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Evaluation of Cancer Chemopreventive Potentials of *Bixa orellana* L. Leaf Extract

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ABSTRACT

Bixa orellana L. (Bixaceae) is used in ethnomedicine for its anticancer/antitumor effects. This study evaluated the cancer chemopreventive mechanisms of *B. orellana* leaf extract. The methanol extract and fractions of the leaf extract were subjected to phytochemical screening. Inhibition of cancer initiation mechanisms was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity and metal chelation *in vitro* assays. Protection against oxidative damage induced cancer was evaluated using carbon tetrachloride (CCl_4)-induced oxidative stress animal model. Inhibition of inflammation-mediated carcinogenesis was evaluated using Complete Freund's adjuvant (CFA)-induced chronic inflammation. Characterization of the most active fraction was done using high-performance liquid chromatography diode-array detector (HPLC-DAD) technique. Among phytochemicals detected in the extract, phenolic compounds and alkaloids were the most abundant. Ethyl acetate fraction (EAF) showed the best DPPH scavenging activity with 73.16 µg/mL IC_{50} value. This fraction was also the most effective in chelating metal ions with 860 EDTA Eq/g. The extract and EAF at 200 and 400 mg/kg produced significant ($p<0.05$) inhibition of CCl_4-induced lipid peroxidation with significant ($p<0.05$) increase in catalase and superoxide dismutase antioxidant enzymes activity. Significant ($p<0.05$) inhibition of chronic inflammatory edema was also produced by treatment with ethyl acetate fraction from the 7th day. Serum nuclear factor-kappa-b (NF-KB) and interleukin (IL)-6 were significantly ($p<0.05$) suppressed by the extract and EAF. HPLC fingerprint of the purified EAF revealed Isofistularin-1 alkaloid as the most abundant compound. Phenolic compound – methyl gallate was also identified. *B. orellana* exhibited cancer chemopreventive potentials which can be explored in anticancer drug discovery.

Keywords: *Bixa orellana*, Cancer chemoprevention, Isofistularin-1, Inflammation, Oxidative stress.

Introduction

Irrespective of data disparities in the exact incidence and prevalence of cancer, it is globally accepted that morbidity and mortality associated with cancer are on a steady increase.[1] Given the spiraling cost of treatment with their associated side effects, increased insight on the mechanisms of carcinogenesis, and on the conclusion that most cases of cancer are preventable; efforts have been focused on strategies of interrupting and/or reversing the carcinogenic process.
One of the promoted approaches in cancer prevention with enormous potentials is chemoprevention.[2] This is the use of natural, synthetic, or biochemical agents to reverse, suppress or prevent the multiple pathways and processes among the three stages of carcinogenesis: initiation, promotion, and progression.[3]
Compounds or biomolecules that inhibit cancer initiation are referred to as "blocking agents". These agents may interfere with the interaction between chemical carcinogens or endogenous free radicals and DNA, thereby reducing the level of damage and resulting mutations that contribute not only to cancer initiation but also progressive genomic instability and overall neoplastic transformation.[4]

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Protection from cancer can also be achieved through induction of repair pathways, decreased cellular uptake of carcinogens, metabolic activation of pro-carcinogens and/or enhanced detoxification of reactive substances and free radical scavenging.[5] Downregulation of chronic inflammatory responses and their associated production of reactive oxygen and nitrogen species may also contribute to the prevention of cancer initiation.[6]
Remarkable differences in the incidence of cancer around the world have been greatly attributed to dietary patterns.[7] Based on this idea, and numerous epidemiological findings, attention has centered on dietary phytochemicals as an effective intervention in cancer development. Among the numerous natural dietary plants used in protecting against cancer is *Bixa orellana* L. (Bixaceae). Following a purported increased incidence of new cancer cases in Brazil, *Bixa orellana* was among the dietary plants proposed as a viable chemopreventive strategy for Brazilians.[8] Extract and compounds from *B. orellana* have been documented to possess anticancer/antitumor effects.[9] The main effective compound was thought to be Bixin. These activities and compounds have been greatly associated with the seed with little or no scientific studies on the cancer chemopreventive potentials of the leaf extract.[9] A 6-month chronic toxicity study with the leaf extract in humans demonstrated no significant or obvious adverse effect.[10] This study evaluated the possible cancer chemopreventive mechanisms of *Bixa orellana* leaf extract and characterization of major phytoconstituents responsible for this activity.

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Materials and Methods

The leaves of *B. orellana* were collected from Nsukka, Enugu State, Nigeria in December, 2018; and were authenticated by Mr. Alfred Ozioko of Bioresource Development and Conservation Project, Nsukka, Enugu State, Nigeria. A voucher specimen (PCG/474/A/021) has been deposited at the herbarium of the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Enugu State University of Science and Technology, Enugu State, Nigeria. The leaves were air-dried and pulverized with a mechanical grinder (Gx160 Delmar 5.5HP, Honda Motor CO., LTD, Japan).

Animals

Swiss-male Albino rats (200 – 220 g) were used. The animals were obtained from the Animal House of the Department of Pharmacology, Faculty of Pharmaceutical Sciences, Enugu State University of Science and Technology, Enugu State, Nigeria. The animals were maintained in standard laboratory animal conditions of 12 h light, room temperature, 40-60% relative humidity and fed with rodent feed (Guinea Feeds, Ibadan, Oyo State, Nigeria). They were allowed free access to water *ad libitum*. All animal experiments were conducted in compliance with NIH guide for the care and use of laboratory animals (Pub No: 85-23 Revised 1985) as described in the animal experimental protocol reviewed, approved and supervised by the Faculty Animal Care and Use Committee (ESUT/FPS/PHA/2018/012).

Extraction

About 1 kg of the pulverized leaves was cold macerated in 7.5 L of methanol for 48 h with intermittent shaking. The resulting solution was filtered and concentrated *in vacuo* using a rotary evaporator (RE300 Model, United Kingdom) at 40°C to obtain the methanol extract.

Liquid-liquid fractionation

A 10% methanol preparation of 30.2 g extract was subjected to liquid-liquid partition successively with 2.5 L of n-hexane, ethyl acetate, butanol, and then water using separating funnel to give the n-hexane, ethyl acetate, butanol and water soluble fractions, respectively. The n-hexane and ethyl acetate fractions were concentrated using a rotary evaporator at 40°C while other fractions were concentrated *in vacuo* at 60°C.

Phytochemical Analyses

The phytochemical analyses of the extract and fractions obtained were carried out using standard methods.[11]

Purification and HPLC analyses

The most active fraction was further purified through vacuum liquid and Sephadex LH-20 chromatographic techniques. HPLC analysis was performed on the purified most active fraction with a Dionex P580 HPLC system coupled to a photodiode array detector (UVD340S, Dionex Softron GmbH, Germany). Diode array detection was done at 235, 254, 280, and 340 nm. The separation column (125 × 4 mm; length × internal diameter) was prefilled with Eurospher-10 C18 (Knauer, Germany), and a linear gradient of nanopure water (adjusted to pH 2 by addition of formic acid) and methanol was used as eluent.

DPPH antioxidant assay

The DPPH test of the extract and fractions were evaluated using the method described by Ajaghaku *et al*.[12] DPPH solution (0.6 Mmol) was freshly prepared using methanol as solvent and 0.5 mL of this solution was mixed with 0.5 mL serial concentration (7.8125, 15.625, 31.25, 62.5, 125, 250, 500, 1000 µg/mL) of the extract and fractions. Methanol was used to adjust the final volume of the resulting solution to 5 mL and absorbance taken at 520 nm after 30 minutes incubation in the dark at room temperature. Ascorbic acid was used as a standard. The absorbance of the test substances and the negative control (0.5 mL of DPPH solution and 4.5 mL of methanol) were compared and the value was used as a parameter for the evaluation of the antioxidant property. The free radical scavenging activity was obtained using the relationship shown below

DPPH Radical Scavenging Activity = 100 {(Ac – AS)/AC}
Where; AC = Absorbance of Control, AS = Absorbance of Sample

Metal chelation assay

The method previously described by Badria *et al*.[13] was used for this assay. Briefly, 250 µL of 3 Mm FeSO4, 1 mL of 0.2 M 2,2-bipridyl solution, 1 mL of 0.2 M Tris HCl, 400 µL of 10% hydroxylamine, 2.5 mL of methanol and 100 µL of distilled water were added to each test sample (250 µL, 0.5 mg/mL). The absorbance was determined at 522 nm and used to evaluate the metal (Fe^{2+}) chelating activity using ethylenediamine tetraacetate (EDTA) as a standard. The result was expressed as mg EDTA eqv/g of extract.

CCl_4-induced oxidative stress assay

The method reported by Obioha *et al*.[14] was used for this study. Seven groups of 5 animals per group were used. Groups 1and 2 received 200 and 400 mg/kg of *B. orella* extract, 3 and 4 received 200 and 400 mg/kg ethyl acetate fraction. Group 5 received 50 mg/kg silymarin, while group 6 received 5 mL/kg of 5% Tween 80 and group 7 healthy control untreated. Doses of the extract and fractions were chosen based on preliminary study on their acute toxicity which was above 5000 mg/kg. Treatments lasted for 14 days and were administered orally once a day. Two hours after the 14th-day treatment, the animals were administered a single intraperitoneal dose of CCl_4 (2 mL/kg), in a 1:1 ratio with olive oil except group 7 that was administered 2 mL/kg olive oil (i.p). Blood samples were collected from the animals through the retro-orbital plexus 8 h after CCl_4 administration for the determination of serum antioxidant enzymes (catalase and superoxide dismutase) activity and serum lipid peroxidation. Serum catalase and superoxide dismutase enzyme activities were estimated as described by Weydert and Cullen,[15] using their assay kits (Elabscience Biotechnology Co. Ltd., South Africa). Malondialdehyde (MDA) was used as an index of lipid peroxidation and was estimated by a modified thiobarbituric acid method as described by Draper and Hadley,[16] using a malondialdehyde assay kit (Elabscience Biotechnology Co. Ltd., South Africa).

Complete Freund's Adjuvant (CFA)-induced chronic inflammation

Chronic inflammation was induced according to the method previously described by Tekieh *et al*.[17] Briefly, a single subcutaneous injection (0.1 mL) of heat-killed Mycobacterium tuberculosis suspended in sterile mineral oil (10 mg/mL), CFA (Sigma, St. Louis, MO/USA), into the left hind paw was performed for all the animals except animals on a healthy control group that received 0.1 mL of sterile mineral oil.

Animal grouping

Group 1 (healthy control) received no treatment and no injection of CFA but injected with sterile mineral oil (0.1 mL) on the left hind paws, Group 2 (CFA control) received vehicle (5 mL/kg 5% Tween 80), Group 3 (positive control) received Diclofenac sodium (15 mg/kg), Groups 4 and 5 received methanol extract of *B. orellana* (200 and 400 mg/kg), Groups 6 and 7 n-Hexane fraction (200 and 400 mg/kg), Groups 8 and 9 ethyl acetate fraction (200 and 400 mg/kg), groups 10 and 11 butanol fraction (200 and 400 mg/kg) and groups 12 and 13 water fraction (200 and 400 mg/kg). All treatments were administered orally thirty minutes before CFA induction (day 0), then the animals were treated daily for up to 21 days.

Measurement of paw edema

The first day after CFA injection unilateral inflammation was established in injected hind paw. Inflammation due to CFA injection was assessed by measurements of paw volumes pre- and post-injection (on days 0, 7, 14, and 21). Water displacement in a plethysmometer (Ugo Basil, Italy) was used to measure paw volume. Quantification of edema was done by measuring the difference in foot volume between day 0 and successive time points. Post-induction volumes were calculated as the percentage of the day 0 volume.

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Assays for Nuclear Factor Kappa B (NF-kB) and Interleukin (IL)-6

After measurement of the last paw edema, blood samples were collected from the animals through retro-orbital venous plexus into plain tubes which were allowed to clot for 30 mins and centrifuged at 3000 g for 10 minutes at 10°C (using refrigerated centrifuge) to obtain the serum. Serum NF-kB activity was estimated by the quantitative sandwich enzyme immunoassay (ELISA) technique using rat specific NF-kB assay kit (Elabscience Biotechnology Co. Ltd., South Africa) while quantitative detection of serum IL-6 was done using Invitrogen™ rat specific IL-6 ELISA kit. These assays were performed following the manufacturer's procedure.

Statistical analysis

The result was presented as mean + SEM (n = 5). One-way analysis of variance was done using SPSS version 18. Post-hoc analysis was done using Tukey's Test. Differences between mean were considered significant at $p < 0.05$. Graphical plots were done using Microsoft Excel version 2010.

Results and Discussion

Yield and phytochemical contents of the extract and fractions

The methanol extractive yield of Bixa orellana pulverized leaves and its different solvent polarity fractions are presented in Table 1. Out of 500 g pulverized dried leaves, 80.4 g extract was obtained representing 8.04% yield. Further partitioning of 30.2 g of this extract into the different solvent of varying polarities showed the intermediate polar solvent -ethyl acetate gave the highest yield with 40.4% while n-hexane was the least with 6.62% yield. Alkaloids and tannins were abundantly present in the powdered leaves (Table 2). The extract showed a positive test for all the tested phytocompounds (Table 3). Differential distribution of the phytocompounds was observed in the fractions. Alkaloids re dominated in the n-hexane and ethyl acetate fractions. A moderate quantity of flavonoids was recorded in the ethyl acetate and butanol fractions while tannins were partitioned in the butanol and methanol fractions. Multiple scientific studies have indicated the capacity of plant-derived compounds to modify cell transformation and cancer growth.[18,19] These potentials suggest that they could serve as an important tool in cancer chemopreventive interventions.

HPLC fingerprint of the most active fraction

The HPLC fingerprint of the ethyl acetate fraction (most active fraction) is as shown in Figure 1. There are 8 major peaks numbered 1 (Rt – 11.04 min), 2 (Rt – 12.85 min), 3 (Rt – 14.91 min), 4 (Rt- 16.13 min), 5 (Rt – 39.61 min), 6 (Rt – 42.54 min), 7 (45.15 min) and 8 (Rt – 55.17 min). Other spectra characteristics of these peaks were as shown in Table 4. Of the 8 peaks, only two (peaks numbers 2 and 3) hits were found in the library. Peak 3 with spectra hit as isofistularin-1 was shown to be the most abundant phytocompound. The structures of the detected phytocompounds are shown in Figure 2.

Numerous alkaloids from medicinal plants have demonstrated antiproliferative and anticancer effects on wide categories of cancers both *in vitro* and *in vivo*.[20] Vinblastin, Vinorelbine, vincristine and vindesine have already been successfully developed as anticancer drugs.[30] Alkaloids have been reported as an anti-inflammatory agent – an activity that supports their exploration as cancer chemopreventive phytocompound.[21] Brominated alkaloid – isofistularin has been documented to inhibit DNA methyltransferase enzyme activity which is an epigenetic antiproliferative mechanism.[22] Through this mechanism, this compound has been shown to restore mRNA re-expression of a ligand-activated transcription factors known for its role in the detoxification from environmental carcinogens.[22] Although the epigenetic mechanism of action *B. orellana* is still ongoing in our laboratory, the identification of isofistularin as the most abundant phytocompound in the HPLC fingerprint of the most active fraction of *B. orellana* leaf extract suggests that *B. orellana* may possess additional epigenetic properties that may be important in its potentials as a lead candidate in the discovery of plant-based cancer chemopreventive agent.

Antioxidant activity

The extracts and fractions exhibited antioxidant activity as reflected by their inhibition of DPPH radical. The extract gave IC_{50} value of 412 μg/mL while the IC_{50} values of n-hexane, ethyl acetate, butanol and water fraction were 162.6 mg/mL, 73.16 μg/mL, 91.06 μg/mL and 629.3 μg/mL, respectively. Ascorbic acid (reference drug) gave an IC_{50} value of 6.30 μg/mL.

Pretreatment with the extract and ethyl acetate fraction of *B. orellana* protected against CCL_4-induced oxidative damage. Their protective effect was evident by significant ($p < 0.05$) reduction in serum malondialdehyde concentration (an index of lipid peroxidation) at 400 mg/kg of the extract and fraction compared to the CCL_4-induced control (Figure 3). Significant ($p < 0.05$) reduction was also recorded at a lower dose (200 mg/kg) of the ethyl acetate fraction. The antioxidant serum enzyme system was adversely affected by CCL_4 induction. However, pretreatment with the extract and ethyl acetate fraction produced significant ($p < 0.05$) increase in both serum catalase and superoxide dismutase enzyme activity just like silymarin at 50 mg/kg (Figure 4).

Different reports have indicated that diet rich in fruits and vegetables have an important risk reduction of developing cancer, mainly due to their antioxidant contents.[18] Antioxidants have shown considerable promise as effective agents for cancer prevention by reducing oxidative stress which has been implicated in the development of many diseases, including cancer.[23] Antioxidants are capable of donating electrons to stabilize reactive oxygen species and inhibit their detrimental effects. Phytocompounds can also produce antioxidant effects through the induction of antioxidant enzymes like catalase and superoxide dismutase enzymes capable of scavenging endogenously produced reactive species.[24] *Bixa orellana* showed good antioxidant activity through both donations of electron and increased activity of antioxidant enzymes.

Phenolic compounds are best known for their antioxidant activity due to their structural features. Several types of flavonoids have been identified as having anti-proliferative efficacy in various cancer models through inhibition of reactive oxygen species formation and their associated oxidative effect on DNA.[25] Tannins is another class of polyphenolic compounds that have been shown to exert broad cancer chemoprotective activity in several animal models.[26] This activity has been explained by its ability to inhibit carcinogen activation, besides its antioxidant and anti-inflammatory properties.[27] Although the antioxidant activity of alkaloids has been documented, these compounds do not have a reputation as good electron-donating agents but act mainly through induction of endogenous antioxidant enzymes as well as inhibition of metal-induced free radical formation.[28] The presence of these phytocompounds in *B. orellana* particularly in its ethyl acetate fraction may have strengthened their antioxidant activity and may thus inform the potential chemopreventive effect of *B. orellana*.

Metal chelation activity

The chelation activity of the EDTA (standard) was inversely proportional with colour degree resulting from binding reagent and iron. The plot of the EDTA concentrations against their activity produced a linear graph with regression equation $Y = -0.1417x + 0.7291$ ($R^2 = 0.9958$). The chelating activity of extract and fractions were derived from this equation. The extract produced 36.1 ± 0.64 mg EDTA Eq/g while the order of activity of the fractions was ethyl acetate >water >butanol >hexane (Figure 5).

Table 1: Extractive yield of the extract and its fractions

Sample	Weight (g)	Yield (%)¶
Extract	80.4	8.04
N. Hexane F.	2.0	6.62
E. acetate F.	12.2	40.40
Butanol F.	7.6	25.17
Methanol F.	8.4	27.81

¶ Yield of the extract was calculated from 1 kg pulverized dried leaves and the fractions from 30.2 g of the extract. F = fraction.

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Table 2: Qualitative phyto-constituents of the extract and fractions

Phytoconstituents	Extract	N. Hexane F.	E. acetate F.	Butanol F.	Methanol F.
Alkaloids	+	+	+	-	-
Saponins	+	-	-	-	+
Tannins	+	-	+	+	+
Flavonoids	+	-	+	+	+
Steroids	+	+	+	+	_
Terpenoids	+	-	+	+	+
Glycoside	+	+	+	+	+

+ = Present , - = absent.

The ethyl acetate fraction produced the highest chelating activity that was similar to the standard.

A very promising direction in the development of cancer chemopreventive drugs is inhibiting the molecular pathway that keeps cancer cells alive and able to metastasize. Metals particularly copper and iron are two essential metals that play a significant role in this effect.[29] Due to the chemical properties of metals, they are useful in industrial areas which increase their opportunity for exposure to humans. Some of the heavy metals like arsenic, cadmium, chromium and nickel are classified as group 1 carcinogens by the International Agency for Research on Cancer.[30] Although some of these metals perform crucial biological functions they are under tight regulation; the excess amount of which results in cell malfunction and ultimately toxicity.

Various reports have found that exposure to heavy metals leads to disruption in tumor suppressor gene expression, damage repair processes and enzymatic activities concerned with metabolism via oxidative damage.[30] Due to these effects, metal chelators originally designed for other applications are being repurposed as anticancer agents.[31] Among the various mechanisms through which chelating agents act including binding the metal extracellularly, depleting the metal ions from cancer cells or from intracellular sites that regulate cell function; the common thread in all of these mechanisms is decreasing the bioavailability of functional forms of these metals.[29]

Table 3: Quantitative estimation of phyto-constituents in *B. orellana*

Phyto-constituents	Percentage yield (% w/w)¶
Alkaloids	7.8
Tannins	6.4
Flavonoids	4.8
Saponins	3.4

¶ Yield calculated from 2 g of air dried samples

Many polyphenolic compounds like flavonoids and tannins possess more than one metal-binding site and therefore are capable of polymerization. This aggregation effect reduces the ability of the complex to partition into membranes and reduces access of these metals to intracellular compartments. Similarly, alkaloids have been documented for their metal chelating property.[32] The metal chelating activity of *B. orellana* adds to its potential cancer chemopreventive effect.

Anti-inflammatory activity

Complete Freund's Adjuvant administration provoked chronic inflammation as demonstrated by persistent paw edema (Figure 6). The CFA induced chronic edema was significantly ($p < 0.05$) inhibited by 400 mg/kg of the extract from the 14th day when compared with CFA control. Significant ($p < 0.05$) inhibition at a lower dose of the extract (200 mg/kg) was produced on the 21st day. Ethyl acetate fraction at 200 mg/kg produced significant ($p < 0.05$) inhibition of paw edema from the 7th day just like diclofenac sodium at 15 mg/kg. The inhibitory effect of the extract and ethyl acetate fraction on inflammatory edema was accompanied by significant ($p < 0.05$) reduction in serum NF-κB and IL-6 concentrations at all the tested doses except at 200 mg/kg of the extract for NF-κB serum concentration (Figures 7 and 8). Apart from the deleterious free radical oxidative effect and excessive heavy metal mediated cellular malfunctions; inflammation is another important predisposing condition for carcinogenesis. Inflammation fuels malignant transformation of cells via many mechanisms which include but not limited to persistent production of growth factors, production of reactive oxygen and nitrogen species that interact with DNA resulting in genomic alterations.[33] Cancer preventive literature is full of reports of anti-inflammatory agents of plant-derived origin most of which were reported as NF-κB inhibitors.[34] NF-κB is a transcriptional factor that is constitutively activated in many types of cancers. Its activation increases the expression of genes whose products promote cell survival and proliferation. Some of these products include inflammatory mediators like cytokines.[35] Of the inflammatory cytokines, IL-6 emerges as a most promising potential target for cancer prevention due to its involvement in the activation of cancer signaling pathways, induction of the expression of other inflammatory cytokines and suppression of apoptosis.[36] The anti-inflammatory effect exhibited by *B. orellana* particularly its inhibition of NF-κB and IL-6 further strengthens its cancer chemopreventive potentials.

Table 4: Ethyl acetate HPLC spectra specifications

Spectral number	Ret. Time (min)	Spectral hit	Peak height (mAU)	Peak area (mAU*min)
1	11.04	Not available	13.250	15.034
2	12.85	Methyl gallate	19.877	11.247
3	14.91	Isofistularin-1	111.877	82.762
4	16.13	Not available	14.770	7.951
5	39.61	Not available	27.464	21.285
6	42.54	Not available	13.716	9.201
7	45.15	Not available	14.938	6.201
8	55.17	Not available	26.415	64.866

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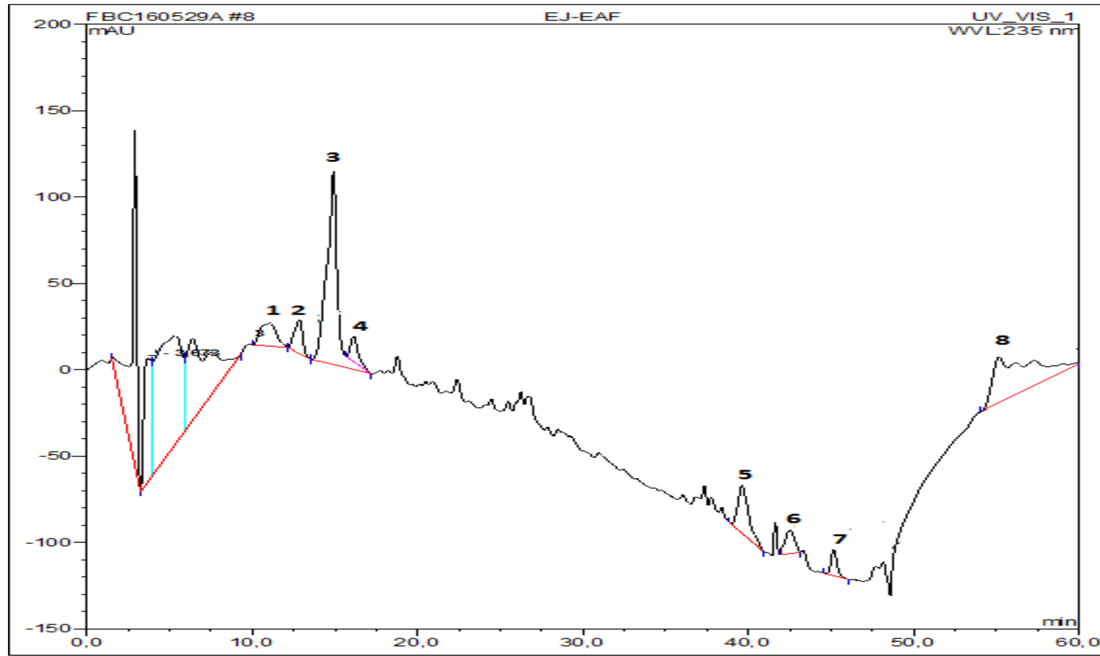

Figure 1: HPLC spectra of ethyl acetate fraction

1(spectra hit not available, Rt = 11.04 min); 2 (methyl gallate, Rt = 12.85 min); 3 (Isofistularin-1 Rt = 14.91 min); 4 (spectra hit not available, Rt = 16.13 min); 5 (spectra hit not available, Rt = 39.61 min); 6 (spectra hit not available, Rt = 42.54 min); 7 (spectra hit not available, Rt = 45.15 min); 8 (spectra hit not available, Rt = 55.17 min).

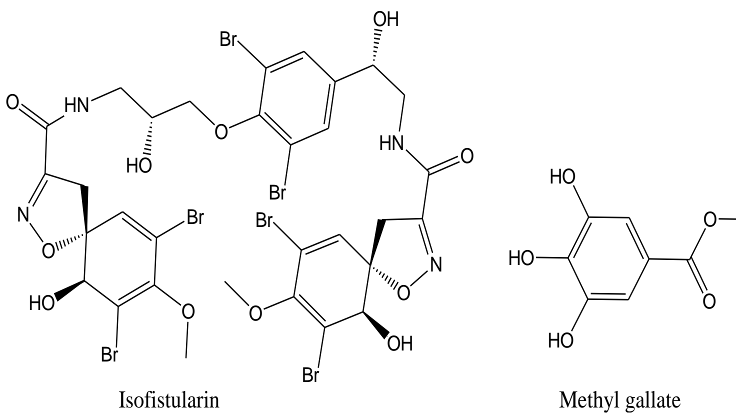

Figure 2: Chemical structures of the phytocompounds detected by HPLC-DAD

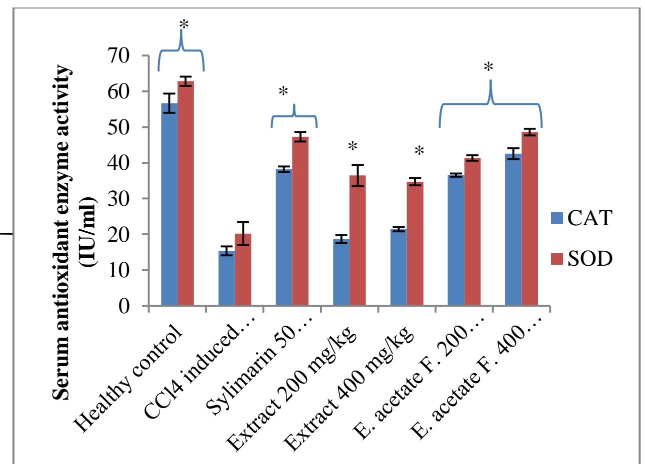

Figure 4: Effect of the extract and ethyl acetate fraction on serum catalase and superoxide dismustase

* $P < 0.05$ compared to CCl4 induced control. F = fraction

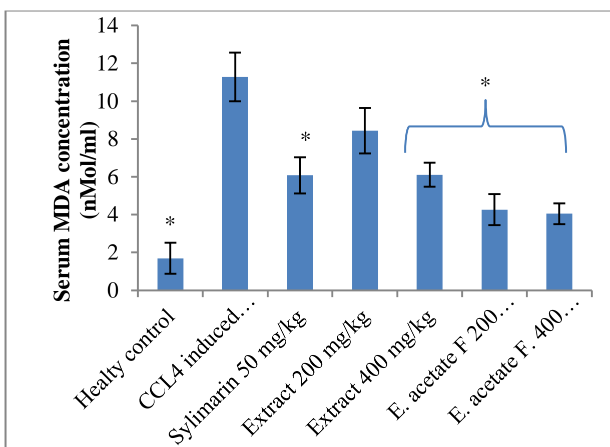

Figure 3: Effect of the extract and ethyl acetate fraction on lipid peroxidation

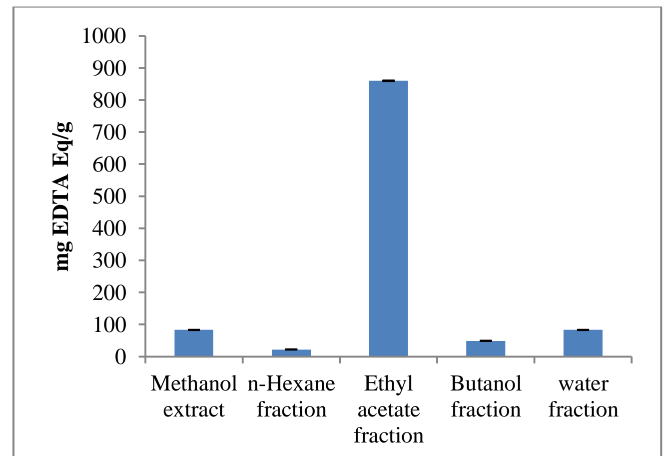

Figure 5: Metal chelation activity of the extract and fractions of *B. orellana* leaf

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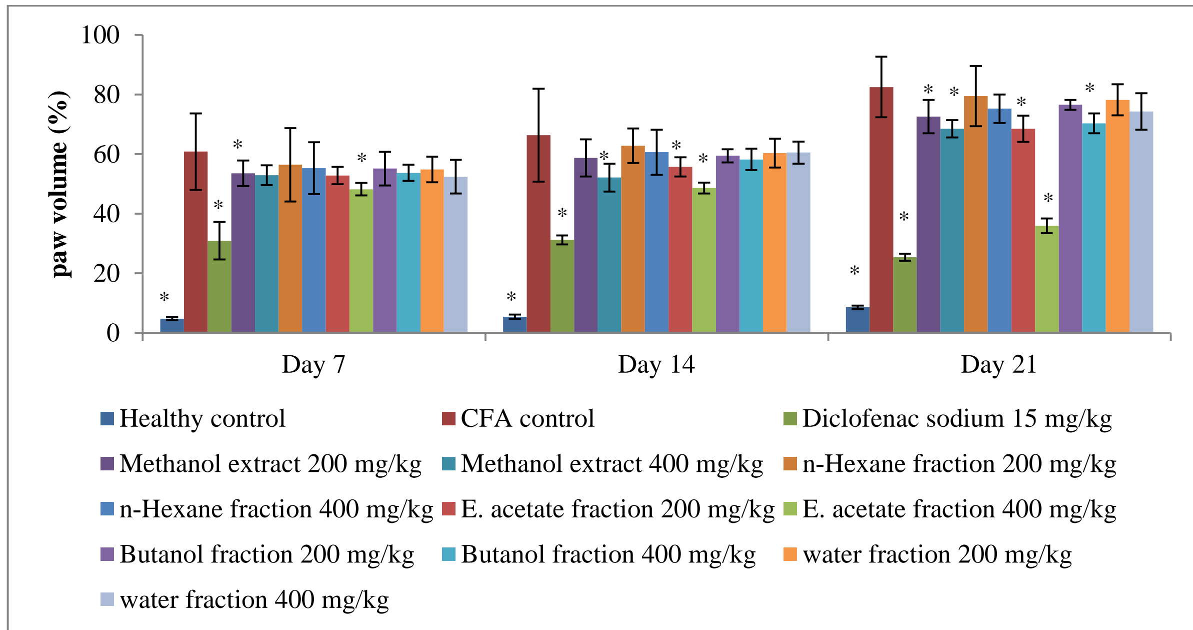

Figure 6: Effect of the extract and fractions on chronic inflammation
* P < 0.05 compared to CFA control

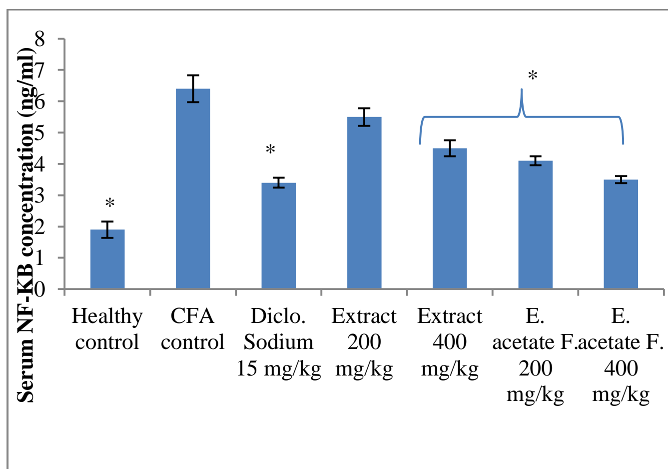

Figure 7: Effect of the extract and ethyl acetate fraction on NF-κB. * P < 0.05 compared to CFA control. F = fraction

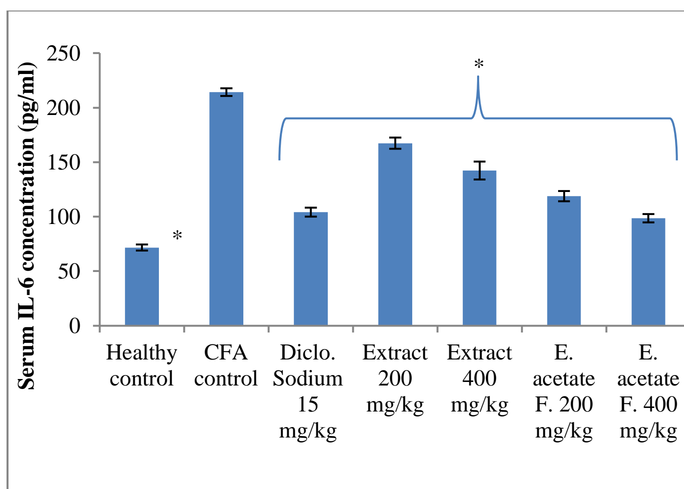

Figure 8: Effect of the extract and ethyl acetate fraction on IL-6.
* P < 0.05 compared to CFA control. F = fraction

Conclusion

B. orellana exhibited cancer chemopreventive potentials which can be explored in anticancer drug discovery.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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