# EXTRACTION, ISOLATION AND PURIFICATION OF VARIOUS COMPONENTS OF CASSIA UNIFLORA AND IT'S IN VITRO STUDIES

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**ABSTRACT - Cassia species are one among the plants to possess great therapeutical value due to their wide rage of pharmacological activities. Hence, in our present study, we have isolated the extract of Cassia uniflora and isolated some important constituents using various solvents among which methanolic extract was optimal. The isolated compounds were also characterized by mass and IR spectroscopy. The extract was further checked for anti-oxidant as well as anti-diabetic activity. From the results obtained, the leaves and fruit extract were found to have good alpha-glucosidase as well as 5-lipoxygenase inhibition.**

**Key words:** Cassia uniflora, extract, characterization, anti-oxidant, anti-diabetic

## 1. INTRODUCTION

Cassia species are one among the plants to possess great therapeutical value.[1] Leaves and pods of few species of cassia such as *fistula, spectabilis* and *podocarpa* possess laxative as well as antimicrobial activities. [2,3] The seeds and flowering extracts of *C. auriculata* possess antidiabetic activity.[4] Antioxidant activity of *C.fistula* was evaluated by Manonmania, leaves of *C. nigricans* as appetizers, febrifuges and to treat skin infections such as scabies, ringworm and eczema.[5,6] Hepato-protective and anti-inflammatory activity of *C. occidentalis*, *C. sophera, and C. fistula* were also present in the literature.[7] *C. italica, C. sophora, C. pumila* were reported to possess CNS depressant, anxiolytic as well as hypnotic activity.[8] Barakol was isolated from *Cassia siamea* having anxiolytic and purgative activity.[9] *C. mimosoides* was reported to possess anti-obese activity.[10] *C. auriculata, C. tora, C. fistula, C. alata* were also reported to possess antioxidant activity and anti-diabetic activity.[11] Among these, another species of cassia (C. uniflora) has has attracted attention for its properties. Nivedita G *et al.,* in 2010 identified various phyto-constituents present in *C. uniflora* and determined the larvicidal activity against *Aedes aegypti*.[12] Petroleum ether, methanolic and ethyl acetate extracts of *C. uniflora* were reported to possess analgesic, anti-arthritic as well as anti-inflammatory property.[13] In the present investigation, the plant parts such as fruit, leaf, stems and bark of *C. unioflora* as shown in figure 1, were considered for the study where in we have identified the solvent which extract the components in large quantities and further characterized. The extracts were also studied for their antioxidant as well as anti-diabetic activity.

Figure 1: Image of *C. uniflora*

## 2. MATERIALS AND METHODOLOGY

### 2.1 Plant collection, drying, pulverization and extraction

Stem, fruit and leaves of *C. uniflora* were obtained from Rajahmundry region of East Godavari district. They were identified and confirmed by Dr. K. N. Reddy (Taxonomy department) of Laila impex R&D Centre located in vijayawada. All the plant materials were later deposited in raw drug museum of Laila impex. The plant materials were air dried under shade powdered mechanically to coarse or fine powder. The components were extracted with methanol, water and dil. aq alcohol (60%) to obtain aqueous, methanolic and hydro alcoholic extracts of various parts of *Cassia uniflora.* Codes of extracts were named as below and in the further discussion the extracts were mentioned as their codes as seen in table 1.

TABLE 1: EXTRACTION OF *C. UNIFLORA*

| Extract | Crude drug of | Quantity | Extract obtained | Solvent used | Volume |
|---|---|---|---|---|---|
| CulMeOH | leaf | 100g | 20.39g | Methanol | 600ml |
| CulH2OAlc | leaf | 100g | 25.71g | 60 % methanol in water | 600ml |
| CulWat | leaf | 100g | 30.89g | Water | 600ml |
| CusMeOH | stem | 45g | 4g | Methanol | 300ml |
| CusH2OAlc | stem | 45g | 5.55g | 60% methanol in water | 300ml |
| CusWat | stem | 45g | 6.86g | Water | 300ml |
| CufMeOH | fruit | 35g | 4g | Methanol | 250ml |
| CufH2OAlc | fruit | 35g | 6g | 60% methanol in water | 250ml |
| CufWat | fruit | 35g | 4.43g | Water | 250ml |

As methanolic extract of the leaf was obtained in large quantities, various solvent was used to isolate the compounds which include CH3Cl, 2%, 6%, 10%, 20%, and 50 acetone in chloroform, acetone and methanol. Chloroform extract was obtained in large quantity (2g) which were further subjected to resolute the components.

### 2.2 ABTS radical scavenging activity [14]

Radical scavenging activity was performed by following the method described by Re et al., with certain modifications. ABTS (Hi-Media) radical cation was generated by reacting ABTS stock solution 7mM with 2.45mM potassium persulfate (final concentration) by dissolving in distilled water (1:1) ratio and the mixture was allowed to incubate 16-20 h for the formation of ABTS radical cation at room temperature. Prior usage, the prepared solution was diluted using ethanol to get absorbance of 0.700 at 734 nm. Dilutions of plant extracts in various solvents were prepared to obtain concentrations of 100-400 µg/ml. The plant extract of varying concentrations with 1ml of ABTS solution was homogenized and its absorbance was recorded spectrophotometrically (Sican 2301, Inkarp) at 734nm by keeping ethanol as a blank and Trolox as positive control. The antiradical activity, ABTS scavenging activity was expressed as IC50 (µg/ml). The % inhibition of radical scavenging activity was then calculated using the following formula:

$$ABTS\ scavanging\ activity\ (\%) = \frac{\text{Negative control absorbance} - Sample\ absorbance}{A\text{Negative control absorbance}} X100$$

### 2.3 Alpha amylase inhibition [16]

The α-amylase inhibition was measured by following the method of Adisakwattana et al. with some modification. Porcine pancreatic α-amylase (4 units/ml) MP Biomedicals (Cat. No: 191239) was dissolved in 0.1M phosphate buffer of pH 6.9. Plant extract's stock solutions for inhibition assay were prepared in various concentrations of 100–400 µg/ml and added to the solution containing starch and phosphate buffer of 1g/L and 165µl respectively. Initiation of the reaction was by adding enzyme solution (75µL) to the incubation medium. After 10 min of incubation, termination of the reaction was performed by adding 250ml dinitrosalicylic (DNS) reagent (which contains mixture of 1% 3,5- DNS acid, 0.05% $Na_2SO_3$, 0.2% phenol and 1% aq. NaOH) and heating at 100°C for 10 min. Thereafter, 250µl of 40% of potassium sodium tartrate (PST) solution was added to the mixtures in order to stabilize the color. After the temperature was dropped down to room temperature, the absorbance was recorded at 540nm using a microplate reader (EnSpire® Multimode Plate Reader). Acarbose was taken as positive control. Inhibitory activity (inhibition %) was calculated by the following formula:

$$\%\ inhibition\ of\ \pm amylase = (Abs\ of\ control) - \frac{(Abs\ of\ sample)}{Abs\ of\ sample}\ X\ 100$$

### 2.4 Determination of α-glucosidase inhibitory activity

α-glucosidase inhibition was determined using 4-nitrophenyl-alpha-D-glucopyranoside as the substrate. α-glucosidase (Sigma Chemical Co St. Louis M.O. USA) solution (0.006%) was prepared in 0.02M phosphate buffer (pH 6.3). 0.13ml of enzyme solution was incubated with 0.13ml of extract and 0.45ml of 0.02 M phosphate buffer for 1 h at 25°C. After pre-incubation, 0.67ml of 2 M 4-nitrophenyl-alpha-D-glucopyranoside was added to the reaction. The mixture was further incubated for at least 30 min at the temperature of 30°C. Reaction termination was done by adding 1 M Na2CO3 solution (2 ml). Determination of the amount of 4-nitrophenol formed was recorded at 405nm using a microplate reader. Inhibitory activity (inhibition %) was calculated by the following formula:

$$\%\ inhibition\ of glucosidase = (Abs\ of\ control) - \frac{(Abs\ of\ sample)}{Abs\ of\ sample}\ X\ 100$$

## 3. RESULTS AND DISCUSSION

*Cassia uniflora* stem, fruit and leaves were extracted with methanol, hydroalcohol and water. The *Cassia uniflora* leaf methanolic extract was selected to carry out further work. The leaf methanolic extract was fractionated by column chromatography and 4 compounds were isolated by fractionation which were identified by mass and IR spectroscopic methods. All the details of the extraction are tabulated in table 2.

### 3.1 COMPOUND CulMeOH-1

The compound **CULMEOH-1** was obtained as colourless white crystalline solid mp 180-182°C. The molecular formula of compound **CULMEOH-1** was shown to be $C_7H_{14}O_6$ based on the Negative ion LC-MS [*m/z* 193(M-H)$^-$] analysis as in spectrum as can be seen in figure 2. The IR spectrum showed bands at 3403.65,3318 (hydroxyl), 1511.47, 1452, 1129, 1072.47, 1002 and 961 cm$^{-1}$ (ether). From the above spectral data, physical constants the compound **CULMEOH-1** have found to corroborate well with reported data of 3-O methyl D chiro inositol.

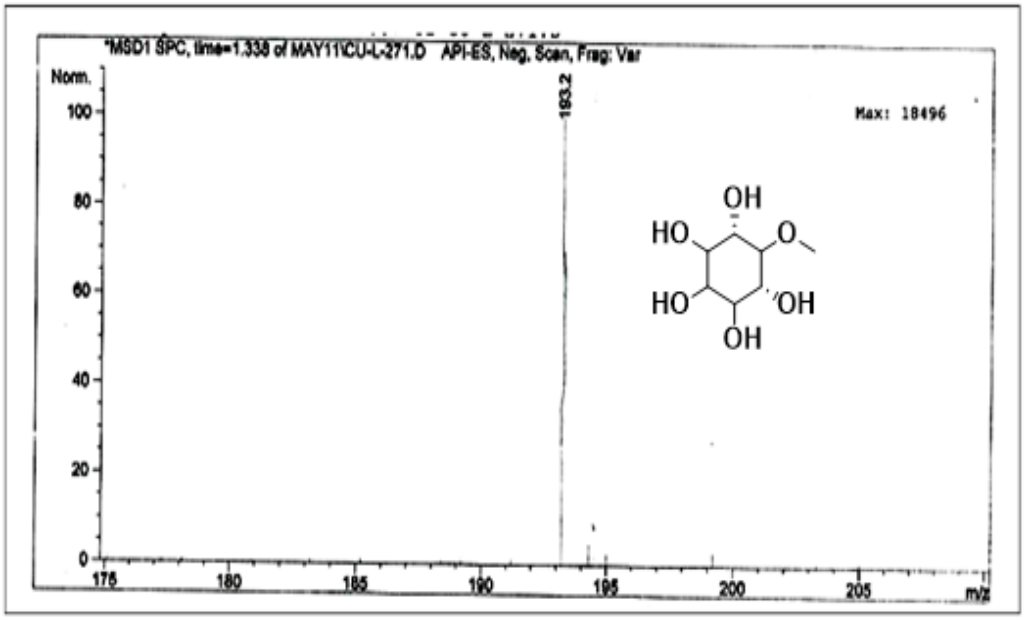


Figure 2: Mass spectra of CulMeOH-1

### 3.2 COMPOUND CULMEOH-2

Compound **CULMEOH-2** was obtained as a pale yellow powder from methanol mp 328-330°C and analyzed for $C_{15}H_{10}O_6$ based on the Negative ion LC-MS [m/z 285.2(M-H)$^-$] analysis as in the spectrum shown in figure 3. The IR spectrum showed bands at 3419 (hydroxyl), 2923, 2855.94 symmetric and asymmetric stretching 1655.23 (carbonyl) 1608.83 alkene, 1569.89, 1511.78,1445 (aromatic),1265.43 and 1166 (C-O). The spectral data and physical constants of compound **CULMEOH-2** are well in agreement with reported data of luteolin

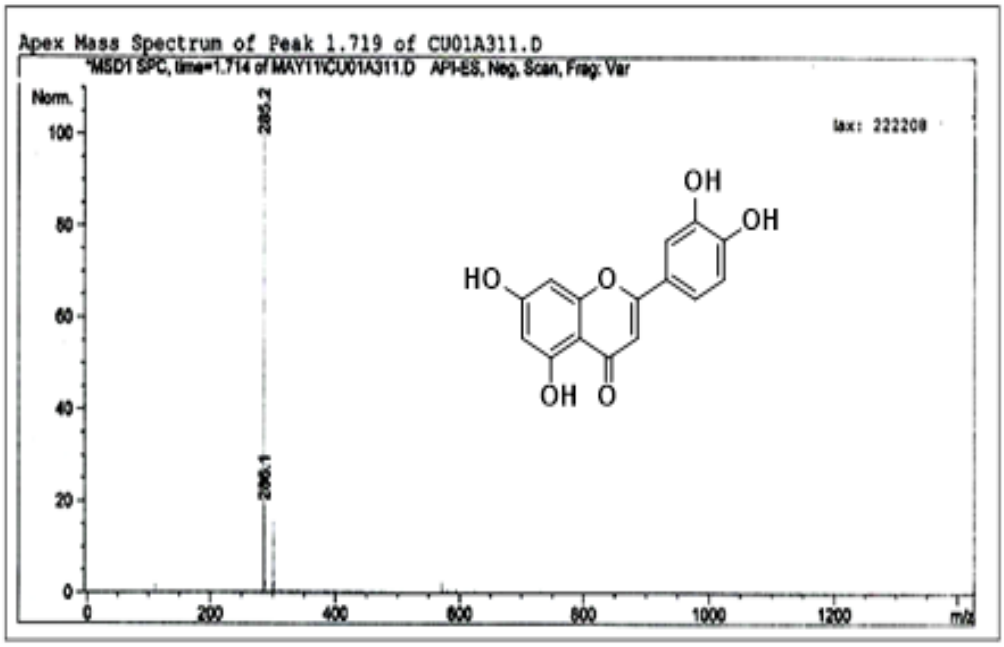


Figure 3: Mass spectra of CulMeOH-2

### 3.3 COMPOUND CULMEOH-3

Compound **CULMEOH-3** was obtained as solid; from methanol mp > 52-55°C. Based on the positive ion LC-MS [m/z 353.4(M+H)$^+$] analysis as in the spectrum shown in figure Z. The IR spectrum showed bands at 2914, 2846 (C-H stretching symmetric & asymmetric) 1469 11407 (C-H bending) 1218 and 1120cm$^{-1}$ (C-O stretching) as in the spectrum as shown in figure 4. The spectra were found to be long chain alkane pentacosane.

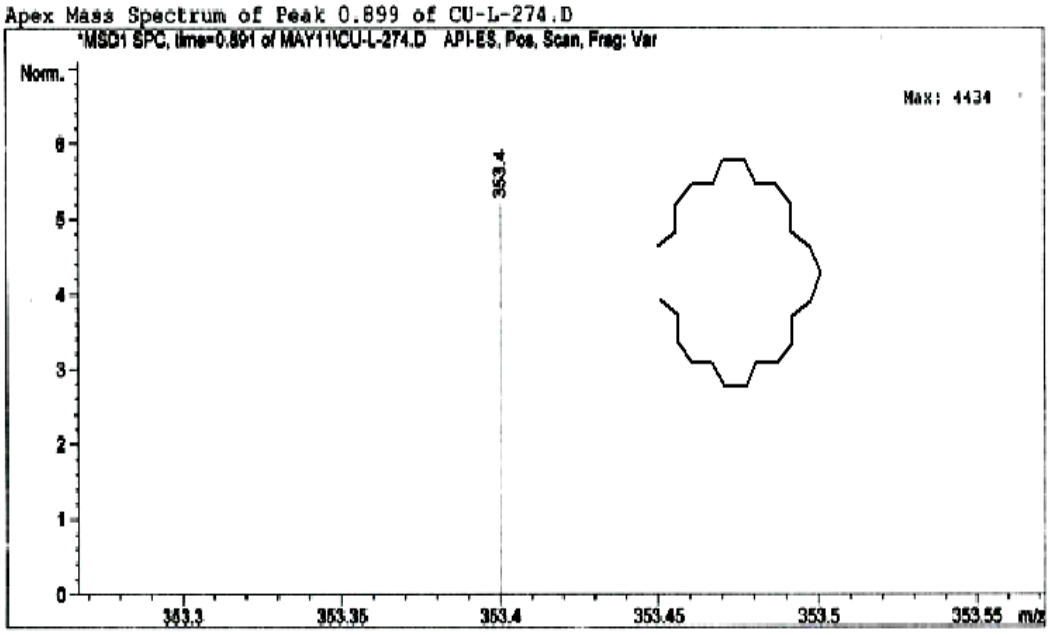


Figure 4: Mass spectra of CulMeOH-3

### 3.4 COMPOUND CULMEOH-4

Compound **CULMEOH-4** was obtained as solid; mp 82-84°C. The IR spectrum showed bands at 3398 (hydroxyl), 2914, 2847 cm$^{-1}$ for CH strectching 1436 1359 and 1120cm$^{-1}$ (C-O) as in the spectrum as shown in figure ZA. The molecular formula of compound **CULMEOH-4** was shown to be $C_{30}H_{62}O$ based on the positive ion LC-MS [*m/z* 437.8(M-H)$^-$]. The spectra as shown in figure 5, were in accord with those published previously for 1-tricontanol.

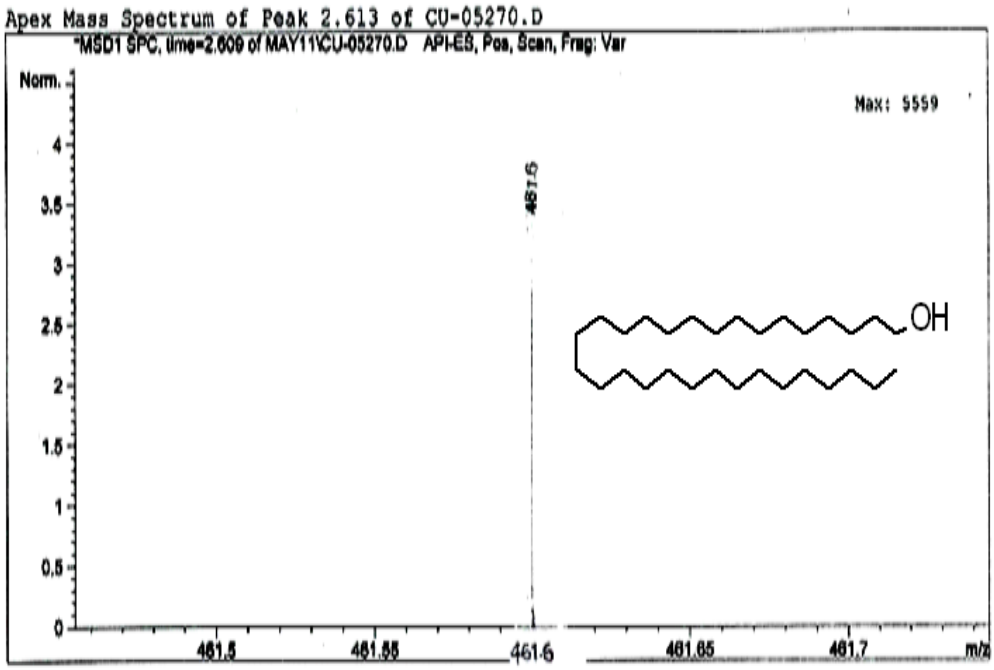


Figure 5: Mass spectra of CulMeOH-4

TABLE 2: DATA OF ISOLATED COMPOUNDS FROM CULMEOH

| | C-1 | C-2 | C-3 | C-4 |
|---|---|---|---|---|
| **Chemical name:** | (1S,2S,4S,5R)-6-methoxycyclohexane-1,2,3,4,5-pentol | 2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-4-chromenone | n-pentacosane | triacontan-1-ol |
| **Common name:** | Methyl Inositol | Luteolin | Pentacosane | Melissyl alcohol |
| **Physical state:** | White crystals | Pale yellow powder | White powder | White powder |
| **Melting point:** | 180-182 $^{o}$C | 328-330 $^{o}$C | 52-55 $^{o}$C | 82-84 $^{o}$C |
| **$R_f$ (cm):** | 0.5 (solvent system chloroform:methanol 6:4) | 0.75 (solvent system-chloroform:methanol 8:2) | 0.87 (solvent system-hexane:ethyl acetate 8:2) | 0.37 (solvent system-Hexane:chloroform 8:2) |

### 3.5 Biological activity

Methanolic, hydro alcoholic and aqueous extracts of *Cassia uniflora* (leaves, fruits and stem parts) were screened for various *in-vitro* pharmacological assays such as antioxidant, anti-diabetic and anti-inflammatory.

Methanolic, hydro alcoholic and aqueous extracts of leaves, fruits and stems of *Cassia uniflora* was found to possess good radical scavenging activity with superoxide radical scavenging with $IC_{50}$ value 25.59 µg/ml against ascorbic acid and gallic acid (DPPH radical scavenging activity) with $IC_{50}$ value 3.56 µg/ml and 38.41 respectively; whereas, 8.85 µg/ml against ascorbic acid (3.09 µg/ml) and gallic acid (8.85 µg/ml) in case of ABTS scavenging activity as can be seen in figure 6.

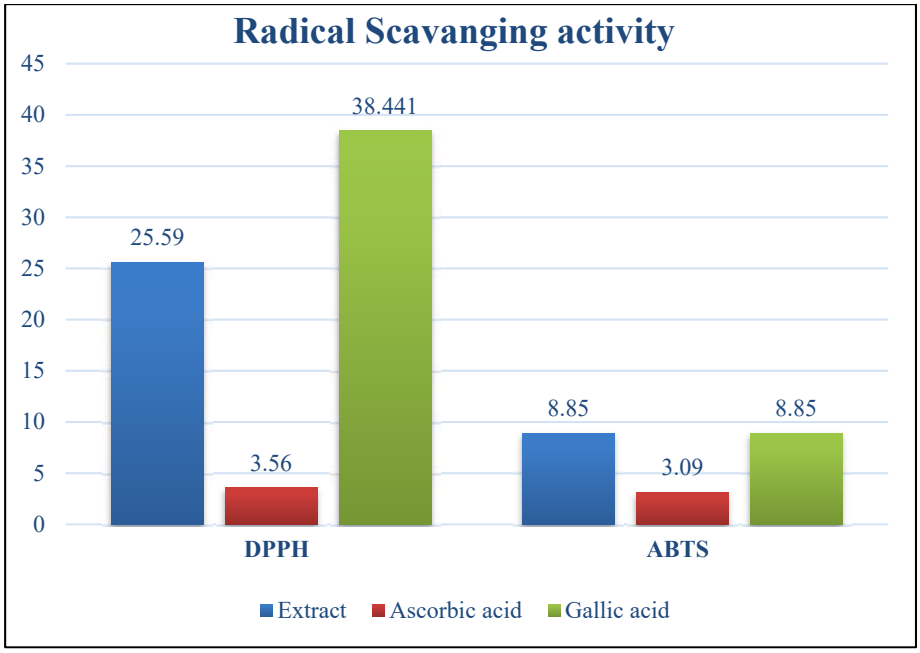


Figure 6: Radical scavenging activity of CulMeOH

Methanolic, hydroalcoholic and aqueous extracts of leaves, fruits and stems of *Cassia uniflora* were screened for *in-vitro* antidiabetic activity with alpha-amylase and alpha-glucosidase assay. Amongst leaves methanolic and fruit hydro alcoholic extracts of *Cassia uniflora* found to possess good alpha-glucosidase activity and alpha-amylase activity with $IC_{50}$ value 5.65 µg/ml, 5.04 µg/ml, 275 µg/ml 264 µg/ml, respectively, by comparing the results with standard as Acarbose $IC_{50}$ value 57 µg/ml(alpha-glucosidase) and 103.27 µg/ml (alpha-amylase) as can be seen in figure 7 and 8 respectively.

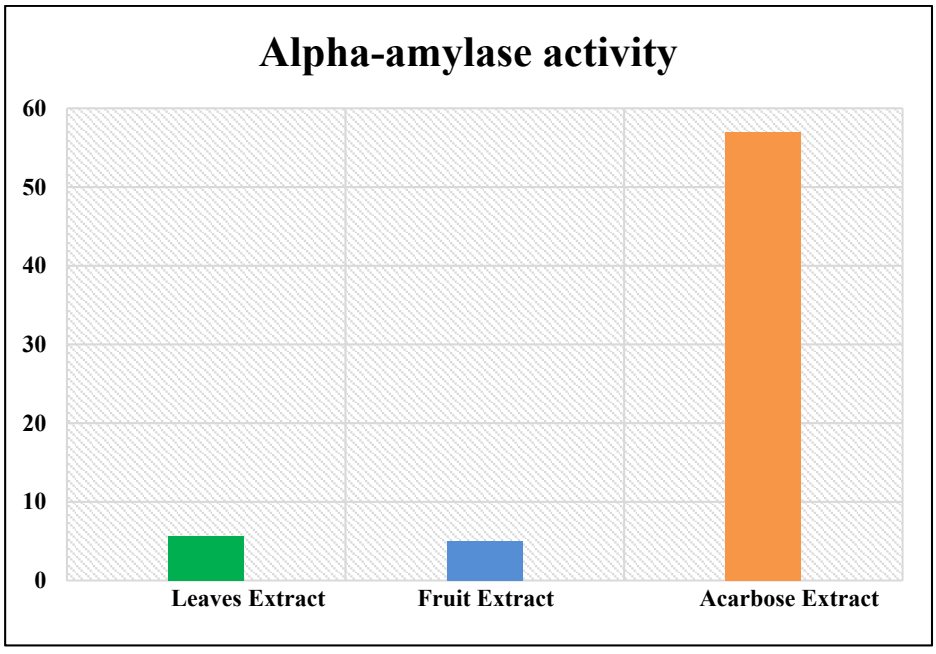


Figure 7: Alpha amylase scavenging activity of CulMeOH

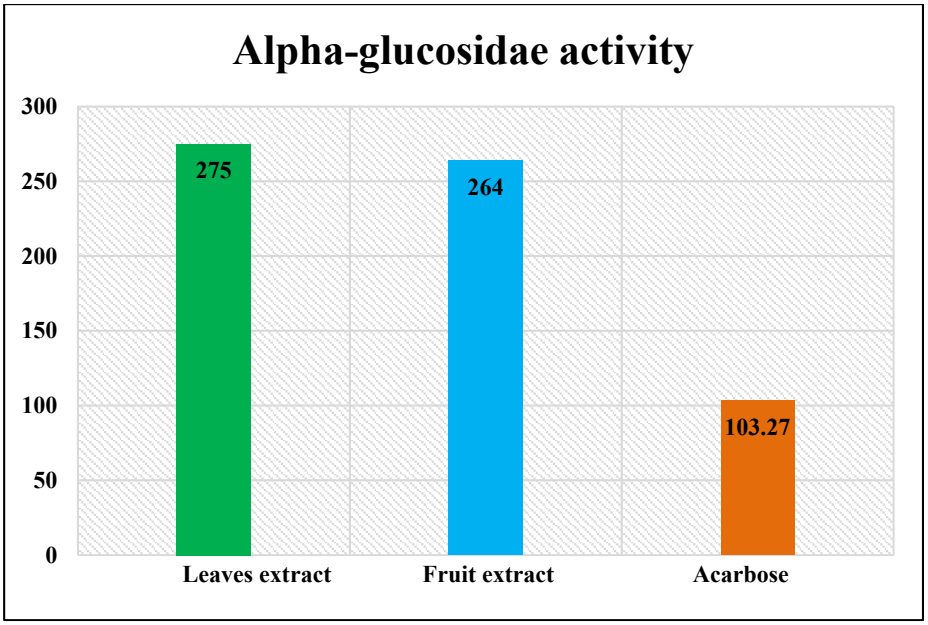


Figure 8: Alpha glucosidase scavenging activity of CulMeOH

Hydroalcoholic extract of leaves, stems and fruits of *Cassia uniflora* was found to have good 5-lipoxygenase inhibitory activity with $IC_{50}$ value 47.85µg/ml, 46.97µg/ml and 46.58µg/ml by comparing the results with standard demethylated curcumin with $IC_{50}$ value 6.13 µg/ml as can be seen in figure 9.

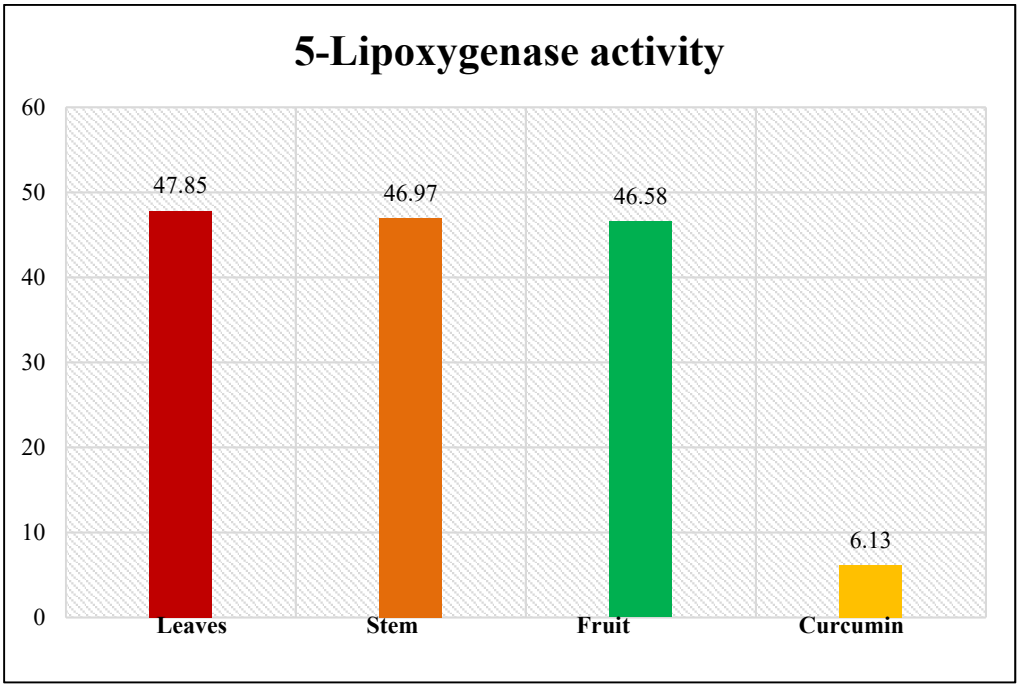


Figure 9: 5-lipoxygenase inhibitory activity of CulMeOH

## 4. CONCLUSION

In consideration with extractive value and *In-vitro* antioxidant assay, methanolic leaf extract of *Cassia uniflora* was forwarded to fractionation and column chromatography for isolation of phytoconstituents. The isolated fractions were also screened for ABTS radical scavenging assay and alpha-glucosidase assay. In case of *Cassia uniflora* leaf 50% acetone in chloroform and methanol fraction was found to have good ABTS radical scavenging activity, so it was chromatographed for isolation. Compound C-2 (luteoline) was isolated from 50% acetone in chloroform fraction and compound C-1(methyl inositol) was isolated from methanol fraction. Chloroform fraction was also forwarded to column chromatography and compound C-3(pentacosane), C-4(triacontane-1-ol) was isolated. By comparing the data with standard library these four compounds were identified by IR and LC-MS.

## 5. ACKNOWLEDGEMENT

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## 6. CONFLICT OF INTEREST

Nil.

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