ginseng induce growth-inhibitory effect on melanoma cells (B16). *Artif Cells Nanomed Biotechnol*. 2019 Dec;47(1):3297–305.
10. Chen F, Tang Y, Sun Y, Veeraraghavan VP, Mohan SK, Cui C. 6-shogaol, a active constituents of ginger prevents UVB radiation mediated inflammation and oxidative stress through modulating Nrf2 signaling in human epidermal keratinocytes (HaCaT cells). *J Photochem Photobiol B*. 2019 Aug;197:111518.
11. Li Z, Veeraraghavan VP, Mohan SK, Bolla SR, Lakshmanan H, Kumaran S, et al. Apoptotic induction and anti-metastatic activity of eugenol encapsulated chitosan nanopolymer on rat glioma C6 cells via alleviating the MMP signaling pathway [Internet]. Vol. 203, *Journal of Photochemistry and Photobiology B: Biology*. 2020. p. 111773. Available from: <http://dx.doi.org/10.1016/j.jphotobiol.2019.111773>
12. Babu S, Jayaraman S. An update on  $\beta$ -sitosterol: A potential herbal nutraceutical for diabetic management. *Biomed Pharmacother*. 2020 Nov;131:110702.
13. Malaikolundhan H, Mookkan G, Krishnamoorthi G, Matheswaran N, Alsawalha M, Veeraraghavan VP, et al. Anticarcinogenic effect of gold nanoparticles synthesized from *Albizia lebbek* on HCT-116 colon cancer cell lines. *Artif Cells Nanomed Biotechnol*. 2020 Dec;48(1):1206–13.
14. Han X, Jiang X, Guo L, Wang Y, Veeraraghavan VP, Krishna Mohan S, et al. Anticarcinogenic potential of gold nanoparticles synthesized from *Trichosanthes kirilowii* in colon cancer cells through the induction of apoptotic pathway. *Artif Cells Nanomed Biotechnol*. 2019 Dec;47(1):3577–84.
15. Gothai S, Muniandy K, Gnanaraj C, Ibrahim IAA, Shahzad N, Al-Ghamdi SS, et al.

Pharmacological insights into antioxidants against colorectal cancer: A detailed review of the possible mechanisms. *Biomed Pharmacother.* 2018 Nov;107:1514–22.

16. Veeraraghavan VP, Hussain S, Balakrishna JP, Dhawale L, Kullappan M, Ambrose JM, et al. A Comprehensive and Critical Review on Ethnopharmacological Importance of Desert Truffles: *Terfezia claveryi*, *Terfezia boudieri*, and *Tirmania nivea* [Internet]. *Food Reviews International.* 2021. p. 1–20. Available from: <http://dx.doi.org/10.1080/87559129.2021.1889581>
17. Sathya S, Ragul V, Veeraraghavan VP, Singh L, Niyas Ahamed MI. An in vitro study on hexavalent chromium [Cr(VI)] remediation using iron oxide nanoparticles based beads. *Environmental Nanotechnology, Monitoring & Management.* 2020 Dec 1;14:100333.
18. Yang Z, Pu M, Dong X, Ji F, Priya Veeraraghavan V, Yang H. Piperine loaded zinc oxide nanocomposite inhibits the PI3K/AKT/mTOR signaling pathway via attenuating the development of gastric carcinoma: In vitro and in vivo studies. *Arabian Journal of Chemistry.* 2020 May 1;13(5):5501–16.
19. Rajendran P, Alzahrani AM, Rengarajan T, Veeraraghavan VP, Krishna Mohan S. Consumption of reused vegetable oil intensifies BRCA1 mutations. *Crit Rev Food Sci Nutr.* 2020 Oct 27;1–8.
20. Barma MD, Muthupandiyani I, Samuel SR, Amaechi BT. Inhibition of *Streptococcus mutans*, antioxidant property and cytotoxicity of novel nano-zinc oxide varnish. *Arch Oral Biol.* 2021 Jun;126:105132.
21. Samuel SR. Can 5-year-olds sensibly self-report the impact of developmental enamel defects on their quality of life? *Int J Paediatr Dent.* 2021 Mar;31(2):285–6.
22. Samuel SR, Kuduruthullah S, Khair AMB, Shayeb MA, Elkaseh A, Varma SR. Dental pain, parental SARS-CoV-2 fear and distress on quality of life of 2 to 6 year-old children during COVID-19. *Int J Paediatr Dent.* 2021 May;31(3):436–41.
23. Tang Y, Rajendran P, Veeraraghavan VP, Hussain S, Balakrishna JP, Chinnathambi A, et al. Osteogenic differentiation and mineralization potential of zinc oxide nanoparticles from *Scutellaria baicalensis* on human osteoblast-like MG-63 cells [Internet]. Vol. 119, *Materials Science and Engineering: C.* 2021. p. 111656. Available from: <http://dx.doi.org/10.1016/j.msec.2020.111656>
24. Yin Z, Yang Y, Guo T, Veeraraghavan VP, Wang X. Potential chemotherapeutic effect of betalain against human non-small cell lung cancer through PI3K/Akt/mTOR signaling pathway. *Environ Toxicol.* 2021 Jun;36(6):1011–20.
25. Veeraraghavan VP, Periadurai ND, Karunakaran T, Hussain S, Surapaneni KM, Jiao X. Green synthesis of silver nanoparticles from aqueous extract of *Scutellaria barbata* and coating on the cotton fabric for antimicrobial applications and wound healing activity in fibroblast cells (L929). *Saudi J Biol Sci.* 2021 Jul;28(7):3633–40.
26. Mickymaray S, Alfaiz FA, Paramasivam A, Veeraraghavan VP, Periadurai ND, Surapaneni KM, et al. Rhaponticin suppresses osteosarcoma through the inhibition of

- PI3K-Akt-mTOR pathway. Saudi J Biol Sci. 2021 Jul;28(7):3641–9.
27. Teja KV, Ramesh S. Is a filled lateral canal – A sign of superiority? [Internet]. Vol. 15, Journal of Dental Sciences. 2020. p. 562–3. Available from: <http://dx.doi.org/10.1016/j.jds.2020.02.009>
  28. Theertha M, Sanju S, Priya VV, Jain P, Varma PK, Mony U. Innate lymphoid cells: Potent early mediators of the host immune response during sepsis. Cell Mol Immunol. 2020 Oct;17(10):1114–6.
  29. T T, Thilagavathi T, Arvindganth R, Vidhya D, Dhivya R. PRELIMINARY PHYTOCHEMICAL SCREENING OF DIFFERENT SOLVENT MEDIATED MEDICINAL PLANT EXTRACTS EVALUATED [Internet]. Vol. 6, International Research Journal of Pharmacy. 2015. p. 246–8. Available from: <http://dx.doi.org/10.7897/2230-8407.06455>
  30. Sofowora A. Medicinal Plants and Traditional Medicine in Africa. 2000. 256 p.
  31. Hudaib MM, Tawaha KA, Mohammad MK, Assaf AM, Issa AY, Alali FQ, et al. Xanthine oxidase inhibitory activity of the methanolic extracts of selected Jordanian medicinal plants. Pharmacogn Mag. 2011 Oct;7(28):320–4.
  32. Golomb BA, Evans MA. Statin adverse effects : a review of the literature and evidence for a mitochondrial mechanism. Am J Cardiovasc Drugs. 2008;8(6):373–418.