Anti-inflammatory and cytotoxic activities of *Bursera copallifera*

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ABSTRACT

Background: The plant species *Bursera copallifera* (DC) bullock is used in traditional medicine to treat inflammation.The leaves of this plant can be prepared as an infusion to treat migraines, bronchitis, and dental pain. **Objective:** The purpose of this study was to determine the anti-inflammatory and cytotoxic activities of organic extracts from the stems, stem bark, and leaves of *B. copallifera*, which was selected based on the knowledge of its traditional use. **Materials and Methods:** We evaluated the ability of extracts to inhibit mouse ear inflammation in response to topical application of 12-O tetradecanoylphorbol-13-acetate. The extracts with anti-inflammatory activity were evaluated for their inhibition of pro-inflammatory enzymes. In addition, the *in vitro* cytotoxic activities of the organic extracts were evaluated using the sulforhodamine B assay. **Results:** The hydroalcoholic extract of the stems (HAS) exhibited an anti-infl ammatory activity of 54.3% (0.5 mg/ear), whereas the anti-infl ammatory activity of the dichloromethane-methanol extract from the leaves (DMeL) was 55.4% at a dose of 0.1 mg/ear. Methanol extract from the leaves (MeL) showed the highest anti-inflammatory activity (IC_{50} = 4.4 μg/mL), hydroalcoholic extract of leaves, and DMeL also reduce the enzyme activity, (IC_{50} = 6.5 μg/mL, IC_{50} = 5.7 μg/mL), respectively, from stems HAS exhibit activity at the evaluated concentrations (IC_{50} = 6.4 μg/mL). The hydroalcoholic extract of the stems exhibited the highest cytotoxic activity against a breast adenocarcinoma cell line (MCF7, IC_{50} = 0.90 μg/mL), whereas DMeL exhibited an IC_{50} value of 19.9 μg/mL. **Conclusion:** In conclusion, extracts from leaves and stems inhibited cyclooxygenase-1, which is the target enzyme for nonsteroidal anti inflammatory drugs, and some of these extracts demonstrated substantial antiproliferative effects against the MCF7 cell line. These results validate the traditional use of B*. copallifera*.

Key words: Anti-inflammatory, *Bursera copallifera,* cyclooxygenase-2, cytotoxic, phospholipase A2, traditional medicine

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INTRODUCTION

Inflammatory cascades can lead to the development of diseases such as chronic asthma, rheumatoid arthritis, multiple sclerosis, inflammatory bowel disease, and psoriasis.[1] The discovery of two different cyclooxygenase (COX) isoforms, COX-1, and COX-2, and evidence that arachidonic acid derivatives other than those formed in the classical COX pathway (for example, leukotrienes and lipoxins) exhibitor modulate inflammatory properties, has contributed to a better understanding of the cell mediators and effects triggered an inflammatory response.[2] Phospholipases A_2 (PLA_2s) represent a family of phospholipid (PL)-hydrolyzing enzymes that participate in various pathological processes, particularly including inflammation. Thus, the control of PL derivatives produced by the action of PLA_2 has long been used to treat these types of diseases.[3] Lipoxins (lipoxygenase interaction products (LXs) are yet another group of lipid mediators formed during arachidonic acid metabolism; they are generated during the cellular interactions that occur as part of the multicellular host response to inflammation.[2] They are synthesized not only through the 5-LO pathway, but also by the action of two other enzymes, 12-LO, and 15-LO. Classical nonsteroidal anti-inflammatory drugs and selective COX-2 inhibitors block the cascade originating

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from arachidonic acid that leads to the production of prostaglandins (PGs).[2] Nevertheless, COX-2-specific inhibitors are used to reduce the development of colon cancer in high-risk patients because adenocarcinoma cells in the colon overexpress COX-2.[4] Thus, there is a continuing need to develop safer and more effective anti-inflammatory inhibitors.

Cancer is the second leading cause of death after heart disease. Frequently diagnosed cancers include lung, colorectal, breast, and prostate cancers; and a total of 16,60,290 new cancer cases and 5,80,350 cancer deaths were reported in 2013 in the USA alone.[5] Cancer remains one of the most devastating diseases in the world, and new and improved cancer therapies have produced only a modest improvement in 5 years survival rates.[6] Numerous challenges must be overcome in cancer treatment, the most frequent of which includes drug resistance, toxicity, and low specificity.[7]

Bursera copallifera (DC) Bullock is popularly known as "Copal ancho" in Mexico.[8] This native tree grows up to 8 m high and has a resinous bark, with small leaves shaped such as topknots, small white flowers, and small red fruits.[9] In prehispanic times, these resins played a central role in daily life and were used in social, economic, and religious ceremonial activities. The "copal" that we currently encounter is a solid resin that is produced from several species of the genus *Bursera*,[10] including *B. copallifera*. The leaves can be prepared like an infusion to treat migraines and bronchopulmonary diseases (e.g., bronchitis and cough), and the resin can be used as a medicinal ointment to treat stroke and dental pain.[11] Insecticidal activity against *Spodoptera frugiperda* larvae has been reported for an acetone extract from the leaves of *B. copallifera*.[11] A previous study on the cytotoxic activity of *B. copallifera* demonstrated the activity of a chloroform extract prepared from the stems against nasopharyngeal carcinoma (KB, IC_{50} = 13.98 ± 0.9 μg/mL) and breast adenocarcinoma (MCF7, IC_{50} = 7.1 ± 1.4 μg/mL) cell lines, whereas a chloroform extract of the fruits was only active against the MCF7 cell line (IC_{50} = 5.9 ± 0.4 μg/mL),[12] where IC_{50} denotes the concentration that inhibited 50% of the control growth after the incubation period. In our study, we performed an extensive screening of the cytotoxic activities of the aerial parts of *B. copallifera* against additional cell lines. However, the anti-inflammatory properties of *B. copallifera* have not been reported.

Natural products have played a fundamental role in biomedical research and drug development in recent decades. Although *B. copallifera* is widely used in Mexico, its anti-inflammatory effects remain unknown, and its cytotoxic effects have not been fully characterized. Therefore, in the present study, we sought to validate the use of this species in traditional medicine. These studies demonstrated that *B. copallifera* exhibited potent anti-inflammatory activity in an acute dermatitis model (12-*O*-tetradecanoylphorbol-13-acetate [TPA]) via the inhibition of COX-1 and also exhibited significant cytotoxic activities against the KB and MCF7 cell lines.

SUBJECTS AND METHODS

Preparation of extracts from *Bursera copallifera*

The stems, stem bark, and leaves of *B. copallifera* were collected in Sierra de Huautla (N 18°31'16.5"), Morelos, Mexico, in August 2011. Voucher specimen No. 31809 was deposited at the Herbarium of the University of Morelos in the Centro de Investigación en Biodiversidad y Conservación Conservación (CIByC) at the Universidad Autónoma del Estado de Morelos. The air-dried and powdered parts of *B. copallifera* (stems, 289.2 g; stem bark, 613.6 g; and leaves, 305.5 g) were subjected to exhaustive extractions (5 g of dry tissue/100 mL) using *n*-hexane (degreased), dichloromethane-methanol (1:1), methanol, and a 70% hydromethanolic solution in a maceration process at room temperature. The crude extracts were obtained following evaporation of the solvents under reduced pressure at 40°C.

Animals

Groups of six male mice (CD1) weighing 25–30 g were maintained on a 12:12 h light-dark cycle with food and water available *ad libitum*. The animals were allowed to adapt to the laboratory for at least 3 h prior to testing and were only used once. The animals were provided by the Facultad de Medicina at the Universidad Autónoma del Estado de Morelos. The experiments were conducted in accordance with the federal regulations for animal experimentation and care (SAGARPA, NOM-062-ZOO-1999, Mexico) and were approved by the Institutional Animal Care and Use Committee. All of the experiments were performed using groups of six animals each. All of the animals in the study were sacrificed by cervical dislocation.

Administration of extracts

The hydroalcoholic, methanolic, and dichloromethane-methanol crude extracts were dissolved in mixtures of H_2O-MeOH (3:7), MeOH and CH_2Cl_2-MeOH (1:1), respectively, immediately prior to the experiments.

Mouse model of acute inflammation

The mouse model of acute inflammation that was used in this study was a slight modification of a previously reported procedure.[13,14] Edema was induced in the right ear of each

mouse by the topical application of 2.5 μg of TPA in 20 μL acetone. The effects of the organic extracts of *B. copallifera* on the ear edema were examined by the topical application of the respective extracts to the ears (20 μL/ear, 10 μL on each surface). The mice were sacrificed 4 h later by cervical dislocation. Ear punch biopsies (8 mm in diameter) were obtained and immediately weighed. The weight increase of the ear punches was directly proportional to the degree of inflammation. Indomethacin (INDO) and TPA were dissolved in acetone. The vehicle was the solvent used in the assay, and the negative control was the vehicle with TPA (2.5 μg/ear).

Inhibitory activity against cyclooxygenase-1

The positive control (INDO, 99%, Sigma-Aldrich Co., St. Louis MO 63103 USA) and the organic extracts were subjected to a full inhibition curve versus COX-1 using a commercially available COX (ovine) colorimetric inhibitor screening assay kit (catalog No. 701050; Cayman Chemical, Ann Arbor, Michigan 48108 USA) according to the manufacturer's instructions. INDO and the organic extracts were serially dissolved in dimethyl sulfoxide (DMSO) to produce a series of logarithmic final concentrations (200, 20, and 2 μg/mL), which were subsequently assayed.

Inhibitory activity against cyclooxygenase-2

The positive control (INDO, 99%, Sigma-Aldrich Co., St. Louis MO 63103 USA) and the organic extracts were subjected to a full inhibition curve versus COX-2 using a commercially available COX (human) colorimetric inhibitor screening assay kit (catalog No. 7010500111; Cayman Chemical, Ann Arbor, Michigan 48108 USA) according to the manufacturer's instructions. INDO and the organic extracts were serially dissolved in DMSO to produce a series of logarithmic final concentrations (200, 20, and 2 μg/mL), which were subsequently assayed.

Inhibitory activity against phospholipases A_2

The most active extract in the TPA-induced edema model was evaluated for its inhibition of PLA_2 activity. $AnsPLA_2$ (secretory PLA_2, type V) inhibitor screening assay kit (catalog No. 10004883; Cayman Chemical) was used according to the manufacturer's instructions. The inhibitors (extracts) were dissolved in DMSO to produce solution concentrations of 200, 20, and 2 μg/mL. Palmitoyl trifluoromethyl ketone was obtained from Sigma-Aldrich and was used as the positive control; this compound was dissolved in ethanol at concentrations of 12.3, 1.2, and 0.12 μg/mL (40, 4 and 0.4 μM, respectively).

Inhibitory activity against 15-lipoxygenase

The positive control (Nordihydroguaiaretic acid [NDGA], catalog No. 760717; Cayman Chemical) and the organic extracts were subjected to a full inhibition curve versus 15-LO using a commercially available lipoxygenase inhibitor screening assay kit (catalog No. 760700; Cayman Chemical, Ann Arbor, Michigan 48108 USA) according to the manufacturer's instructions. NGDA positive control was used at 100 μM final concentration, the organic extracts were serially dissolved in DMSO to produce a series of logarithmic final concentrations (200, 20, and 2 μg/mL), which were subsequently assayed.

Cytotoxic assay

All of the extracts that were obtained from the different parts of *B. copallifera* were evaluated against a panel of five human cancer cell lines: KB, MCF7, colon carcinoma, prostate carcinoma, and cervical carcinoma (Ca Ski).

The method employed was based on the sulforhodamine B assay, as reported by Skehan, *et al.*[15] All of the stock cultures were grown in T-25 flasks (containing 5 mL of RPMI1640 medium that was supplemented with L-glutamine, 25 mM HEPES, 0.25% sodium bicarbonate, 10% fetal bovine serum, penicillin, and streptomycin [at 5000 units/mL of medium]). Freshly trypsinized cell suspensions were dispensed in 96-well microplates at densities ranging from 30,000 to 40,000 cells per well together with the plant extracts that were dissolved in DMSO (at a final concentration of 10%) at concentrations of 20–4 μg/mL. Following a 3 days culturing period at 37°C in a humidified atmosphere with 5% CO_2, the cells that had adhered to the plastic substratum were fixed with cold trichloroacetic acid (30%). The optical density was measured at 590 nm using a microplate reader (SpectraMaxPlus 384, Molecular Devices). The results are expressed as IC_{50} values. Podophyllotoxin and paclitaxel were included as positive controls. According to the National Cancer Institute (NCI) guidelines, the extracts were considered active when the IC_{50} values were <20 μg/mL.[16]

Statistical analysis

The results are expressed as the means ± standard deviation. The data were analyzed using the (OriginLab, Massachusetts USA), version 8.0, and the IC_{50} values were obtained by nonlinear regression. Significance was determined using Student's *t*-test, and values of $P < 0.001$, $P < 0.01$, and $P < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Table 1 summarizes the physical characteristics of the collected plant species, including the weights and yields of the extracts that were obtained from different parts of the *B. copallifera* plant. The following extracts were obtained from various parts of the plant: Methanolic extract from the stems (MeS), hydroalcoholic extract from

the stems (HAS), dichloromethanolic extract from the leaves (DMeL), methanolic extract from the leaves (MeL), hydroalcoholic extract from the leaves, dichloromethanolic extract from the stem bark (DMeSB), methanolic extract from the stem bark (MeSB), and hydroalcoholic extract from the stem bark (HASB). The anti-inflammatory activities of these extracts were evaluated on TPA-induced auricular edema in mice. All of the evaluated extracts exhibited anti-inflammatory effects at doses of 0.1, 0.5, and 1.0 mg/ear [Table 2].

Dichloromethanolic extract from the leaves was the organic extract with the highest yield and activity (55.4%) and pharmacological potency (0.1 mg/ear) in this mouse model of acute inflammation. Thus, all the extracts (except MeSB, which showed no activity on TPA test) were used in subsequent *(in vitro)* tests to determine the mechanism of their anti-inflammatory activity.

Effects of *Bursera copallifera* extracts on 12-*O*-tetradecanoylphorbol-13-acetate-induced cutaneous inflammation

We assessed the anti-inflammatory activities of the organic extracts of *B. copallifera* in a murine model of TPA-induced acute irritant contact dermatitis. Table 2 summarizes the results that were obtained from treatment with the organic extracts of the stems, leaves, and stem bark at doses of 1, 0.5, and 0.1 mg/ear. DMeL was the most effective extract and significantly inhibited 55.4% of the edema at a dose of 0.1 mg/ear compared with the negative control group ($P < 0.001$). However, this extract did not exhibit dose-dependent behavior, that is, activity was observed at doses of 0.5 and 1 mg/ear with inhibition values of 25.4% and 23%, respectively [Figure 1]. The positive control INDO exhibited significant inhibition (77.8%, $P < 0.01$) at a dose of 0.1 mg/ear. MeS moderately inhibited the inflammation, reaching 25.9% at the highest dose (1 mg/ear). MeS exhibited significant inhibition at all doses compared with the negative control group ($P < 0.01$). HAS exhibited the highest inhibition (54.3%) at a dose of 0.5 mg/ear, which was significantly different from the negative control group ($P < 0.01$). However, DMeSB exhibited moderate activity (21.6%) only at a dose of 1 mg/ear ($P < 0.01$) [Table 2], whereas MeSB did not exhibit activity at the evaluated concentrations. HASB exhibited the considerable activity (35% inhibition) at a dose of 0.5 mg/ear, which was significantly different from the negative control group ($P < 0.01$). Finally, INDO was effective against inflammation at the evaluated concentration.

These results directly illustrate the anti-inflammatory effects of *B. copallifera*, providing additional evidence that some of the organic extracts reduced TPA-induced contact

Table 1: Organic extracts of the aerial parts of *Bursera copallifera* and the corresponding yields

Part	Weight (g)	Organic extract	Weight (g)	Yield (%)
Stems	289.2	MeS	13.52	4.6
		HAS	7.5	2.5
Leaves	305.5	DMeL	22.1	7.8
		MeL	12.9	4.2
		HAL	6.1	2.0
Stem bark	664.1	DMeSB	15	2.2
		MeSB	5.5	0.82
		HASB	5.8	0.87

MeS: Methanolic extract of stems; HAS: Hydroalcoholic extract of stems; DMeL: Dichloromethanolic extract of leaves; MeL: Methanolic extract of leaves; HAL: Hydroalcoholic extract of leaves; DMeSB: Dichloromethanolic extract of stem bark; MeSB: Methanolic extract of stem bark; HASB: Hydroalcoholic extract of stem bark

Table 2: Organic extracts from *Bursera copallifera* and their acute anti-inflammatory activities against TPA induced-mouse ear edema

Extract	Inhibition of ear edema (%)		
	1 mg/ear	0.5 mg/ear	0.1 mg/ear
MeS	25.9**	17.1**	17.6**
HAS	36.1*	54.3**	NA
DMeL	23.0*	25.4*	55.4***
MeL	NA	NA	18.6*
HAL	28.7*	30.6*	NA
DMeSB	21.6**	NA	NA
MeSB	NA	NA	NA
HASB	15**	35**	NA
INDO			77.8**

***P<0.001; **P<0.01; and *P<0.05 (n=6) indicate significant differences compared with the negative control group (vehicle + TPA at 2.5 µg/ear). NA: Not active (i.e., no activity was observed at the evaluated concentration); TPA: 12-*O*-tetradecanoylphorbol-13-acetate; MeS: Methanolic extract of stems; HAS: Hydroalcoholic extract of stems; DMeL: Dichloromethanolic extract of leaves; MeL: Methanolic extract of leaves; HAL: Hydroalcoholic extract of leaves; DMeSB: Dichloromethanolic extract of stem bark; MeSB: Methanolic extract of stem bark; HASB: Hydroalcoholic extract of stem bark; INDO: Indomethacin

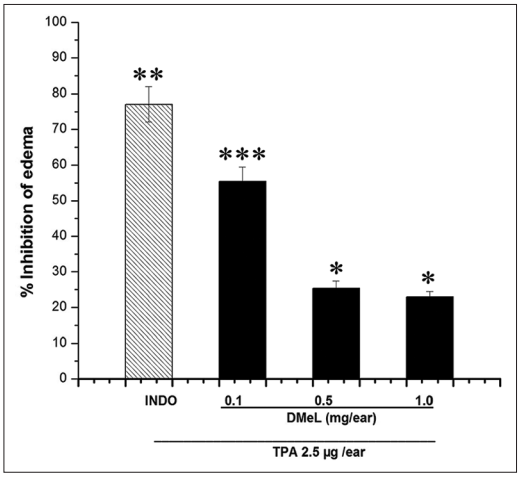

Figure 1: Inhibitory effect of dichloromethanolic extract of leaves of *Bursera copallifera* at 0.1, 0.5, and 1 mg/ear and indomethacin at 0.1 mg/ear; statistically significant difference from the negative control group is expressed at ***$P < 0.001$, **$P > 0.01$, and *$P < 0.05$ ($n = 6$)

dermatitis. Although the TPA-induced mouse ear model of inflammation is nonspecific, it is widely used for acute anti-inflammatory screening because TPA activates PLA_2,[17] and the resulting edema is primarily mediated by PG E_2.[18] Thus, both PLA_2 and COX are involved in this model, and the organic extracts of *B. copallifera* may have interfered with these enzymes to inhibit TPA-induced inflammation.

In vitro inhibition of phospholipases A_2, cyclooxygenase-1, cyclooxygenase-2, and 15-LO enzymes

Organic extracts that exhibited anti-inflammatory activity in the TPA-induced model were evaluated for its *in vitro* inhibitory enzyme activity of PLA_2s, 15-LO, COX-1, and COX-2. The extracts exhibited dose-dependent inhibition of COX-1 and COX-2 [Table 3], MeL showed the most considerable COX-1 inhibition (IC_{50} = 4.4 μg/mL, r = 0.9752), whereas the positive control INDO exhibited (IC_{50} = 4.9 μg/mL, r = 0.9915), while DMeSB did not exhibit activity at the evaluated concentrations. COX-2 inhibitors screening, exhibited lower activity than COX-1, nevertheless HAS exhibited the highest inhibition (IC_{50} = 19.9 μg/mL, r = 0.9906), compared to the positive control INDO (IC_{50} = 33.1 μg/mL, r = 0.9743), is noted that INDO nonselective inhibits the enzymes COX-1 and COX-2, that is why, we used it as positive control. The extracts did not show considerable activity against PLA_2 and 15-LO at the tested concentrations (200, 20 and 2 μg/mL), whereas the positive control palmitoyltrifluoromethylketone[19] produced 25% inhibition of PLA_2 at a concentration of 1.2 μg/mL (4 μM), and NDGA exhibited 62.7% inhibition of 15-LOX at a concentration of 330 μg/mL (100μM).

In vitro cytotoxic activity

The cytotoxic activities of the organic extracts of *B. copallifera* are presented in Table 4. The results indicate that *B. copallifera* exhibited the cytotoxic activity (IC_{50} < 50 μg/mL) against at least two cancer cell lines: KB and MCF7. MCF7cells were most sensitive to HAS (IC_{50} = 0.90 ± 0.06 μg/mL) and DMeL (IC_{50} = 19.9 ± 0.01 μg/mL). The least sensitive cancer cell lines were PC3, Ca Ski, and HF-6. MeL exhibited moderate activity against KB cells (IC_{50} = 22.2 ± 0.06 μg/mL). One of the criteria that the NCI uses to identify a crude extract as promising for further purification is an IC_{50} value <20 μg/mL.[20] HAS and DMeL exhibited selective activity against two cancer cell lines indicating that these extracts are candidates for further isolation of their active constituents.

Anti-inflammatory and cytotoxic natural products

Natural products are promising substances for the identification and development of bioactive compounds to develop drugs for the treatment of inflammatory diseases. These plant-based medicines that have traditionally been used in crude forms such as tinctures, teas, poultices, powders, and other herbal formulations, currently serve as sources to discover novel drugs. There are several reports of traditional medicines providing relief from pain and inflammation.[7] For example, the methanol extract of the leaves of *Salix matsudana* demonstrated substantial inhibitory activity against COX-1 and COX-2.[21] *Trichilia catigua* (catuaba) was found to inhibit completely PLA_2 activity at a concentration of 120 μg/mL.[22] For the *Bursera* genus, the *n*-hexane extract and secondary fractions of *B. simaruba* exhibited anti-inflammatory activity in a mouse paw edema model.[23]

Since the 1940s, approximately 175 small molecules have been identified as being effective agents for cancer therapy. Of these, 131 (74.8%) are not synthetic, whereas 85 (48.6%) are natural products or directly derived from natural products.[24] For the *Bursera* genus, the roots of

Table 3: *In vitro* cytotoxic assay of plant extracts from *Bursera copallifera* against five tumor cell lines using the sulforhodamine B assay

Part	Extract	Cytotoxic activity IC_{50} (μg/mL)				
		HF6	MCF7	PC-3	Ca Ski	KB
Stems	MeS	>50	>50	>50	>50	>50
	HAS	>50	0.90±0.06	>50	>50	>50
Leaves	DMeL	>50	19.9±0.01	>50	>50	>50
	MeL	>50	>50	>50	>50	22.2±0.06
	HAL	>50	>50	>50	>50	$33.4±9.0^{-3}$
Stem bark	DMeSB	>50	>50	>50	>50	>50
	MeSB	>50	>50	>50	>50	>50
	HASB	>50	>50	>50	ND	>50
	Paclitaxel	1.85±0.06	$0.0157±1.0^{-3}$	0.6976±0.05	1.3±0.96	$1.0^{-2}±5^{-3}$
	Podophyllotoxin	$1.6^{-3}±5.0^{-4}$	$1.2^{-3}±7.0^{-4}$	$0.0315±4.0^{-4}$	$3.2^{-2}±9.1^{-4}$	$4.0^{-4}±2.8^{-4}$

Cytotoxic effect on human cancer cell lines: KB: Nasopharyngeal carcinoma; HF6: Colon carcinoma; MCF7: Breast adenocarcinoma; PC-3: Prostate carcinoma; Ca Ski: Cervical carcinoma; MeS: Methanolic extract of stems; HAS: Hydroalcoholic extract of stems; DMeL: Dichloromethanolic extract of leaves; MeL: Methanolic extract of leaves; HAL: Hydroalcoholic extract of leaves; DMeSB: Dichloromethanolic extract of stem bark; MeSB: Methanolic extract of stem bark; HASB: Hydroalcoholic extract of stem bark; ND: Not determined; IC_{50}: Inhibitory concentration 50%; SRB: Sulforhodamine B

Table 4: IC_{50} determination of *Bursera copallifera* extracts as inhibitors of COX-1 and COX-2[a]

Extract	IC_{50} (μg/mL)			
	COX-1	*r*	COX-2	*r*
MeS	12.2	0.9803	38.9	0.9753
HAS	6.4	0.9985	19.9	0.9906
DMeL	6.5	0.9752	47.8	0.9906
MeL	4.4	0.9942	41.2	0.9888
HAL	5.7	0.9968	38.9	0.9784
DMeSB	NA	-	NA	-
MeSB	ND	-	ND	-
HASB	13.8	0.9905	128.8	0.9980
INDO	4.9	0.9915	33.1	0.9743

[a]Values are means of three determinations. MeS: Methanolic extract of stems; HAS: Hydroalcoholic extract of stems; DMeL: Dichloromethanolic extract of leaves; MeL: Methanolic extract of leaves; HAL: Hydroalcoholic extract of leaves; DMeSB: Dichloromethanolic extract of stem bark; MeSB: Methanolic extract of stem bark; HASB: Hydroalcoholic extract of stem bark; ND: Not determined; NA: Not activity; *r*: Linear correlation; IC_{50}: Inhibitory concentration 50%; COX-1: Cyclooxygenase-1; COX-2: Cyclooxygenase-2; INDO: Indomethacin

Bursera tonkinensis exhibited cytotoxic activity against KB cells, with an IC_{50} value of 4.1 μg/mL.[25] In addition, the hydroalcoholic extract of the stem bark of *Bursera fagaroides* var. *Fagaroides* exhibited potent cytotoxic activity against four cancer cell lines. This extract also exhibited antitumor activity in mice inoculated with L5178Y lymphoma cells and in zebra fish.[26] Lautié, *et al*.,[12] have also reported cytotoxic activity for *B. copallifera* (using a chloroformic extract of stems and fruits).

However, the novelty of our contribution lies in the broad panel of evaluated cell lines using several organic extracts (aerial parts). HAS and the DMeL exhibited cytotoxic activity against MCF7 cells, and the anti-inflammatory activity of *B. copallifera* (stems and leaves) is in general mediated by the inhibition of COX-1. The effectiveness of these plant extracts used in folk medicine to suppress inflammatory responses may be due to their inhibitory activity on COX enzymes. Further studies are considerate to determine the mechanism of the cytotoxic or antiproliferative effect of these extracts and other inflammatory parameters.

CONCLUSIONS

The results obtained in this study motivate future studies on extracts of *B. copallifera* to isolate the bioactive secondary metabolites that possess anti-inflammatory and cytotoxic activity (MCF-7 and KB). The associated anti-inflammatory mechanism is mediated by the direct inhibition of COX-1 and moderate of COX-2, primarily associated with inflammatory disease. The active extracts were obtained from the leaves and stems of *B. copallifera* not only to advance our scientific knowledge beyond that of traditional medicine but also to preserve the integrity and life of the tree, which has considerable ecological and cultural significance. Thus, our work may conclude that one of the main anti-inflammatory mechanisms is COX inhibition, underlying the popular use of this plant species is the fast inhibition of proinflammatory eicosanoids.

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