

# Comparative Evaluation of Anti-Cholesterol Potential of Apple Cider Vinegar and Its Herbal Formulation with Allium Sativum and Honey-An *In-Vitro* Assay

Fathima Hinaz Z, Gayathri R, J Selvaraj, V Vishnu Priya, Kavitha S, Gayathri R\*

Department of Biochemistry, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, India

## ABSTRACT

**Introduction:** Apple cider vinegar, Allium sativum and honey are natural herbal compounds generally used in households and it's also a folk medicine. Allium sativum and honey are rich in various phytochemicals. High LDL cholesterol over time can damage arteries and contribute to heart diseases. Oxidative stress is one of the major etiology in the pathogenesis and complications of atherosclerosis. As per Ayurveda there exhibit a huge collection of plants with anti-cholesterol potential, but only few of them have been scientifically proven.

**Materials and methods:** The herbs were collected from a local herbal store and then the extraction of the herbs was done. Phytochemical screening test was done followed by the evaluation of antioxidant and anti-cholesterol potential. The data were analysed statistically by a one-way analysis of variance (ANOVA) followed by Duncan's multiple range test was used to see the statistical significance among the groups. The results with the  $p < 0.05$  level were considered to be statistically significant.

**Results:** The Apple cider vinegar and its formulation exhibited a significant antioxidant potential (IC-50 at  $280\mu\text{g/ml}$ ) and anti-cholesterol potential (IC-50 at  $305\mu\text{g/ml}$ ).

**Conclusion:** The synergistic effect and the benefits of Apple cider Vinegar and its formulation was studied. The study has created an awareness on the importance of including herbal formulation in the prevention of cholesterol when consumed on a daily basis.

**Key words:** Anti-cholesterol, folk medicine, antioxidant, synergism, phytochemicals, Innovative technology, Novel method

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**Corresponding author:** Gayathri R  
**e-mail** ✉: gayathri.sdc@saveetha.com  
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## INTRODUCTION

Apple cider vinegar is considered to be a type of vinegar which is made from cider or apple must with a pale colour. It is also noted that unpasteurized or organic Apple cider vinegar contains the mother of vinegar. Just like that in apple juice, Apple cider vinegar also contains pectin, vitamin B1, vitamin B2, vitamin B6, folic acid, biotin, vitamin C, etc. [1] There are various other research already being conducted to identify its various other therapeutic effects on blood glucose level, infections, satiety, hypertension, cancer and many other clinical purposes [2]. In a study conducted on Apple cider vinegar, it has been revealed that apple cider vinegar shows promising effects on patients with type 2 diabetes as well as diabetes related clinical conditions [2,3]. Many medicinal components that show various health benefits have been reported in natural vinegar. Various kinds of vinegar are

produced from various other regional foods according to some customs. The balsamic vinegar of Modena, Italy is obtained from their local white Trebbiano grapes. Traditional rice wine Vinegar is having its roots from Asia. While coconut and cane vinegar is very common in India, and other vinegars such as date vinegar is more famous in the middle Eastern countries. Vinegar is also very famous for its antimicrobial properties. [4].

Garlic is one of the major food sources in the Asian kitchen, which shows various beneficial medical properties, starting with anti-CVD, high blood pressure, dyslipidemia etc. Garlic can be consumed on a daily basis just like that of Apple cider vinegar in the form of raw Garlic, powdered Garlic tablets, or extracted oil. Among various other foods that show anti-inflammatory properties, and cardiovascular parameters, the use of Garlic is quite common [5]. Animal studies have revealed that the hesperidin and eryosytryn have antioxidant properties and that they can decrease oxidative stress [6].

Honey has been reported to show a broad range of beneficial effects. While all of these three herbs

individually already exhibit, anti-cholesterol properties, when combined together it can act as a super strong anti-cholesterol agent that can be consumed on daily basis; as you can have a look into our history of food habits, all three, Apple cider vinegar, Honey and Garlic have been consumed by our ancestors on a daily basis, which is why they maintained a healthy life, and also did they had no need of any sort of synthetic drugs. So by analyzing the anti-cholesterol potential of Apple cider vinegar, Garlic and Honey, the side effects of the standard drug for cholesterol can be prevented.

Atherosclerosis is a chronic disease occurring in the arterial walls [7]. Multiple risk factors are associated along with atherosclerosis, that are associated along with atherosclerosis, that are associated along with atherosclerosis, that are associated along with atherosclerosis, that involves oxidative stress, hypercholesterolemia, hypertension, diabetes, inflammatory responses etc [7,8]. Accumulating evidence reveals that plant originated antioxidant sources are far better effective than synthetic ones [9-11]. Our team has extensive knowledge and research experience that has translate into high quality publications [12-28]. The aim of the study is to evaluate the anti-cholesterol potential of apple cider vinegar and its herbal formulation with Garlic and Honey.

## MATERIAL AND METHOD

### Chemicals

All chemicals and reagents used for this research work were purchased from sigma chemical company St. Louis, MO, USA; Invitrogen, USA; Eurofins Genomics India Pvt Ltd, Bangalore, India; New England Biolabs (NEB), USA

### Collection of plant material

Apple cider vinegar, allium sativum and honey were purchased from a local herbal health care centre, Chennai District, Tamil Nadu, India. The species were identified and authenticated at the Department of Centre for Advanced study in Botany, University of Madras, Chennai, India. Garlic cloves were finely peeled, chopped and crushed using mortar and pestle. The coarse paste was used for the extraction with ethanol.

### Preparation of formulation of apple cider vinegar with garlic and honey

Garlic was crushed and juice was extracted. Equal volume of Apple cider vinegar, Honey and Garlic extract was added. Antioxidant and Anti-cholesterol potential of Apple cider vinegar was evaluated and compared with the formulation of Apple cider vinegar with Garlic and Honey.

### 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 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 millilitre 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 millilitre 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.

#### Antioxidant activity

##### DPPH free radical scavenging activity

Scavenging of 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radicals was assessed by the method of [29]. 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 517 nm. Ascorbic acid at the same concentrations was used as standard. The

capability to scavenge the DPPH radical was calculated and expressed in percentage (%) using following formula:

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

#### In vitro anti-cholesterol activity

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. Ten microliter of the extract was pipetted into a microtiter plate followed by the addition of 2000  $\mu\text{L}$  of R1 reagent and 10  $\mu\text{L}$  of cholesterol as sample. Twenty microliter of distilled water and 2000  $\mu\text{L}$  of R1 reagent were used as blank. Negative control consisted of 20  $\mu\text{L}$  cholesterol and 2ml R1; standard consisted of 20  $\mu\text{L}$  simvastatin and 2000  $\mu\text{L}$  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:

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

#### Statistical analysis

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

## RESULTS

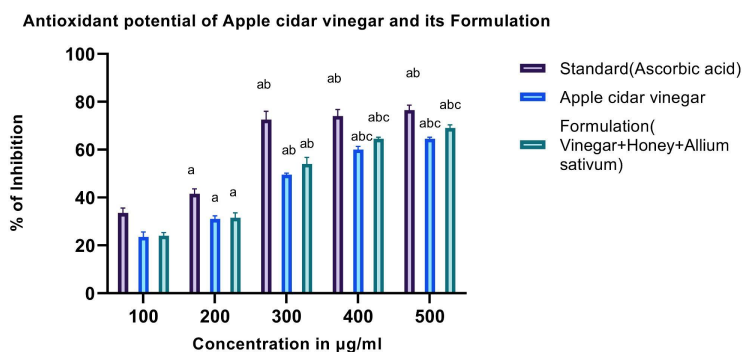
From study, it was evident that the formulation showed a strong presence of phytochemicals such as alkaloids, flavonoids, terpenoids and steroids.

On the contrary, Apple cider vinegar showed the presence of flavonoid alone. From this finding it is evident that formulation increases the efficiency of the drug by increasing the phytonutrients. The antioxidant analysis of the apple cider vinegar (IC-50 at 310  $\mu\text{g}/\text{ml}$ ) and its formulation with extracts of Garlic and honey (IC-50 at 280  $\mu\text{g}/\text{ml}$ ) (Figure 1) was analysed and compared with the standard vitamin C. When compared to standard drugs the extract contained lesser antioxidant capacity (Table 1).

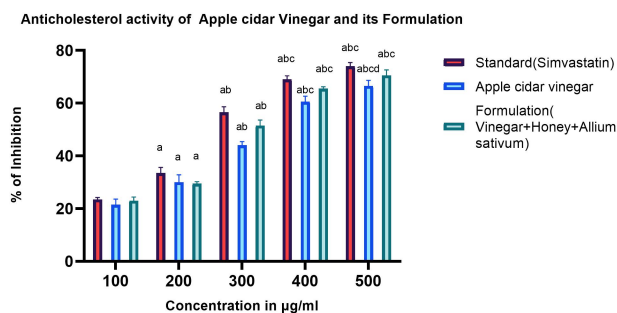
Anti-cholesterol activity of both Apple cider vinegar and its formulation was analysed. IC50 was found to be 355  $\mu\text{g}/\text{ml}$  and 325  $\mu\text{g}/\text{ml}$  respectively (Figure 2). Anti-Cholesterol potential of the extracts increased in a dose dependent manner as compared to the standard (statins).

**Table 1: Phytochemical Analysis of Apple cider vinegar and its formulation with Allium sativum and honey.**

S.NO	Phytochemicals	Apple cider vinegar	Apple Cider Vinegar, Garlic, Honey
1	Alkaloids	-	++
2	Flavonoids	+	++
3	Terpenoids	-	+
4	Carbohydrates	-	++
5	Saponins	-	++
6	Phenols	-	++
7	Proteins	+	+++
8	Tanans	-	++
9	Steroids	-	+



**Figure 1: Represents the antioxidant potential of apple cider vinegar and its formulation (Vinegar +Honey+Garlic) compared with the standard Vitamin C-DPPH Assay." X" axis represents the different concentrations of the herbal extracts and the "Y" axis represents the % of inhibition. Purple colour denotes standard drug (Vitamin-C), blue colour represents apple cider vinegar and green colour represents formulation (Vinegar+Honey+Garlic). Each bar represents Mean  $\pm$  SEM of 3 independent observations. Significance at  $p < 0.05$ ."**



**Figure 2: Represents the Anti-Cholesterol potential of Apple cider vinegar and its Formulation (Vinegar +Honey+AlliumSativum) compared with the standard drug (Simvastatin).” X” axis represents various concentrations of the herbal extracts and “Y” axis represents the % of inhibition. Red colour denotes standard drug (Simvastatin), blue colour represents Apple cider vinegar and green colour represents formulation (Vinegar+Honey +Garlic).Each bar represents Mean  $\pm$  SEM of 3 independent observations . Significance at  $p < 0.05$ .”**

## DISCUSSION

Phytochemicals present in formulation (Apple cider vinegar, Garlic and Honey) was found to be Alkaloids, Terpenoids, Saponins, Phenols, Tanans, Flavonoids and Steroids whereas in the Apple cider Vinegar only slight traces of flavonoids and proteins were found. This finding clearly shows that herbal drugs when taken as a formulation increases the phytonutrients which can be the key determinant of its medicinal potential.

The antioxidant analysis of the extract exhibited radical scavenging capacity at (IC-50=280µg/ml). For Apple cider vinegar and (IC-50=305µg/ml) for the formulation. The increased antioxidant potential of the formulation clearly indicates the synergistic role of the herbal extracts. The presence of phytonutrients like flavonoids, Alkaloids, Phenols indicates that the extract can be a potential cholesterol reduction property. The extracts were prepared and analysed for antioxidant property by DPPH free radicals scavenging assay. In an oxidative stress setting, free radicals are molecules with an unpaired electron. Phenolic compounds have greater importance in free radical scavenging activity. The effect of antioxidants on DPPH free radical scavenging was considered to be due to their hydrogen donating ability. The results obtained in this study. Apple cider vinegar, Garlic, Honey has a significant antioxidant activity.

The extracts were prepared and analyzed for anti-cholesterol properties. Simva-statin was used as positive control and inhibitory action was found for the extract. The formulation of Apple cider vinegar, Garlic and Honey extract exhibited a significant anti-cholesterol property as compared to Apple cider vinegar when taken alone. Aged garlic extract (AGE) is known to have a protective effect against immune system, endothelial function, oxidative stress and inflammation [30]. It has been found that honey ameliorates cardiovascular risk factors in

healthy individuals and in patients with elevated risk factors [31]. Liver and kidney of diabetic rats showed significant ( $p < 0.05$ ) elevation in the levels of cholesterol, TGs, FFAs and PLs, when compared with normal rats. Upon oral administration of DS there was a significant ( $p < 0.05$ ) reduction in the content of cholesterol, TGs, FFAs and PLs in both the tissues, when compared with diabetic control rats [32]. Animals treated with diosmin exhibited significant dwindled levels of lipid peroxidation in the liver [33]. Both normal and diabetic animals were fed with standard animal food containing apple cider vinegar (6% w/w) for 4 weeks. Fasting blood glucose did not change, while HbA1c significantly decreased by apple cider vinegar in diabetic groups ( $p < 0.05$ ) [34]. Natural medicines always garner more attention in diabetic pharmaceuticals due to their long term efficacy and safety [35].Vinegar, honey and Garlic are components of ancient and indigenous medicines, people use these as natural remedies at home. High cholesterol levels trigger mitochondrial oxidative stress on cartilage cells; oxidative stress plays a major role in the development of insulin resistance [36]. Phytosterols are bioactive compounds that are naturally present in plant cell membranes with chemical structure similar to the mammalian cell- derived cholesterol [12].Though much research has been done on individual extracts and its activity, further research is needed to study the herbal drug as a formulation. These research can create an awareness and fill the gap in the current drug requirements.

Further in-vivo study may be done to analyze the potential health benefits in the prevention and generation of reactive oxidant species cholesterol associated disorder. The sugar components determine the energy value and the physicochemical properties of honey which are its critical aspects for technological functions of honey [37]. Several other studies have already been conducted concerning the connection between Garlic and honey in reducing inflammatory biomarkers and lipid levels. Flavonoids like quercetin can be introduced into RBCs and have an antioxidant effect on the membranes of the RBCs. The research's key purpose was to provide treatment options for patients who can't take statins due to side effects. Additional research is needed on the topic, as there is not enough evidence to say how well they work and for which patients they'll work best. This study depicts that these remedies from herbal extract are generally safe, with no adverse effects, and cholesterol lowering benefits, these alternatives are reasonable to consider in certain conditions. Natural remedies are recommended for patients who cant tolerate statins, but however natural remedies cannot replace statin therapy, while this study can help in further study for better therapeutic effects on cholesterol. Synergism is the interaction or the cooperation between these three herbs to produce a combined effect of anti-cholesterol properties that sum up their separate effects into one.

### CONCLUSION

Herbal extracts which are taken on a daily basis as an indigenous medicines prove to be more potent in curing ailments like hyper cholesterol and other disorders arise due to oxidative stress. Compared to individual extracts, formulations of indigenous medicine needs lots of research to explore its medicinal value. From the current study, a formulation with apple cider vinegar with Garlic and Honey proves to be more efficient in decreasing cholesterol levels. In future more research is required to explore the activity of more such formulation and synergistic role of herbal extracts.

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### CONFLICT OF INTEREST

The author declares that there was no conflict of interest in the present study.

### AUTHORS CONTRIBUTION

- Fathima Hinaz: Literature search, data collection, Analysis, Manuscript drafting
- Gayathri R: Data verification, Manuscript drafting.

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