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A Comparative Analysis on the Anti-Cholesterol Activities of *Allium cepa* and *Allium sativum*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Background: Genus *Allium* produces compounds of sulfur which is an important component for medical use. Studies have The *Allium* species and their extracts have the effect on cardiovascular disease risk factor. Both *Allium cepa* and *Allium sativum* are used as one of the spices in food preparation. *Allium sativum* or garlic is employed in the treatment of many diseases like blood pressure, atherosclerosis, high cholesterol, heart attack and coronary heart disease. Many biological properties like antioxidant, antimicrobial and antidiabetic are attributed to the abundance of *Allium cepa*.
Aim: The study aimed to compare the *in vitro* anti-cholesterol activities of *Allium sativum* and *Allium cepa.*
Methods: The phytochemical analysis, in vitro antioxidant activity and anti-cholesterol activity of both the extracts *Allium cepa* and *Allium sativum* were analysed using standard protocols. 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 $p<0.05$.

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Results: Phytochemical screening showed that both the plant extracts are rich in phytochemicals like phlobatannin, carbohydrate, flavonoids, alkaloids, terpenoids, proteins and steroids and detection of saponin was done. DPPH radical scavenging activity showed the potent antioxidant activity of both the plant extracts. A comparative analysis on the anti-cholesterol activities of *Allium cepa and Allium sativum* revealed that Allium cepa showed more anti-cholesterol activity compared to *Allium sativum*.
Conclusion: The study revealed the potent antioxidant and anticholesterol activity of *Allium cepa* compared to *Allium sativum*.

Keywords: Allium cepa, Allium sativum, hyperlipidemia, Anti-cholesterol activity, Antioxidant activity, innovative technology, novel method.

1. INTRODUCTION

Hyperlipidemia is a condition where excess fatty substances, lipids are present in the blood. Abnormal level of lipids in blood (hyperlipidemia) is a symptom of different disorders of lipoprotein metabolism. Extra amount of lipid circulating in blood is attached to the protein and is known as hyperlipoproteinemia [1]. Hypercholesterolemia is a genetic disorder of metabolism of lipoprotein characterized by high plasma concentrations of deposition of cholesterol in extravascular tissues and increased risk of heart disease [2]. One of the leading causes of deadly disease in the world is hyperlipidemia. Increased levels of lipids circulating in blood develops cardiovascular and metabolic syndrome diseases [3]. Chemically active atoms which use oxygen to generate energy in the form of ATP are known as free radicals. Antioxidants are helpful in preventing harmful effects of free radicals [4].

Medical plants such as *Allium cepa* and *Allium sativum* have become the main focus of intense study to gather conversation and potential pharmacological effects. Allium sativum not only has a wide spectrum of actions in antibacterial, antiviral, antifungal and antiprotozoal, but also has beneficial effects on the cardiovascular systems [5]. It is used as an herbal medicine and prevents deadly diseases like cardiovascular disease. In epidemiological studies and animal experiments it lowers the plasma lipid and cholesterol [6]. Many recognised Allium sativum as diuretic and be used for treatments like stomach aches, etc [7]. Allium sativum is used to prevent wound infection and food spoilage. *Allium cepa* is used to evaluate DNA damages and assess a greater number of chemical agents. It is widely used to assess the impacts caused by xenobiotics which is an important tool for environmental monitoring studies. Allium cepa is a common and rich source of dietary flavonoids, and has three highly valuable phytochemicals: flavonoids, fructans, and organosulfur compounds which provide beneficial effects for human health [8]. Both Allium cepa and Allium sativum are used all over the world for cure infections, diseases, injuries etc [9].

Free radicals contribute to many disorders in humans like arthritis, central nervous system injury, and cancer. Recent research confirms that antioxidants are the most effective tools to eliminate free radicals which cause oxidative stress and are possible protective agents that protect the cells from reactive oxygen species and retard the progress of many diseases as well as lipid peroxidation [10]. This research is needed to find a cost-effective and safe drug for anti-cholesterol activity. Since a comparative anti-cholesterol activity is not studied in *Allium cepa* and *Allium sativum,* this study will fulfill this deficiency. Our team has extensive knowledge and research experience that has translate into high quality publications [11-30]. The aim of this study is to have a comparative analysis on the anti-cholesterol activities of *Allium cepa* and *Allium sativum.*

2. MATERIALS AND METHODS

2.1 Phytochemical Screening Test

2.1.1 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.

2.1.2 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 red or dull violet rings at the junction of the liquids showed the presence of carbohydrates.

2.1.3 Test for Flavonoid

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.

2.1.4 Test for Alkaloids

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

2.1.5 Test for Terpenoids

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

2.1.6 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.

2.1.7 Detection of saponins

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

2.1.8 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.

2.2 DPPH Free Radical Scavenging Activity of *Allium sativum* and *Allium cepa*

Scavenging of 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radicals was assessed by the method of Hatano et al, (1989). 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 the following formula:

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

2.3 *In vitro* Anti-cholesterol Activity of *Allium sativum* and *Allium cepa*

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 microliters of the extract were 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:

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

2.4 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 $p<0.05$.

3. RESULTS

The antioxidant activity of the extract *Allium cepa* showed that there is an increase in the *in vitro* antioxidant activity as the concentration increases. The percentage of inhibition of *Allium cepa* is more compared to *Allium sativum* for the anti-cholesterol activity (Fig1). Both *Allium cepa* and *Allium sativum* have anti-cholesterol activities. But compared to both plant extracts,

Allium cepa has more anti-cholesterol activity compared to *Allium sativum* (Fig.2). Preliminary phytochemical screening analysis showed presence of phytochemicals like, protein, amino acid, carbohydrate, terpenoids, flavonoids, alkaloids steroids and saponins. Amino acids were high present in both the plant extracts studied (Table 1).

Table 1. Phytochemical screening of *Allium cepa* and *Allium sativum*

PHYTOCHEMICAL SCREENING OF ALLIUM CEPA (ONION) AND ALLIUM SATIVUM (GARLIC)

PHYTOCHEMICAL	ALLIUM CEPA	ALLIUM SATIVUM
Protein	+	+
Amino acid	+++	+++
Carbohydrate	++	+
Terpenoids	+	+
Flavonoids	+	++
Alkaloids	+	++
Steroids	++	+
Saponin	++	+

The phytochemical screening of Allium cepa and Allium sativum is tabulated ('+' indicate presence and '-'indicate absence)

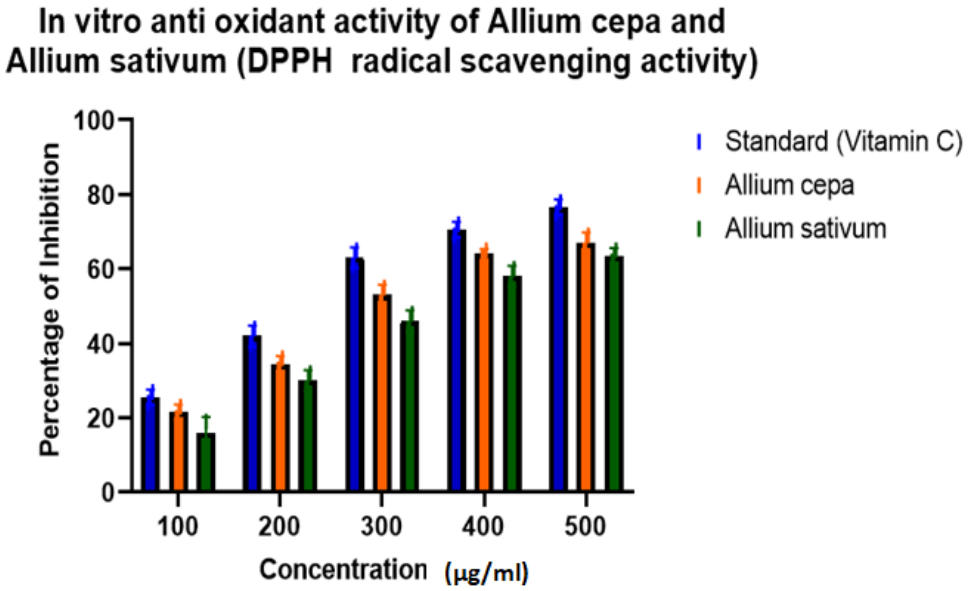

Fig. 1. Bar graph depicts the association between the concentration and scavenging activity. X-axis represents the concentration of "Standard (Vitamin C)", "*Allium cepa*", and "Allium *sativum*". Y-axis represents the percentage of inhibition. Green colour represents Standard (Vitamin C), Yellow colour denotes *Allium sativum* and Blue colour denotes *Allium cepa*. The percentage of inhibition of *Allium cepa* is more for DPPH radical scavenging activity. Significance p<0.05

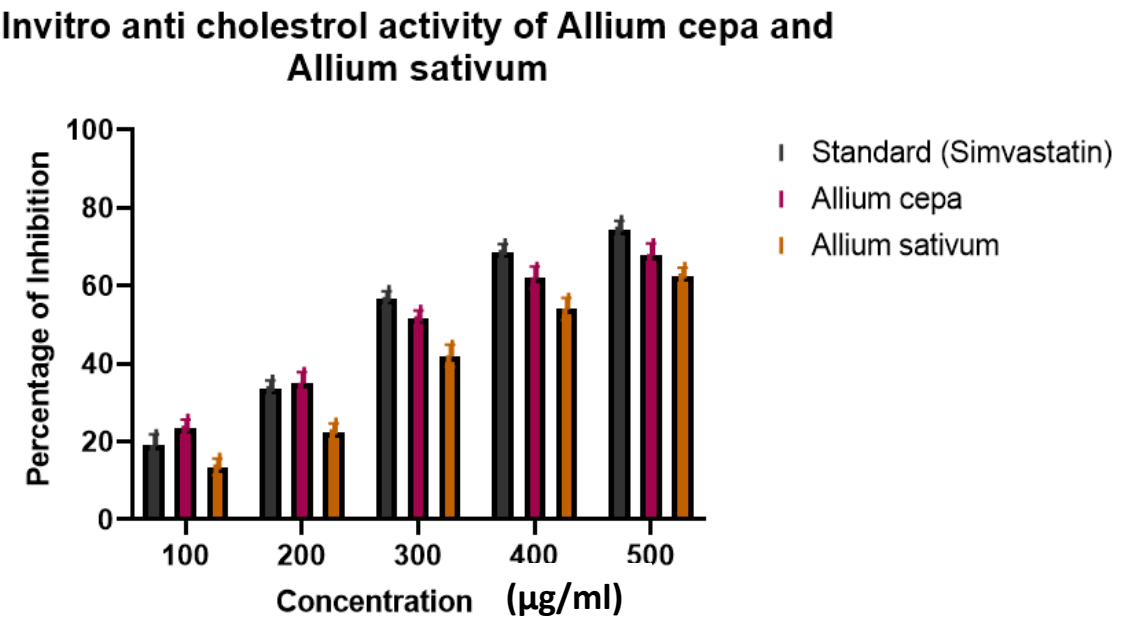

Fig. 2. Bar graph depicts the association between the concentration and anticholesterol activity. X-axis represents the concentration of "Standard (Simvastatin)", "*Allium cepa*" and "*Allium sativum*". Y-axis represents the percentage of inhibition. Blue colour represents Standard (Simvastatin), Orange colour denotes *Allium sativum* and Grey colour denotes *Allium cepa*. The percentage of inhibition of *Allium cepa* is more for anti-cholesterol activity. Significance p<0.05

4. DISCUSSION

The results of the phytochemical screening indicates that *Allium sativum* is rich in amino acids, flavonoids, alkaloids and moderately rich in proteins, carbohydrates, terpenoids, steroids, and saponin. Phytochemical screening of Allium *cepa revealed that it* is rich in amino acid, carbohydrate, steroids and saponin and it is moderately rich in protein, terpenoids, flavonoids, and alkaloids. DPPH radical scavenging activity showed that both the plants possess in vitro antioxidant activity. The plant also possessed anti-cholesterol in vitro. In another study by [31], screening test of *Allium cepa* and *Allium sativum* resulted that water and ethanol extracted more components than other substances like acetone in this study .The plant is rich in phytochemicals which might be the underlined reason for the beneficial activities of the plant. The medicinal value of plant is related to their phytochemical component content and secondary metabolites, including: phenolic compounds, flavonoids, alkaloids, tannins, and other stress gene response products [32].

The in vitro antioxidant activity of the plant extracts was assayed by DPPH radicals scavenging activity by using vitamin C as standard. The results showed that the extracts possessed in vitro antioxidant activity in a concentration-dependent manner. Due to antioxidant activity, *Allium cepa* and *Allium sativum* have a role in decreasing risk factors of chronic diseases and by reducing the oxidative stress [33]. Due to the presence of organosulfur and flavonoid compounds these beneficial effects are present in these plant extracts [32]. Hence it was found that *Allium cepa* and *Allium sativum* possess potent antioxidant activity. The ethanolic extracts of *Allium cepa* and *Allium sativum* are found to have in vitro anti-cholesterol activity in a dose dependent manner although its activity is not as potent as the standard drug statin.

Allium sativum gained substantial interest by many researchers because of its impact on lipid levels. *Allium sativum* is discovered to have useful cardiovascular effects including reduction in cholesterol [34]. Hyperlipidemia is caused by the abundance of fatty substances or lipids in blood. This may be caused due to genetic factors or metabolic diseases like diabetes mellitus etc. Since the prolonged exposure of synthetic anti-cholesterol drug, statin is having severe side effects, the search for a new cost effective and

natural drug is an urgent need and our results showed in vitro anti-cholesterol activity. Hence these plants can be used for the treatment of hypercholesterolemia. Only preliminary in vitro studies are done on anti-cholesterol activities. To develop a therapeutic drug for hypercholesterolemia for these drugs, further in vitro and in vivo studies should be performed.

5. CONCLUSION

A comparative analysis on the anti-cholesterol activities of *Allium cepa* and *Allium sativum* was done. Both *Allium cepa* and *Allium sativum* have anti-cholesterol activities. But *Allium cepa* possessed more anti-cholesterol activity compared to *Allium sativum.*

DISCLAIMER

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.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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