The medicinal plant *Calpurnia aurea* leaves act as an anti-inflammatory source of 5-Lipoxygenase / Cyclooxygenase-2 dual inhibitors

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**ABSTRACT**
*Calpurnia aurea* is a medicinal plant belonging to the family of Fabaceae. Traditionally in Ethiopia the leaves have been used for the treatment of diarrhea, leishmaniasis, swelling, cough and snake bite. Based on the traditional use the leaves of *C. aurea* might be a promising source for development of anti-inflammatory agents. The objective of the current research was to explore the 5-Lipoxygenase (5-LOX), Cyclooxygenase-1 & Cyclooxygenase-2 (COX-1 & COX-2) inhibitory activity of inflammatory enzymes of organic leaves crude extract of *C. aurea* and its phytochemical analysis. The dried leaves powder of the *C. aurea* (100 g) was extracted with successive soxhlet extraction by solvents of low polarity (Petroleum ether) to a high polar solvent (Ethanol). These organic leaf extracts *C. aurea* were assessed for the *in vitro* anti-inflammatory activity by 5-Lipoxygenase, Cyclooxygenase-1 & Cyclooxygenase-2 inhibitory activities by ELISA method and further subjected to phytochemical screening. The ethanolic leaves extract of *C. aurea* showed significant 5-LOX inhibitory activity of 51.07% at 100 µg/ml with IC₅₀ of 95.30 µg/ml and the *C. aurea* leaves acetone extract showed the selective COX-2 index (2.35) inhibitory activity with an IC₅₀ of 200.75 µg/ml. The current work revealed that the *C. aurea* leaf acetone extract (CALAC) is shows potential source for the isolation and development of 5-LOX and COX-2 dual inhibitory compounds for management of inflammatory related diseases and inflammatory cancers.

**INTRODUCTION**
Inflammation is an innate immune protective response of vascular tissues and innate immune cells against infection and to restore tissue injury. Dysregulation of acute inflammation leads to chronic inflammatory-related diseases such as diabetes, obesity, arthritis and inducing malignant cell-transformation in different types of cancers (*Kim et al., 2018; Clark et al., 2017*). The Arachidonic acid (AA) is a 20 carbon polyunsaturated fatty acid extensively present in mammalian membrane systems involved in different inflammatory pathways (*Sevanian and Kim, 1985*) and A. A act as
precursor for three major inflammatory pathways by both prostaglandins and leukotrienes synthesis by cytochrome P-450 monoxygenase (Montellano and Paul, 2015), 5- Lipooxygenase (Piomelli et al., 1987) and Cyclooxygenase (Santos et al., 2017) pathways.

During inflammation, the 5-Lipooxygenase (5-LOX) activation leads to the active synthesis of the Leukotrienes (LTs) such as 5-hydroperoxy-eicosatetraenoic acid (5-HPETE) and LTC4, LTD4, and LTE4 are known as “cysteinyl leukotrienes” implicated in the pathogenesis of various inflammatory diseases, asthma, progression of tumors (Epstein et al., 1990). Cyclooxygenases (COX) are prostaglandin-endoperoxide synthase (E.C. 1.14.99.1) act on arachidonic acid (AA) and produce different types of effective pro-inflammatory prostaglandins such as PGE2 and PGF2a (Shaikh et al., 2015). In mammalian cells, COX exists as COX-1 and COX-2 isoforms, these are close in their structure around 60-65% of the sequence are nucleotide sequences are conserved (Hwang et al., 2010). COX-1 is a house keeping enzyme that expressed all cell types of mammalian tissues such as vascular endothelium, platelets stomach, kidney, and uterine epithelium and maintained the normal physiological functions (Samuelsson et al., 2010).

Several clinical and molecular reports on chronic inflammatory diseases and cancers proven that the Arachidonic Acid metabolites are up-regulated and plays a vital role in Auto-inflammation and tumorigenesis. Pharmacological inhibition of the Arachidonic Acid metabolic inflammatory pathway by natural as well as synthetic molecules have been considered as novel approaches to the advancement of dual 5-LOX and COX-2 inhibitors without any side effects for treating various inflammatory disorders and cancer (Weletnsae et al., 2019).

The NSAIDs are anti-inflammatory drugs, but non-specific inhibition of COX-1 and long term administration causes severe side effects such as gastrointestinal (GI) ulceration, obstruction, and has restricted the therapeutic usage of NSAIDs (Harirforoosh et al., 2013). Conventional NSAIDs is being replaced by selective COX-2 inhibitors, such as Celecoxib, suggestive for inflammation and cancer. The selective inhibition of COX-2 enzyme “shunt” the A.

A metabolism pathway towards the 5-LOX pathway, which can induce production excess production of Leukotrienes and cysteinyl-leukotrienes. These Leukotrienes and cysteinyl-leukotrienes are acting as pro-inflammatory increase micro-vascular permeability and induce cell proliferation by inhibiting apoptosis (Ziboh et al., 2004).

Plant derived natural products act as cancer preventive and anti-inflammatory agents by targeting COX-2 and 5-LOX, for reducing both gastric and cardiovascular side effects by balancing AA metabolism in the body (García-Lafuente et al., 2009). Hence, researchers are considering an alternative for synthetic dual 5-LOX and COX-2 inhibitor from medicinal plants.

*Calpurnia aurea* is belongs to the family of Fabaceae and well known medicinal plant in Axum town, Tigray region, Ethiopia. This flowering plant commonly known in Amric Digita and Hitsawts in Tigrigna. This plant species are extensively disseminated in Africa and Southern Asia (Asres et al., 1986). Literature survey brings to light that, the leaf and stem of *C.aurea* have been used for treatment of human and live stock infectious and non-infectious disease (Jäger et al., 1986).

Since long time in Ethiopia the leave of *C. aurea* is used for the treatment of infectious protozoan, bacterial, fungal and viral diseases such as syphilis, malaria, leishmaniasis, elephantiasis, diarrhoea, trachoma, rabies and non-infectious diseases such as diabetes, hypertension, different swellings, stomach- ache, bowel, and bladder disorders (Umer et al., 2013). Therefore, current research focused on investigated inhibitory effects of the organic leaf extracts *C. aurea* on 5-LOX and COX-I & COX-2 inflammatory enzymes and its phytochemical analysis.

**MATERIALS AND METHODS**

**Study area**

The leaves of selected Medicinal plant *C. aurea* were collected from Axum city, in the Central Zone of region Tigray, Ethiopia. It has an elevation of 2,131 meters (6,991 ft) above sea level latitude of 14° 07' 15.92" N longitude 38° 43' 24.13". The average annual temperature in Axum is 18.3 °C and the rainfall averages is 652 mm.

**Plant material collection and authentication**

The Leaves of *C. aurea*, were collected in Axum city, Central Zone of Tigray region. The plant was authenticated by the Department of Biology, National Herbarium, Addis Ababa University, and voucher specimen number of TW 002 was deposited in the National Herbarium for future reference.

**Preparation of extract and Percentage of yield**

The leaves were rinsed carefully with running tap water; and one time with distilled water, then the leaves were shade dried for 15 days. The dried leaves were coarsely powdered with the electrical...
grinder and powdered leaves of *C. aurea* (100 g) was taken for successive extraction by using various polarities solvents such as petroleum ether, chloroform, acetone, and ethanol by using soxhlet apparatus (*Das et al., 2010*).

The organic leaf extracts of *C. aurea* were subsequently concentrated to dryness and stored desiccated at 4 °C until further use for qualitative phytochemical analysis, *In vitro* screening of dual 5-LOX/COX-2 inhibitors, the extracts were placed in weighed flasks before drying. The remaining plant parts residues were extracted with other solvents sequentially. Then the yield value was calculated as:

\[
\text{Percentage of Yield} = \left( \frac{\text{Extract obtained}}{\text{Total amount of plant material}} \right) \times 100
\]

**In vitro 5-Lipoxygenase, Cyclooxygenase-1 & 2 activity 5-Lipoxygenase inhibitory assay (5-LOX)**

*In vitro* 5-LOX inhibitory activity of various concentrations of (20-100 µg/ml) organic leaf extracts *C. aurea* (CALPE, CALCH, CALAC and CALET) were determined according to modified procedure of (*Bisht et al., 2014; Kumar et al., 2016*) by monitored change in absorption at 405 nm by using UV-Vis spectrophotometer.

According to modified method of (*Bisht et al., 2014; Kumar et al., 2016*) COX-I & COX-II initial and inhibitory tubes were prepared by taking 950 µL of reaction buffer, 10 µL of heme and COX-I and COX-2 enzymes in the tubes. In inhibitor tubes 20 µL of the tested plant extracts at various concentrations (20-100 µg/mL) in each tube in addition to the above ingredients. The COX-I & COX-II enzymatic reactions were initiated by adding 100 µL of arachidonic acid in each tube and quenched with 50 µL of 1 M HCl. PGH₂ thus formed was reduced to PGF2α by adding 100 µL of SnCl₂, and reading the plate at 405 nm. IC₅₀ values of tested plant extracts were determined by using graph pad prism Graph pad prism 6.0 software.

\[
\% \text{ of COX-I & COX-II Inhibition} = \left( \frac{\text{Ab Control} - \text{Ab Test}}{\text{Ab Control}} \right) \times 100
\]

**Cyclooxygenases inhibitory assay**

*In vitro* COX-1 and COX-2 inhibitory activity of various concentrations of (20-100 µg/ml) organic leaf extracts *C. aurea* (CALPE, CALCH, CALAC and CALET) were determined according to modified procedure of (*Bisht et al., 2014; Kumar et al., 2016*) by monitored change in absorption at 405 nm by using UV-Vis spectrophotometer.

According to modified method of (*Bisht et al., 2014; Kumar et al., 2016*) COX-1 & COX-2 inhibitors were prepared by taking 950 µL of reaction buffer, 10 µL of heme and COX-I and COX-2 enzymes in the tubes. In inhibitor tubes 20 µL of the tested plant extracts at various concentrations (20-100 µg/mL) in each tube in addition to the above ingredients. The COX-I & COX-II enzymatic reactions were initiated by adding 100 µL of arachidonic acid in each tube and quenched with 50 µL of 1 M HCl. PGH₂ thus formed was reduced to PGF2α by adding 100 µL of SnCl₂, and reading the plate at 405 nm. IC₅₀ values of tested plant extracts were determined by using graph pad prism Graph pad prism 6.0 software.

\[
\% \text{ of COX-I & COX-II Inhibition} = \left( \frac{\text{Ab Control} - \text{Ab Test}}{\text{Ab Control}} \right) \times 100
\]

**Phytochemical analysis**

The phytochemical analysis of tested extracts were carried out by using the standard methods (*Audu et al., 2007*).

**Statistical analysis**

The results were represented as the standard error of the mean ± (SEM). The statistical difference between the tested extracts were analyzed by one-way analysis of variance followed by Turkey ‘multiple comparison test Graph pad prism 6.0 software and followed by Dunnett’s t-test.*p?<0.05, **p?<0.01, ***p?<0.001 represents a significant difference between the extracted test group.

**RESULTS AND DISCUSSION**

**Percentage yield**

One hundred grams of the dried leaves of *C. aurea* were extracted with petroleum ether, chloroform, acetone and ethanol solvent by soxhlet’s extraction. The colour, consistency and obtained percentage yields of these crude extracts were shown in Table 1.

As presented in Table 1 the leaves of *C. aurea* reported the highest percentage of yield in acetone extract (6.1g/100g) and ethanol extract (5.7g/100g) followed by least percentage of yield.
Table 1: Colour, consistency and percentage yield (w/w) of different solvent leaf extracts of *C. aurea*

| Solvent       | Colour     | Consistency | Percentage yield (g/100g) |
|---------------|------------|-------------|---------------------------|
| Petroleum Ether | Green      | Sticky      | 1.4                       |
| Chloroform    | Dark green | Non – Sticky| 1.7                       |
| Acetone       | Dark green | Non – Sticky| 6.1                       |
| Ethanol       | Dark green | Sticky      | 5.7                       |

Table 2: Inhibitory effect of *C. aurea* leaf extracts on 5-LOX activity

| Plant extracts/Standard | Concentration (µg/ml) | % Inhibition | IC$_{50}$ (µg/ml) |
|-------------------------|-----------------------|--------------|-------------------|
| CALPE                   | 20                    | 1.07±0.16    | 223.11            |
|                         | 40                    | 7.12±0.12    |                   |
|                         | 60                    | 10.32±0.20   |                   |
|                         | 80                    | 16.19±0.10   |                   |
|                         | 100                   | 20.46±0.12   |                   |
| CALCH                   | 20                    | 27.58±0.32   | 182.74            |
|                         | 40                    | 29.54±0.21   |                   |
|                         | 60                    | 31.67±0.12   |                   |
|                         | 80                    | 35.23±0.24   |                   |
|                         | 100                   | 38.97±0.12   |                   |
| CALAC                   | 20                    | 32.21±0.33   | 112.22            |
|                         | 40                    | 34.52±0.12   |                   |
|                         | 60                    | 38.97±0.21   |                   |
|                         | 80                    | 44.13±0.09   |                   |
|                         | 100                   | 47.69±0.41   |                   |
| CALET                   | 20                    | 38.26±0.14   | 95.30             |
|                         | 40                    | 42.53±0.21   |                   |
|                         | 60                    | 45.20±0.03   |                   |
|                         | 80                    | 46.80±0.09   |                   |
|                         | 100                   | 51.07±0.54   |                   |
| NDGA                    | 2.5                   | 27.19±0.45   | 5.54              |
|                         | 5                     | 44.8±0.35    |                   |
|                         | 10                    | 66.81±0.86   |                   |

The results obtained from 5-LOX inhibitory activities of *C. aurea* leaf extracts exhibiting dose-dependent inhibitory activities on 5-LOX enzyme, with lowest IC$_{50}$ value

were in petroleum ether (1.4g/100g) and chloroform(1.7g/100g) respectively. The leaves of *C. aurea* showed the highest percentage of yield in non-polar solvent acetone, which indicated that the leaves of *C. aurea* phytococonstituents were non-polar in nature.

**Effect of *C. aurea* leaf petroleum ether (CALPE), *C. aurea* leaf chloroform (CALCH), *C. aurea* leaf acetone (CALAC) and *C. aurea* leaf ethanol (CALET) on 5-Lipoxygenase (5-LOX) activity**

The percentage of 5- Lipoxygenase inhibitory activity of *C. aurea* leaf extracts at concentrations of 20, 40, 60, 80 and 100µg/ml were shown in Table 2. As shown in Figure 1, the 5-LOX inhibitory activities of CALPE, CALCH, CALAC and CALET at 100µg/ml were found to be 20.46%, 38.97%, 47.69% and 51.07% respectively, with significant difference, i.e. (**p<0.001 and (**p<0.01) among all extracts, except between CALAC, CALET i.e. (*p<0.05) and the Nordihydroguaiareticacid (NDGA) was taken as a positive control and % of inhibition at 10µg/ml was found to be 66.81%. The IC$_{50}$ values of CALPE, CALCH, CALAC, CALET and NDGA were shown in
### Table 3: Inhibitory effect of *C. aurea* leaf extracts on COX-1 and COX-2 activity

| Plant extracts | Concentration (µg/ml) | % Inhibition COX-1 | % Inhibition COX-2 | IC_{50} (µg/ml) COX-1 | IC_{50} (µg/ml) COX-2 | COX-2 Selectivity (COX1/COX-2) |
|----------------|-----------------------|-------------------|-------------------|----------------------|----------------------|-----------------------------|
|                |                       | COX-1             | COX-2             |                      |                      |                             |
| **CALPE**      | 20                    | 13.41±0.04        | 14.99±0.05        | 265.83               | 405.55               | 0.66                        |
|                | 40                    | 18.23±0.11        | 17.47±0.02        |                      |                      |                             |
|                | 60                    | 21.49±0.30        | 18.93±0.57        |                      |                      |                             |
|                | 80                    | 23.52±0.12        | 21.10±0.12        |                      |                      |                             |
|                | 100                   | 25.17±0.24        | 22.26±0.01        |                      |                      |                             |
| **CALCH**      | 20                    | 16.23±0.21        | 15.38±0.01        | 191.73               | 273.71               | 0.70                        |
|                | 40                    | 21.49±0.94        | 20.32±0.03        |                      |                      |                             |
|                | 60                    | 26.72±0.05        | 22.26±0.01        |                      |                      |                             |
|                | 80                    | 29.13±0.02        | 24.73±0.02        |                      |                      |                             |
|                | 100                   | 31.47±0.01        | 26.43±0.02        |                      |                      |                             |
| **CALAC**      | 20                    | 7.02±0.01         | 11.13±0.01        | 472.28               | 200.75               | 2.35                        |
|                | 40                    | 8.12±0.03         | 13.91±0.02        |                      |                      |                             |
|                | 60                    | 10.07±0.02        | 19.01±0.01        |                      |                      |                             |
|                | 80                    | 12.77±0.21        | 24.03±0.02        |                      |                      |                             |
|                | 100                   | 14.38±0.33        | 28.05±0.02        |                      |                      |                             |
| **CALET**      | 20                    | 18.45±0.03        | 29.98±0.16        | 168.90               | 176.84               | 0.96                        |
|                | 40                    | 22.27±0.05        | 31.61±0.49        |                      |                      |                             |
|                | 60                    | 28.63±0.12        | 33.15±0.09        |                      |                      |                             |
|                | 80                    | 31.59±0.04        | 37.09±0.01        |                      |                      |                             |
|                | 100                   | 34.83±0.03        | 40.57±0.02        |                      |                      |                             |
| Indomethacin   | 2.5                   | 18.57±0.55        | 27.68±0.52        | 7.43                 | 4.89                 | 1.51                        |
|                | 5                     | 45.12±0.57        | 63.28±0.43        |                      |                      |                             |
|                | 10                    | 60.93±0.45        | 75.12±0.58        |                      |                      |                             |

### Table 4: Qualitative Phytochemical analysis of different organic solvent crude leaves extract of *C. aurea*

| Phytoconstituents | Petroleum ether | Chloroform | Acetone | Ethanol |
|-------------------|-----------------|------------|---------|---------|
| Alkaloids         | +               | +          | +       | ++      |
| Flavonoids        | -               | +          | ++      | +++     |
| Tannins/ Phenols  | -               | +          | ++      | +++     |
| Cardiac Glycosides| -               | -          | +       | ++      |
| Terpenoids        | +               | ++         | +       | ++      |
| Saponins          | -               | -          | +       | ++      |
| Steroids          | -               | -          | +       | ++      |
| Quinones          | +               | -          | -       | +       |
| Coumarins         | -               | +          | +       | ++      |
| Carbohydrates     | -               | -          | -       | +       |
| Volatile oils     | -               | -          | -       | -       |
| Anthraquinones    | -               | -          | -       | -       |
| Amino acid and protein | -     | +          | ++      | ++      |

*"++" indicates presence of Primary and Secondary metabolites*

*"-" indicates absence of Primary and Secondary metabolites*
Table 2, found to be 223.11 µg/ml, 182.74 µg/ml, 112.22 µg/ml, 95.30 µg/ml and 5.54 µg/ml, respectively.

Based on the results, it was observed that CALET showed significant 5-LOX inhibitory activity than other extracts. The 5-LOX inhibitory activity of C. aurea leaf extracts exhibiting the following order, 

CALPE < CALCH < CALAC < CALET

Effect of C. aurea leaf extracts (CALPE, CALCH, CALAC and CALET) on Cyclooxygenase (COX-1 and COX-2) activity

The percentage of COX 1 and COX 2 enzyme inhibitory activity of C. aurea leaf extracts at concentrations of 20, 40, 60, 80 and 100 µg/ml were shown in Table 3. The results obtained from the COX 1 and COX 2 inhibitory activities of C. aurea leaf extracts revealed that CALPE, CALCH, CALAC and CALET inhibited COX-2 mediated prostaglandin biosynthesis with a significant IC\textsubscript{50} values, compared with COX-1 derived prostaglandin synthesis.

As shown in Figure 2, the COX-1 inhibitory activities of CALPE, CALCH, CALAC, and CALET at 100 µg/ml were found to be 25.17%, 31.47%, 14.38%, and 34.83% respectively, with a significant difference i.e. (**p ≤ 0.01) among CALPE with CALCH, CALAC and CALET, except between CALCH with CALET and CALAC with CALET i.e. (*p ≤ 0.05) and the Indomethacin was taken as a positive control, for comparing the COX-1 inhibitory activity of C. aurea leaf extracts and its percentage of inhibition at 10 µg/ml was found to be 60.93%. The IC\textsubscript{50} values of CALPE, CALCH, CALAC, CALET and Indomethacin were found to be 265.83 µg/ml, 191.73 µg/ml, 1274 © International Journal of Research in Pharmaceutical Sciences
472.28μg/ml, 168.90μg/ml and 7.43μg/ml respectively.

As shown in the Figure 3, the COX-2 inhibitory activities of CALPE, CALCH, CALAC, and CALET at 100μg/ml were found to be 22.26%, 26.43%, 28.05%, and 40.57% respectively with significance difference i.e. (**p ≤ 0.001) among CALPE with CALET, CALCH with CALET, except between CALCH with CALAC, CALAC with CALET(*p ≤ 0.05), there was no significance (NS) difference between remaining tested extracts. Indomethacin was used as a positive control, and its percentage of inhibition at 10μg/ml was found to be 75.12%. The IC_{50} values of CALPE, CALCH, CALAC and CALET and Indomethacin were shown in the table-3, found to be 405.55μg/ml, 273.71μg/ml, 200.75μg/ml, 176.84 μg/ml and 4.89μg/ml respectively.

As shown in Table 3 the COX-2 selective Index (SI = IC_{50} COX-1 / IC_{50} COX-2) of *C. aurea* leaf extracts CALPE, CALCH, CALAC, CALET and Indomethacin were 0.66, 0.70, 2.35, 0.96 and 1.51 respectively. The COX-2 selectivity, ratio of CALAC and Indomethacin were greater than (> 1) but CALPE, CALCH CALET shown less than (< 1).

**Selective index of COX-1 versus COX-2 inhibition**

The COX-1/2 inhibitory activities of *C. aurea* leaf crude organic extracts (CALPE, CALCH, CALAC and CALET) and standard (Indomethacin) were evaluated using the enzyme linked immunosorbent assay (ELISA) method against ovine COX-1 and human recombinant COX-2. As shown in the Table 3 and Figure 4 the selectivity index of *C. aurea* leaf crude organic extracts and standard were calculated as the ratio of IC_{50} COX-1/IC_{50} COX-2. The tested *C. aurea* leaf crude organic extracts and standard showed the different potential of COX-1/2 inhibition. *C. aurea* leaf petroleum ether extract, chloroform extract, ethanolic extract, showed COX-1 inhibition and *C. aurea* leaf acetone extract (CALAC) shown twofold COX-2 inhibition, with potent COX-2 inhibitory effect with IC_{50} of 200.75μg/ml. The chief capable 5-LOX and COX-2 dual inhibitors found to be in CALAC, which has a significant point of selectivity towards COX-2, and shown twofold COX-2 selectivity than the standard. From Table 3, it was revealed that (CALPE, CALCH, and CALET) shown COX-1 selective inhibitory activity.

Based on the results, it was revealed that CALAC exhibited significant COX-2 selectivity inhibitory activity than other tested extracts and Indomethacin. The COX-2 selectivity ratio of *C. aurea* leaf extracts exhibiting the following order,

CALPE < CALCH < CALET < CALAC

**The qualitative phytochemical analysis of petroleum ether, chloroform, acetone and ethanol leaves extract of *C. aurea***

The qualitative analysis of phytochemicals present petroleum ether, chloroform, acetone, and ethanol leaves extract of *C. aurea* was presented in Table 4, and the result showed that all the four leaves extract of *C. aurea* contained alkaloids, tannin and phenolic compounds. The petroleum ether leaves extract of *C. aurea* was screening its phytochemical constituents as shown in Table 4, the result indicated that petroleum ether extracts shown the presence of alkaloids, terpenoid, quinones and remaining both primary and secondary metabolites were showing absent. The chloroform leaves extract of *C. aurea* was screening its phytochemical constituents as shown in Table 4, the result indicated that the presence of primary metabolites amino acids, proteins and secondary metabolites such as alkaloids, flavonoids, terpenoid, tannin and phenolic compounds, and coumarins, remaining phytoconstituents were showing absent.

The acetone leaves extract of *C. aurea* was screening its phytochemical constituents as shown in Table 4, the result indicated that the presence of alkaloids, flavonoids, saponins, tannin and phenolic compounds, terpenoids, coumarins, amino acids and proteins, remaining phytoconstituents were showing absent. The ethanolic leaves extract of *C. aurea* was screening its Phytochemical constituents as shown in Table 4. The result indicated, all primary and secondary metabolites such as carbohydrates, amino acids and proteins and alkaloids, flavonoids, saponins, tannin and phenolic compounds, glycosides, terpenoids, quinones, coumarins, the remaining anthraquinones and volatile oils were showing absent.

From Tables 1 and 4 it was concluded that the highest percentage of yield was registered in ethanol extract (4.4g /100g) and maximum number of secondary metabolites were found in ethanolic leaf extracts of *C. aurea*.

Among all tested organic extracts, ethanolic leaf extract of *C. aurea* displayed, potent 5-LOX inhibition i.e. 51.07 % at 100 μg/ml with (IC_{50} = 95.30μg/ml), which was relatively similar to standard NDGA 66.81% with (IC_{50} = 5.54 μg/ml) as shown in the Table 2, the 5-LOX inhibitory activity was credited to the anti-inflammatory potential of the ethanolic leaf extract of *C. aurea*. (Akula and Odhav, 2013) reported that among the eighteen selected plants studied against 5-LOX inhibitory activity, *Bidens pilosa* is a flowering plant belongs to family aster and native to America (IC_{50} = 21.8 μg/ml) showed highest anti-
inflammatory activity while *Emex australis* indigenous medicinal plant of South Africa belongs to the family Polygonaceae (IC\textsubscript{50} 81.4 µg/ml) showed least inhibition of 5-LOX activity and they concluded that the anti-inflammatory activities of *Biden spilosa* could be explained by the potent inhibitory effects of their phenolic compounds on arachidonic acid metabolism through the lipoxygenase pathway.

The 5-LOX inhibitory activity of ethanolic leaf extracts of *C. aurea* (CALET) owing of flavonoids, tannin and phenolic compounds, terpenoids, quinones and coumarins. It is possible correlation observed between the anti-inflammatory activities of the studied plant was extracts is due to the considerably high amounts of phenolic compounds. From in vitro cyclooxygenase inhibitory study confirmed that the *C. aurea* leaf acetone extracts (CALAC) showed twofold selective inhibitory activities against COX-2 as compared to COX-1, than other organic leaf extract of *C. aurea* (CALPE, CALCH, and CALET) and (Burnett et al., 2007) reported that *Baicalensis* extract and isolated baicalin showed about twofold more COX-2 versus COX-1 inhibition when IC\textsubscript{50} values were compared.

The current work indicated that CALAC shown selective index (2.35) and inhibited COX-2 mediated synthesis of PGE\textsubscript{2} derivatives with an IC\textsubscript{50} value of 200.75µg/ml, compared with COX-1 with an IC\textsubscript{50} value of 472.88µg/ml.

At the same time, the COX-2 selective index of CALAC was twofold to the standard Indomethacin (1.51). A low COX-1/COX-2 ratio specified a preferential COX-2 inhibitor; which is pharmacologically valuable. The COX-2 selectivity, ratio of CALAC and Indomethacin were greater than (> 1) but CALPE, CALCH CALET shown less than (< 1).

Present results revealed that the dual inhibitory activity of organic leaf extracts of *C. aurea* on 5-LOX/COX enzymes was found to be the presence of phytochemicals such as flavonoids, tannins/phenols, terpenoids, coumarins, and quinones. Among the all tested plant extracts of *C. aurea*, leaves acetone extract shown significant 5-LOX and COX dual inhibitory activities, due to the presence of a highest number of secondary metabolites, such as flavonoids, tannins/phenols, terpenoids.

Our results also correlated with other anti-inflammatory work of medicinal plants Catechin, a natural flavonoid isolated from *Acacia catechu* L, shown in vitro COX-2 and 5-LOX inhibitory activity (Burnett et al., 2007) and also inhibited both ovine COX-1 and COX-2 at IC\textsubscript{50} of 15mg/ml, by decreased production of PGE\textsubscript{2}.

**CONCLUSIONS**

Based on the results it was concluded that the *C. aurea* leaf ethanolic extract of (CALET) confirmed the promising 5-LOX inhibitory activity which is comparable to standard NDGA. The *C. aurea* leaf acetone extract (CALAC) showed promising 5-LOX and COX-2 selectivity inhibitory activity than other tested extracts and standard NDGA & indomethacin. From the current study the *C. aurea* leaf acetone extract is a potential source for the isolation and development of 5-LOX and COX-2 dual inhibitory compounds for the management of inflammatory-associated diseases.

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**Conflict of Interest**

The authors declare that they have no conflict of interest for this study.

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