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Inhibitory activity of xanthine oxidase by fractions *Crateva adansonii*

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ABSTRACT

Objective: To study the inhibitory effect of various extracts from *Crateva adansonii* (*C. adansonii*) used traditionally against several inflammatory diseases such as rheumatism, arthritis, and gout, was investigated on purified bovine milk xanthine oxidase (XO) activity. **Methods:** Xanthine oxidase inhibitory activity was assayed spectrophotometrically and the degree of enzyme inhibition was determined by measuring the increase in absorbance at 295 nm associated with uric acid formation. Enzyme kinetics was carried out using Lineweaver-Burk plots using xanthine as the substrate. **Results:** Among the fractions tested, the chloroform fraction exhibited highest potency (IC_{50} 20.2±1.6 μg/mL) followed by the petroleum ether (IC_{50} 30.1±2.2 μg/mL), ethyl acetate (IC_{50} 43.9±1.4 μg/mL) and residual (IC_{50} 98.0±3.3 μg/mL) fractions. The IC_{50} value of allopurinol used, as the standard was 5.7±0.3 μg/mL. **Conclusions:** Enzyme inhibition mechanism indicated that the mode of inhibition was of a mixed type. Our findings suggest that the therapeutic use of these plants may be due to the observed Xanthine oxidase inhibition, thereby supporting their use in traditional folk medicine against inflammatory-related diseases, in particular, gout.

1. Introduction

Gout and hyperuricemia are the common metabolic disorders in human, associated with an elevated uric acid level in the blood[1], leading to the deposition of urate crystals in the joints and kidneys, leading to gouty arthritis and uric acid nephrolithiasis. Xanthine oxidase (XO) and xanthine dehydrogenase (XDH) are the two interconvertible forms of same gene product known as xanthine oxidoreductase (XOR). It catalyses the oxidation of hypoxanthine to xanthine and then to uric acid, the final reactions in the metabolism of purine bases[2,3]. The treatment for gout is either increasing the excretion of uric acid or reducing the uric acid production. Xanthine oxidase inhibitors (XOIs) are much useful, since they possess lesser side effects compared to uricosuric and anti-inflammatory agents. Allopurinol is the only clinically used XOI, which also suffers from many side effects such as hypersensitivity syndrome, Stevens Johnson syndrome and renal toxicity[4]. Thus, there is a need to develop compounds with XOI activity which are devoid of the undesirable side effects of allopurinol. A potential source of such compounds can be obtained from medicinal plants[5,6]. Flavonoids and polyphenolic crude extracts have been reported to possess xanthine oxidase inhibitory activity[1,7,8]. *Crateva adansonii* (*C. adansonii*) belonging to the family *Capparaceae* and phylum *Magnoliophyta* is small tree of forest and savanna woodland,often on river-banks, widely distributed in Nigeria,and Across Africa[9,10]. The leaves are applied externally to relieve pain in joints; the fresh juice of leaves is used for the relief of ear ache, eye infection and anodyne in toothache. Powder of bark is used in rheumatism, itch, epilepsy, and asthma[11]. Many medicinal plants have been used for the prevention and treatment of gout and related inflammatory disorders,but they lack sufficient scientific evidence. Hence, the objective of the present study is to determine the *in vitro* xanthine oxidase inhibitory activity of the fractions of the leaves *C. adansonii* and its enzyme inhibition mechanism.

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2. Materials and methods

2.1. Plant material

The plant material consists of dried powdered leaves of *C. adansonii* belonging to the family *Capparaceae* and phylum *Magnoliophyta*. The leaves were collected in and around Minna. Niger State of Nigeria and was authenticated by the staff of Biological Science Department of Federal University of Technology, Minna.

2.2. Preparation of the extract and fractionation

The air-dried powdered leaves of *C. adansonii* (500 g) were extracted with methanol-water (7:3) mixture using a mechanical shaker for 4 h. The resultant extract was concentrated under reduced pressure to yield a residue. The hydromethanolic extract was then extracted successively with equal volumes of petroleum-ether, chloroform and ethyl acetate. Each fraction was then concentrated under reduced pressure to obtain petroleum-ether fraction (PEF), chloroform fraction (CF), ethyl acetate fraction (EAF) and residual fraction (RF).

2.3. Chemicals

XO (source: Bovine Milk) XO was purified from bovine milk using the method of Atmani et al[12] and XO was kept frozen and thawed just before use. The buffer used in our study was 0.1 M monopotassium phosphate-disodium phosphate (KH_2PO_4/Na_2HPO_4), and xanthines were obtained from Department of Biochemistry Laboratory Federal University of Technology, Minna. Allopurinol was obtained from Zagbayi pharmaceutical Minna. All other drugs and chemicals used in the study were obtained commercially and were of analytical grade.

2.4. Phytochemical screening

Preliminary phytochemical screening of the plant extract was performed for the presence of alkaloids, phenolics, tannins, flavonoids, saponins, carotenoids, carbohydrates and glycosides[13].

2.5. In vitro xanthine oxidase inhibitory activity

All the fractions of *C. adansonii* were assayed for their *in vitro* xanthine oxidase inhibitory activity.The XOI activity was assayed spectrophotometrically using xanthine as the substrate[14]. The assay mixture consisted of 1 mL of the fraction (5-100 μg/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 ℃ for 15 min, the reaction was initiated by the addition of 2 mL of the substrate solution (150 mM xanthine in the same buffer). The assay mixture was incubated at 25 ℃ for 30 min. The reaction was then stopped by the addition of 1 mL of 1 N hydrochloric acid and the absorbance was measured at 290 nm using a UV spectrophotometer[15,16]. Different concentrations of the fractions (5-100 μg/mL) were dissolved in dimethyl sulfoxide (DMSO) and the final concentration of DMSO was 5%, which did not affect the enzyme assay.

Proper controls with DMSO were carried out. Allopurinol (5-100 μg/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 1mmol of uric acid/min at 25 ℃. XOI activity was expressed as the percentage inhibition of XO in the above assay system calculated as

Inhibition (%) = (1-[B/A])×100, where A represents the activity of the enzyme without plant extract and B is the activity of XO in the presence of plant extract.

The enzymatic inhibitory mode of action of different extracts from both plants that exhibited the highest inhibition of XO activity was determined by the Lineweaver-Burk plot using varying concentrations of xanthine.

2.6. Enzyme inhibition mechanism

To determine the mode of inhibition by the most active chloroform fraction, Lineweaver-Burk plot analysis was performed. The assay was carried out in the absence and presence of the chloroform fraction with varying concentrations of xanthine as the substrate, using the XO assay methodology. The mode of inhibition was compared with that of the positive control allopurinol. The Lineweaver-Burk transformed valueswere plotted to determine the mode of inhibition[17,18].

3. Results

Preliminary phytochemical screening of the plant extract revealed the presence of phenolics, alkaloids, flavonoids and saponins (Table 1). All the fractions of *C. adansonii* elicited a dose dependent inhibition of xanthine oxidase enzyme activity. Inhibition of xanthine oxidase resulted in a decreased production of uric acid, which was measured spectrophotometrically.

At a concentration of 100 μg/mL, highest activity was observed in the chloroform fraction followed by the petroleum-ether, ethyl acetate and residual fractions. The concentration of the fractions at which 50% of the xanthine oxidase inhibitory activity (IC_{50}) was calculated.

These results were compared with the standard drug allopurinol, which showed (92.25±0.05)% inhibition at 100 μg/mL concentration with IC_{50} value (5.7±0.3) μg/mL (Table 2).

Table 1
Phytochemical screening of *C. adansonii*.

Ingredient	Petroleum-ether	Chloroform	Ethyl acetate
Alkaloids	+	+	+
Phenolics	+	+	+
Tannins	–	–	–
Flavonoids	+	+	+
Saponins	+	+	+
Carotenoids	–	–	–
Carbohydrates	–	–	–
Glycosides	–	–	–
Balsams	–	–	–

Key: (+) present, (–) – absent.

Table 2
Percentage yield and xanthine oxidase inhibitory activity of the fractions of *C. adansonii*.

Fraction	% Yield fraction (w/w)	Percentage xanthine oxidase inhibition					IC_{50} (μ g/mL)
		5 μ g/mL	10 μ g/mL	25 μ g/mL	50 μ g/mL	100 μ g/mL	
Petroleum-ether	1.8	28.35 ± 0.90	39.53 ± 1.20	48.32 ± 0.90	66.73 ± 1.57	78.59 ± 1.60	30.10 ± 2.20
Chloroform	1.7	19.55 ± 1.30	36.00 ± 0.80	55.00 ± 1.00	76.70 ± 1.10	84.75 ± 0.50	20.20 ± 1.60
Ethyl acetate	0.9	11.85 ± 0.30	23.75 ± 0.70	39.50 ± 1.00	56.35 ± 1.20	64.55 ± 1.50	43.90 ± 1.40
Residual	5.1	5.90 ± 1.10	28.80 ± 1.20	35.70 ± 0.50	41.50 ± 1.20	50.05 ± 1.80	98.00 ± 3.30
Allopurinol		44.91 ± 1.80	66.91 ± 0.80	75.00 ± 1.40	85.46 ± 0.80	92.25 ± 0.50	5.70 ± 0.30

The inhibition mechanism of the chloroform fraction of *C. adansonii*, which is a potent inhibitor of xanthine oxidase, was studied by kinetic analysis using double-reciprocal plotting. Lineweaver-Burk plots of reactions in the presence and absence of the chloroform fraction in a xanthine oxidase reaction mixture is shown in Figure 1.

The mode of inhibition was investigated and compared with the standard drug, allopurinol. The data indicates that the mode of xanthine oxidase inhibition for the chloroform fraction is of mixed type (between uncompetitive and non-competitive type of inhibition).

Therefore, the fraction inhibited XO by binding either with the free enzyme or the enzyme-substrate complex.

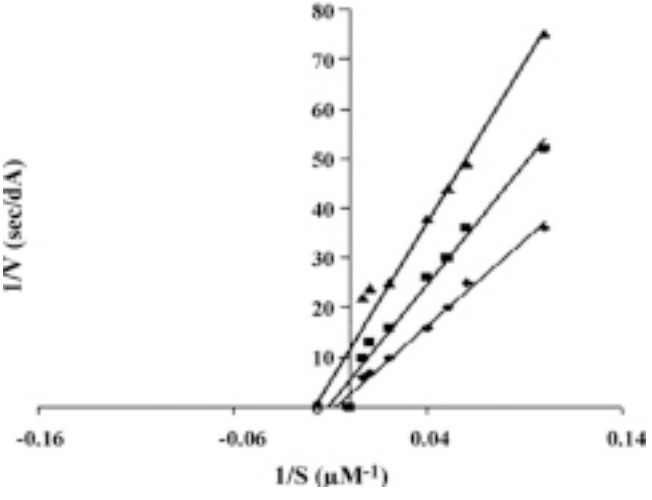

Figure 1. Lineweaver-Burk plot of inhibition of xanthine oxidase by chloroform fraction of *C. adansonii* (▬) and allopurinol (▲). The symbol (◆) represents negative control.

4. Discussion

Recent findings show that the occurrence of gout is increasing worldwide, possibly due to the changes in dietary habits like intake of food rich in nucleic acids, such as meat and seafoods. Hypouricemic agents are commonly employed for the treatment of chronic gouty arthritis, which includes xanthine oxidase inhibitors and uricosuric agents[19]. In general, allopurinol is the drug of choice; however it has serious side effects. Thus, new alternatives with increased therapeutic activity and lesser side effects are desired. We thus began our program to look for xanthine oxidase inhibitors of phytochemical origin from the various fractions of the hydromethanolic extract of the leaves of *C. adansonii*. The leaves of *C. adansonii* are being frequently used in Nigeria traditional medicinal system for the treatment joint pain and related inflammatory disorders.

All the fractions of *C. adansonii* inhibited xanthine oxidase in a concentration-dependent manner. The *in vitro* inhibition of xanthine oxidase by the chloroform fraction is moderate when compared to allopurinol. However, at higher doses of the fraction, xanthine oxidase would be significantly inhibited. Flavonoids are a group of polyphenolic compounds, which have been reported to possess xanthine oxidase inhibitory activity[20].

Hence, the presence of phenolic and flavonoid content in the extract would have contributed towards xanthine oxidase inhibition.

In conclusion, the study suggests that *C. adansonii*

leaves possess xanthine oxidase inhibitory activity that might be helpful in preventing or slowing the progress of gout. Further *in vivo* experiments and the isolation and identification of active compounds present in the leaves should be carried out to identify a potential chemical entity for clinical use in the prevention and treatment of gout and related inflammatory disorders.

Conflict of interest statement

We declare that we have no conflict of interest.

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