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Phytochemical and Biological Evaluation on Leaf and Rhizome Extracts of *Curcuma caesia* Growing in Nepal

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Abstract

Curcuma caesia (Black Turmeric) is a perennial medicinal herb native to Southeast Asian countries of India and Nepal. It is known for its rich phytochemical profile and therapeutic properties. This study investigated the phytoconstituents, biological activities, and toxicity, and performed GC-MS analysis of rhizome and leaf extracts and fractions of *C. caesia.* Total phenolic content and total flavonoid content were quantified using the Folin-Ciocalteu reagent and $AlCl_3$ colorimetric methods, respectively. The ethyl acetate fraction of the leaf exhibited the highest TPC (91.14 ± 1.55 mg GAE/g), while the aqueous fraction of the rhizome recorded the highest TFC (155.62 ± 0.61 mg QE/g). The ethyl acetate fraction of the rhizome showed the most potent antioxidant activity (IC_{50} 7.58 ± 0.41 µg/mL), and the methanol extract of the leaf showed the highest antidiabetic potential (IC_{50}: 296.69 ± 0.48 µg/mL). Antibacterial activity was observed against *Klebsiella pneumoniae, Bacillus subtilis*, and *Staphylococcus aureus.* Toxicity assessment revealed an LC_{50} of 458.58 ± 20.23 µg/mL for the ethyl acetate fraction of the leaf in the brine shrimp lethality assay. GC-MS analysis identified 9, 17-octadecadienal, wilfortrine, and phytol as major compounds. These findings highlight the pharmacological potential of C. caesia and its application in traditional medicine for managing ailments such as stomach aches, migraines, and wounds. Further research is recommended to elucidate its mechanisms of action and develop novel therapeutic agents.

Keywords: Antibacterial; Antidiabetic; *Curcuma caesia*; Phytoconstituents

Introduction

Plants are known to contain different types of pharmaceutical activities that arise due to the presence of plant secondary metabolites [1, 2]. The bioactive compounds from plants have the potential to be developed into novel antibacterial and antioxidant agents [3]. Antibacterial activity in plants can also be used as a natural food preservative [4]. The extent of cytotoxicity in plants is related to its bioactivity. The plants with high cytotoxicity may contain potential anticancer and insecticidal compounds.

Curcuma caesia Roxb. (family Zingiberaceae) prominently known as 'Kalo Haledo' or 'Black Turmeric' is a perennial herb native to Southeast Asian countries, India, and Nepal [5]. The plant is cultivated in the hilly regions of Nepal. The genus *Curcuma* includes more than 80 species of plants that are recognized as affluent sources of valuable phytoconstituents. Traditional healers have been using rhizomes and leaves of *Curcuma caesia* in ethnomedicinal procedures from the beginning of human civilization [5]. The plant is used to treat digestive complaints, colds, coughs, and abdominal gas [2]. People of the Adi tribe from Arunachal, India sought relief from stomach aches through the consumption of a decoction prepared from fresh rhizomes of this

plant [6]. The plant is included in the Indian traditional medicine to treat cancer [7]. Dried rhizomes and leaves are used for piles, wounds, leprosy, impotency, fertility, toothaches, vomiting, and allergies [8]. *Curcuma caesia* is also used in rheumatic arthritis, tonsils, and asthma relief [9]. This plant is also used for gastric disorders, sprains, and bruises as well as migraine relief [8]. Previous scientific investigations have searched into the anti-diabetic, anticancer, antioxidant, anti-inflammatory, analgesic, CNS depressant, and anticonvulsant properties of black turmeric extract [10-12]. The species is also conceded for alleviating properties towards fever, tumors, leucoderma, bronchitis, epilepsy, hemorrhoids, diabetes, and inflammation [12].

Phenols, flavonoids, alkaloids, terpenoids, glycosides, tannins, saponins, and sterols are reported from *C. caesia* [12]. The leaves produced 0.7% essential oil after hydro-distillation and the major components of the essential oil included camphor (11%) and, eucalyptol (16.43%) [1]. In another study, GC-MS analysis of the essential oil of *C. caesia* revealed tropolone (15.86%) as the major bioactive substance [2]. The traditional uses and the findings of previous studies establish *C caesia* as an important medicinal plant. Available literature has not explored its full potential in pharmaceutical research. Hence, this research is the first attempt regarding the biological activities and toxicity studies in this plant which will help to explore and preserve this plant globally for isolating the future drug candidates.

Materials and Methods

Plant Sample Collection and Extraction

The rhizome and leaf samples of *C. caesia* were collected from two different sites in Bhaktapur district, Nepal. The coordinates were 27◦ 38’ 43” North, 85◦ 27’ 32” East with an elevation of 1,413 m, and 85◦ 24’ 14” North, 27◦ 39’ 51” East with an elevation of 1,339 m. Photographs of fresh samples from the collection site are provided in **Fig. 1**. The plant was identified by the Central Department of Botany, Tribhuvan University, Kirtipur, Nepal with voucher code number: 210077. Plant samples were cleaned, shade-dried to a constant weight, and ground into powder using a mechanical grinder. Phytochemicals were extracted from 250 g powder of *C. caesia* leaves and rhizome with 500 mL methanol solvent using a cold percolation method. The extracts were filtered with Whatman No. 1 filter paper and concentrated in a rotary evaporator at a controlled temperature (40 ℃) and reduced pressure (650 mm of Hg). Solvent fractions were isolated from methanol extract using a series of solvents (n-hexane, dichloromethane, ethyl acetate, water) with increasing polarity.

Fig. 1: *Curcuma caesia* (a) leaves (b) rhizomes

Gas Chromatography

GC analysis was performed in the Analytical Service Centre, National Academy of Science and Technology (NAST), Lalitpur, Nepal. The instrument used was Agilent 7890 A GC- Agilent 5975 C with triple axis detector included with Aux heater and inlet heater 230, Oven temperature 32, Ionization voltage 70 eV, with injection volume 2 µL, Helium flow 1 mL/min, pressure 6.6018 psi and total run time 59.6 minutes.

Estimation of Total Phenolic Content (TPC)

The Folin-Ciocalteu reagent method was used to estimate TPC [13]. Gallic acid solutions

(20 µL) of concentrations 10, 20, 30, 40, 50, 60, 70, and 80 µg/mL and plant extracts (500 µg/mL in 50% DMSO) were reacted with 100 µL Folin-Ciocalteu phenol reagent (1:10 v/v diluted with distilled water) and 80 µL of Na_2CO_3 (1 M) solutions in triplicates in the wells of a 96-well plate. The 96-well plate was kept in the dark for 15 minutes after which absorbance of the reaction mixtures was measured at 765 nm with a microplate reader. The standard gallic acid calibration curve was used to calculate TPC, and it was expressed as milligrams of gallic acid equivalent per gram of dry extract (mg GAE/g).

Estimation of Total Flavonoid Content (TFC)

The $AlCl_3$ colorimetric method was used to estimate TFC in plant extracts and fractions [14]. Firstly, 130 µL quercetin solutions at concentrations of 15.4, 30.8, 46.2, 61.6, 77, 92.4, 107.8, 123.2, 138.6, and 154 µg/mL were added to 96 well plates. The remaining wells received 20 µL of plant extract solutions and 110 µL of distilled water. Then 60 µL ethanol, 5 µL $AlCl_3$, and 5 µL sodium acetate were added to each well. The reaction mixture was left in the dark for 30 minutes and absorbance was taken at 415 nm using a microplate reader. TFC was calculated from a standard quercetin calibration curve and expressed as milligrams of quercetin equivalent per gram of dry extract (mg QE/g).

Antioxidant Activity

The DPPH assay was used to estimate the antioxidant activities in crude extract and fractions [15]. The stability of DPPH allows batch testing and long-term studies. This method is quantitative, and the degree of antioxidant activity can be quantified by the measurement of absorbance. 100 µL of plant extract solutions (1.5625, 3.125, 6.25, 12.5, 25, and 50 µg/mL in 50% DMSO) were loaded in the wells of 96 well plates in triplicate. 100 µL of 0.1 mM DPPH reagent was also added to each well and then, the plate was placed in the dark. After 30 minutes, the absorbance was taken at 517 nm with a microplate reader. Quercetin and 50% DMSO were used as positive standard and control respectively. The following relation was used to calculate the percentage of DPPH free radical scavenging.

$$\text{Percentage scavenging} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Median inhibitory concentration (IC_{50}) is defined as the concentration of plant extract that scavenges 50% of free radicals. It was calculated from GraphPad Prism 9 software using graph plots of [inhibitor] vs normalized response – variable slope least square fit.

Antidiabetic Activity

The antidiabetic activity was determined using α-amylase inhibition assay according to the DNS method [16]. 250 µL solution of α-amylase enzyme (3.75 unit) was prepared in 20 mM phosphate buffer solution (pH 6.95). The enzyme was premixed with 250 µL solutions of plant extracts and fractions at concentrations from 250 to 2000 µg/mL. The reaction mixture was incubated at 25 °C for 10 minutes followed by the addition of 250 µL starch solution. The mixture was incubated at 25 °C for another 10 minutes. Then, 0.5 mL of 1% DNS reagent was added to the mixture. The contents were kept in boiling water for 10 minutes, cooled, and then diluted to 5 mL by adding distilled water. Absorbance was taken at 540 nm. Acarbose and a reaction mixture in which the volume of plant extract solution is substituted by an equal volume of buffer solution were used as positive standard and control respectively. The following relation was used to calculate the percentage of enzyme inhibition.

$$\text{Percentage inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

IC_{50} is defined as the concentration of plant extracts or fractions that inhibit 50% of enzyme activity. It was calculated by using Graph Pad Prism 9 software.

Antibacterial Activity

The agar well diffusion method was used to estimate the antibacterial activities in plant extracts and fractions. Zone of inhibition (ZOI) measurement was used to assess the antibacterial properties. The procedure was carried out by adhering to the accepted protocol [17]. The organisms used in the study are *Klebsiella pneumoniae* (ATCC 700603), *Bacillus subtilis* (ATCC 35021), *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923). Ampicillin (1 mg/mL) and 50% DMSO were used as positive standard and control respectively.

Toxicity

Preliminary analysis of cytotoxicity in plant extracts and solvents was carried out using brine shrimp lethality bioassay according to standard protocols [18]. It is a rapid, inexpensive, and simple screening technique to evaluate the toxicity in plants. 2 mL solutions of plant extracts and fractions at different concentrations (10, 100, and 1000 µg/mL in methanol) were added to different test tubes. 2 mL of 100% methanol was used as blanks. The solvent in each test tube is evaporated using a hot water bath. The leftover residues were re-dissolved in 5 mL of artificial seawater. 10 healthy brine shrimp nauplii were added to each test tube. The number of surviving larvae in each test tube was counted after 24 hours. The concentration of extract lethal to 50% of the test organism (LC_{50}) was calculated from the percentage mortality versus concentration curve.

Statistical Analysis

All the tests were performed in triplicates and results were expressed as mean ± standard error (SE). GraphPad Prism 9 software was used to analyze data and plot graphs. Values were compared using One-Way ANOVA followed by Tukey's test. Values with $p < 0.05$ were considered statistically significant.

Results and Discussion

Chemical Composition of Leaf and Rhizome Extracts

Gas chromatography was used to analyze the major chemical constituents in methanol extracts of leaf and rhizome. The chromatograms for leaf and rhizome extracts are given in **Fig. 2(a)** and **Fig. 2(b)**.

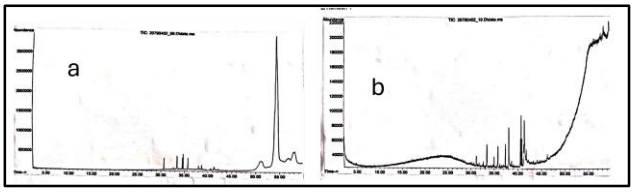

Fig. 2. Chromatogram obtained for methanolic extracts of *C. caesia* (**a**) leaf (**b**) rhizome.

Principal components detected in leaf extract included 9,17-octadecadienal (19.75%), wilfortrine (12.97%), phytol (5.84%), 3-fluorobenzoic acid (5.84%), hexadecanoic acid (4.41%), and pentadecanoic acid (4.41%) (**Table 1**).

Table 1 Major chemical constituents in methanol extract of leaf of *C. caesia* as detected by GC analysis.

Name of compound	Retention time (min)	Area%	Molecular formula	Molecular weight
Hexadecanoic acid	37.33	4.41	$C_{16}H_{32}O_2$	256.42
Pentadecanoic acid	37.33	4.41	$C_{15}H_{30}O_2$	242.40
Wilfortrine	38.045	12.97	$C_{41}H_{47}NO_{20}$	873.80
Phytol	40.829	5.84	$C_{20}H_{40}O$	296.53
3-Fluorobenzoic acid	40.829	5.84	$C_7H_5FO_2$	140.11
9,17-Octadecadienal	41.206	19.75	$C_{18}H_{32}O$	264.40

Likewise, the major components detected in rhizome extract were dodecanoic acid (80.88%), imidazol-4-one (0.78%), and 9-methyltetracyclo [7.3.1.0(2.7).1(7.11)] tetradecane (0.57%), 2-cyclopenten-1-one (0.57%), phenol (0.54%), 4-

[[dimethylamino) sulfonyl] methylamino]-2,4,6-cycloheptatriene-1-one (0.54%), cis-vaccenic acid (0.44%), and octadecanoic acid (0.44%) (**Table 2**). The structures of compounds are provided in **Fig. 3** and **Fig. 4**. Phytochemicals detected in leaves and rhizomes are responsible for many biological and pharmacological activities. For instance, wilfortrine detected in the crude extract of the leaves induces apoptosis in cancer cell lines [19]. Phytol displays antihyperalgesic, anti-inflammatory, and antiarthritic properties [20]. Pejin et al., (2014) observed cytotoxic activity of phytol against various cancer cell lines [21]. It also displayed potent activities against bacterial and fungal strains in a separate study [22].

Table 2 Major chemical constituents in methanol extract of rhizome of *C. caesia* detected by GC analysis.

Name of compound	Retention time (min)	Area %	Molecular Formula	Molecular weight
Phenol	30.623	0.54	C_6H_5OH	94.11
4-[[(dimethylamino) sulfonyl] methylamino]-2,4,6-cycloheptatriene-1-one	30.623	0.54	$C_{10}H_{14}N_2O_3S$	242.29
9-Methyltetracyclo [7.3.1.0(2.7).1(7.11)] tetradecane	33.406	0.57	$C_{15}H_{24}$	204.36
2-Cyclopenten-1-one	34.539	0.57	C_5H_6O	82.10
Imidazol-4-one	34.712	0.78	$C_3H_2N_2O$	82.06
Cis- Vaccenic acid	41.304	0.44	$C_{18}H_{34}O_2$	282.47
Octadecanoic acid	41.304	0.44	$C_{18}H_{36}O_2$	284.48
Dodecanoic acid	54.455	80.88	$C_{12}H_{24}O_2$	200.32

Antimicrobial activity is reported in 9,17-octadecadienal [23]. 2-cyclopenten-1-one selectively induces 70-kDa heat shock protein (HSP70) associated with antiviral activity against vesicular stomatitis [24]. The use of plants for toothaches, stomach aches, migraine, sprains, and bruises in traditional medicine may be associated with the antihyperalgesic, anti-inflammatory, and antinociceptive properties of phytol [6, 8]. The antimicrobial activity of 9,17-octadecadienal and phytol make the plant a suitable antiseptic agent in the treatment of wounds. Phytol may also be the principal component that exerts pharmacological activity in the treatment of rheumatic arthritis [9]. The effectiveness of the plant in treating cancer in traditional medicine may be due to the presence of wilfortrine and phytol [7].

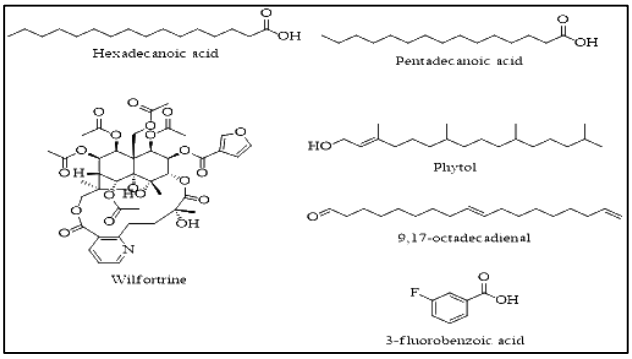

Fig. 3. Structure of the compounds detected by gas chromatography in methanol extract of leaf of *C. caesia.*

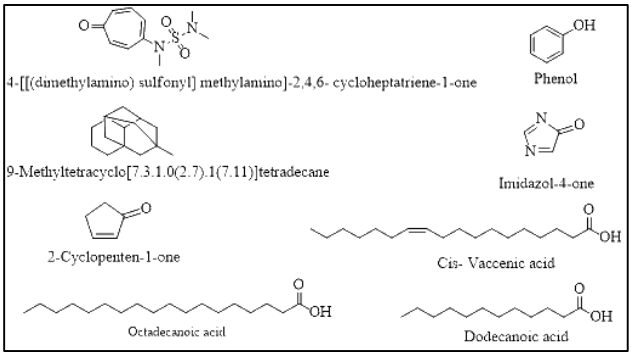

Fig. 4. Structures of compounds detected by gas chromatography in methanol extract of rhizome of *C. caesia.*

Total Phenolic and Flavonoid Content

Total phenolic content was calculated using a standard gallic acid calibration curve (y = 0.035x - 0.136, R^2 = 0.987) given in **Fig. 5(a)**. The amount of phenolic content ranged from 17.88 ± 0.32 in CLA to 91.14 ± 1.55 mg GAE/g in CLE (**Table 3**). The descending order of TPC was CLE > CRE > CLD > CRD > CLCr > CRH > CRCr > CLH > CRA > CLA. Similarly, the standard quercetin calibration curve with the regression equation y = 0.0131x – 0.013, R^2 = 0.9803 was used to calculate the total flavonoid content (TFC). The curve is provided in **Fig. 5(b)**.

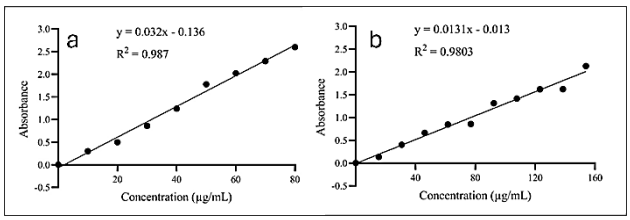

Fig. 5. Standard calibration curves for (**a**) gallic acid and (**b**) quercetin.

CRA contained the highest amount of flavonoid content with 155.62 ± 0.61 mg of QE/g, whereas the lowest amount of 43.43 ± 1.09 mg QE/g was found in CRE (**Table 3**). The descending order of flavonoid content was CRA > CLH > CLD > CLA > CRH > CLCr > CRD > CRCr > CLE > CRE. Crude extract of leaf contained higher TPC and TFC than crude extract of rhizome.

Table 3 TPC, TFC, Antioxidant activity (IC_{50}), and Antidiabetic activity (IC_{50}) in extracts and fractions of *C. caesia.*

Plant extracts and fractions	TPC (mg GAE/g)	TFC (mg QE/g)	Antioxidant activity IC_{50} (µg/mL)	Antidiabetic activity IC_{50} (µg/mL)
CRCr	40.15 ± 0.75^{a,b}	52.51 ± 1.27^{a,b}	23.6 ± 1.29	806.68 ± 1.83
CRH	43.58 ± 1.29^{a}	59.93 ± 0.58^{a}	Nm	983.67 ± 0.45
CRD	75.80 ± 2.31^{c}	58.82 ± 2.01^{a}	11.78 ± 0.42^{a}	Nm
CRE	87.75 ± 0.47^{d,e}	43.43 ± 1.09^{c}	7.58 ± 0.41^{b}	Nm
CRA	35.16 ± 0.88^{b}	155.62 ± 0.61^{d}	Nm	719.92 ± 1.90
CLCr	55.69 ± 0.15	59.74 ± 0.84^{a}	Nm	296.69 ± 0.48
CLH	39.69 ± 1.55^{a,b}	152.01 ± 0.44^{d}	Nm	610.84 ± 0.93
CLD	82. 41 ± 0.87^{c,d}	111.30 ± 0.76^{e}	11.22 ± 0.69^{a}	Nm
CLE	91.14 ± 1.55^{e}	46.41 ± 2.07^{b,c}	9.55 ± 0.43^{a,b}	Nm
CLA	17.88 ± 0.32	106.09 ± 1.73^{e}	Nm	Nm
#Quercetin	Nm	Nm	3.46 ± 0.19	Nm
#Acarbose	Nm	Nm	Nm	49.54 ± 0.47

Key: C, *Curcuma caesia*; R, root; L, leaf; Cr, crude; H, hexane; D, dichloromethane; E, ethyl acetate; A, aqueous. # positive standard; Nm, values not measured. Values are the mean ± SE (n=3). Values followed by different letters or no letters in the same columns are significantly different from each other at $p < 0.05$.

The concentrations of phenolics and flavonoids were higher in leaf extract than in rhizome extract. Similarly, phenolic content increased with an increase in the polarity of solvent fractions as the compounds themselves are polar due to their hydroxyl functional group. A similar trend of more polar solvents extracting higher concentrations of phenolics was also reported by Chaturvedi et al., (2021) in *C. caesia* rhizome extracts [1]. TFC and TPC of 19.95 ± 0.12 mg QE/g and 59.29 ± 0.42 mg GAE/g in CRCr reported in that study were comparable to 52.51 ± 1.27 mg QE/g and 40.15 ± 0.75 mg GAE/g observed in present study [1]. Borah et al., (2019) reported TPC at 2.13 ± 0.02 mg/mL and IC_{50} at 1.48 µg/mL in the DPPH assay for leaf oil of the plant and suggested the antioxidant activity was due to high phenolic content [2]. TPC and IC_{50} values in the DPPH assay displayed a significant correlation with $p < 0.01$ in the present study. The correlation is graphically presented in **Fig. 6(b)**. Such type of correlation is also reported by Budha Magar et al., (2023) in *Chromolaena odorata* [25]. Free radical scavenging mechanisms in phenolic compounds involve donating hydrogen atoms, chelating with metal ions involved in free radical production, and interfering with enzyme actions [26].

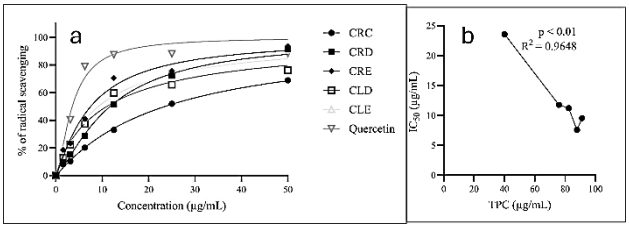

Fig. 6. (**a**) Plot of the percentage of radical scavenging vs concentration of plant extract and fractions of *C. caesia* (**b**) Plot of a correlation between antioxidant activity (IC_{50}) and TPC.

Antioxidant Activity

The percentage of free radical scavenging increased with the increase in concentrations as shown in **Fig. 6(a)**. Antioxidant activity is inversely related to the IC_{50} value [27]. The highest antioxidant activity with the lowest IC_{50} of 7.58 ± 0.41 µg/mL was recorded in CRE (**Table 3**). CLE, CLD, CRD, and CRCr displayed IC_{50} values of 9.55 ± 0.43, 11.22 ± 0.69, 11.78 ±

0.41, and 23.6 ± 1.29 µg/mL respectively. The IC_{50} value for quercetin used as a positive standard was 3.46 ± 0.19 µg/mL. Antioxidant activities were found to be higher in ethyl acetate fraction than dichloromethane fraction and crude extract, whereas activities were comparable between respective fractions of leaf and rhizome. Observed IC_{50} values of 7.58 ± 0.41 and 9.55 ± 0.43 µg/mL in ethyl acetate fractions of leaf and rhizome respectively were slightly higher than 3.46 ± 0.19 µg/mL recorded for pure quercetin. IC_{50} of 23.60 ± 1.29 µg/mL for CRCr in DPPH was lower than the 441.9 µg/mL reported in the literature [11]. Such variation may have been caused by differences in the number and the concentrations of secondary metabolites due to plant growth, environmental stress, and genetics. Antioxidant molecules in plants prevent various chronic diseases such as diabetes, cancer, and neurodegenerative disorders [3].

Antidiabetic Activity

Plant extracts and fractions displayed significant α-amylase inhibitory activities. Concentration-dependent increments in the percentage of enzyme inhibition were observed in the study (**Fig. 7**). The observed IC_{50} values were 296.69 ± 0.48 (CLCr), 610.84 ± 0.93 (CLH), 719.92 ± 1.90 (CRA), 806.68 ± 1.83 (CRCr), 983.67 ± 0.45(CRH), and 49.54 ± 0.47 µg/mL for acarbose used as positive standard (**Table 3**). α-Amylase used in the present study are digestive enzymes that hydrolyze α-1,4-glycosidic linkage in carbohydrates [28]. They are secreted by salivary glands and the pancreas. α-Amylase inhibitors display their antidiabetic activity by blocking and delaying the digestion of carbohydrates and subsequent absorption of glucose. Antidiabetic activities were found to be higher in leaves than in rhizomes. Crude extracts displayed lower IC_{50} values than hexane and aqueous fractions. The inability of those solvents to extract antidiabetic agents or loss of synergism due to the separation of metabolites in different fractions may be a plausible explanation. Crude extract of leaves displayed the lowest IC_{50} of 296.69 ± 0.48 µg/mL which was still higher than 49.54 ± 0.47 µg/mL recorded for the antidiabetic drug acarbose. Antihyperglycemic and antioxidant activities observed in plant extracts and fractions can be attributed to secondary metabolites like phytol and high concentrations of phenolic and flavonoid compounds.

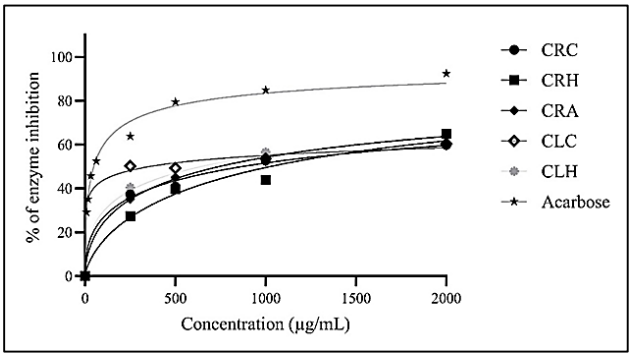

Fig. 7. Percentage of enzyme inhibition vs concentration plot of plant extracts, fractions, and acarbose.

Antibacterial Activity

Crude extracts of rhizome and leaf and dichloromethane fraction of leaf were active against *B. subtilis, S. aureus*, and *K. pneumoniae*. The ethyl acetate fraction of the leaf was active against *B.* subtilis and *S. aureus*. CLA did not display any significant ZOI against test organisms. Plant extracts and fractions were inactive against *E. coli*. CLD recorded the highest ZOI of 11.33 ± 0.33 µg/mL against *S. aureus*. Observed ZOIs for plant extracts and fractions were lower compared to ampicillin used as a positive standard. The ZOI displayed by plant extracts and fractions are given in **Table 4**. Photographs of Petri plates are given in **Fig. 8**. Reports of antibacterial activities in *C. caesia* are also found in the literature. Harit et

al., (2013) reported ZOIs of 13 and 12 mm against *B. subtilis* and *S. aureus* for ethanol extract of rhizome at 20 mg/mL [29]. Borah et al., (2019) recorded ZOIs of 10, 7, and 11 mm against *B. subtilis, B. cereus,* and *S. aureus* respectively for 100 µg/mL of leaf essential oil from the plant [2]. The observed antibacterial activities are due to various secondary metabolites including wilfortrine, phytol, and 9,17-octadecadienal detected by GC analysis.

Table 4 Zone of inhibition shown by extracts and fractions of *C. caesia* against *K. pneumoniae, B. subtilis, S. aureus,* and *E. coli.*

Plant extracts	ZOI (mm) of plant extracts and fractions			
	S. aureus	*K. pneumoniae*	*B. subtilis*	*E. coli*
CRCr	8.00 ± 0.57[a]	8.33 ± 0.33	8.33 ± 0.33	-
CLCr	9.67 ± 0.33	7.33 ± 0.33	7.33 ± 0.33[a]	-
CLD	11.33 ± 0.33[a, b]	8.0 ± 0.57	9.33 ± 0.33[a]	-
CLE	8.33 ± 0.33[b]	-	8.67 ± 0.33	-
CLA	-	-	-	-
[#]Ampicillin	37	25	14	20

Key. [#] positive standard; -, absence of significant ZOI. Values are the mean ± SD (n=3). Values followed by the same letters in the same column are significantly different from each other at $p < 0.05$.

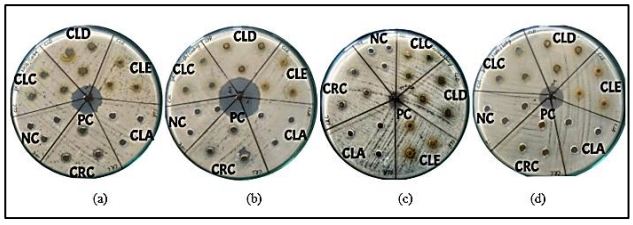

Fig. 8. ZOI inhibition shown by plant extracts and fractions against (a) *Klebsiella pneumoniae*, (b) *Staphylococcus aureus*, (c) *Bacillus subtilis*, and (d) *Escherichia coli*. CRCr (Crude extract of rhizome), CLCr (Crude extract of the leaf), CLD (Dichloromethane fraction of leaf), CLE (Ethyl acetate fraction of leaf), CLA (Aqueous fraction of leaf), PC (Positive control, ampicillin), and NC (Negative control, 50% DMSO)

Brine Shrimp Lethality

Plant extracts and fractions were mildly toxic towards brine shrimp nauplii as the LC_{50} value ranged from 458.58 ± 20.23 µg/mL in CLE to 854.77 ± 56.68 in CLD (**Table 5**). The decreasing order of the toxicity is given as; >CLCr >CLA >CLH >CRCr >CRD >CRE >CR H >CRA >CLD. The lethality of plant extracts and fractions increased with an increase in concentration which is graphically presented in **Fig. 9(a)** and **Fig. 9(b)**. The brine shrimp lethality assay used in the study is a reliable technique for the preliminary determination of cytotoxicity [30]. Thus, the toxicity observed in plant extracts and fractions should correspond to the anticancer activity of the plant reported in the literature [10]. All the plant extracts and fractions displayed LC_{50} less than 1000 µg/mL. Leaf extract and fractions were found more cytotoxic than their corresponding rhizome extract and fractions.

Table 5 Calculation of LC_{50} value of various crude extracts and solvent fractions of leaf and rhizome of *C. caesia.*

Plant extracts	LC_{50} (µg/mL)	Plant extracts	LC_{50} (µg/mL)
CRCr	602.86 ± 33.32[a]	CLCr	491.25 ± 41.10[e]
CRH	646.27 ± 78.68	CLH	600.22 ± 0.41[f]
CRD	608.72 ± 43.46[b]	CLD	854.77 ± 56.68[a, b, e, f, g, h]
CRE	624.51 ± 64.04	CLE	458.58 ± 20.23[c, g]
CRA	715.06 ± 53.39[c, d]	CLA	543.51 ± 49.06[d, h]

Key. Values are the mean ± SE (n=3). Values followed by the same letters are significantly different from each other at $p < 0.05$.

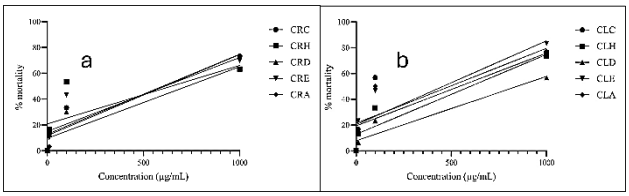

Fig. 9. Percentage mortality versus concentration curve in brine shrimp assay for(a) rhizome and (b) leaf of the plant.

Conclusions

The findings of this study revealed the chemical composition of the leaf and rhizome extracts of *Curcuma caesia*. The research confirmed the plant's remarkable antioxidant activity and significant antibacterial potential. Furthermore, *C. caesia* exhibited promising

anti-diabetic properties. The extracts and their fractions showed mild toxicity toward brine shrimp nauplii. The observed biological activities are attributed to the synergistic effects of the plant's bioactive chemical constituents. Notably, compounds such as phytol, wilfortrine, and 9,17-octadecadienal are likely responsible for the traditional medicinal applications of this plant. Nevertheless, further studies are necessary to validate these findings at molecular and pharmaceutical levels to ensure their reliability and application in medical research. These results underscore the importance of exploring *C. caesia* further, focusing on characterizing and isolating its active principles to develop potential drug candidates.

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Author's Contribution Statement

Tinky Sharma: Methodology, Writing: original draft, Data analysis **Akash Budha Magar:** Formal analysis, Statistical analysis, and Writing: review and editing, **Sabita Khatri:** Formal analysis. **Ismita Lohani:** Data analysis and Writing: review and editing **Sangita Pakka:** Data analysis and Literature review. **Khaga Raj Sharma:** Conceptualization, Supervision, and Writing: review and editing

Conflict of Interest

The authors do not have any conflict of interest throughout this research work.

Data Availability Statement

The data supporting this study's findings are available from the corresponding authors upon reasonable request.

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