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In Vitro Xanthine Oxidase Inhibitory Potentials of Garlic Oil

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ABSTRACT

Gout is a disorder that is caused due to the accumulation of uric acid crystals in the joints and various tissues of the body. Xanthine oxidase inhibitors are one of the important ways for controlling gout. Garlic (Allium sativum) is a member of the Liliaceae family.Garlic oil is rich in organosulfides compounds. Garlic and its derivatives have less side effects which resulted in the upcoming of medicine for various diseases .The therapeutic properties of garlic oil are reduction in the risk factors of cardiovascular disease, antioxidant effect, antimicrobial effect. Garlic oil is a useful compound in treatment of arthritis, toothache, chronic cough, constipation, parasitic infestation. The aim of the present study is to screen the phytochemical constituents and to assess the in vitro xanthine oxidase inhibitory potential of garlic oil. The oil was purchased online from DEVES HERBES. Invitro activity was assessed by using allopurinol as standard and phytochemical screening was done using standard phytochemical procedures. The result showed that garlic oil has xanthine oxidase inhibitory potential, the activity increases with increases in concentration. The phytochemical analysis showed that garlic oil is rich in alkaloids, and has moderate amounts of carbohydrates, terpenoids, flavonoids, and phlobatannin. This study shows potent oxidase inhibitory activity and presence of phytochemical constituents in garlic oil. This oil can be suggested for the treatment of gout if further detailed in vivo confirmatory studies are done.

KEY WORDS: ALLOPURINOL; XANTHINE OXIDASE; INHIBITORS; GARLIC OIL; GOUT.

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INTRODUCTION

Gout distinguished itself in the history of Homo sapiens since time immemorial (Ragab, Elshahaly and Bardin, 2017). Excess of urate in the body will cause gout which will lead to the accumulation of crystals of monosodium urate in various tissues. Hereditary disorders of uric acid secretion and purine metabolism are the main causes of gout (Meltzler, 2014). Accumulation of crystals are mainly found around the joints (Ragab, Elshahaly and Bardin, 2017). Asymptomatic hyperuricemia, acute gouty arthritis, inter critical gout, chronic tophaceous gout are the four clinical stages of gout. The major risk factors for the developing gout is serum uric acid level (SUA) (Schlesinger, 2004). Gout is predominantly found in males in olden days but they now gout has been increased in females particularly after menopause (Nuki and Simkin, 2006). When compared to the other Asian populations Tamilians have a higher level of uric acid (Talbott, 1958; Paul and James, 2017).

Xanthine oxidase is a versatile enzyme, ubiquitous among species i.e. bacteria to man and they are found in various types of tissues in mammals. It belongs to a group of enzymes known as molybdenum iron-Sulphur flavin hydrolases. Compared to uricosuric and anti-inflammatory agents, xanthine oxidase inhibitors have less side effects (Nuki and Simkin, 2006; Umamaheswari et al., 2007). Catalyzing oxidation of hypoxanthine to xanthine and xanthine to uric acid are the major functions of xanthine oxidase. But overproduction of uric acid will lead to hyperuricemia which will further lead to gout. Excess of uric acid can be controlled by xanthine oxidase inhibitors which is used in therapeutics (Unno, Sugimoto and Kakuda, 2004; Nuki and Simkin, 2006).

Allopurinol is the important drug used for the treatment of gout (Pacher, Nivorozhkin and Szabó, 2006). Uric acid production and serum uric acid rate are decreased by allopurinol (Saag et al., 2017). Gout patients who use allopurinol for treatment have improvement in their glomerular functions (Gibson et al., 1982). In addition to the xanthine oxidase inhibitory effect, allopurinol is also involved in preventing reperfusion during ischemia. (Manning, Coltart and Hearse, 1984). Allopurinol the xanthine Oxidase inhibitor is the only drug which is used for prevention of gout (Umamaheswari et al., 2007). But it has a few side effects that affects some portion of the population which will lead to gastrointestinal upset,skin rashes, liver dysfunction etc. (Sathisha et al., 2011).

Garlic (Allium sativum) is a member of the Liliaceae family and is an important spice that is used all over the world (Raghu, Lu and Sheen, 2012). Use of garlic in modern medicine is widespread in the world (Banerjee and Maulik, 2002). Garlic oil is rich in organo sulphur compounds like alliin, trace elements (zinc, magnesium, copper, selenium and iodine), protein content, dietary fibre, tocopherols, ascorbic acid and polyphenols (Raghu, Lu and Sheen, 2012). Organophosphate compounds are the vital substance that are extracted from garlic oil (Arifah et al., 2020). Garlic and its derivatives have less side effects which resulted in the development of medicine for various diseases (Davis, 2004). Garlic oil is used in treatment of arthritis, toothache, chronic cough, constipation, parasitic infestation, snake and insect bites, gynecologic diseases, etc. The therapeutic properties of garlic oil are reduction in the risk factors of cardiovascular disease, antioxidant effect, antimicrobial effect, reduction of cancer risk, enhancement of detoxification of foreign compounds (Bayan, Koulivand and Gorji, 2014). Therefore, the aim of the present study is to assess the in vitro xanthine oxidase inhibitory potential of garlic oil to check whether it can be used for the treatment of gout.

MATERIALS AND METHODS

Garlic oil was purchased from DEVES HERBES.

2.1. In vitro xanthine oxidase inhibitory activity: In vitro xanthine oxidase inhibitory of garlic oil 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 garlic oil (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^{0}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 ^{0}C. XOI activity was expressed as the percentage inhibition of XO in the above assay system calculated as percentage of inhibition as follows.

$$\% \text{ of inhibition} = (Ac-At) / Ac \times 100$$

where Ac is the absorbance of control reaction and At is the absorbance of test reaction. The assay was done in triplicate for each concentration. Allopurinol (0.1 to 0.5 µg/ml) was used as standard.

2.2. Phytochemical screening test

2.2.1. Test for phlobatannins: 2 ml of oil and 1 ml of conjugated HCl. The formation of the red colour complex indicates the presence of phlobatannin.

2.2.2. Test for carbohydrates: a) Fehling's test - 1 ml of sample is mixed with Fehling's solution a and b and boiling it for 3 minutes, reddish brown colour will be formed which indicates the presence of carbohydrates
b) Benedict's test -1ml of sample is mixed with 2 ml benedict's reagent. The formation of red, green or brown colour will indicate the presence of carbohydrate

2.2.3.Test for flavonoids: 1 ml of sample mixed with 2 ml of liquid ammonia solution. The formation of creamish

yellow colour indicates the presence of flavonoids.

2.2.4. Test for alkaloids: 1 ml of sample mixed with 1 ml of concentrated HCL and 6 drops of hexane and 3 drops of picric acid. The formation of creamish pale yellow proves the presence of alkaloids.

2.2.5. Test for terpenoids: To 2ml of sample, 1 ml of chloroform, 3 ml of conc H2SO4 were added. The formation of red colour represents the presence of terpenoids.

Figure 1: Xanthine oxidase inhibitory activity of garlic oil. Allopurinol used as standard. Each bar represents the mean±SEM of 3 independent observations. P value <0.05 is considered to be significant.

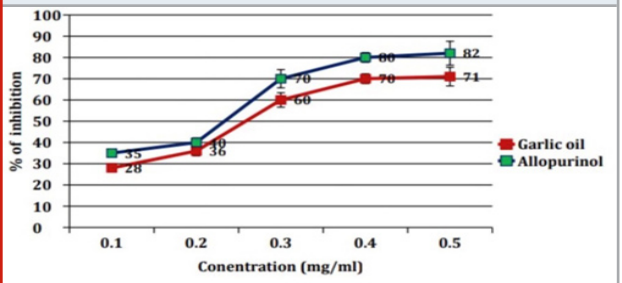

Table 1. Phytochemical screening of garlic oil

Phytochemical constituents	Results
Phlobatannins	(+)
Carbohydrates	(+)
Flavonoids	(+)
Alkaloids	(++)
Terpenoids	(+)

2.3. Statistical analysis: The data were subjected to statistical analysis using one way analysis 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 $p<0.05$.

RESULTS AND DISCUSSION

The results showed that garlic oil has inhibited the activity of xanthine oxidase and the activity increases with increase in concentration (Figure 1). The inhibitory activity of the oil was compared to the standard drug allopurinol. The results revealed that the inhibitory activity of garlic oil is less when compared to the standard drug. In clinical practice allopurinol was the most frequently used drug to treat hyperuricemia (Nuki and Simkin, 2006). Xanthine oxidase inhibitors which cause inhibition in the synthesis of uric acid, are effective in reducing the plasma and urinary urate levels which helps to reduce the development of tophaceous deposits (Nuki and Simkin, 2006). The allopurinol has a short life span in plasma. Patients who consume allopurinol continuously have decreased renal functions (Pacher, Nivorozhkin and Szabó, 2006). In order to reduce the side effects of the standard drug allopurinol, a drug of natural origin can be an alternative. Thus garlic oil which is a natural product that acts as an inhibitor can be used to reduce hyperuricemia.

The biologically active components pave way for the scientists and researchers to find more medicine. The phytochemical analysis showed that the oil is rich in alkaloids. The carbohydrates, flavonoids, terpenoids, phlobatannin and carbohydrates are found in moderate levels (Table 1). Phytochemicals, plant-derived non-nutritive compounds, are one of the many different types of the dietary factors which play an important role in various functions of the animal body (Abbas et al., 2015). The protective effects of these phytochemicals were found in many human diseases and ailments. (Barnes, 2001). A large number of natural compounds present in food materials have been reported to possess antioxidant properties (Abbas et al., 2015). the significant biological actions such as subduing oxidative stress, protection from degenerative disease, and reducing risk of cardiovascular disease could be attributed to their intrinsic antioxidant capabilities. Hence the presence of these phytochemicals might be the underlying reason for the protective effects of garlic oil including the xanthine oxidase inhibitory potential.

CONCLUSION

This study shows the potent inhibitory effect of garlic oil against xanthine oxidase enzyme whose hyperactivity is the reason behind the development of gout. Hence if detailed in vivo studies are conducted in this oil in this aspect, this oil can be used for the development of drugs to treat gout.

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Conflicts of Interest: None declared

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