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Analysis of phytochemicals and biological activities of rhizome of Curcuma longa, aerial parts of Centella asiatica, and corn silk of Zea mays

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Abstract

Nepal is a storehouse of medicinal plants. Medicinal plants like the rhizome of Curcuma longa, the aerial part of Centella asiatica, and corn silk of Zea mays were used traditionally as medicine for diseases like inflammation, hepatic disorders, cough, etc. In this study, these selected plants were subjected to the analysis of phytochemical constituents, and biological activities following standard methods. Phytochemical analysis of the methanolic extract of these selected plants revealed the presence of different chemical constituents such as polyphenols, flavonoids, glycosides, quinones, saponins, and tannins. C. longa rhizomes also showed the strongest DPPH radical scavenging activity with IC_{50} of 55.06 $\mu g/mL$ which was very close to standard ascorbic acid (49.09 $\mu g/mL$) than that of the aerial part of C. asiatica (72.56 $\mu g/mL$) and corn silk of Z. mays (131.96 $\mu g/mL$). Total phenolic and total flavonoid content was found highest in C. longa with the values of 195.95 ± 0.899 mg GEA/g and 56.45 ± 4.056 mg QE/g respectively. The phenolic and flavonoid content of methanolic extract of aerial parts of C. asiatica was found to be 110.78 \pm 1.984 mg GEA/g and 30.00 \pm 2.358 mg QE/g and corn silk of Z. mays were found to be 65.92 \pm 1.244 mg GEA/g and 18.50 \pm 1.424 mg QE/g respectively. The methanolic extract of rhizomes of C. longa exhibited high α -amylase inhibitory activity with IC₅₀ values of 382.30 μ g/mL than that of C. asiatica with IC_{50} value of 520.48 $\mu g/mL$ and Z. mays with IC_{50} value 593.09 $\mu g/mL$.

Keywords

Curcuma longa, Centella asiatica, Zea mays, phytochemical, antioxidant, anti-diabetic.

Article information

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Introduction

significant biodiversity, making it a perfect place to find novel medicines. According to World Health Organization (W.H.O.) research from 2008, traditional medicine is the major source of primary healthcare for the majority of people in the Asian

Nepal has a huge number of medicinal plants and region [1]. Plants have played a significant role in maintaining human health and improving the quality of human life. As plants contain therapeutic components, they have been used for ages to treat human illnesses [2].

Curcuma Longa is a perennial herb grown in trop-

ical southeast Asia [3]. It is commonly known as turmeric in English and Besar in Nepali. Mainly rhizome of *C. Longa* has great medicinal value. In addition to flavoring and coloring food, *C. longa* rhizome is used for a variety of other uses [4]. Rhizome is used in the treatment of various diseases like diabetes, inflammation, Alzheimer, analgesic, biliary disorders, anorexia, cough, hepatic disorders, and sinusitis [5,6]. Biological activities of *C. longa* include those that are anti-bacterial, anti-inflammatory, anti-oxidant, anti-coagulant, and anti-diabetic, according to research by Bhat et al. (2015) [7].

Centella asiatica is a perennial, prostrate, stoloniferous creeper herb that can have an average length of 15 cm. It is also known as "gotu kola" in many other regions of the world and "ghotapre" in Nepali. Its common names include "madhukaparni" in Sanskrit, "Brahmi" in Hindi. It grows in tropical and subtropical regions and grows widely in different habitats. In Nepal, it is found at a height of 96-2200 m above sea level [8]. Many pharmacological effects of C. asiatica are thought to exist, including immunomodulatory, memory-improving, cardioprotective, anti-cancer, antibacterial, anti-inflammatory, antidiabetic, and antioxidant characteristics [8, 9].

Zea mays is the second most significant crop grown in Nepal [10]. Z. mays presents with a long and yellowish stigma known as corn silk is mostly used in clinical practice to treat gonorrhea, prostatitis, urethritis, cystitis, nephritis, and urinary stones [11]. According to B. Thoudam et al 2011, the methanolic extract of corn silk had the highest level of antioxidant activity (85.2 mg/mL), while the ethyl acetate extract had the lowest (45.5 mg/mL) [2]. Plants in different geographical areas show different biological activities. The extraction process as well as the methodology also impact the result of the research.

A literature survey revealed that not much work has been reported on the biological activities of the rhizome of C. longa, the aerial part of C. asiatica, and corn silk of Z. mays in Nepal. Plants differ as per climate which results in different physiological metabolites. So, we intend to choose these three plants for analysis in this study.

2 Material and Methods

2.1 Materials

The plant samples (rhizome of Turmeric, aerial part of the Ghodtapre, and Corn silk of maize) were collected from Bhaktapur and Kathmandu valley based on traditional medicinal value from March to July 2018. The plants were identified by literatures and compared with the voucher specimens

deposited at National Herbarium and Plant Laboratories, Godavari, Kathmandu.

2.2 Extraction

The gathered rhizome of C. longa, the aerial part of C. asiatica, and corn silk of Z. mays were rinsed with distilled water, shaded dried, powered being stored in a clean plastic bag until needed. Cold percolation was used for the extraction of methanolic samples of selected plants. About 100 g shade dried ground powder of rhizome of C. longa, the aerial part of C. asiatica, and corn silk of Z. mays were kept in a conical flask separately soaked in methanol. The sample was allowed to stand at room temperature for a few days before being filtered and concentrated with a rotatory evaporator. The concentrated filtrate was air-dried to obtain a solid or semisolid residue. The same process was repeated for all other selected plants. After that, the extracts were kept in air-tight vials and stored in a room at cold and dry place.

2.3 Phytochemical screening

The phytochemical constituent of the selected plant extract was determined using standard protocol [12]. The presence of different phytochemicals was analyzed using different specific reagents.

2.4 Antioxidant activity

DPPH free radical scavenging activity assay was carried out. The ability of the rhizome of $C.\ longa$, the aerial part of $C.\ asiatica$, and corn silk of $Z.\ mays$ extracts to scavenge DPPH free radicals was estimated using protocol [13]. In brief, the stock solution of each extract was prepared in methanol (10 mg/mL). By serial dilution of the stock solution, the plant samples at various concentrations (10–100 μ g/mL) were added to a 100 μ M solution of DPPH in methanol. The absorbance of each solution was determined at 517 nm after 30 min of incubation at 37 °C using a UV-visible spectrophotometer. The measurement was performed in triplicates.

The efficiency of the DPPH free radical scavenging activity was determined by using the following equation:

$$\% \ scavenging = \frac{Ac - As}{Ac} \times 100 \ \%$$

where, As is Absorbance of sample solution, Ac is Absorbance of control (DPPH solution + methanol). The IC $_{50}$ value is the effective sample concentration required to neutralize 50% of the DPPH free radicals.

2.5 Determination of Total Phenol Content (TPC)

The total phenolic content of all extracts was determined using the Folin-phenol reagent as described by the standard protocol [12]. The plant samples at various concentrations of 0.125, 0.25, 0.5, and 1.0 mg/mL, were prepared by serial dilution of stock solution of plants $10~\rm mg/mL$. These diluted solutions were then incubated for 30 minutes with $10\%~\rm FCR$ and $7\%~\rm Na_2CO_3$, and absorbance was measured at 760 nm about a blank for each concentration. Gallic acid was used as a reference compound. The measurement was performed in triplicates.

2.6 Determination of Total Flavonoid Content (TFC)

Using the aluminum chloride colorimetric method with quercetin as a reference, the TFC of each extract was calculated [14]. The plant samples at concentrations of 0.125–1.0 mg/mL, were prepared by serial dilution of stock solution. The absorbance of each quantity of extract was measured at 510 nm against a blank. The measurement was performed in triplicates.

2.7 α -amylase inhibition assay

To determine the antidiabetic potential of selected plants, an α -amylase inhibition assay was carried out using a standard protocol with slight modification [13]. The stock solution of each plant extract was prepared in DMSO (1 mg/mL). Six distinct concentrations of each extract, 1000 μ g/mL, 640 μ g/mL, 320 μ g/mL, 160 μ g/mL, 80 μ g/mL, and 40 μ g/mL were made by serial dilution of the resulting stock solution. Similar methods were created for the commonly used acarbose as a standard compound. The blue starch iodine complex, which was detected at 630 nm, was used to identify the undigested starch as a result of enzyme inhibition. The measurement was performed in triplicates.

3 Results and Discussion

3.1 Phytochemical analysis

The micro-chemical analysis of a crude extract of the rhizome of *C. longa*, the aerial part of *C. asiatica*, and corn silk of *Z. mays* in methanol extract depicted the presence of a class phytochemical as shown in Table 1.

Table 1: Result of Phytochemical screening of the rhizome of C. longa, the aerial part of C. asiatica, and corn silk of Z. mays

| S. N. | Phytochemicals | Curcuma longa | Centella asiatica | Zea mays |
|-------|--------------------|---------------|-------------------|----------|
| 1. | Alkaloids | + | + | + |
| 2. | Flavonoids | + | + | + |
| 3. | Reducing sugar | + | + | + |
| 4. | Terpenoids | + | + | + |
| 5. | Saponins | _ | + | _ |
| 6. | Phenolic compounds | + | + | + |
| 7. | Tannis | + | + | + |
| 8. | Glycosides | + | + | + |
| 9. | Coumarins | _ | + | + |
| 10. | Sterols | + | _ | + |

(+) means presence, and (-) means absence

The crude extract of the rhizome of *C. longa*, the aerial part of *C. asiatica*, and corn silk of *Z. mays* in methanol extract revealed the existence of a class of phytochemicals in the phytochemical study as indicated in Table 1. The appearance of certain hues as viewed by a microscope confirmed the existence of phytochemical. Literature reveals that the methanolic extract of *C. longa* consists of bioactive compounds like tannin, glycosides, flavonoid alkaloid saponin, steroids, glycosides, carbohydrates, proteins, starch, and amino acid [15, 16]. Similarly, *C. asiatica* methanolic extract comprise bioactive compounds like alkaloids, tannin, flavonoids, phenolics, saponin, glycosides, terpenoids, and steroids [17]. And methanolic extract of corn silk of *Z. mays*

contained alkaloids, tannin, flavonoids, phenolics, glycosides, terpenoids, and steroids [2]. The result of preliminary phytochemical screening for the same samples may show some variation due to different environmental factors, methods of collection of samples, time of collection, time for grinding, percolation, lab setup, and chemical grades.

3.2 Antioxidant activity

The DPPH radical assay was carried out for all extracts by using Ascorbic acid as standard according to the standard procedure and absorbance was recorded at 517 nm by a spectrophotometer (Table 2).

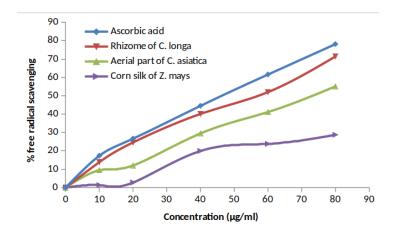


Figure 1: A plot of free radical scavenging of methanolic extracts and concentrations of plants, and ascorbic acid

Table 2: Absorbance of DPPH radical in different concentrations of Ascorbic acid

| S.N. | Concentration $(\mu g/mL)$ | Absorbance |
|------|----------------------------|------------|
| 1 | 10 | 0.639 |
| 2 | 20 | 0.567 |
| 3 | 40 | 0.429 |
| 4 | 60 | 0.297 |
| 5 | 80 | 0.169 |
| 6 | 100 | 0.061 |

(Each value is a mean of triplicate data)

the concentration of DPPH free radicals since there is a transfer of hydrogen radicals from ascorbic acid to DPPH free radicals to form stable DPPH-H molecule resulting in decolorization from violet to pale yellow. The plant extracts showed antioxidant properties in the preliminary tests therefore further test of all those extracts were carried out. The control used involved DPPH and methanol omitting the sample extracts.

Accordingly, the % radical scavenging of each

The decrease in absorbance was due to a decrease in plant extract at different concentrations was calculated and listed in Table 3.

> The IC_{50} values of methanolic extracts of C. longa, C. asiatica, and Z. mays were found as 55.06 $\mu g/mL$, 72.56 $\mu g/mL$, and 131.96 $\mu g/mL$ respectively (Figures 1 and 2). Since these values are lower than 100 μ g/mL and comparable with IC_{50} values of the standard; ascorbic acid (49.09 $\mu g/mL$), these extracts show remarkable antioxidant activity may be due to the presence of a phenolic group.

Table 3: Percentage of radical scavenging with different concentrations of plants extracts

| Concentration | Rhizome of | Aerial part of | Corn silk of | Ascorbic acid |
|--------------------|------------|----------------|--------------|---------------|
| $(\mathrm{mg/mL})$ | C. longa | C. asiatica | Z. mays | |
| 10 | 13.84 | 9.44 | 1.29 | 17.33 |
| 20 | 24.58 | 12.03 | 2.71 | 26.65 |
| 40 | 40.10 | 29.49 | 19.92 | 44.51 |
| 60 | 52.00 | 41.13 | 23.80 | 61.57 |
| 80 | 71.41 | 55.11 | 28.71 | 78.13 |

IC₅₀ values of the standard; ascorbic acid (49.09 est DPPH scavenging activities were shown by the

The IC₅₀ values of methanolic extracts of C. longa, $\mu g/mL$), these extracts shows remarkable antioxwas found as 55.06 µg/mL. Since these values idant activity. This result is in agreement with are lower than 100 μ g/mL and comparable with the literature study which showed that the high-

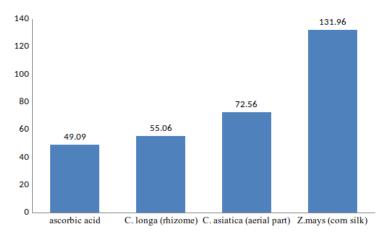


Figure 2: Bar graph showing IC_{50} values of various methanolic plant extracts

methanolic extract of C. longa. These scavenging activities of the extract could be related to the lipid-oxidation process, thus contributing to their electron transfer/ hydrogen donating ability [18]. The ethanolic extract of C. asiatica stem extract

showed greater free radical scavenging than leaves extract [19]. But in this present study aerial part (stem + leaves) exhibited an anti-oxidant capacity less than $C.\ longa$ but greater than $Z.\ mays$.

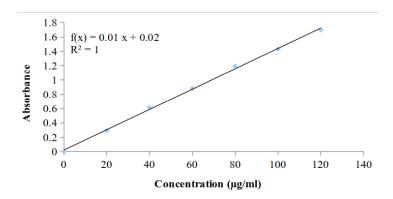


Figure 3: Calibration curve of Gallic acid

The literature revealed that the methanolic extract of corn silk of Z. mays showed strong antioxidant activity at 85.2 mg/mL which may be due to the presence of phenolic and flavonoid constituents [2]. But the study showed the lower antioxidant capacity of corn silk which may be due to variations in environment, time of collection and methodology.

3.3 Total Phenolic Content (TPC)

The total phenolic compound present in the methanolic extract of two different plants was evaluated by using the Folin-cocalteu reagent (FCR) according to the standard procedure given involving the gallic acid as standard. The absorbance graph for standard gallic acid is shown in Figure 3. TPC values of methanolic extracts of *C. longa* rhizome, *C. asiatica* aerial parts, and *Z. mays* corn silk were determined using a calibration curve and

absorbance which are shown in the Figure 4.

From the result obtained from TPC, it has been found that phenolic compounds are a class of antioxidant that acts as the free radical terminator. The highest value of rhizome of C. longa methanolic extract (TPC 195.95 \pm 0.89) showed high antioxidant activity than the methanolic extract of C. asiatica, and Z. mays (Figure 4). This result is in agreement with the literature study which showed that the highest TPC was shown by methanolic extract of rhizome of C. longa (260 \pm 0.025 mg/g) [20]. The literature reviewed that the methanolic extract of C. longa was reported to be TPC of 39.38 mg GAE/g. The study value is slightly lower than the reported value of literature review which could be due to variations in environmental conditions or may be due to the presence of other secondary metabolites or may be due to differences in

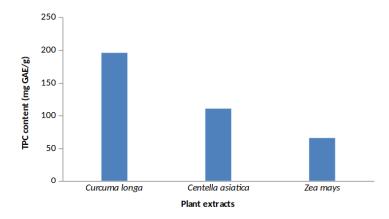


Figure 4: TPC values of three plant samples

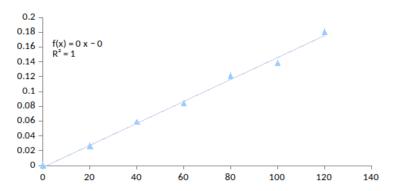


Figure 5: Calibration curve of quercetin

extraction methodology [18]. The therapeutic properties of *C. asiatica* may potentially be attributed to the presence of phenolic compounds as research in the literature revealed that phenolic compounds are the major contributors to the antioxidant activity in plants and that these compounds are also effective hydrogen donors, making them good antioxidants [21]. It was discovered that the TPC of the corn silk extract in this investigation (TPC 272.81 mg GAE/100mg) was lower than the corn silk extracts reported by literature studies. This difference in TPC could be attributed to an environmental variable [22].

3.4 Total Flavonoid Content (TFC)

According to the standard protocol and using quercetin as the reference standard, the total flavonoid compound found in the methanolic extract of selected plants was calculated. The absorbance vs concentration curve for the standard is shown in Figure 5.

TFC values of methanolic extracts of *C. longa* rhizome, *C. asiatica* aerial parts, and *Z. mays* corn silk were determined using a calibration curve and absorbance which are shown in Table 5.

From the result obtained from TFC, it has been found that phenolic compounds are a class of an-

tioxidant that acts as the free radical terminator. The highest value of rhizome of C. longa methanolic extract (TFC 56.45 ± 4.05) showed high antioxidant activity than the methanolic extract of C. asiatica, and Z. mays (Figure 6).

The literature revealed that the methanolic extract of C. longa indicated the most abundant flavonoid content which supports to have several biological activities such as antimicrobial and protective compounds against plant disease [18]. Methanolic extract of the rhizome C. longa contained TFC (79.36 \pm 0.01), which is slightly higher than the value of this study [20]. This may be due to variations in the time of collection and environment. The presence of rutin, catechin, and quercetin in the C. asiatica leaf, root, and petiole resulted in a higher concentration of flavonoids, according to the literature [23].

Higher TFC in methanolic extract contributed to a strong scavenging activity which is thought to give higher antioxidant activity. Methanolic extract of corn silk showed higher TFC than water extract [22]. But in this study, the methanol extract of corn silk of Z. mays showed lower TFC. The difference in result may be due to variations in altitude, and in time of collection of samples.

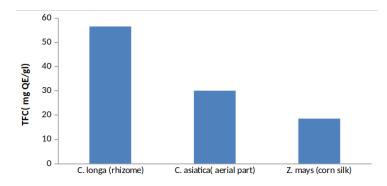


Figure 6: Total flavonoid content of different methanolic plant extracts

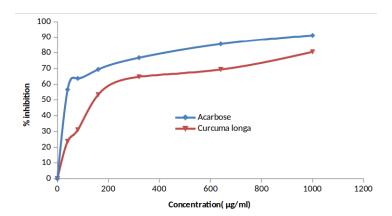


Figure 7: Comparison of α -amylase inhibition % between Acarbose and methanol extract of C. longar

3.5 α -Amylase inhibition activity

The anti-diabetic properties of methanolic extract of $C.\ longa,\ C.\ asiatica,\ and\ Z.\ mays$ were measured by taking a carbose as standard, and IC $_{50}$ values were also calculated. To assess the anti-diabetic potential of chosen samples, a starch-iodine assay for α -amylase inhibition was performed. Percentage inhibition of α -amylase by different concentrations of plant extracts and Acarbose were calculated and these data were tabulated below Table 4.

The above result in table 4 demonstrated that selected plant extracts show amylase inhibition activity and there is concentration-dependent increase in inhibition percentage. $C.\ longa$ extract had higher % inhibition of 80.62 at 1000 $\mu g/mL$ than the $C.\ asiatica$ and Z.myas. Acarbose as standard drug had % inhibition of 91.23 at 1000 $\mu g/mL$.

 IC_{50} values were calculated using graph obtained by plotting % inhibition against concentration and results are shown in Table 7.

Thus, the methanolic extract of C. longa showed

a lower IC₅₀ value than that of C. asiatica and Z. mays. The IC₅₀ value of the standard was found as 85.43 μ g/mL. The IC₅₀ value of C. longa was found as 382.30 μ g/mL which showed a higher anti-diabetic property than that of C. asiatica and Z. mays.

The literature revealed that some bioactive compounds such as flavonoids, phenolic acid, and steroids are known to be bioactive antidiabetic principles. *C. longa* having terpenes, alkaloids, flavonoids, phenols, and sterols showed potent inhibitory activity towards alpha/beta-glucosidase [4].

In the previous study, the triterpenes compound is responsible for the biological activity of C. asiatica, and asiaticoside one of triterpene showed an activity as an antidiabetic agent [24]. The literature revealed that C. asiatica leaves possess significant antidiabetic activity [25]. But the research did not show the antidiabetic activity of the aerial part C. asiatica this may be due to variations in environment and time of collection.

The methanolic extract of corn silk of Z. mays showed lower antidiabetic activity than other plant extracts. This result correlates well with the fact that it possessed lower antioxidant capacity and

lower phenolics. However, the percentage inhibition (64.94 \pm 0.26) was not bad at 1000 $\mu g/mL$ concentration. This may be due to the availability of phytochemicals like alkaloids, tannins, flavonoids,

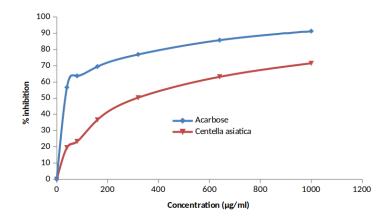


Figure 8: Comparison of α -amylase inhibition % between Acarbose and methanol extract of C. asiatica

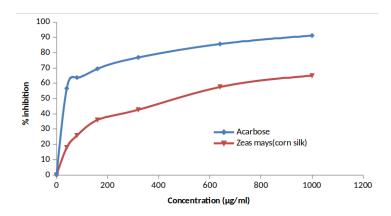


Figure 9: Comparison of α -amylase inhibition % between Acarbose and methanol extract of Z. mays

phenolics, glycosides, terpenoid, and steroids which could be responsible for the antidiabetic potential of corn silk. The literature revealed that hexane and methanolic extract of corn silk inhibited the $\alpha\text{-glucosidase}$ with IC50 value ranges of 31.6 \pm 0.4 $\mu\mathrm{g/mL}$ to 35.7 \pm 0.6 $\mu\mathrm{g/mL}$ [26].

4 Conclusion

The methanolic extracts of the three chosen plants were subjected to phytochemical analysis in this study, which revealed several chemical constituents, including polyphenols, flavonoids, glycosides, saponins, and tannins. Thus, it can be con-

cluded that the selected plants are rich in secondary metabolites. The methanolic extract of rhizome of $C.\ longa$ demonstrated the strongest DPPH radical scavenging action with an IC₅₀ value of 55.06 $\mu \rm g/mL$ which is similar to standard ascorbic acid (49.09 $\mu \rm g/mL$). The outcome showed that the highest total phenolic content was in $C.\ longa$ (195.95 \pm 0.899 mg GAE/g) extract followed by the aerial part of C. asiatica (110.78 \pm 1.984 mg GAE/g extract). The extract which showed the lowest content of total phenol was $Z.\ mays$ (65.92 \pm 1.244 mg GAE/g). The total flavonoid content (56.45 \pm 4.056 mg QE/g) was highest in the extract of $C.\ longa$ among other plant extracts. Among the se-

Table 4: α -amylase inhibition % by different concentrations of plant extracts and acarbose

| Concentration | Acarbose | C. longa | C. asiatica | Z. mays |
|---------------|------------------|------------------|---------------------------|------------------|
| (mg/mL) | | (rhizome)(in %) | (aerial part) inhibition | (corn silk) |
| 1000 | 91.23 ± 0.31 | 80.62 ± 1.04 | 71.47 ± 0.55 | 64.94 ± 0.26 |
| 640 | 85.69 ± 0.23 | 69.39 ± 0.02 | 63.12 ± 0.16 | 57.55 ± 0.21 |
| 320 | 76.89 ± 0.34 | 64.74 ± 1.35 | 50.3 ± 0.45 | 42.67 ± 0.09 |
| 160 | 69.43 ± 0.65 | 53.41 ± 0.50 | 36.63 ± 1.03 | 36 ± 0.25 |
| 80 | 63.69 ± 0.54 | 31.11 ± 0.84 | 23.30 ± 0.53 | 25.82 ± 0.40 |
| 40 | 56.60 ± 0.31 | 23.75 ± 0.50 | 19.49 ± 1.20 | 17.94 ± 0.16 |

(Each value is a mean of triplicate data)

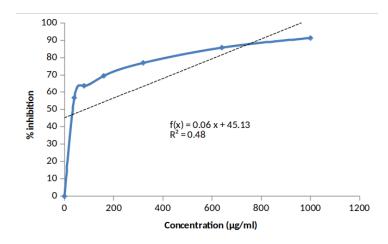


Figure 10: Inhibition % vs Concentration graph for Acarbose

Table 5: Comparison of IC₅₀ values of plant extracts with the standard for α -amylase inhibition

| S.N. | Sample | $ m IC_{50}~(\mu g/mL)$ |
|------|--------------|-------------------------|
| 1 | Acarbose | 85.43 |
| 2 | $C.\ long a$ | 382.30 |
| 3 | C. asiatica | 520.48 |
| 4 | Z. mays | 593.09 |

lected plants, the methanolic extract of the rhizome of $C.\ longa$ exhibited high α -amylase inhibitory activity with an IC₅₀ value of 382.30 $\mu g/mL$. As a result, the rhizome of $C.\ longa$ demonstrated significant biological activities, although they cannot be used directly for medicinal purposes. For their in-vivo application as well as to characterize and isolate the unknown component to introduce their

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pharmacological qualities, additional comprehensive phytochemical and pharmacological research along with the mechanism of action are essential.

Conflict of interest

The authors do not have any conflict of interest pertinent to this work.

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