Study of antioxidant and xanthine oxidase inhibitory activities of *Cascabela thevatia* and *Coriandrum sativum*

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Abstract: Two traditionally used medicinal plants, *Cascabele. thevatia* and *Coriandrum sativum*, were chosen for this investigation, and their bioactive components and xanthine oxidase inhibitory potential were examined. The phytochemical screening of the investigated plant leaf extracts revealed that these plants contain a wealth of secondary metabolites such as alkaloids, flavonoids, phenols, saponins, and terepenoids, among other things. Calculations of total phenol content (TPC) and total flavonoids content (TFC) values in plant extracts revealed that *C. thevatia* contained the greater values of TPC (75.21 mg GAE/g) and TFC (69.09 mg QE/g). Both leaf extracts exhibited significant antioxidant activity comparable to that of standard ascorbic acid, as evidenced by the DPPH and FRAP assays.The methanol leaf extract of *C. thevatia*, with an IC_{50} value of 26.1±1.51 mg/mL, had the highest xanthine oxidase inhibitory activity between studied plants, making it an effective antigout agent. The findings provide scientific support for the use of *C. thevatia* and *C. sativum*, both of Nepalese origin, in traditional medicine to treat gout. This is the first study to show that *C. thevatia* leaf extract inhibits xanthine oxidase. This study may provide useful data for future research.

Keywords: Xanthine; Xanthine oxidase; Antioxidant; Traditional medicine; Antigout.

Introduction

Plants and their derivatives have historically served as fundamental components of healing systems in a developing country such as Nepal, where traditional knowledge and biodiversity synergistically enhance medical practices[1]. An estimated 80–90% of the population relies on indigenous medicinal plant harvesting, which are the foundation of the ayurvedic healthcare system and remains integral to both traditional and modern medical frameworks[2]. Globally, plant-derived compounds continue to play a pivotal role in pharmaceutical innovation, with approximately 25% of contemporary drugs originating directly from botanical sources, while many synthetic medications are modeled on isolated plant constituents[3]. Plants have a wide range of biochemical enzymes that make medicinal compounds like alkaloids, flavonoids, and phenolic acids. These compounds are very important for treating conditions like infectious diseases, cancer, and metabolic disorders[4].

The shift toward plant-based medicines in recent decades reflects by growing public preference for safer, cost-effective alternatives to synthetic drugs, despite limited regulatory standardization or clinical validation. Numerous studies demonstrate that phenolic compounds found in plants have higher levels of antioxidant activity than synthetic antioxidants such as vitamins or butylated hydroxytoluene (BHT), making them essential in the fight against diseases linked to oxidative stress[5]. In rural areas, remedies derived from plants frequently serve as the main resources for addressing health issues like cancer, bacterial infections, and inflammatory diseases, influenced by cultural traditions and challenges in accessing cancer, bacterial infections, and inflammatory diseases, influenced by cultural traditions and challenges in accessing modern healthcare[6]. These days, a lot of people are interested in

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these natural substances since xanthine oxidase (XO) inhibitors, like the drug allopurinol, have the ability to treat hyperuricemia and gout.

Recent investigations indicate that plant-derived flavonoids and phenolics serve as potent xanthine oxidase inhibitors, with efficiency comparable to allopurinol, so offering a promising avenue for the development of novel treatments[7,8].

This study focuses on two important plants, namely *C. thevetia* (Apocynaceae family) and *C. sativum* (Apiaceae family). *C. thevetia* usually grown as an ornamental plant, native to Mexico. It's antifungal, antibacterial, and antitermite properties has been reported despite its toxic component. According to reports, *C. thevatia* leaves are used in traditional treatments to treat diabetes, cardiovascular disease, and rheumatism[9,10]. In a similar vein, *C. sativum* is a widely used medicinal herb that has both therapeutic and nutritional advantages. The herb is used in traditional medicine. Its seeds, which are rich in coumarins, flavonoids, polyphenols, fatty acids, and essential oils, are the main reason it is grown. The chemical makeup and biological activity of coriander seed and plant essential oils, which include antibacterial, antioxidant, hypoglycemic, hypolipidemic, analgesic, anti-inflammatory, and anticancer properties, have been well investigated[11]. It was found that xanthine oxidase is inhibited by the ethanol extract of *C. sativum* leaves[12]. Thus far, no evidence of *C. thevatia* blocking xanthine oxidase has been reported. Therefore, the purpose of this study is to determine the effectiveness of crude extracts from both Nepalese species in blocking XO in order to expand the range of natural substitutes for allopurinol. This approach aims to help develop long-lasting medications and solve healthcare issues in areas with limited resources by combining traditional knowledge with biochemical confirmation.

Methods and Methodology

Collection of plant materials

Leaves of plant *C. thevetia* were collected from Banke,, Nepalgung, and *C. sativum* from Kathmandu in April 2019. The plants were authenticated at the national herbarium and plant laboratories in Godavari, Lalitpur, Nepal. The collected plant materials were dried under shade and grinded separately to a fine powder and collected in airtight plastic bags. The collected specimens were identified with the help of available literature.

Chemicals, reagents and enzymes

Analytical grade chemicals and enzymes were employed in this investigation. Methanol, ethanol, and dichloromethane (DCM) were supplied by Merck Company, India. Ascorbic acid, gallic acid, xanthine oxidase, xanthine, quercetin, and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) were all brought from Sigma Aldrich Chemicals Pvt. Ltd. India branch.

Extract preparation

The collected leaves from the research plants were dried at room temperature. They are shaded to avoid direct sunshine. After it had completely dried, it was powdered in a grinder. 100 grams of each dry plant were extracted at room temperature using the cold percolation method with methanol as a solvent. The solvent was then evaporated under low pressure using a Rotary evaporator (EYELA Co, Ltd, USA) while the temperature remained below the solvent's boiling point. The resultant crude extract was stored in vials and refrigerated at 4 ^{o}C.

Phytochemical screening

Phytochemical constituents were analyze by Culie's procedure[13]. He described it in "Methods for Studying Drugs". Plant materials were extracted with solvents of increasing polarity.

Estimation of total phenolic content (TPC) and total flavonoids content (TFC)

To measure the total phenolic content in the plant extract, the Folin-Ciocalteu colorimetric method based on an oxidation-reduction reaction was used in a 96-well plate method[14]. The total flavonoid content in the plant extract was quantified using an aluminum chloride colorimetric

assay in 96-well plates[15]. A microplate reader from Synergy, BioTek, Instruments, Inc., USA, was used for this.

Antioxidant assay

The antioxidant activity of selected plant extracts was evaluated using the DPPH and FRAP assays. A 96-well plate method, adapted from the colorimetric approaches described by Sabudak et al. (2013) and Subedi et al. (2012), was used to assess the extracts ability to scavenge DPPH free radicals[16, 17]. The percentage of DPPH radical scavenging activity was calculated using the following formula:

DPPH scavenging activity (%) = $\{1-(A_{sample} - A_{blank})/A_{control}\} \times 100\%$

Where, A_{sample} = is the absorption of the sample, While A_{blank} = is the absorption of extract without DPPH, and $A_{control}$ = is the absorption of a sample without plant extract.

The IC_{50} value was determined by plotting the percentage of inhibition against concentration. All experiments were performed in triplicate.

The FRAP (Ferric Reducing Antioxidant Power) assay was performed following the method of Benzie and Strain which evaluates the reducing power of the extracts, (1996)[18]. The FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ in a 10:1:1 ratio. Sample extracts and $FeSO_4$ standard solutions were prepared in suitable solvents. For the assay, 200 µL of the sample or standard was added to 3 mL of freshly prepared FRAP reagent, mixed thoroughly, and incubated at 37 °C for 4 minutes. The absorbance was then measured at 593 nm using a spectrophotometer. Antioxidant reducing power was determined by comparing the absorbance values to a standard $FeSO_4$ calibration curve and expressed as µM Fe^{2+} equivalents.

In vitro xanthine oxidase inhibition assay

Xanthine oxidase inhibition activity was performed spectrophotometrically in 96 well plates described previously by Noro et al. (1983) with minor modification[19].25 mg/mL stock solutions of all the extract was prepared by dissolving 25 mg plant extract in 1 mL 50% DMSO. Allopurinol (100 µg/mL) was used as a positive control. 75 µL of 50 mM sodium phosphate buffer (pH 7.5), 20 µL of plant extract dissolved in DMSO (final concentration maintain less than 5 %) and 20 µL of freshly prepared enzyme solution (0.1 units/mL of xanthine oxidase in phosphate buffer) were mixed in a well plate and pre-incubated at 37 ^{0}C for 15 min, and then 60 µL of substrate solution (0.15 mM of xanthine) was added into the mixture. The mixture was incubated at 37 ^{0}C for 30 min. And the reaction was stopped with the addition of 25 µL of 0.5 M HCL solution prior to measuring the absorbance at 290 nm on a BioTek microplate spectrophotometer. A blank was prepared in the same way; however, the order of adding the substrate and HCL solution was reversed. All samples assay for XO inhibitory activity were in concentrations of 50, 25, 12.5, 6.25, 3.125 µg/mL respectively, but for allopurinol concentrations are 0.1, 0.05, 0.025, 0.0125, and 0.0625 µg/mL. Percentage inhibition was calculated according to the following equation:

Inhibition (%) = $\{(A_{control} - A_{sample})/A_{control}\} \times 100\%$

Where Acontrol stands for enzyme activity without plant extract and Asample is XO activity in the presence of plant extract.IC_{50} values were obtained through linear regression analysis, the plot of concentration against percent inhibition.

Statistical analysis

All the analysis was carried out in triplicate and the result are expressed as mean +SD.

Results and Discussion

Phytochemical screening

The phytochemical analysis of crude methanol leaf extracts from *C. thevetia* and *C. sativum* revealed the absence of glycosides and steroids, as indicated in Table 1,

but the presence of several significant phytochemical constituents, including volatile oil, alkaloids, flavonoids, polyphenols, saponins, and terpenoids. Where**, (+)**indicates the presence of phytochemicals, while (-) indicates their absence.Tannin is only found in *C. thevetia*, while diterpenes are only found in *C. sativum* Saponins were found to have anti-arthritic properties *in vitro* due to their capacity to inhibit xanthine oxidase, which interferes

Table 1: Results of phytochemical screening of methanol extract of tested plants.

Phytohemicals	*Cascabela thevatia*	*Coriandrum sativum*
Volatile Oil	+	+
Polyphenols	+	+
Flavonoids	+	+
Glycosides	-	-
Tannin	+	-
Saponins	+	+
Alkaloids	+	-
Terpenoids	+	+
Carbohydrates	+	+
Diterpenes	-	+
Steroids	-	-

with uric acid metabolism[20]. Flavonoids are a class of secondary metabolites with xanthine oxidase inhibitory properties. Researchers have extensively studied the structure-activity connection for xanthine oxidase inhibition of these secondary metabolites[21]. The results are consistent with earlier literatures.

Estimation of total phenolic and flavonoid contents

The extracts total phenolic content (TPC) and total flavonoid content (TFC) were calculated using a calibration curve for gallic acid and quercetin, and expressed as GAE/g and QE/g of dry weight, respectively. The TPC in the test plant extracts were calculated by using regression equation y=0.0113, R^2 = 0.972, obtained from above calibration curve of Gallic acid followed by formula cV/m.

The total phenolic content (TPC) in *C. thevetia* was 75.21 mg GAE/g, while in *C. sativum* it was 61.06 mg GAE/g (Table 2). Tang et al. (2013) discovered that the total phenolic content in *C. sativum* extracts varied from 1.73±0.49 mg GAE/g to 31.38±2.75 mg GAE/g[22]. This study exhibited a greater total phenolic content (TPC) than the aforementioned experiment; however, Nhut et al. (2020) reported a TPC of *C. sativum* at 113.08 mg GAE/g, which aligns more closely with this research. Both plants demonstrate significant potential for TPC value[23].

The TFC in the plant extracts taken under study was calculated by using regression equation y = 0.0011x, R^2= 0.992, of the curve obtained from the graph, followed by the formula $\frac{cV}{m}$ and expressed as mg QE/g of extract in dry weight. The total flavonoid content of the methanol extracts from two plants was analyzed, revealing that the methanol extract of *C. thevetia* contains 69.09 mg QE/g, whereas that of *C. sativum* has 48.18 mg QE/g. The data above demonstrates that *C. thevetia* possesses a comparatively greater quantity of TFC than *C. sativum*. Literature reviews indicate that the total flavonoid content (TFC) of the ethanolic extract of *C. sativum* was measured at 72.64 mg quercetin equivalents per gram (QE/g). *C. thevetia*exhibited a comparatively lower concentration of total flavonoid content (TFC) in relation to total phenolic content (TPC), consistent with the findings of this study[8].

Antioxidant assay

Methanol extracts were investigated for antioxidant activity *in vitro* against DPPH and FRAP. Different concentrations of plant extracts were used and the results

Table 2: Total Phenol and Total Flavonoid content of test plant extracts.

Plants Name	TPC (mg GAE/g)	TFC (mg QE/g)
C. thevetia	75.21	69.09
C. sativum	61.06	48.18

are presented in the figure1–2. Both the tested plants in this study showed significant antioxidant activity through DPPH and FRAP assay methods (Table 3-4). *C. thevetia*

and *C. sativum* had similar IC_{50} values, which were higher than the standard ascorbic acid IC_{50} value of 27.74 µg/mL, indicating lower antioxidant potency. The literature review revealed the ethanolic extract of *C. sativum* showed a much lower IC_{50} value (78.30 µg/mL) compared to its aqueous extract (1335.0 ± 37.7 µg/mL), suggesting that the ethanolic extract is more effective[24]. Additionally, earlier studies, such as Seetharaman et al. (2017), have noted that *C. thevetia* has strong antioxidant properties,which supports this study's claim that it has antioxidant potential[9].

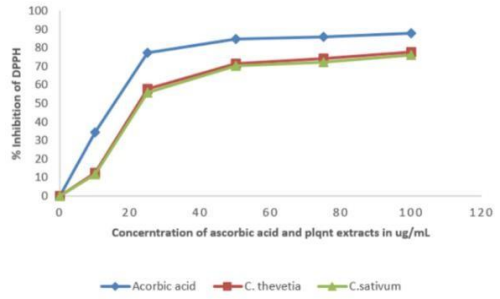

Figure 1: A plot of % of inhibition versus concentration of all sample extracts and ascorbic acid.

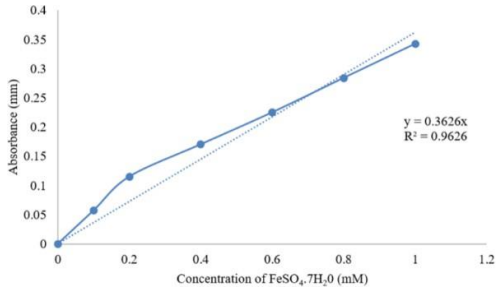

Figure 2: Calibration curve of standard ferrous sulphate ($FeSO_4$).

A similar result was also observed in the FRAP assay method which also showed *C. thevetia* and *C. sativum* as a good antioxidant agents. The above data shows (table 4) that *C. thevetia* has more antioxidant power with 496.41 mM Fe (II)/L as compared to *C. sativum* having antioxidant power of 413.67 mM Fe (II)/L. This result is comparable to the data obtained from the DPPH assay also. Literature review reveals that the root extract of *C. sativum*, the ethyl acetate extract, had the highest FRAP value of 0.129 ± 0.007 mmoL/g[25]**.** Higher amounts of phenolic and flavonoid could be responsible for the highest antioxidant potential[26]. A positive correlation was identified between the antioxidant activity of the tested plants and their total phenolic and flavonoid content.

Table 3: IC_{50} values of tested plants.

Sample Name	IC_{50} (ug/mL)
Ascorbic acid	27.74
C.thevatia	44.76
C.sativum	46.41

Table 4: Antioxidant power of different plant extracts by FRAP assay.

Plants Name	Concentration (mg/mL)	Antioxidant Power(mM Fe(II)/L)
C.thevetia	1	496.41
C.sativum	1	413.67

Xanthine oxidase inhibition activity

Using UV-visible spectrophotometry, a well-established method for assessing the antitigout characteristics of plant extracts, the inhibitory efficiency of the extracts on xanthine oxidase was evaluated. Table 5 below displays the results of the xanthine oxidase inhibition activity. Few studies conducted in the past have acknowledged that ethanol and methanol are more effective than other solvents in extracting xanthine oxidase inhibitors from plant materials.

In this case, the inhibitory action on xanthine oxidase was enhanced as the concentration of the methanol extract was increased. Both extracts inhibited xanthine oxidase in a nearly same manner. A graphic representation of xanthine inhibition of Allopurinol and both plant extracts is given in the figure 3-4.

The tested plant extract exhibited high IC_{50} value as shown in table 6 means low xanthine oxidase inhibitory activity compared to the standard with low IC_{50} value (table 6).

The IC_{50} value was calculated for both plant extracts and allopurinol which are mentioned in table 6. *in-vitro* anti-arthritic activity of *C. thevatia* leaves has been reported against Bovine Serum Albumin (BSA), utilizing

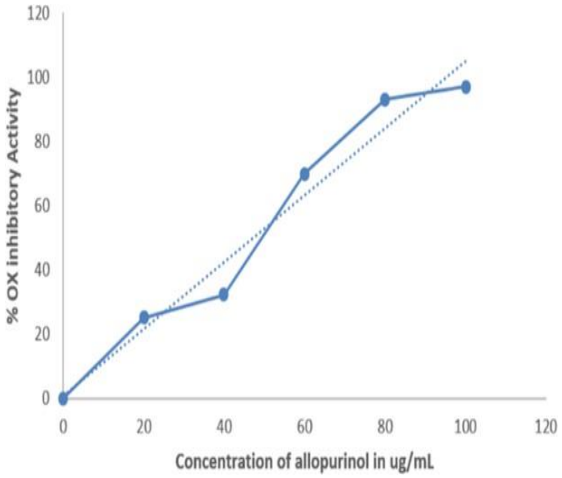

Figure 3: Graphical representation for the % of xanthine oxidase inhibition by allopurinol.

Table 5: % of xanthine oxidase inhibitory activity with different concentrations of Allopurinol and plant extracts.

Allopurinol Concentration (µg/mL)	6.25	12.5	25	50	100
Plant extracts concentration (mg/mL)	3.125	6.25	12.5	25	50
Sample name	% of xanthine oxidase inhibition(mean ± SD)				
Allopurinol		32.1	70.0	93.0	96.9
	25.14±14	3±0.44	7±1.02	9±0.64	3±0.39
C. thevatia	21.99±2.55	28±0.89	41.51±8.86	54.56±0.82	61.38±1.11
C. sativum	26.17±0.53	30.17±4.54	35.29±3.87	47.31±1.11	63.25±3.23

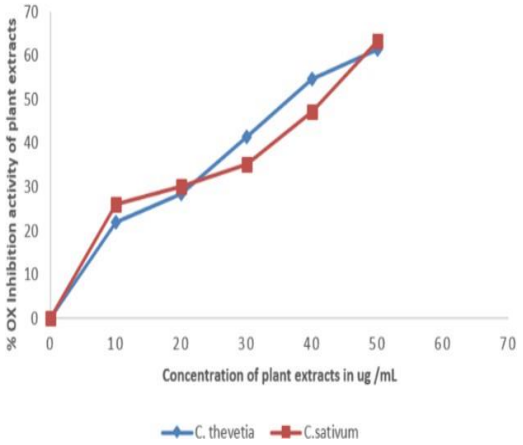

Figure 4: A graphical representation for the % inhibition of xanthine oxidase by the *C. thevatia* and *C. sativum* plant extracts.

Table 6: IC_{50} values for xanthine oxidase inhibition assay of different plant extracts and standard allopurinol.

Sample Name	IC_{50} (mg/mL) value with SD
Allopurinol	0.015±0.15
C.thevatia	21.98±3.10
C.sativum	26.1±1.51

diclofenac as the reference standard through the protein denaturation inhibition method[25]. Our findings confirmed the xanthine oxidase inhibitory activity of *C. thevatia* extract, as predicted by Khan et al., (2024), based on in-silico molecular docking investigations of the GC-mass spectra of *C. thevatia* extract[26].The inhibitory activity of xanthine oxidase in vitro by *C. sativum* was found to be equivalent to the value reported by Rao (2019)[27].

Conclusions

This work revealed a noteworthy concentration of phytochemicals and their kinds in the methanol extracts of *C. thevetia* and *C. sativum* leaves. *C. thevetia* methanol extract contained more phenols and flavonoids than *C. sativum*. There is a positive link between the total phenol and flavonoid content in *C. thevetia*, as this study found that the leaves of *C. thevetia* contain more antioxidants and xanthine oxidase inhibitory compounds than *C. sativum*. It shows potential for the creation of novel phyto-pharmaceuticals. These drugs are considered safe for human use because they are plant-based. Still, more

research including *in vivo* tests and toxicity assessments isneeded to fully understand its safety and feasibility for human use.

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