Therapeutic Potential of Methanolic Stem Extract of *Costusafer*on Serum Uric Acid, Tumor Necrosis Factor-alpha (TNF-α), and Interleukin-6 (IL-6) Levels in Monosodium urate (MSU)-Induced Gouty Arthritis in Wistar Rats

Abstract

The therapeutic potential of methanolic stem extracts of *Costusafer* was investigated to evaluate their effects on serum uric acid, TNF-α, and IL-6 levels, which are the key biomarkers associated with inflammatory and metabolic disorders like gouty arthritis. This study involved administering varying doses (100 mg/kg, 200 mg/kg, and 400 mg/kg) of the extract to a Wistar rat model with induced gouty arthritis and assessing its impact compared to a control group and an untreated MSU group. The results revealed that the extracts significantly reduced ($p < 0.05$) serum uric acid levels in a dose-dependent manner, with the high dose achieving a reduction below the control level. Additionally, the extracts effectively decreased ($p < 0.05$)TNF-α and IL-6 levels, indicating potent anti-inflammatory properties. The high dose exhibited the most substantial reduction in these biomarkers, underscoring the extract's therapeutic potential. These findings support the traditional use of *C.afer* and provide scientific validation for its application in managing hyperuricemia and inflammatory conditions.

Keywords

Costusafer, serum uric acid, TNF-α, IL-6, anti-inflammatory, hyperuricemia, therapeutic potential

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1.0 Introduction

Gouty arthritis is a type of inflammatory arthritis that is fundamentally linked to disturbances in purine metabolism and uric acid levels. It is characterized by the deposition of urate crystals in the joints and soft tissues, which leads to intense inflammatory reactions [1].Gouty arthritis, characterized by elevated serum uric acid levels (hyperuricemia)and severe joint inflammation,

poses significant health challenges [2, 3].The condition is often associated with the accumulation of monosodium urate (MSU) crystals in the joints. The presence of MSU crystals in the joints activates the NLRP3 inflammasome in macrophages, leading to the release of pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF-α)[4]. Tumournecrosis factor-alpha (TNF-α) and interleukin-6(IL-6) are both cytokines involved in inflammation and immune responses[5, 6].TNF-α is a pro-inflammatory cytokine produced by various cell types, including macrophages and T-cells, while interleukin-6(IL-6), on the other hand, is produced by various cells, including macrophages, T-cells, and fibroblasts[7]. Tumour necrosis factor-alpha (TNF-α) plays a key role in systemic inflammation and is involved in the regulation of immune cells [6, 8]. Elevated levels of TNF-α are associated with various inflammatory diseases and conditions [9]. Likewise, interleukin-6 (IL-6) levels are often elevated in response to infection, injury, or inflammation [10]. Both tumour necrosis factor-alpha(TNF-α) and interleukin-6(IL-6)result in an acute inflammatory response in gouty arthritis, characterized by pain, redness, warmth, and swelling in the affected joints. Chronic cases of gouty arthritis may involve the formation of tophi and persistent joint damage[11].They are targets for therapeutic intervention in gouty arthritis[12]. Therapeutic agents that inhibit these cytokines can help reduce inflammation and alleviate symptoms [13].

Over the years the vast treasures inherent in medicinal plants are constantly harnessed to treat and manage diverse health challenges plaguing mankind. These nature's gift to mankind is rich in phytochemicals [14]. The increased patronage of plant medicine arises from the fact that they are perceived as having minimal side effects[15] in comparison to synthetic drugs [16, 17] nearly all year round availability [18], and general affordability because of their low cost [19].*C.afer* has been studied for its potential health benefits, including its antimicrobial[20], anti-inflammatory [21], and antioxidant properties[22]. These attributes make it a subject of interest for investigating its efficacy in treating inflammatory diseases like gouty arthritis.

This study aims to evaluate the therapeutic potential of methanolic stem extracts of *C. afer*on inflammatory markers and serum uric acid levels in Wistar rats with MSU-induced gouty arthritis.

2.0 Materials and Methods

2.1 Plant Material

Fresh stems of *C. afer* wereprocured from a local market in Port Harcourt, Rivers State, Nigeria. The plant material was authenticated to confirm itsspecies identity at the herbarium unit of the Biology Department, Federal University Otuoke, Bayelsa, Nigeria. After authentication, the stems were air-dried in the shade to preserve the integrity of the active compounds. Once dried, the stems were ground into a fine powder using a mechanical grinder. The powdered material was then stored in airtight containers to maintain its quality and prevent moisture absorption until it was needed for extraction.

2.2 Extraction Procedure

A 200 g sample of the powdered stem material was subjected to extraction using methanol (1:5 w/v) for 72 hours at room temperature. The mixture was filtered, and the solvent was evaporated using a rotary evaporator. The resulting extract was stored at 4°C until use.

2.3 Phytochemical Analysis

2.3.1 Qualitative Phytochemical Screening:

Qualitative phytochemical screening of the methanolic stem extracts of *C. afer* was conducted to identify the presence of various bioactive compounds. The screening included tests for flavonoids, tannins, saponins, alkaloids, phenols, anthraquinones, cardiac glycosides, terpenoids, phlobatannins, and cardenolides. These tests were performed following the standard procedures outlined by Anyasor*et al.*[22].

2.3.2 Quantitative Phytochemical Analysis

The procedures for the quantitative determination of tannins, alkaloids, flavonoids, saponins, and phenols, were adapted from the methods described by Kpomah*et al.*[23].The procedures for the

quantitative determination of anthraquinones, cardiac glycosides, terpenoids, phlobatannins, and cardenolideswere adapted from the methods describedby Wu*et al.*[24],Ikeda *et al.*[25]and Malik [26] respectively.

2.3.3 Antioxidant Activity

The antioxidant activity was estimated using the procedure described by Anyasor*et al.*[22].Rapid thin layer chromatography (TLC) was employed by applying a concentrated methanolic solution of the extract onto silica gel plates. These plates were then developed using methanol: ethyl acetate mixture (2:1), allowed to air dry and sprayed with a 0.2% w/v DPPH solution in methanol. The presence of yellow spots on the plates indicated antioxidant activity. For further evaluation, the DPPH radical scavenging activity of the extracts was measured using the spectrophotometric method outlined by Anyasor*et al.*[22]. Specifically, 1 mL of a 0.3 mM DPPH methanol solution was mixed with 2.5 mL of the extract or standard solutions (at concentrations of 100, 200, 300, and 400 µg/mL) and incubated at room temperature for 30 minutes. The absorbance of the resulting solution was recorded at 518 nm and used to calculate the percentage antioxidant activity (AA %), using the formula:

$$AA\ \% = \frac{[100-\left(\left(Abs_{sample-}\ Abs_{blank}\right)\times 100\right)]}{Abs_{control}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ (1)$$

Methanol (1.0 mL) combined with the extract solution (2.5 mL) served as the blank, while 1 mL of 0.3 mM DPPH plus methanol (2.5 mL) was the negative control. Ascorbic acid and gallic acid were used as positive controls. The assay was conducted in triplicate for each concentration.The IC_{50} values for methanol extract, aqueous extract, ascorbic acid, and gallic acid were calculated.

2.4 Animal Model

Twenty five Wistar rats averagely weighing 235.67 ± 6.88 g were used for the study. The animals were housed under controlled conditions with a 12-hour light/dark cycle and provided with standard rodent chow and water *ad-libitum*. The study was approvedby the Directorate of Research and Quality Assurance, Federal University Otuoke, Bayelsa State vide a letter referenced DRQA/FUO/0113/11/06/24.

2.5 Induction of Gouty Arthritis

Gouty arthritis was induced in rats by intra-articular injection of monosodium urate (MSU) crystals (0.1 mg/rat) into the knee joint under anesthesia (ketamine 60 mg/kg and xylazine 10 mg/kg).

2.5 Experimental Design

The rats were divided into five groups (n = 5 per group):

Group 1: Control (no treatment)

Group 2: MSU-induced arthritis + allopurinol tablet (5 mg/kg of body weight)

Group 3: MSU-induced arthritis + low-dose *C. afer* extract (100 mg/kg body weight)

Group 4: MSU-induced arthritis + medium-dose *C. afer* extract (200 mg/kg body weight)

Group 5: MSU-induced arthritis + high-dose *C. afer* extract (400 mg/kg body weight)

Treatment was administered orally once daily for 7 days, starting 24 hours after MSU injection.

2.6 Assessment of Serum Uric Acid, TNF-α, and IL-6 Levels

2.6.1 Collection of blood

At the end of the treatment period, blood samples were collected from the rats via cardiac puncture under anaesthesia into the sample blood bottles.

2.6.2Determination of Serum Uric Acid

Serum uric acid (SUA) level was measured by using the enzymatic colourimetricmethod of Fossate *et al.* [27] with slight modification done by Hussain*et al.*[28].

2.6.3 Determination of Serum TNF-α and IL-6 Levels

The serum level of TNF-α was measured using ELISA kits as reported by Acar*et al.*[29]. In this method, blood samples collected in the plain tubes were centrifuged for around 10 min at ×1500 g which was followed by serum extraction. TNF-α levels were estimated by using a human "ELISA kit (Diaclone, Besancon Cedex, France) following the manufacturer's protocol.Similarly, the serum level of IL-6 was measured using ELISA kits as reported bySaxena*et al.*[30].IL-6 levels were determined from the serum samples extracted by centrifuging the blood collected in the plain vial at ×1500 g for 10 min by using a commercially available human IL-16 ELISA kit from Krishgen Biosystems (United Kingdom) following the manufacturer's instructions.

2.7 Statistical Analysis

Data were analyzed using one-way ANOVA followed by Tukey's post hoc test to compare the means between groups. A p-value of <0.05 was considered statistically significant. Descriptive statistics (mean ± standard deviation) were used to summarize the data.

3.0 RESULTS

3.1 Phytochemical Analysis

The methanolic stem extracts of *C.afer* were analyzed for their phytochemical content. Qualitative analysis revealed the presence of flavonoids, phenols, tannins, saponins, and alkaloids, while anthraquinones, cardiac glycosides, terpenoids, phlobatannins, and cardenolides were not detected (Table 1). Quantitative analysis showed concentrations of flavonoids (0.774 ± 0.036 mg GAE/g), alkaloids (0.368 ± 0.02 mg GAE/g), and phenols (0.29 ± 0.01 mg GAE/g), along with lower levels of anthraquinones, cardiac glycosides, and terpenoids (Table 2).

Table 1: Qualitative Phytochemical Screening Results of Methanolic Stem Extracts of *C.afer*

Phytochemical	Result
Flavonoids	+++
Tannins	-
Saponins	-
Alkaloids	+
Phenols	+++
Anthraquinones	++
Cardiac Glycosides	+
Terpenoids	++
Cardenolides	-
Terpenoids	-
Phlobatannins	-

Key: +: Present in trace amounts

++: Present in moderate amounts

++: Present in high amounts

Table 2: Quantitative Determination of the Phytochemical content of methanolic stem extracts of *C. afer*

Phytochemical	Concentration (mg GAE/g)
Flavonoids	0.774 ± 0.036
Alkaloids	0.368 ± 0.02
Phenols	0.29 ± 0.01
Anthraquinones	0.012 ± 0.003
Cardiac glucosides	0.011 ± 0.002

Terpenoids	0.17 ± 0.04

3.3 Antioxidant Activityof Methanol Extract of *C.afer*

Table 3 presents the quantitative determination of antioxidant activity and the IC_{50} values for the methanol extract of C. *afer* and standard antioxidants (gallic acid and ascorbic acid).The antioxidant activity of the methanol extract of *C.afer* was assessed across different concentrations, demonstrating a dose-dependent increase in antioxidant activity. At 100 µg/ml, the extract exhibited 67.14% antioxidant activity, which increased to 74.85% at 400 µg/mL.The IC_{50} value of 92.33 µg/mL indicates the concentration required to inhibit 50% of free radical activity, suggesting a moderate antioxidant potential compared to the standards used.

Gallic Acid, a known potent antioxidant, showed higher activity across all concentrations with an IC_{50} value of 37.92 µg/ml, reflecting its superior antioxidant capacity relative to the methanol extract of *C. afer*. Ascorbic Acid also displayed significant antioxidant activity with an IC_{50} value of 51.40 µg/ml.Overall, while the methanol extract of *C. afer* demonstrates promising antioxidant activity, its effectiveness is lower compared to the standard antioxidants used in this study.

Table 3. Quantitative Determination of Antioxidant Activity and IC_{50} of Methanol Extract of *Costusafer* and Standards

Sample Extract	Concentration (µg/ml)	Percentage Antioxidant Activity (%)	IC_{50} (µg/ml)
Methanol Extract of *C. afer*			**92.33**
	100	67.14 ± 1.58	
	200	68.67 ± 0.26	
	300	72.29 ± 0.26	
	400	74.85 ± 0.25	
Gallic Acid			**37.92**
	100	78.36 ± 1.05	
	200	82.24 ± 0.66	
	300	84.73 ± 0.66	
	400	86.67 ± 1.18	
Ascorbic Acid			**51.40**
	100	71.87 ± 2.21	
	200	76.43 ± 3.03	
	300	78.77 ± 2.91	
	400	88.97 ± 1.11	

3.2 Serum Uric Acid, TNF-α Levels, and IL-6 Levels

The results of the effects of methanolic stem extracts of *C. afer* on serum uric acid, TNF-α, and IL-6 Levels are presented in Table 4.

Table 4: Effects of Methanolic Stem Extracts of *Costusafer* on Serum Uric Acid, TNF-α, and IL-6 Levels

Parameter	Control Group	Untreated MSU Group	Low Dose (100 mg/kg)	Medium Dose (200 mg/kg)	High Dose (400 mg/kg)
Serum Uric Acid (mg/dL)	5.26 ± 0.45	9.32 ± 0.50**	7.58 ± 0.40*	6.32 ± 0.30**	4.78± 0.35***
TNF-α (pg/mL)	15.7 ± 1.2	28.4 ± 1.6**	22.5 ± 1.3*	17.8 ± 1.2**	12.4 ± 1.1***
IL-6 (pg/mL)	32.1 ± 2.0	58.9 ± 3.1**	48.2 ± 2.8*	38.6 ± 2.4**	28.1 ± 2.2***

*Significant difference from untreated MSU group ($p < 0.05$)
**Significant difference from the control group ($p < 0.05$)
***Highly significant difference from the control group ($p < 0.01$)*

Serum Uric Acid

The results of the effect of methanolic stem extracts of *Costusafer* on serum uric acid levels are presented in Table 5. The untreated MSU group exhibited significantly elevated serum uric acid levels (9.32 ± 0.50 mg/dL) compared to the control group (5.26 ± 0.45 mg/dL), indicating increased uric acid levels due to the condition being studied. Treatment with the low dose (100 mg/kg) of the extracts reduced serum uric acid to 7.58 ± 0.40 mg/dL. The medium dose (200 mg/kg) resulted in a further decrease to 6.32 ± 0.30 mg/dL, while the high dose (400 mg/kg) achieved the most substantial reduction, lowering serum uric acid to 4.78 ± 0.35 mg/dL. This high dose resulted in levels significantly($p < 0.05$) below those of the control group, demonstrating a potent effect of the extracts in reducing serum uric acid.

3.3 Serum TNF-α Levels

The results of the effect of methanolic stem extracts of *C.afer* on TNF-α levels are also detailed in Table 5. The untreated MSU group showed significantly higher TNF-α levels (28.4 ± 1.6 pg/mL) compared to the control group (15.7 ± 1.2 pg/mL), indicating increased inflammation. Treatment with the low dose (100 mg/kg) of the extracts reduced TNF-α to 22.5 ± 1.3 pg/mL. The medium dose (200 mg/kg) further decreased TNF-α to 17.8 ± 1.2 pg/mL, while the high dose (400 mg/kg) led to the most significant reduction, with TNF-α levels dropping to 12.4 ± 1.1 pg/mL. This high dose resulted in TNF-α levels significantly($p < 0.05$) lower than those of the control group, suggesting a strong anti-inflammatory effect of the extracts.

3.4 Serum IL-6 Levels

The effect of methanolic stem extracts of *Costusafer* on IL-6 levels, as presented in Table 5, showed that the untreated MSU group had elevated IL-6 levels (58.9 ± 3.1 pg/mL) compared to the control group (32.1 ± 2.0 pg/mL). Treatment with the low dose (100 mg/kg) reduced IL-6 to 48.2 ± 2.8 pg/mL. The medium dose (200 mg/kg) further decreased IL-6 to 38.6 ± 2.4 pg/mL, and the high dose (400 mg/kg) achieved the most significant reduction, lowering IL-6 to 28.1 ± 2.2 pg/mL. This high dose resulted in IL-6 levels significantly ($p < 0.05$) lower than those of the control group, highlighting the effectiveness of the extracts in reducing inflammatory cytokine levels.

4.0 Discussion

4.1 Discussion

The study aimed to evaluate the therapeutic potential of methanolic stem extracts of *C. afer* in modulating serum uric acid, TNF-α, and IL-6 levels, which are key biomarkers in gouty arthritis and other inflammatory and metabolic disorders. The results demonstrate a notable dose-dependent effect of the extract on these parameters, highlighting its potential as a therapeutic agent.

The antioxidant activity of the methanol extract of *C. afer* was assessed, showing a dose-dependent increase with percentages ranging from 67.14% at 100 µg/ml to 74.85% at 400 µg/ml. The IC_{50} value (92.33 µg/ml) of the extract indicates its moderate antioxidant potential compared to standards like Gallic Acid and Ascorbic Acid, which exhibited IC_{50} values of 37.92 µg/ml and 51.40 µg/ml, respectively. These findings suggest that while *C. afer* has significant antioxidant activity, it is less potent than the standard antioxidants. This result is consistent with other studies on *C. afer*, which have reported moderate antioxidant properties due to its phytochemical content, including flavonoids and phenolic compounds [22]. Antioxidants are implicated in decreasing morbidity and mortality arising from oxidative stress [31].

The significant reduction in serum uric acid levels observed with *C. afer* extracts supports its potential in managing hyperuricemia [32]. This finding aligns with research on other plant-based interventions such as *Hibiscus sabdariffa*, and *Aster glehni*which have demonstrated similar uric acid-lowering effects attributed to their ability to inhibit xanthine oxidase, an enzyme involved in uric acid production [33, 34]. The high dose of *C. afer* extracts achieved a reduction in uric acid levels below the control, suggesting a potent effect comparable to or exceeding that observed with other plant extracts known for their uric acid-lowering properties.

The observed reduction in TNF-α levels with *C. afer* extracts highlights its potential anti-inflammatory properties. Previous research supports this finding, with various plant extracts such as *Curcuma longa* (turmeric) and *Zingiber officinale* (ginger) showing reductions in TNF-α levels in inflammatory models [35, 36]. The high dose of *C. afer* extracts significantly reduced TNF-α levels, indicating its effectiveness in modulating inflammation, consistent with findings from other studies on anti-inflammatory plant extracts.

Similarly, the reduction in IL-6 levels observed with *C. afer* extracts is consistent with research on other anti-inflammatory agents. For example, *Panax ginseng* and *Andrographis paniculata* have been reported to reduce IL-6 levels in animal models of inflammation [37]. The high dose of *C. afer* extracts achieved a significant reduction in IL-6 levels, suggesting a strong anti-inflammatory action comparable to other plant-based therapies.

The anti-inflammatory property of *C. afer* may be attributed to its high content of flavonoids. Many studies have demonstrated that flavonoids can reduce cytokine expression and secretion,

providing therapeutic benefits in inflammation-associated diseases as cytokine modulators. Additionally, flavonoids exhibit wide pharmacological effects by inhibiting various enzymes involved in inflammatory processes, such as cyclooxygenase, aldose reductase, and xanthine oxidase [13, 38, 39]. The phytochemical screening and quantitative analysis of *C. afer* support this hypothesis, suggesting that the observed anti-inflammatory effects may be mediated through these flavonoids and other active compounds present in the extract. This implies that the methanol extract of *C.afer* demonstrates notable antioxidant and anti-inflammatory properties, with significant effects on serum uric acid, TNF-α, and IL-6 levels. These findings highlight the potential of *C. afer* as a therapeutic agent for managing conditions such as gouty arthritis, though further research is needed to fully elucidate its mechanisms and optimize its therapeutic applications

4.2 Conclusion

The study successfully demonstrated that methanolic stem extracts of *C.s afer* exert significant effects on serum uric acid, TNF-α, and IL-6 levels in a dose-dependent manner. The high dose of the extract achieved the most pronounced reductions in these biomarkers, suggesting a potent therapeutic potential.The reduction in serum uric acid levels indicates that *C.afer* may be effective in managing conditions associated with hyperuricemia, such as gout. The significant decrease in TNF-α and IL-6 levels highlights the] potential anti-inflammatory properties of the extract, which could be beneficial in treating inflammatory disorders like gouty arthritis.Overall, the findings support the traditional use of *C. afer* and provide scientific validation for its therapeutic applications in managing inflammatory and metabolic conditions.

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