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Exploring the Anti-Inflammatory Potential: Phytochemical Screening and *In Vitro* Dual Inhibition of 5-LOX and COX by *Vernonia amygdalina* Leaf Extract"

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Abstract

The clinical management of inflammatory diseases often relies on NSAIDs and selective COX-2 inhibitors, which shift the arachidonic acid pathway towards 5-LOX, leading to the production of cysteinyl-leukotrienes associated with cancer and inflammation. To develop more effective and safer anti-inflammatory medications, dual inhibitors targeting both COX-2 and 5-LOX are being pursued. *Vernonia amygdalina* a medicinal plant in the Asteraceae family, historically used for treating skin infections and inflammatory diseases, was investigated in this study. It focused on screening *V. amygdalina* leaf organic extracts *in vitro* to develop anti-inflammatory agents by assessing their inhibitory effects on 5-Lipoxygenases, Cyclooxygenase-1, and Cyclooxygenase-2 enzymes. The study involved successive Soxhlet extraction of powdered dried leaves using solvents of varying polarity (from Diethyl ether to Ethanol). The resulting extracts were evaluated for *in vitro* anti-inflammatory activity through ELISA assays. Results showed that the ethanolic leaf extract of *V. amygdalina* (VALET) exhibited significant 5-LOX inhibition (71.63% at 100 µg/ml) with an IC_{50} of 52.99 µg/ml and potent COX-2 inhibition (69.84% at 100 µg/ml) with an IC_{50} of 41.35 µg/ml, demonstrating one-fold COX-2 versus COX-1 inhibition. This activity was attributed to the presence of secondary metabolites like flavonoids, tannins, and polyphenolic compounds. In conclusion, *V. amygdalina* ethanolic leaf extracts represent a promising source for dual inhibitory compounds against 5-LOX and COX-2, highlighting the potential of traditional remedies in managing inflammatory diseases and cancers

Keywords: Phytochemical analysis, *Vernonia amygdalina*, 5-LOX inhibitors; Anti-inflammatory agents; Indomethacin.

Introduction

Excessive production of mediators in the Arachidonic acid (AA) cascade, especially from the cyclooxygenase (COX) and lipoxygenase (LOX) pathways is a key factor in many inflammatory diseases. Precise regulation of these pathways is crucial for initiating and sustaining inflammatory responses (Rudrapal *et al.,* 2023).Non-steroidal anti-inflammatory drugs (NSAIDs) are essential pharmacological agents widely used to intervene in various inflammatory conditions. They provide short-term relief for common pain and are used for managing chronic inflammatory diseases like osteoarthritis and rheumatoid arthritis (Bindu *et al.,* 2020).NSAIDs primarily work by inhibiting LOX and COX enzymes, by reducing the synthesis of pro-inflammatory prostaglandins in the AA cascade. Their versatile use highlights their importance in both acute and chronic inflammatory situations, making them a cornerstone in managing inflammatory disorders Gunaydin and Bilge (2018).Nonselective traditional NSAIDs (tNSAIDs), by inhibiting prostaglandins, are associated with diverse adverse effects, including gastrointestinal bleeding, stroke, and renal failure, while the introduction of COX-2 selective inhibitors (Coxibs) to address NSAID-related gastrointestinal issues has led to an elevated cardiovascular risk due to reduced endothelial prostaglandin I_2 (PGI_2) and increased platelet aggregator thromboxane A_2 (TXA_2), resulting in the withdrawal of certain Coxibs like Celecoxib and valdecoxib from the market due to their adverse cardiovascular effects, collectively highlighting an increased risk of cardiovascular problems and stroke associated with both tNSAIDs and Coxibs Brune and Patrignani, (2015).

Hence, the problem highlighted in this research paper is that despite advancements in medicinal research, there is a crucial need for new therapeutic agents to address inflammation and enhance the safety of NSAIDs. Current drugs targeting individual molecular pathways may result in undesired effects, toxicity, and drug resistance, whereas those acting on multiple targets simultaneously are less prone to resistance and better suited for managing complex disease systems (De Gaetano *et al.,* 2003). The severe side effects observed with 'Coxibs' suggest that inhibiting one biosynthetic pathway could shift metabolism to another, leading to potentially fatal consequences. Therefore, the concept of dual inhibition of COX-2/5-LOX enzymes is proposed as a rational approach for developing new anti-inflammatory agents with an improved safety profile. Dual COX-2/5-LOX inhibition concept has also gained significance in a number of disease conditions such as cancers and neurodegenerative disorders like Alzheimer's and Parkinsonism disease (Dos *et al.,*2012). We previously reported that the selected medicinal plants *Calpurnia aurea* leaves and *Cucumis ficifolius* root have been found to act as a source of 5-Lipoxygenase/Cyclooxygenase-2 dual inhibitors, demonstrating *in vitro* COX-2 and 5-LOX inhibitory activity (Tesfay *et al.,* 2019; Tesfay *et al.,* 2020).

Vernonia amygdalina shrub from Asteraceae family and also commonly called 'Bitter Leaf' because of bitter taste of its leaves (Gautam *et al.,* 2016) *V. amygdalina* have many nutritive properties that can cure various diseases. It is compared to other plant parts, leaves are the most used in disease treatment such as hypertension, measles, constipation, induction of uterine mobility, control of post-partum hemorrhage, fever, viral disease, hypercholesterolemia, voluntary skin depigmentation, nausea, loss of appetite-induced ambrosia, schistomiasis, amoebic dysentery and other gastrointestinal tract problems (Erasto *et al.,* 2007)

While *V. amygdalina* has been traditionally used as an anti-inflammatory agent, current research has not explored its potential as a inhibitor of 5-LOX, leaving a significant gap in our understanding of its anti-inflammatory mechanisms. The lack of attempts to elucidate these specific inhibitory properties limits the exploration of *V. amygdalina* as a source for screening

and developing plant-derived COX-2 and 5-LOX inhibitors. Addressing this gap could unveil novel therapeutic avenues and contribute to the development of alternative anti-inflammatory agents with potential benefits over existing treatments. Despite the historical use of *V. amygdalina* as an anti-inflammatory agent, there have been no efforts to explore its potential as a dual inhibitor of COX-2 and 5-LOX. Thus the main objective of this current research is to assess the phytochemicals and evaluation of *in vitro* 5-LOX and COX-1/2 dual inhibitory activities of *V. amygdalina* leaf extracts.

Materials and Methods

The leaves of *V. amygdalina was* collected from Axum, Central Zone of Tigray, Ethiopia in the month of January 2022. The plant material was authenticated in National herbarium center of Ethiopia department of Biology, Addis Ababa University, and a voucher number of NB 001 was assigned to the authenticated specimen.

Preparation of the extracts

The leaves of *V. amygdalina* was dried at 25° C for 10 days in the absence of sunlight and powdered was using a mixer and stored in an air tight container. The powdered medicinal plant material was taken and subject to serial exhaustive solvent extraction, during this solvents of increasing polarity from a low polar (diehyl ether) to a high polar solvent (ethanol) to ensure that wide polarity range of compounds could be extract, during extraction solvents are diffuse in to the plant material and solubilize the phytochemical compounds with similar polarity. For qualitative determination, the extracts were placed in pre-weighed flasks before drying. The remaining plant parts residue was extracted with other solvents sequentially (Das *et.al.*, 2010)

Qualitative phytochemical analysis

To distinguish diverse phyto-constituents, including alkaloids, flavonoids, tannins, terpenoids, glycosides, steroids, triterpenoids, saponins, carbohydrates, amino acids, and proteins in various leaf extracts of *V. amygdalina*, phytochemical screening was conducted using established standard procedures (Nortjie *et al.*,2022)

5-Lipoxygenase inhibitory assay

According to Bisht *et al.,* 2014; Kumar *et al.,* 2016 the 5-lipoxygenase inhibitory activity was used as an indicator of the anti-inflammatory activity. The assay was done using linoleic acid as substrate and 5-lipoxygenase as enzyme. Inhibition studies in the presence of various concentrations of leaf extracts of *V. amygdalina* (20-100µg/ml) were recorded at 234nm using UV-Vis spectrophotometer. In the assay protocol, 160µl of 100mM sodium phosphate buffer (pH 8.0), 10µl of test samples and 20µl of soybean Lipoxygenases solution (167 U/ml) were mixed and incubated at 25°C for 10 min. The reaction was then initiated by the addition of 10µl of the substrate in the form of sodium linoleic acid solution. The enzymatic conversion of sodium linoleic acid to form (9Z, 11E)-(13S)-13-hydroperoxyoctadeca-9, 11-dienoate was measured by monitoring the change of absorbance at 234 nm over a period of six min using UV-vis spectrophotometer. Another reaction mixture (a negative control) was prepared by replacing 10µl samples with 2.47 ml mixture of sodium phosphate buffer (5 ml) and DMSO (25µl) into the quartz. The percentage inhibition was calculated by the following formula:

Inhibition (%) = [Ab Control – AbTest /Ab Control] × 100

A graph was drawn with the Percent Inhibition as a function of the inhibitor concentration to determine the IC_{50} value (concentration at which there was 50% inhibition).

Cyclooxygenase I &2 Inhibitory Assay

According to Bisht *et al.,* 2014; Kumar *et al.,* 2016 the *in vitro* COX-1/2 inhibitory activities of various concentrations of leaf extracts of *V. amygdalina* (20-100µg/ml) were evaluated using

'COX (Ovine/Human) inhibitor screening kit' (Cayman Chemical Company, Ann Arbor, MI) with 96-well plates. All the reagents added were prepared just before use. This screening assay directly measures PGF2α produced by $SnCl_2$reduction of COX-derived PGH_2. COX-2 initial activity tubes were prepared taking 950µL of reaction buffer, 10µL of heme and 10µL of COX-2 enzymes in tubes. Similarly, COX-2 inhibitor tubes were prepared by adding 20µL of the leaf extracts of *V. amygdalina* at various concentrations (20-100µg/mL) in each tube in addition to the above ingredients. The background tubes corresponding to inactivated COX-2 enzymes obtained after keeping the tubes containing enzymes in boiling water for 3 min along with vehicle control. Reactions were initiated by adding 10µL of arachidonic acid in each tube and quenched with 50µL of 1 M HCl. PGH2 thus formed was reduced to PGF2α by adding 100µL of $SnCl_2$, and reading the plate at 405 nm. A graph was drawn with the Percent Inhibition as a function of the inhibitor concentration to determine the IC_{50} value (concentration at which there was 50% inhibition. The percentage inhibition was calculated by the following formula:

Inhibition (%) = [Ab Control – AbTest /Ab Control] × 100

Statistical Analysis

The results were expressed as the Standard error of the mean ± (SEM). The statistical difference between the test extracted were evaluated by one-way analysis of variance followed by Turkey's multiple comparison test using Graph pad prism 6.0 software and followed by Dennett's t-test. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$ represents a significant difference between the extract test group.

Results and Discussion

The percentage yield

One hundred grams of the dried leaves of *V. amygdalina* was extracted with diethyl ether chloroform, ethyl acetate and ethanol solvent by Soxhlet's extraction. The colour, consistency and obtained percentage yields of these crude extracts were calculated shown in table 1.

As shown in the table 1**,** the dried leaves of *V. amygdalina* with different organic solvents yield, and extract residues ranged from 1.7 to 9.1g/100g. As presented in the table 4.1 the leaves of *V. amygdalina* shown the highest percentage of yield were obtained in ethanol (9.1g/100g) and ethyl acetate (7.4g/100g) followed by least percentage of yield were in diethyl ether (1.7g/100g) and chloroform(3.4g/100g) respectively.

Table 1: Colour, Consistency and Percentage yield (w/w) of different solvent leaf extract of *V. amygdalina*

Solvent	Colour	Consistency	Percentage yield (g/100g)
Diethyl Ether	Green	Sticky	leaves of V. amygdalina 1.7
Chloroform	Dark green	Non – Sticky	3.4
Ethyl Acetate	Dark green	Non – Sticky	7.4
Ethanol	Dark green	Sticky	9.1

As shown in the table 1**,** the dried leaves of *V. amygdalina* with different organic solvents yield, and extract residues ranged from 1.7 to 9.1g/100g. As presented in the table 4.1 the leaves of *V. amygdalina* shown the highest percentage of yield were obtained in ethanol (9.1g/100g) and ethyl acetate (7.4g/100g) followed by least percentage of yield were in diethyl ether (1.7g/100g) and chloroform(3.4g/100g) respectively.

This result is ethanol showed that the percentage yield of crude leaves of *V. amygdalina* extracts increased as polarity of the solvent of extraction used increased. The result of the percentage yield of selected plant recommended that ethanol was a better solvent for the extraction leaves of *V. amygdalina*

Qualitative phytochemical analysis

The qualitative analysis of phytochemical present diehyl ether, chloroform, ethyl acetate and ethanol leaves extract of *V. amygdalina* were presented in table 2, and the results showed that all the four leaves extract of *V. amygdalina* contained alkaloids, flavonoids, tannins/ phenols and terpenoids.

The diehyl ether leaves extract of *V. amygdalina* was screening for its phytochemical constituents as shown in the table 2, the results indicated that diehyl ether extracts shown the presence of alkaloids, flavonoids, tannin and phenolic compounds, terpenoids, saponins and steroids, amino acids and remaining cardiac glycosides and carbohydrates were showing the absent. The chloroform leaves extract of *V. amygdalina* was screening for its phytochemical constituents as shown in the above table 2, the results indicated that the presence of both primary metabolites and secondary metabolites, except sponins were showing absent. The ethyl acetate leaves extract of *V. amygdalina* was screening for its phytochemical constituents as shown in the above table 2, the result indicated that the presence all the secondary metabolites. The ethanolic leaves extract of *V. amygdalina* was screening for its phytochemical constituents as shown in the above table 2, the result indicated that the presence all the secondary metabolites, our results were agreement with Alabi and Adeyemi reported the presence of flavonoids (luteolin 7-O-b-glucuronide, luteolin 7-O-b-glucoside) and a number of phytochemicals (alkaloids, anthraquinone, steroid, phenol, phytate, oxalate, cyanogenic glycoside, tannins, and saponins) in ethanolic preparations of *V. amygdalina* and particularly luteolin (3´,4´,5,7 tetrahydroxyflavone) exhibits high antioxidant qualities among the three flavones.

Table 2: Qualitative phytochemical analysis

Phytoconstituents	**Solvents**			
	Diehyl ether	**Chloroform**	**Ethylacetae**	**Ethanol**
Alkaloids	+	+	++	++
Flavonoids	+	+	++	+++
Tannins/ Phenols	+	+	++	+++
Cardiac Glycosides	-	+	+	++
Terpenoids	+	+	+	++
Saponins	+	-	++	++
Steroids	+	+	++	++
Carbohydrates	-	+	+	++
Amino acid & protein	+	+	++	++

"+" Indicates moderate presence of primary and secondary metabolites.
"++" Indicates significant presence of primary and secondary metabolites.
"+++"Indicates promising presence of primary and secondary metabolites
"-" indicates absence of Primary and Secondary metabolites

The effect of *V. amygdalina* leaf organic extracts on 5-Lipoxygenase (5-LOX) activity.
The 5-lipoxygenase (5-LOX) enzyme inhibitory activity of *V. amygdalina* leaf extract was assessed at concentrations ranging from 20 to 100 µg/ml, as detailed in table 3 and figure 1.

Table 3: Inhibitory effect of *V. amygdalina* leaf extracts on 5-LOX activity

Solvent extracts/Standard	**Concentration (µg/ml)**	**% Inhibition**	**IC_{50}(µg/ml)**
VALDE	20	24.87±0.30	141.9
	40	31.17±0.11	
	60	35.03±0.13	
	80	37.13±0.28	
	100	41.51±0.02	
VALCH	20	31.52±0.13	118.76
	40	34.33±0.14	
	60	37.13±0.80	
	80	43.26±0.10	
	100	46.58±0.02	
VALEA	20	15.06±0.01	70.16
	40	35.73±0.01	
	60	44.83±0.03	
	80	51.13±0.09	
	100	70.93±0.02	
VALET	20	27.85±0.08	52.99
	40	48.34±0.16	
	60	54.47±0.16	
	80	66.20±0.15	
	100	71.63±0.17	
Zileuton	20	27.32±0.13	51.93
	40	47.9± 0.17	
	60	57.09±0.15	
	80	67.08±0.28	
	100	72.68±0.05	

The outcomes reveal a dose-dependent relationship in the inhibitory activities of *V. amygdalina* leaf extract against the 5-LOX enzyme. Notably, the extract exhibited higher inhibitory activity at lower IC_{50} values, indicative of a potent dose-dependent inhibition of the 5-LOX enzyme.
The 5-lipoxygenase (5-LOX) inhibitory activities of VALDE, VALCH, VALEA, and VALET at a concentration of 100 µg/ml were determined, resulting in percentages of inhibition at 41.51%, 46.58%, 70.93%, and 71.63%, respectively. Zileuton served as the positive control, exhibiting a 5-LOX inhibitory activity of 72.68% at 100 µg/ml. The calculated IC_{50} values for VALDE, VALCH, VALEA, VALET, and Zileuton were documented in table 3, yielding values of 141.9 µg/ml, 118.76 µg/ml, 70.16 µg/ml, 52.99 µg/ml, and 51.93 µg/ml, respectively. As shown in the figure 1, the statistical analysis revealed significant differences (***p≤0.001 and (**p≤0.01)) in 5-LOX inhibitory activities among all extracts, with the exception of the comparison between VALDE and VALEA, which exhibited a significant difference at (*p≤0.05)
Upon analysis of the outcomes, it was discerned that VALET demonstrated notably higher 5-lipoxygenase (5-LOX) inhibitory activity compared to the other treated extracts. The ranking of 5-LOX inhibitory activity in *V. amygdalina* leaf extracts was observed as follows:
VALDE < VALCH < VALEA < VALET
The family of flowering plants known as *Asteraceae*is used in traditional medicine for a variety of reasons, including the presence of flavonnds and polyphenols, two of the many phytochemical components found in this family. Numerous plants belonging to this family were tested for their ability to inhibit the lipoxygenase enzyme.

Inhibitory effect of *V. amygdalina* leaf extracts on 5-LOX activity

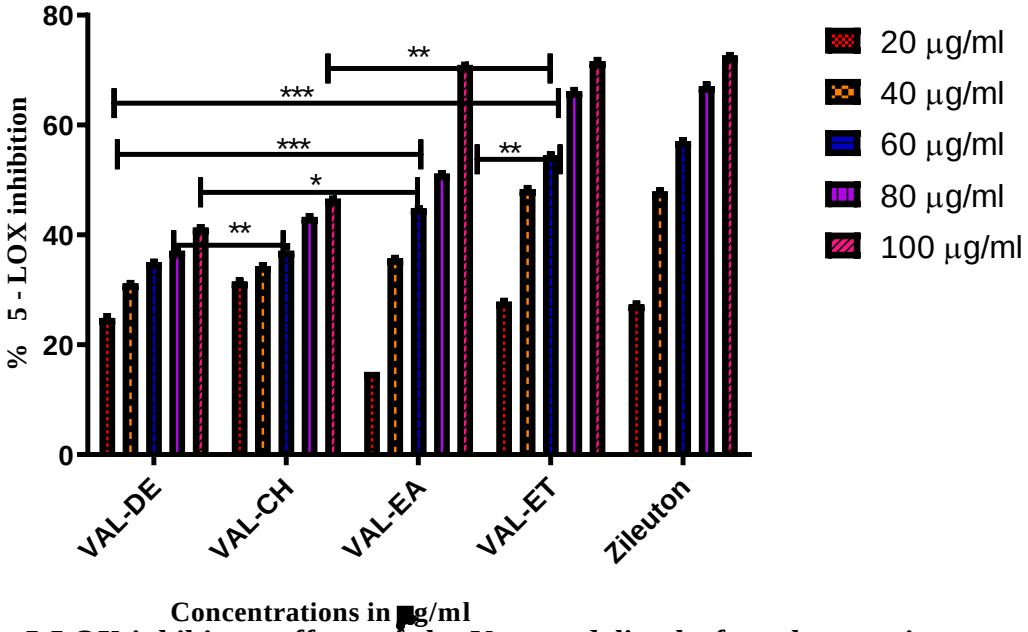

Figure- 1 : 5-LOX inhibitory effects of the *V. amygdalina* leaf crude organic extracts (20-100 µg/ml), values are shown in mean of three replicates ± SEM. The significant variable (*p≤0.05, **p≤0.01, ***p≤0.001) between *V. amygdalina* leaf extracts were analysed by one way ANOVA followed by Tukey's multiple comparison test

Five distinct organic solvents (methanol, dichloromethane, ethyl acetate, n-hexane, and n-butanol) were utilized for the extraction of *Vernonia oligocephala* roots. The lipoxygenase inhibition potential of all extracts was examined. Among the various extracts examined, only the butanol extract exhibited noteworthy LOX inhibition, with an IC_{50} value of 132.20 µg/mL. The roots underwent phytochemical analysis, revealing the presence of saponins, terpenoids, flavonoids, glycosides, phenolic compounds, and steroids. Notably, the methanol extract exhibited the highest content of flavonoids (97.35 mg quercetin equivalent (QE)/g of extract) and phenolic compounds (113.11 mg GAE/g of extract) (Mahmood *et al.,* 2019)

Effect of *V. amygdalina* organic leaf extracts on Cyclooxygenase (COX-1 and COX-2) activity

The COX-1 and COX-2 enzyme inhibitory activities of *V. amygdalina* leaf extracts were assessed at concentrations of 20, 40, 60, 80, and 100 µg/ml, as detailed in table 4. The findings indicated that VALDE, VALCH, VALEA, and VALET demonstrated significant inhibitory effects on COX-2-mediated prostaglandin biosynthesis, as evidenced by their notable IC_{50} values in comparison to COX-1-derived prostaglandin synthesis.

Inhibitory effect of *V. amygdalina* leaf extracts on COX-1 activity

As shown figure 2 the COX-1 inhibitory activities of VALPE, VALCH, VALEA and VALET at 100µg/ml were found to be 25.70 %, 47.81%, 49.16% and 64.02 % respectively, with significant difference i.e. (***p≤0.001 and (**p≤0.01) among all extracts, and the Indomethacin was taken as a positive control for comparing the COX-1 inhibitory activity of *V. amygdalina* leaf extracts and its percentage of inhibition at 100 µg/ml was found to be 48.93 %. The IC_{50} values of VALDE, VALCH, VALEA, VALET and Indomethacin were shown in table 4, found to be 330.5 µg/ml , 199.01 µg/ml, 103.35 µg/ml, 66.94 µg/ml and 84.39 µg/ml respectively. (Jeppesen *et al.,* 2012) reported that the ethanolic extract of *Artemisia persica*, a member of the Asteraceae family, demonstrated the most potent COX-1 inhibitory effect with an IC_{50} of 0.5 µg/mL and this finding highlights the potential anti-inflammatory activity of *A. persica*, supporting its traditional uses in folk medicine. (Krishna Chaithanya *et.al* 2019; 2020) previously reported that the *Cucumisficifolius*root acetone (CFRAC) shown selective inhibitory activity against COX-1 and *Caulpurniaaurea* leaves organic extracts CALPE, CALCH, CALAC and CALET at 100µg/ml were found to be 25.17%, 31.47%, 14.38%, and 34.83% against COX-1 inhibitory activities.

Table 4 : Inhibitory effect of Inhibitory effect of *V. amygdalina* leaf extracts on COX-1 and COX-2 activities.

Solvent extracts /Standard	**Concentration (µg/ml)**	**% Inhibition**		**IC_{50} (µg/ml)**		**COX-2 Selectivity (COX1/COX-2)**
		COX-1	COX -2	COX-1	COX -2	
VALDE	20	17.23±0.	23.16±0.20	330.5	222.35	1.48
	40	20.24±0. 13	27.49±0.11			
	60	21.16±0 17	29.88±0.04			
	80	24.17±0. 12	32.98±0.02	330.5		
	100	25.70±0.	39.83±0.13			
VALCH	20	23.23±0.	26.05±0.33			

	40	28.57±0.	31.98±0.14	199.01	149.00	1.33
	60	31.48±0. 14	37.00±0.55			
	80	33.83±0.	41.19±0.18			
	100	47.81±0.	51.23±0.13			
VALEA	20	14.33±0. 16	28.10±0.54	103.35	78.00	1.32
	40	33.41±0.	39.66±0.19			
	60	39.73±0.	49.05±0.10			
	80	43.37±0.	51.61±0.47			
	100	49.16±0.	53.49±0.82			
VALET	20	29.45±0. 1	32.91±0.04	66.94	41.35	1.61
	40	34.32±0. 1	40.01±0.03			
	60	37.08±0. 3	46.57±0.06			
	80	50.28±0	56.82±0.95			
	100	64.02±0.	69.84±0.57			
Indometh acin	20	17.57±0. 5	43.68±0.52	84.39	40.51	2.08
	40	22.94±0.	50.68±0.06			
	60	39.96±0.	56.31±0.95			
	80	42.12±0.	58.28±0.43			
	100	48.93±0. 4	88.12±0.58			

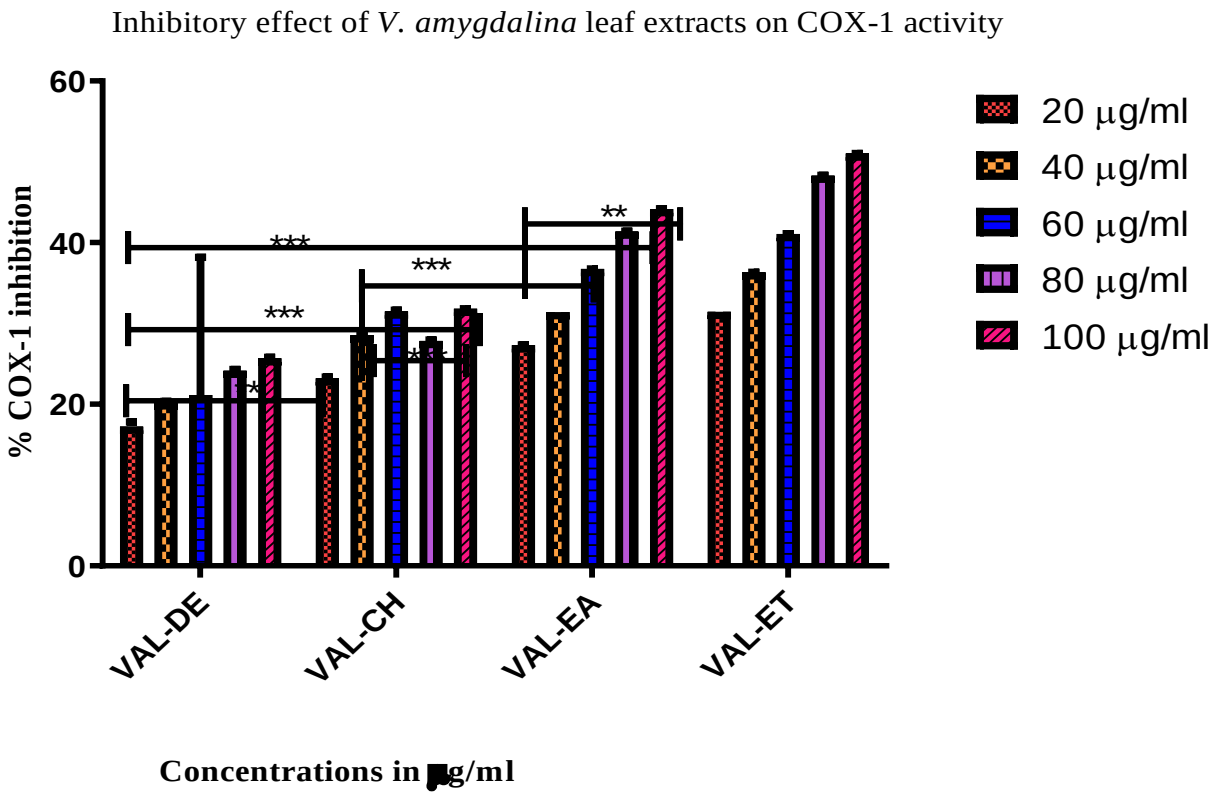

Figure 2: COX-1 inhibitory effects of the *V. amygdalina* leaf crude organic extracts (20-100 µg/ml), values are shown in mean of three replicates ± SEM. The significant variable (*p≤0.05, **p≤0.01, ***p≤0.001) between *V. amygdalina* leaf extracts were analysed by one way ANOVA followed by Tukey's multiple comparison test.

Inhibitory effect of *V. amygdalina* leaf extracts on COX-2 activity

In figure 3, the COX-2 inhibitory activities of VALDE, VALCH, VALEA, and VALET at a concentration of 100 µg/ml were determined to be 39.83%, 51.23%, 53.49%, and 69.84%, respectively, exhibiting significant differences (***p≤0.001 and (**p≤0.01) among all extracts, except between VALDE vs. VALCH, where the difference was (*p≤0.05). Indomethacin served as a positive control, and its COX-2 inhibitory activity at 100 µg/ml was measured to be 88.12%. The IC_{50} values for VALDE, VALCH, VALEA, VALET, and Indomethacin are presented in table 4 were found to be 222 µg/ml, 149 µg/ml, 78 µg/ml, 41.35 µg/ml, and 40.51 µg/ml, respectively. Termer *et al.,* 2021 reported that the leaf extract of *Waltheria indica* and its fractions were found to contain fatty acids, including alpha-linolenic acid, linoleic acid, and oleic acid and exhibited COX-2 inhibition was 41%. Additionally, the plant demonstrated the presence of secondary metabolites in the extract fractions, with identified substances falling within the group of steroidal saponins and triterpenoid saponins. These findings suggest a potential contribution of these secondary metabolites to the COX-2 inhibitory activity.

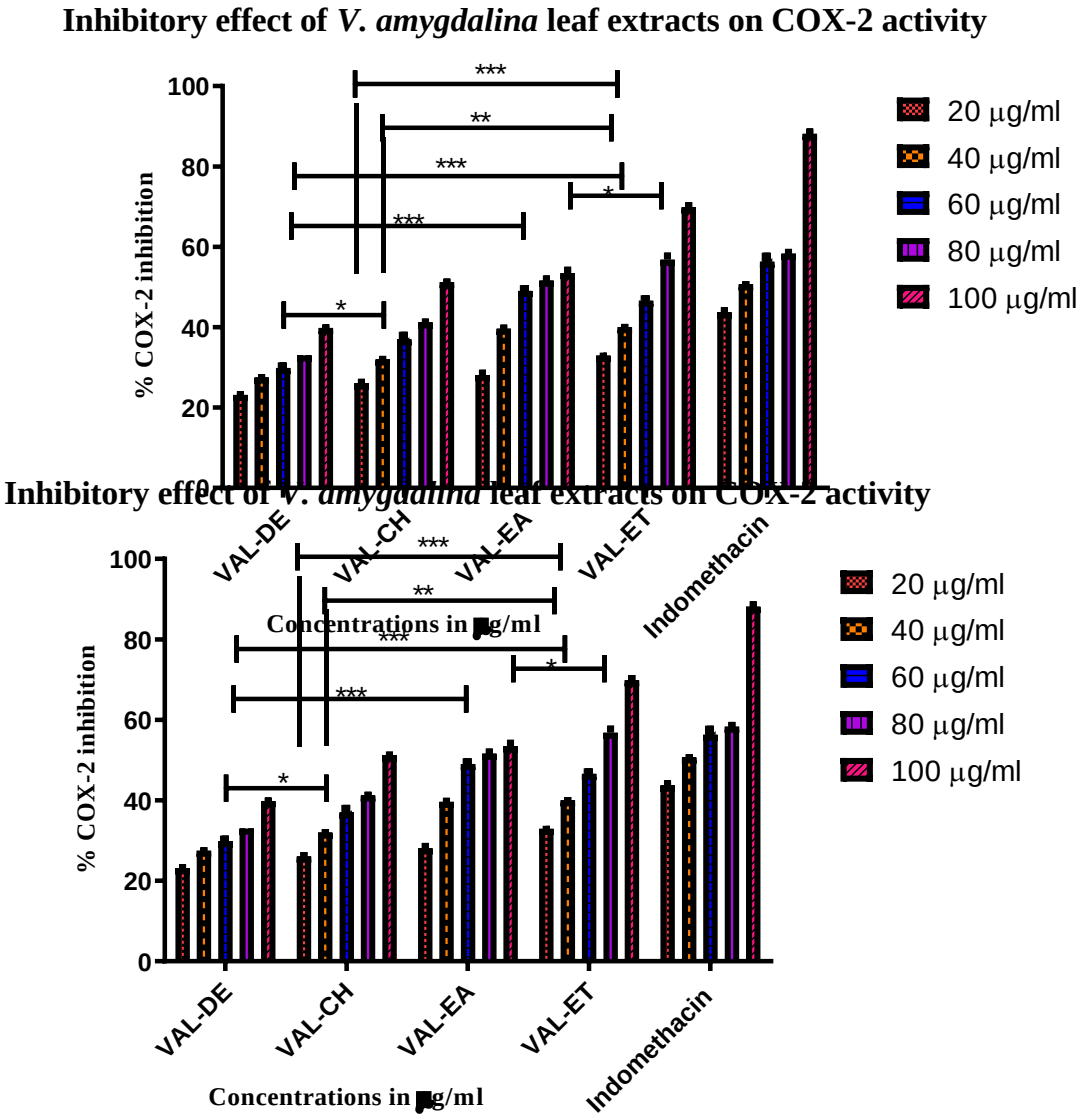

Figure 3: COX-2 inhibitory effects of the *V. amygdalina* leaf crude organic extracts (20-100 µg/ml),values are shown in mean of three replicates ± SEM. The significant variable (*p≤0.05, **p≤0.01, ***p≤0.001) between *V. amygdalina* leaf extracts were analysed by one way ANOVA followed by Tukey's multiple comparison test.

As shown in the table 4, the COX-2 selectivity (SI = IC_{50} COX-1 / IC_{50} COX-2) of *V. amygdalina* leaf extracts VALDE, VALCH, VALEA, VALET and Indomethacin were 1.48, 1.33, 1.32, 1.61 and 2.08 respectively. The COX-2 selectivity, ratio of all extracts and standard Indomethacin were greater than (>) 1

Selective index of COX-1 versus COX-2 inhibition by *V. amygdalina*

The COX-1/COX-2 inhibitory activities of *V. amygdalina* leaf crude organic extracts (VALDE, VALEA, VALCH, and VALET) and the standard (Indomethacin) were assessed through the enzyme-linked immunosorbent assay (ELISA) method against ovine COX-1 and human recombinant COX-2. The resulting half-maximal inhibitory concentrations (IC_{50}) values, as presented in table 4 and figure 4, demonstrated variations in the inhibitory potential. The selectivity index of *V. amygdalina* leaf crude organic extracts and the standard was determined as the ratio of IC50 COX-1/IC50 COX-2. Notably, *V. amygdalina* leaf ethanol extract (VALET)

exhibited an approximate one-fold COX-2 versus COX-1 inhibition, displaying a potent COX-2 inhibitory effect with an IC50 of 41.35 µg/ml. Furthermore, the standard Indomethacin demonstrated a two-fold COX-2 selectivity.

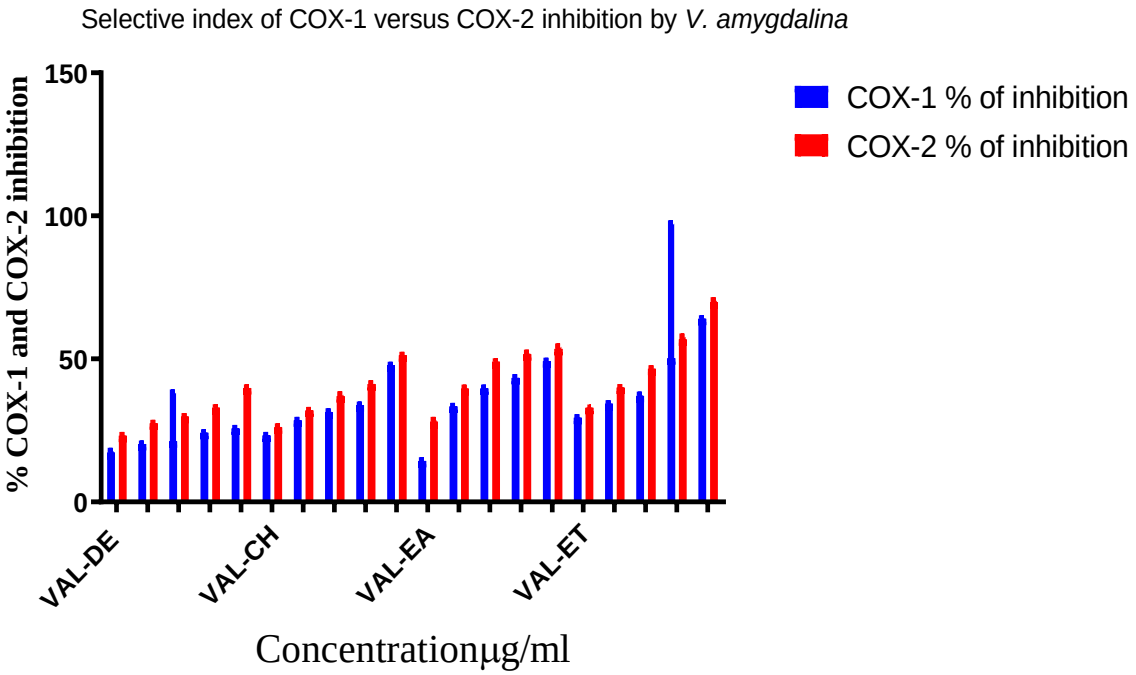

Figure 4 : The percentage inhibition of the COX-1 / COX-2 enzymes and Selective index of COX-1 versus COX-2 inhibition by *V. Amygdalina* leaf, organic extracts (20-100 µg/ml)

Based on the results, it was observed that VALET showed significant COX-2 selectivity inhibitory activity than other tested extracts. The COX-2 selectivity ratio of *V. amygdalina* leaf organic extracts exhibit the following order.

VALEA <VALCH< VALDE< VALET

Conclusion

The leaves of *V. amygdalina* collection served as the foundation for an extensive screening process, with a focus on evaluating inhibitory activity against key arachidonic acid metabolizing enzymes: 5-lipoxygenase (5-LOX), cyclooxygenase-1 (COX-1), and cyclooxygenase-2 (COX-2). Remarkably, all the organic extracts derived from *V. amygdalina* leaves, namely VALDE, VALEA, VALCH, and VALET, exhibited considerable and statistically significant inhibitory activity against 5-LOX and COX-2. The ethanol leaf extract, VALET, particularly stood out, demonstrating a noteworthy one-fold selectivity for COX-2 over COX-1, with a potent COX-2 inhibitory effect boasting an IC_{50} of 41.35 µg/ml. Further analysis indicated that VALET's exceptional anti-inflammatory properties, surpassing those of other solvent extracts, could be attributed to the presence of secondary metabolites such as flavonoids, terpenoids, tannins, and polyphenolic compounds. This study not only underscores the potential of *V. amygdalina* leaves as a source for isolating compounds with dual inhibitory effects on 5-LOX and COX-2 but also emphasizes the rich pharmacological reservoir that traditional remedies hold for discovering novel drugs. Motivated by these findings, subsequent investigations were conducted on the ethanolic extract of *V. amygdalina* leaves, with the aim of isolating and identifying specific bioactive phytochemicals responsible for its impressive 5-LOX and COX-2 dual inhibitory

activity. In conclusion, the comprehensive evaluation of *V. amygdalina* leaves presented in this study opens avenues for further research and development in the field of natural product-based drug discovery. The rich pharmacological potential observed underscores the need for continued investigations to harness the therapeutic benefits of this traditional remedy.

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Conflict of Interest

The authors confirm that this article content has no conflict of interest.

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