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Xanthine Oxidase Inhibitory Effect of Solanum torvum (An in Vitro Study)

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ABSTRACT

Solanum torvum has been used therapeutically for centuries. Pharmacological studies indicate that the stem and root of *S.torvum* have anti-tumor, anti-bacterial, anti-viral, anti-inflammatory and other medicinally important effects. It is also used to treat fever, wounds and tooth decay. Gout, a disease resulting from increased uric acid accumulation in joints and muscles and due to increased activities of xanthine oxidase enzyme in nucleotide metabolism. Xanthine oxidase converts xanthine to hypoxanthine and further to uric acid. The present study is focused on evaluating the in vitro xanthine oxidase inhibitory (XOI) activity of *S. torum* fruit extract and to screen the phytochemical constituent present in it. Ethanolic extract of *S.torvum* (EST) was prepared and xanthine oxidase inhibitory activity was assessed by using Allopurinol as the standard drug. The phytochemical screening of the extract was also done. The results showed that the fruit extract possessed XOI activity in a concentration dependent manner. Also after phytochemical screening showed that EST is rich in phlobatannins, carbohydrates, flavonoids, terpenoids and alkaloids. Thus the study concluded that *S.torvum* possessed potent in vitro XOI activity.

KEY WORDS: XANTHINE OXIDASE; GOUT; SOLANUM TORVUM; PHYTOCHEMICAL SCREENING.

INTRODUCTION

Xanthine Oxidase (XO) is a highly versatile flavoprotein enzyme, ubiquitous among species (from bacteria to human) and within various tissues of mammals (Borges, Fernandes and Roleira, 2002). It is also found in milk. XO is a homodimer with a molecular mass of 290kDa. It belongs to the molybdenum-protein family containing one molybdenum, one of the flavin adenine dinucleotides (FAD) and two iron sulfur (2Fe-2S) center of Ferredoxin type in each of its two independent subunits. It has two substrate binding sites (Kostis et al., 2015). XO is a key enzyme playing a role in hyperuricemia, catalyzing the oxidation of hypoxanthine to xanthine and then to uric acid in nucleotide metabolism (Unno, Sugimoto and Kakuda, 2004). Excess or increased activities of XO leads to excessive production of uric acid which gets accumulated in tissues and results in a diseased condition called Gout and Rheumatoid Arthritis (Sreejith et al., 2013).

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Inhibition of XO decreases uric acid levels and results in an anti-hyperuricemic effect (Borges, Fernandes and Roleira, 2002). Allopurinol, first synthesized as a potential anticancer agent, is nowadays clinically useful as a xanthine oxidase inhibitor used in the treatment of gout. XO serum levels are significantly increased in various pathological stages like hepatitis, inflammation, ischemia-reperfusion, carcinogenesis and ageing (Borges, Fernandes and Roleira, 2002). However, Allopurinol being a chemical has many side effects such as fever, sore throat and allergic reactions. There have been many studies conducted for discovering various xanthine oxidase inhibitors which could be used in treatment of gout and related diseases without any side effects.

Numerous studies have been conducted regarding the XOI activity of plants and plant products. Flacourtia sepiaria was traditionally used as an antidote for snake poisoning, rheumatoid arthritis, gout and kidney diseases. The XOI activity of its methanol extract was found to be significant when compared with standard Allopurinol (Sreejith et al., 2013). Phenolic substances like anthocyanins and polyphenols present in berries of several cultivars of Ribes, Rubus and vaccinium genera had certain inhibitory activity towards XO (Costantino, Albasini and Rastelli, 1992). Erythrina stricta, an evergreen tree, is very effective in treatment of gout due to its XOI activity (Umamaheswari et al., 2009). Ursolic acid also has good hypouricemic activity and therefore has high potential to be used for treatment of gout and hyperuricemia related diseases (Abu-Gharbieh et al., 2018).

S.torvum is a small solanaceous shrub. The plant is found in tropical Africa, Asia, South America. Anciently, it was used in Cameroonian folk medicine for treatment of fever, wounds and tooth decay (Ndebia, Kamgang and Nkeh-ChungagAnye, 2006). The other names of S.torvum include devil's fig, prickly nightshade, shoo-shoo bush, wild eggplant and pea eggplant. It is bushy, spiny and erect. The berries grow in clusters of tiny green spheres which resemble green peas when ripe and later become yellow to brown (Ve et al., 2018). Its taxonomic classification is as follows (Jaiswal, 2012):

Kingdom: Plantae
Division: Magnoliophyta
Class : Magnoliopsida
Order : Solanales
Family : Solanaceae
Genus : Solanum L.
Species : torvum

Among its major chemical constituents are steroids, steroid saponins, steroid alkaloids and phenols (Yousaf, Wang and Baydoun, 2013). It is used in the treatment of coughs and liver diseases. Solanum torvum is used to reduce body heat and strengthen the body. It has good antibacterial activity (Kannan et al., 2012). Many pharmacological studies revealed that this plant exhibits antioxidant activity, cardiovascular, immunomodulatory and nephroprotective activity supporting its traditional uses (Jaiswal, 2012). Aqueous extract from dried fruits of Solanum torvum reduces blood pressure. It also has anti-ulcer properties owing to presence of flavonoids, sterols and triterpenes. It is antiviral, analgesic as well as anti-inflammatory (Agrawal, Bajpei and Patil, 2010). Hence our present study is to assess the inhibitory activity of *S. torvum* on xanthine oxidase in an in vitro model.

MATERIAL AND METHODS

2.1 *S.torvum* fruit extract (EST) preparation: Ethanolic extract of S.torvum was prepared. The fruits were collected, washed and dried thoroughly. Then 30 grams of fruit was weighed out and ground with ethanol. This mixture was kept for one day and then filtered. The extract thus prepared (EST) was dried and used for assessing the XOI activity.

2.2 In vitro XOI activity of *S. torvum* fruit extract: In vitro XOI activity of EST was assessed as per the method of (Nguyen et al., 2004) and (Umamaheswari et al., 2007) . Briefly, the assay mixture consisted of 1ml of the EST (0.1 to 0.5 g/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 25^0 for 15 min, the reaction was initiated by the addition of 2ml of substrate solution (150 M xanthine in the same buffer). The assay mixture was incubated at 25^0 for 30min. The reaction was then stopped by the addition of 1ml of 1N hydrochloric acid and the absorbance was measured at 290 nm using a UV spectrophotometer. Allopurinol (0.1 to 0.5 mg/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. 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.

2.3 Phytochemical Screening test

2.3.1. Test for phlobatannin: 1ml of the EST was treated with 1ml of 1% HCl and boiled for 10 mins. The formation of red color precipitate indicates the presence of phlobatannin.

2.3.2. Test for Carbohydrates

a) Fehling's test: 1ml of the extract was boiled with 1ml of Fehling's A and B for 3min. The formation of brown or red precipitate indicating the presence of carbohydrates (reducing sugar).

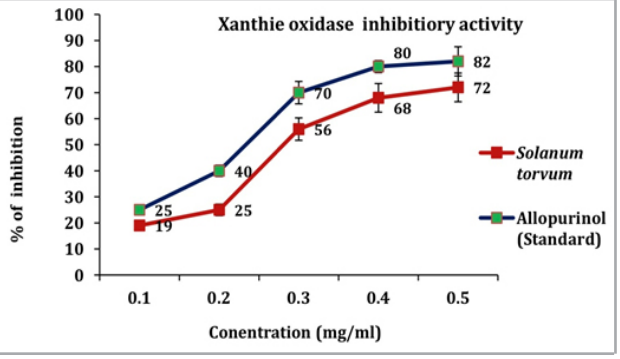
b) Benedict's test: 1ml of the extract was boiled along with 1ml of Benedict's solution. Red, brown or green color precipitate indicates the presence of carbohydrates (reducing sugar).

2.3.3. 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. The formation of yellow color indicates the presence of flavonoids.

2.3.4. Test for Alkaloids: 2ml of extract was mixed with 2ml of HCl. Then 6 drops of HCN was added and further 2 drops of picric acid was added. The formation of a creamish pale yellow precipitate indicates the presence of Alkaloids.

2.3.5. Test for Terpenoids: 2ml of extract along with 2ml of chloroform and 3ml of con. H2SO4 was added. The formation of red precipitate indicates the presence of terpenoids.

Figure 1: Xanthine oxidase inhibitory activity of Solanum torvum. Allopurinol: Was used as a standard drug. Each Bar Represents Mean± SEM of 3 independent observations. p<0.05 is considered to be statistically significant.



2.4 Statistical Analysis: The data was 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.

RESULTS AND DISCUSSION

Natural products provide a vast pool of XO inhibitors that can possibly be developed into clinical products. At present, the potential of developing successful natural products for the management of XO-related diseases is still largely unexplored (Hudaib et al., 2011). Uricosuric drugs which increase the urinary excretion of uric acid, or XO inhibitors which block the terminal step in uric acid biosynthesis, can lower the plasma uric acid concentration, and are generally employed for the treatment of gout (Schlesinger, Dalbeth and Perez-Ruiz, 2009). S.torvum fruit extract was found to have significant XOI activity in a concentration dependent manner (Figure 1). With increasing concentration of EST, the percentage inhibition of xanthine oxidase was also increased, showing its efficacy in a concentration dependent manner. When its XOI activity was compared to that of standard, Allopurinol, the activity was found to be slightly lesser. However, allopurinol causes many side effects such as hepatitis, nephropathy, and allergic reactions (Fagugli et al., 2008). The graph (Figure 1) shows that the activity of allopurinol is high when compared to that of the extract. The XOI activity of the extract could decrease the accumulation of uric acid and its deposition which is the key process in the development of gout.

Table 1. Phytochemical screening of EST

Experiment	Observation	Result	Inference
Test for Phlobatannins:	Red colored complex formed	+++	Presence of Phlobatannins
Fehling's Test:	Reddish brown color formed	+++	Presence of Carbohydrates
Benedict's Test:	Red, brown or green color formed	+++	Presence of Carbohydrates
Test for Flavonoids:	Creamish yellow color formed	++	Presence of Flavonoids
Test for Alkaloids:	Red color formed	+++	Presence of Alkaloids
Test for Terpenoids:	Red color formed	+++	Presence of Terpenoids

Phytochemicals are a powerful group of compounds, belonging to secondary metabolites of plants and including a diverse range of chemical entities such as polyphenols, flavonoids, steroidal saponins, organosulfur compounds, and vitamins (Forni et al., 2019). Most phytochemicals, components of food, beverages, and herbal products are often reported in literature as "nutraceutical", emphasizing their health promoting properties, including the prevention and treatment of pathologies like cancer, cardiovascular diseases, neural disorders, and Alzheimer's disease (Winter et al., 2017). The phytochemical analysis of EST showed that the extract is found to be rich in phlobatannins, carbohydrates, alkaloids, terpenoids and flavonoids, which attribute to its biochemical activities and properties. These secondary metabolites have been documented to possess an array of pharmacological properties attributable to their ability to interfere with multiple signalling cascades that are critical to heterogeneous metabolic diseases (Ramawat, Dass and Mathur, 2009; Forni et al., 2019). The occurrence of these

phytochemicals might have contributed to the beneficial effects of this extract.

CONCLUSION

To conclude the extract showed potent XOI activity and is rich in many phytochemicals. This study provides a platform for the further analysis of the extract in detail about the antigout activity. Detailed in vivo studies are needed for development of this plant fruit as a drug for the treatment of gout.

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Conflicts of Interest: None declared

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