Hindawi Publishing Corporation
Journal of Chemistry
Volume 2013, Article ID 409826, 10 pages
http://dx.doi.org/10.1155/2013/409826

Research Article

Secondary Metabolite Profile, Antioxidant Capacity, and Mosquito Repellent Activity of *Bixa orellana* from Brazilian Amazon Region

Annamaria Giorgi,[1,2] Pietro De Marinis,[1] Giuseppe Granelli,[1] Luca Maria Chiesa,[3] and Sara Panseri[3]

[1] *Department of Agricultural and Environmental Sciences-Production, Landscape, Agroenergy, Via Celoria 2, 20133 Milan, Italy*
[2] *Centre for Applied Studies in the Sustainable Management and Protection of the Mountain Environment, Ge.S.Di.Mont, Università degli Studi di Milano, Via Morino 8, Edolo, 25048 Brescia, Italy*
[3] *Department of Veterinary Science and Public Health, Università degli Studi di Milano, Via Celoria 2, 20133 Milan, Italy*

Correspondence should be addressed to Sara Panseri; sara.panseri@unimi.it

Received 12 November 2012; Revised 12 February 2013; Accepted 20 March 2013

Academic Editor: A. Hamid A. Hadi

Copyright © 2013 Annamaria Giorgi et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The Brazilian flora was widely used as source of food and natural remedies to treat various diseases. *Bixa orellana* L. (Bixaceae), also known as annatto, urucù, or achiote, is a symbol for the Amazonian tribes that traditionally use its seeds as coloured ink to paint their bodies for religious ceremonies. The aim of this study was to investigate the volatile organic compounds (VOCs) profile of *B. orellana* fresh fruits (*in vivo* sampled), dried seeds, wood, bark, and leaves analyzed with Headspace solid-phase microextraction coupled with gas chromatography and mass spectrometry. A screening on phenolic content (the Folin-Ciocalteu assay) and antiradical activity (DPPH assay) of seeds was also conducted. In addition, the repellent properties of seed extracts against *Aedes aegypti* L. were investigated. Volatile compounds detected in *B. orellana* samples consisted mainly of sesquiterpenes, monoterpenes, and arenes: α-humulene is the major volatile compound present in seed extracts followed by D-germacrene, γ-elemene, and caryophyllene. *B. orellana* proved to be a good source of antioxidants. Preliminary data on repellency against *A. aegypti* of three different dried seed extracts (hexane, ethanol, and ethanol/water) indicated a significant skin protection activity. A protection of 90% and 73% for hexane and ethanol/water extracts was recorded.

1. Introduction

The Brazilian Amazon regions represent one of the world's richest pockets of biodiversity. Rich in endemic plants, tropical forests are known to host thousands of edible, medicinal and aromatic plant species [1]. These regions are also populated by numerous Indio tribes renowned for their traditional knowledge in using herbs as a source of food and natural remedies for different illnesses [2]. In 2006, Brazil's Ministry of Health established a policy regulating the use of medicinal plants and phytotherapeutic resources (Política Nacional de Plantas Medicinais e Fitoterápicos), aimed at improving healthcare and sustainable uses of biodiversity [3].

B. orellana L. (Bixaceae), a shrub native to Central and South America, also known as annatto, urucù, or achiote, is a symbol for the Amazonian tribes that traditionally use its seeds as coloured ink to paint their bodies for religious ceremonies [2]. Moreover, it is believed that the original Aztec chocolate beverage contained annatto seeds in addition to cocoa [4]. The term "annatto" in industrialized countries is commonly referred to *B. orellana* seed extract containing carotenoid-type pigments, widely used to dye an assortment of foods, textiles, and body care products [5]. Recently, *B. orellana* extracts have also been shown to be a viable option for commercial exploitation as a replacement for synthetic dyes and pigments in dyeing and finishing of leather [6]. Two carotenoids give annatto its unique red colour: bixin

and norbixin. Bixin (methyl (9-cis)-hydrogen-6,6-diapo-W,W-carotenedioate) is the main responsible for the orange-red colour of the seeds, and extracts of annatto represent approximately 80% of its total carotenoids [5, 7]. Therefore, *B. orellana* is a well-known commercial crop. The main commercial producers are tropical countries including Jamaica, Mexico, and the Philippines, with Peru, Brazil, and Kenya as the main sources of supply [8]. About 80% of the world production, which ranges between 10,000 and 11,000 tons of seeds per year, is utilized by the USA and Western Europe where it is further processed and used to impart the characteristic orange-yellow colour to butter, margarine, and cheese [9].

It is a matter of fact that almost all the organs of *B. orellana* are used in traditional medicine to obtain remedies to treat various diseases [10]. In Brazil, *B. orellana* leaves, roots, and seed extracts are popular as aphrodisiac medicines as well as a remedy to treat fevers, inflammatory conditions, and parasitic diseases. The entire plant is used against dysentery. Extracts of leaves, bark, and roots are reported to be antidotes for poisoning from *Manihot esculenta* (Crantz), *Jatropha curcas* L., and *Hura crepitans* L. [11]. A decoction of the leaves is used to stop vomiting and nausea, as a mild diuretic, to treat heartburn, prostate, urinary difficulties, and stomach problems [10].

Scientific evidences of *B. orellana* bioactivity are nowadays numerous, particularly regarding seed and leaf extracts. Anticonvulsant, antidiabetic, cardioprotective, and antimicrobial activities of different *B. orellana* extracts have been proved [12–14]. Fleischer et al. demonstrated the antimicrobial activity of *B. orellana* leaf and seed extracts against a range of Gram-positive and Gram-negative bacteria and fungi [15]. Moreover, some pharmacological studies have revealed that *B. orellana* extracts possess an antiprotozoal and anthelmintic effect [16]. Due to their broad antioxidant and antibacterial activities, polar extracts from *B. orellana* leaves and seeds have been recently proposed as alternative natural preservatives in food matrices [17]. Phytochemical investigations have revealed the presence of almost twenty-five types of chemical compounds from *B. orellana* different extracts, with the following major components: carbohydrates, carotenoids, steroids, proteins, flavonoids, terpenoids, phenolics, tannins, glycosides, alkaloids detected only in the leaf, and anthroquinones only in the seeds [15, 18].

Despite being widely used and studied, little is known about *B. orellana* volatile organic compounds (VOCs) composition, especially regarding the terpene profile from various plant organs. Moreover, even if *B. orellana* seed extracts claim to repel insects, little data for a clear validation is available. Therefore, the present research provides the first investigation on *B. orellana* chemical composition of different plant organs (fruits, leaves, bark, wood, and seeds) and the volatile profile sampled from living plants growing in an Amazonian Reserve (*in vivo* sampling) during an Italian-Brazilian cooperation project. In addition, a preliminary investigation on the repellent properties of seed extracts against *A. aegypti* L. was conducted.

2. Materials and Methods

2.1. Plant Samples. *B. orellana* plants growing in proximity of the Upper Rio Guamà Reserve located in the municipality of Capitao Poço, Parà State, Brazil (46°59′54.82″ O; 1°52′4.08″ S; 152 m. a.s.l.) were identified, and the volatile compounds emissions of fruits were *in vivo* sampled in February 2011 during a mission of an Italian-Brazilian cooperation project. Dry seeds, roots, leaves, bark, and wood were bought at Belem, at the Ver-o-Peso commercial district, from the most frequented herbalist shop (1°27′8.84″S; 48°30′8.99″O). Identification of the *in vivo* sampled plants and purchased materials was participatory and based on traditional knowledge of Indios Tembè, a tribe living in Itaputyr, a village in the upper Rio Guamà reserve [19, 20].

2.2. Standards and Chemicals. 2,2-diphenyl-1-picrylhydrazyl (DPPH), the Folin-Ciocalteu reagent, sodium carbonate, methanol, and ethanol were purchased from Sigma-Aldrich, Milan, Italy.

Volatile compounds used as references were purchased from Sigma-Aldrich-Fluka, from the General and Flavours and Fragrances catalogue, Milan, Italy.

2.3. Superfine Grinding (SFG). In order to obtain a representative plant sample a superfine powder was prepared from *B. Orellana*'s different organs (seeds, wood, bark, and leaves) using mechanical grinding activation in an energy intensive vibrational mill. Three g of dry plant material samples were ground in a high intensity planetary mill Retsch (model MM 400, Retsch, GmbH, Retsch-Allee, Haan), as previously reported [21]. The mill was vibrating at a frequency of 25 Hz for 4 min using two 50 mL jars with 20 mm stainless steel balls. Precooling of jars was carried out with liquid nitrogen in order to prevent the temperature from increasing during the grinding process. The speed differences between balls and jar resulted in the interaction of frictional and impact forces, releasing high dynamic energies. The interplay of all these forces resulted in the very effective energy input of planetary ball mills.

2.4. Seed Extracts Preparation. Three different *B. orellana* seed extracts were prepared to evaluate antioxidant capacity and phenolic content.

2.5. Traditional Infusion. Aliquots of 1 g from *B. orellana* seed powder were infused for 15 min with 100 mL of freshly boiled distilled water and cooled to room temperature. The infusion was then filtered using Whatman No. 4 filter paper in order to remove plant residues, and the aliquots were stored at +4°C overnight until the analysis.

2.6. Exhaustive Extraction (H_2O and EtOH). Aliquots of 0.3 g of B. *orellana* seed powder were subjected, respectively, to sequential extraction using 8 mL of distilled water, sonicated for 10 minutes, macerated for 1 h, and then sonicated with an ultrasonic cleaning bath (Branson 2510, 20 KHz, 120 W) for a further 30 min at room temperature. The procedure

was repeated eight times to obtain a final volume of 64 mL of extract. This similar procedure was repeated for new samples using 8 mL of EtOH solution. Extracts were then filtered using Whatman No. 4 filter paper, and the aliquots were stored at +4°C until further analysis.

2.7. 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Assay. Antioxidant capacity of the extracts was measured by the stable radical DPPH as previously reported [21]. Each extract was diluted with methanol to five different concentrations between 0.09 and 0.74 mg mL^{-1}. The DPPH concentration in methanol was 0.1 g L^{-1}. One mL of extract solution (sample) or methanol (control) was mixed with 1 mL of DPPH, and the absorption was recorded after 20 min in the dark, at 517 nm (UV/VIS spectrophotometer, model 7800, Jasco, Milan, Italy). Antioxidant capacity (AC) was calculated according to the following equation:

$$\text{AC } [\%] = 100 \times \frac{A_c - A_s}{A_c}, \quad (1)$$

where A_s is the absorbance of the sample and A_c is the absorbance of the control. The concentration of extracts causing a 50% decrease of DPPH solution absorbance (IC_{50}) was calculated by linear regression of the concentration-response plots, where the abscissa represented the concentration (mg mL^{-1}) of the extracts and the ordinate of the average percentage of AC from three separate determinations. The antioxidant capacity of all *B. orellana* extracts was expressed as $1/IC_{50}$ (mean value).

2.8. Determination of Total Phenol Content. The amount of total phenols in *B. orellana* extracts was determined using the Folin-Ciocalteu reagent according to the modified method of Slinkard and Singleton, 1977, using Gallic acid as a standard, as previously reported [21]. Distilled water (1550 μL) was combined and vortexed with 50 μL of sample (4.6 mg dry w/mL) and 100 μL of Folin-Ciocalteu's reagent. Then, 300 μL of Na_2CO_3 (20%) was added to reach a final concentration of 0.05 mg mL^{-1}; the mixture was vortexed. The absorbance of all samples was measured at 765 nm using a UV/VIS spectrophotometer (model 7800, Jasco, Milan, Italy) after incubation at 40°C for 30 min. Quantification was done on the basis of the standard curve of Gallic acid (solution of Gallic acid 10% EtOH, 0.25–10 μg/mL). The calibration equation for Gallic acid was $y = 2.5509x + 0.0096$, $r^2 = 0.9986$. The results were expressed as milligrams of gallic acid equivalents (GAE) per gram of dry weight. Measurements were taken in triplicate and expressed as mean value.

2.9. Seed Extracts for Mosquito Repellency Activity. To evaluate mosquito repellency activity of *B. orellana,* different extracts from powder seeds were prepared using a 5-step extraction/drying procedure (30 mL solvent for each step) using three different solvents: ethanol, ethanol/water (50 : 50 v/v), and hexane, as reported in Table 1. Dry residues were stored at 4°C until resuspension in 5 mL of solvent for skin application (Table 2).

Table 1: Different extraction conditions of *B. orellana* seed powder for mosquito repellency evaluation.

Extraction solvent	Sample weight (g)	Dried extract (g)
Hexane	2.026	0.091
EtOH	2.034	0.178
EtOH/H_2O (50 : 50)	2.002	0.569

Table 2: *B. orellana* seed extracts for mosquito repellency evaluation.

Extraction solvent	Resuspension solvent (5 mL)	Final solution (mg/mL)
Hexane	Acetone	18.2
EtOH	EtOH	35.6
EtOH/H_2O (50 : 50 v/v)	EtOH/H_2O (50 : 50 v/v)	113.8

2.10. Repellent Activity Test. The method of percentage of protection in relation to the dose was used [22]. Hundred blood-starved *Aedes aegypti* females (4–8 days old), reared at 25°C, 80% RH, and an L:D cycle of 12:12 hours, were kept in a net cage (45 × 30 × 45 cm). Before each test, the untreated forearm (control) of some experimenters was exposed to mosquitoes in the test chamber. Once the experimenters observed five mosquito landings on the untreated arm, they removed their arm from the chamber. The arms of the test person were cleaned with ethanol, washed with unscented neutral soap, thoroughly rinsed, and allowed to dry 10 min before extracts application. After air drying the arms of the tested person, only 25 cm^2 ventral side of the skin on each arm was treated and exposed, while the remaining area was covered by rubber gloves and apposite covering bracelets. The different extracts of *B. orellana* seeds (at three different concentrations each) were applied until complete covertures of the skin in three different trials. The control and treated arms were introduced serially into the cage. The number of landings was counted over 3 min at 11:00 a.m, at 2:00 p.m and at 6:00 p.m to evaluate differences in mosquito appetite. The experiment was conducted by 3 human volunteer testers and replicated three times at each concentration. All experiments were carried out at a temperature of 27 ± 1°C and RH 75 ± 5% under laboratory conditions. No skin irritation from the tested seed extracts was observed.

The percentage of protection was calculated by using the formula:

$$\text{protection } \% = \frac{N^\circ\text{of landings control} - N^\circ\text{of landings treated}}{N^\circ\text{of landings control}} * 100. \quad (2)$$

2.11. Headspace Solid-Phase Microextraction (HS-SPME) Volatile Compounds Sampling from B. orellana Living Plants (In Vivo). B. orellana opened fruits were sampled *in vivo* in order to evaluate the VOCs fingerprint emitted by living plants. Each fruit was enclosed in a customised Teflon cage

(manufactured by SNK, Inc., Fullerton, CA: 11 × 21 cm) into which a manual SPME holder was inserted to extract the headspace. VOCs were collected using 50/30 μm Divinylbenzene/Carboxen/polydimethylsiloxane (CAR/PDMS/DVB) StableFlex fibre (Supelco; Bellefonte, PA). The fibre was exposed to the plant headspace for 8 h. Long sampling time were selected in order to obtain a comprehensive VOCs profile emitted by plants. Peaks originating from the cage (Teflon material) were also assessed by extracting a blank sample.

2.12. HS-SPME Volatile Compounds Sampling of Different Dried B. orellana Organs (Seeds, Wood, Bark, and Leaves). The HS-SPME extraction conditions were optimised in our previous study on the characterisation of *Achillea collina* VOCs (selection of SPME fibre, sample amount, and extraction time) [23]. Briefly, all samples were prepared by weighing exactly 1.00 g of *B. orellana* powdered samples of seeds, wood, bark, and leaves, respectively, in a 20 mL glass vial, fitted with cap equipped with silicon/PTFE septa (Supelco, Bellefonte, PA, USA), and by adding 1 mL of the internal standard solution (IS) in water (1,4-cineol, 1 μg/mL, CAS 470-67-7) to check the quality of the fibres.

At the end of the sample equilibration period (1 h), a conditioned (1.5 h at 280°C) 50/30 μm Divinylbenzene/Carboxen/polydimethylsiloxane(CAR/PDMS/DVB) StableFlex fibre (Supelco; Bellefonte, PA) was exposed to the headspace of the sample for extraction (3 h) by CombiPAL system injector auto-sampler (CTC analytics, Switzerland). Temperature of 30°C was selected as extraction temperature in order to prevent possible matrix alterations (oxidation of some compounds, particularly aldehydes). To keep a constant temperature during the analysis, the vials were maintained on a heater plate (CTC Analytics, Zwingen, Switzerland).

2.13. Gas Chromatography-Mass Spectrometry Analysis of VOCs. HS-SPME analysis was performed using a Trace GC Ultra (Thermo-Fisher Scientific; Waltham, MA, USA) Gas Chromatograph coupled to a quadruple Mass Spectrometer Trace DSQ (Thermo-Fisher Scientific; Waltham, MA, USA) and equipped with an Rtx-Wax column (30 m; 0.25 mm i.d.; 0.25 μm film thickness, Restek, USA). The oven temperature programme was from 35°C, hold 8 min, to 60°C at 4°C/min, then from 60°C to 160°C at 6°C/min, and finally from 160°C to 200°C at 20°C/min. Carry-over and peaks originating from the fibre were regularly assessed by running blank samples. After each analysis fibres were immediately thermally desorbed in the GC injector for 5 min at 250°C to prevent contamination. The injections were performed in splitless mode (5 min). The carrier gas, helium, at a constant flow of 1 mL/min. An *n*-Alkanes mixture (C_8–C_{22}, Sigma R 8769, Saint Louis, MO, USA) was run under the same chromatographic conditions as the samples to calculate the Kovats retention indices (KI) of the detected compounds [24]. The transfer line to the mass spectrometer was maintained at 230°C, and the ion source temperature was set at 250°C. The mass spectra were obtained by using a mass selective detector with the electronic impact at 70 eV, a multiplier voltage of 1456 V, and by collecting the data at the rate of 1 scan/s over the *m/z* range of 30–350. Compounds were identified by comparing the retention times of the chromatographic peaks with those of authentic compounds analyzed under the same conditions when available or by comparing the Kovats retention indices with the literature data. The identification of MS fragmentation patterns was performed either by the comparison with those of pure compounds or using the National Institute of Standards and Technology (NIST) MS spectral database. Volatile compounds measurements from each headspace of the plant extracts were carried out by peak area normalization (expressed in percentages). All analyses were done in triplicate.

Data is expressed as mean value and standard deviation.

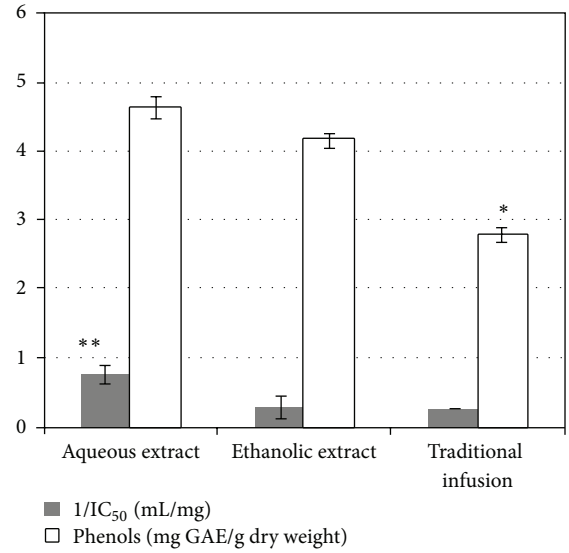

Figure 1: Antioxidant capacity (DPPH scavenging activity)[a] and total phenol content of different extracts of *B. orellana* seed samples (DW)[b]. Data expressed as mean ± standard deviation ($n = 3$). [a]: 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity expressed as inhibitory concentration ($1/IC_{50}$); **: significant $P < 0.05$. [b]: Phenol content expressed as mg GAE/g dry weight; *: significant $P < 0.05$.

3. Results and Discussion

3.1. Antioxidant Capacity and Total Phenol Content Analysis. Results of the antioxidant capacity and total phenol content (TPC) of all *B. orellana* seed extracts (traditional infusion, H_2O, and H_2O/ETOH) are presented in Figure 1. The free radical scavenging activity was evaluated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, which is regarded as an easy, reliable, sensitive, and rapid method. A review by Moon and Shibamoto also reported that almost 90% of antioxidant studies are conducted with this method [25]. We noticed that the DPPH scavenging activity was significantly greater for the seeds extracted with water than for extracts obtained with infusion and ethanol. It is well known that antioxidants react with DPPH free radical and convert it to the stable form. Therefore, *B. orellana* seeds extracted with H_2O seem

to contain a great amount of antioxidant compounds which may act as primary antioxidants able to react with free radicals as efficient hydrogen donors. A broad antioxidant activity of leaf and seed polar extracts has been recently proved using five different test systems by Viuda-Martos et al. [17]. Cardarelli et al. reported that the highest free radical scavenging capacity of annatto extracts was observed in the extract obtained with the most polar solvents, which also showed the highest phenol content [26].

In our conditions, the total phenol content resulted significantly higher for seed extracts obtained with H_2O than for infusion or H_2O/ETOH extraction.

This result clearly indicates that most of the phenols present in the plant are in a polar form. The TPC of *B. orellana* seed extracts, expressed as Gallic acid equivalent, ranged from 2.82 mg/g to 4.67 mg/g. Cardarelli et al. reported a lower TPC content that ranged between 0.30 and 1.84 mg of Gallic acid equivalent per gram of dry seeds (depending on the solvent used for extraction), while Chisté et al. recorded TPC values of 1.7 mg of Gallic acid equivalent per gram of wet seeds [26, 27]. The concentration and type of phenol substances present in the extracts are influenced by seasonal and environmental factors, for example, climate and soil type, genetic factors, and processing methods such as type of solvent used It is noteworthy that the water extractable phenol compounds present in *B. orellana* dry seeds are higher than that of green beans, tomatoes, green cabbage, green collard, and some green leafy vegetables. Bioactive phenols are very interesting as antioxidants because of their natural origin and their ability to act as efficient free radical scavengers, able to protect the cells against the oxidative damage caused by free radicals [28].

3.2. Repellent Activity. Blood-feeding insects are of great medical and veterinary importance, due both to their nuisance value and as vectors of disease [29]. Amongst insects that feed on humans, mosquitoes are of global importance [30]. In Amazonian regions, the use of plants as insect repellents is a common practice among Indio tribes. The majority of Itaputyr interviewed villagers admitted that they could not afford to use synthetic commercial mosquito repellents or insecticides because of high cost and/or discouraged by poor performance of some of the commercial products. Similar findings have been reported elsewhere in Africa, such as Guinea Bissau and Kenya, where the majority of the villagers could not afford synthetic commercial mosquito insecticides due to poverty [31, 32].

The results of mosquito protection in relation to application dose of different *B. orellana* dried seeds extracts are shown in Table 3. Skin repellent test showed the best results using a highly concentrated extract. Dried hexane extract resuspended in acetone provided the highest total percentage protection, followed by hydroalcoholic extract, with 90% and 73% of protection, respectively.

These results support the presence of mosquito repellent compounds in *B. orellana* seeds, particularly in the nonpolar fraction, also suggested by the Indio tradition to use the seeds to paint their bodies during open air religious and war ceremonies.

Table 3: Percentage of protection against mosquitoes (*A. aegypti*) in relation to the dose of the different *B. orellana* seed extracts (DW).

Extraction solvent	Concentration (mg/mL)	Protection (%)	SD (±)
Hexane	18.2	75%	16
	35.6	85%	18
	113.8	90%	18
EtOH	18.2	22%	13
	35.6	45%	14
	113.8	60%	16
$EtOH/H_2O$ (50 : 50 v/v)	18.2	23%	3
	35.6	50%	10
	113.8	73%	13

Data expressed as mean ± standard deviation ($n = 3$).

3.3. Volatile Organic Compounds (VOCs) Analysis. The volatile compounds extracted from headspaces of different *B. orellana* organs are presented in Table 4. At present, this represents the first report on the characterization of volatile compounds from different *B. orellana* organs by using the SPME technique. The SPME technique has the advantage of minimizing the sample handling and consequently decreasing the loss of volatile compounds. It is a simple and fast modern tool used to characterize the VOCs fingerprint of aromatic and medicinal plants and offers a valid alternative to hydrodistillation [33]. Moreover, this technique could represent a valid tool to analyze volatile compounds emitted by *in vivo* plants, sampling them *in situ,* in a nondisruptive way. Overall, 71 volatile compounds were identified in *B. orellana* samples with different proportions amongst the different plant organs.

The majority of volatile compounds present in the headspace from different *B. orellana* organs were sesquiterpenes, monoterpenes, hydrocarbons, and ketones. Simple terpenes have gained attention as agents of communication and defense against insects and can act as attractants or repellants.

Monoterpenes and sesquiterpenes are major components of many spices, fruit, and flower essential oils, having a great economic importance [34]. In the present study, we found a very scarce presence of monoterpenes especially α-pinene, that was absent in the *in vivo* samples. This is in contrast with Galindo-Cuspinera et al. that found α-pinene to be a major component isolated in the oil obtained from *B. orellana* samples. However, the volatile compounds were recovered using dynamic headspace-solvent desorption sampling and analyzed using GC-MS [35].

Sesquiterpenes constituted the major group of volatile compounds found in all the different *B. orellana* extracts analyzed. The major constituents were D-germacrene, δ-elemene, γ-elemene followed by β-caryophyllene, α-caryophyllene, γ-muurolene, and δ-cadinene.

Table 4: Identification of volatile organic compounds (VOCs) by HS-SME-GC/MS from headspace of *B. orellana* plant extracts obtained from different plant parts and from the headspace in living plant fruits (*in vivo* sampling).

RI[a]	Volatile compounds	Seed[b]		Wood[b]		Leaf[b]		Bark[b]		Fruit[b]		"*In vivo*"[c]	
		$n = 5$	s.d.	$n = 5$	s.d.	$n = 5$	s.d.	$n = 5$	s.d.	$n = 5$	s.d.	$n = 5$	s.d.
	Free fatty acids												
1285	Acetic acid	0.03	0.02	nd	—	0.01	—	nd	—	nd	—	nd	—
1628	2-ethylhexanoic acid	nd	—	nd	—	nd	—	nd	—	nd	—	0.12	0.01
	Total	**0.03**		—	—	**0.01**		—	—	—	—	**0.12**	
	Alcohols												
659	Ethanol	1.18	0.03	nd	—	nd	—	0.02		1.06	0.03	nd	—
848	2-methyl-1-propanol	0.01	—	nd	—	nd	—	nd	—	0.03	—	nd	—
919	2-pentanol	nd	—	nd	—	nd	—	nd	—	0.01	—	nd	—
976	1-penten-3-olo	nd	—	nd	—	nd	—	nd	—	0.01	—	nd	—
1040	2-methyl-1-butanol	0.13	0.05	nd	—	nd	—	nd	—	0.05	—	nd	—
1175	3-methyl-2-buten-1-ol	0.01		nd	—	nd	—	nd	—	nd	—	nd	—
1573	2-methoxyphenol	0.06		nd	—	nd	—	nd	—	nd	—	nd	—
1650	Phenol	0.01		nd	—	nd	—	nd	—	nd	—	nd	—
	Total	**1.39**		—	—	—	—	**0.02**		**1.16**		—	—
	Aldehydes												
645	2-methyl-butanal	nd	—	nd	—	0.01		nd	—	nd	—	nd	—
648	3-methyl-butanal	nd	—	nd	—	0.01		nd	—	nd	—	nd	—
687	Pentanal	nd	—	nd	—	0.03		nd	—	nd	—	nd	—
815	Hexanal	nd	—	nd	—	0.01		nd	—	nd	—	0.12	0.01
1034	2-hexenal	nd	—	nd	—	0.01		nd	—	nd	—	nd	—
1243	Nonanal	nd	—	nd	—	nd	—	nd	—	nd	—	0.17	0.02
1332	Decanal	nd	—	nd	—	nd	—	nd	—	nd	—	0.17	0.03
1341	Benzaldehyde	nd	—	2.20	0.04	nd	—	nd	—	nd	—	0.10	—
	Total	**—**	—	**2.20**		**0.07**		—	—	—	—	**0.56**	
	Ketones												
614	2-propanone	nd	—	nd	—	0.02		nd	—	nd	—	0.09	0.01
654	2-methyl-3-butanone	nd	—	0.71	0.01	nd	—	nd	—	nd	—	nd	—
685	3-pentanone	nd	—	2.50	0.02	nd	—	nd	—	0.07		nd	—
701	2-methyl-3-pentanone	nd	—	4.09	0.17	nd	—	nd	—	nd	—	nd	—
712	4-methyl-2-pentanone	nd	—	nd	—	nd	—	nd	—	nd	—	0.29	0.03
852	1,2-cycloctandione	0.01		nd	—	nd	—	nd	—	nd	—	nd	—
984	2-heptanone	nd	—	nd	—	nd	—	nd	—	0.01		nd	—
1121	3-hydroxy-2-butanone	nd	—	0.69	0.03	nd	—	nd	—	nd	—	nd	—
1189	6-methyl-5-hepten-2-one	0.02		nd	—	0.01		nd	—	nd	—	0.14	0.02
1422	4-tert-butylcyclohexanone	nd	—	nd	—	nd	—	nd	—	nd	—	0.50	0.06
	Total	**0.03**		**7.99**		**0.03**		—	—	**0.08**		**1.03**	
	Sulphur compounds												
604	Dimethyl sulfide	0.25	0.02	nd	—	nd	—	0.01	—	0.31	0.02	nd	—
	Total	**0.25**	—	—		—		**0.01**		**0.31**		—	—
	Furans												
639	2-methylfuran	0.02		2.71	0.15	0.01	—	0.01	—	nd	—	nd	—
671	2-ethylfuran	nd	—	nd	—	0.01	—	nd	—	nd	—	nd	—
	Total	**0.02**		**2.71**		**0.02**		**0.01**		—		—	

Table 4: Continued.

RI[a]	Volatile compounds	Seed[b]		Wood[b]		Leaf[b]		Bark[b]		Fruit[b]		"*In vivo*"[c]	
		$n = 5$	s.d.	$n = 5$	s.d.	$n = 5$	s.d.	$n = 5$	s.d.	$n = 5$	s.d.	$n = 5$	s.d.
	Esters												
618	Methyl acetate	0.01	—	nd	—	nd	—	nd	—	nd	—	nd	—
636	Ethyl acetate	0.51	0.12	nd	—	nd	—	nd	—	nd	—	nd	—
895	2-methyl-1-butanol acetate	0.03	—	nd	—	nd	—	nd	—	nd	—	nd	—
898	3-methyl-1-butanol ecetate	0.02	—	nd	—	nd	—	nd	—	nd	—	nd	—
	Total	**0.57**		—		—		—		—		—	
	Hydrocarbons												
746	Toluene	nd	—	nd	—	0.01		nd	—	nd	—	nd	—
884	Ethylbenzene	nd	—	2.24	0.06	nd	—	nd	—	nd	—	2.72	0.03
894	o-xylene	nd	—	nd	—	nd	—	nd	—	0.01		0.70	0.03
909	p-xylene	0.02		2.33	0.02	nd	—	0.01		nd	—	1.51	0.15
979	m-xylene	nd	—	0.73	0.09	nd	—	nd	—	0.01		0.50	0.05
1200	Dodecan	nd	—	nd	—	nd	—	nd	—	nd	—	0.67	0.03
1093	Styrene	0.01	0.01	6.15	0.12	0.01		0.03		nd	—	0.35	0.02
1166	2-ethenyl-1,1-dimethyl-3-methylenecyclohexane	nd	—	nd	—	0.52	0.03	nd	—	nd	—	nd	—
1270	Hydrocarbon	nd	—	nd	—	nd	—	nd	—	nd	—	1.11	0.08
1278	Methyl-(1-methylethenyl)-benzene	0.01	—	nd	—	nd	—	nd	—	0.12	0.08	nd	—
	Total	**0.04**		**11.45**		**0.54**		**0.04**		**0.14**		**7.56**	
	Mono-di-terpenes												
709	Triciclene	0.02		nd	—	nd	—	0.03	—	nd	—	nd	—
726	α-pinene	0.24	0.01	3.97	0.09	0.02	0.01	0.38	0.03	0.15	0.02	nd	—
735	α-thujene	nd	—	nd	—	nd	—	0.02	—	0.06	—	nd	—
776	Camphene	0.23	0.02	4.55	0.12	nd	—	0.98	0.05	0.10	—	nd	—
842	β-pinene	0.10	—	nd	—	nd	—	0.27	0.02	0.05	—	nd	—
876	Sabinene	0.02	—	nd	—	nd	—	0.09	0.01	0.03	—	nd	—
931	α-phellandrene	nd	—	nd	—	nd	—	0.01	—	0.01	—	nd	—
971	β-mircene	0.03	—	nd	—	0.19	0.03	0.13	0.01	0.41	0.02	0.11	0.01
978	α-terpinene	0.05	—	nd	—	0.01	—	nd	—	0.06	0.21	nd	—
1008	D-limonene	0.13		3.60	0.11	0.08	0.01	0.10	0.01	0.61	0.03	0.65	0.03
1081	Cis-ocimene	2.17	0.04	nd	—	3.19	0.05	3.30	0.08	19.99	0.37	3.38	0.08
1099	Trans-ocimene	0.05	0.01	nd	—	0.05	0.01	0.14	0.02	0.69	0.04	0.37	0.02
1111	Cymene	0.14	0.02	nd	—	0.03	0.01	nd	—	2.11	0.03	0.77	0.05
1127	α-terpinolene	0.05	0.01	nd	—	0.01	—	0.01	—	0.28	0.01	nd	—
1223	Allo ocimene isomer	nd	—	nd	—	0.01	—	0.02	—	0.73	0.03	nd	—
1227	Allo-ocimene	0.02		nd	—	nd	—	nd	—	0.10	0.01	0.10	0.02
1494	Naphthalene	nd	—	nd	—	nd	—	nd	—	nd	—	4.85	0.09
	Total	**3.25**		**12.12**		**3.59**		**5.48**		**25.39**		**10.23**	
	Sesquiterpenes												
1301	α-cubebene	0.39	0.13	2.43	0.24	1.91	0.07	1.19	0.06	nd	—	1.16	0.14
1316	δ-elemene	22.01	0.09	5.47	0.18	4.80	0.21	4.39	0.03	18.03	0.27	3.72	0.17
1321	α-ylangene	0.15	0.01	nd	—	0.64	0.02	8.15	0.02	0.57	0.03	nd	—
1328	α-copaene	2.53	0.07	7.39	0.04	11.44	0.15	4.20	0.03	3.25	0.05	2.80	0.08
1355	α-gurjunene	0.09	0.01	nd	—	0.39	0.01	0.99	0.02	nd	—	nd	—
1363	β-cubebene	0.65	0.05	nd	—	0.76	0.02	0.37	0.01	0.68	0.03	nd	—

Table 4: Continued.

RI[a]	Volatile compounds	Seed[b]		Wood[b]		Leaf[b]		Bark[b]		Fruit[b]		"*In vivo*"[c]	
		$n = 5$	s.d.	$n = 5$	s.d.	$n = 5$	s.d.	$n = 5$	s.d.	$n = 5$	s.d.	$n = 5$	s.d.
1387	Sesquiterpene	0.05	0.02	nd	—	0.10	0.02	0.09	0.02	nd	—	3.08	0.08
1390	Sesquiterpene	1.58	0.03	nd	—	1.00	0.07	0.35	0.06	nd	—	nd	—
1402	β-caryophyllene	1.80	0.03	11.02	0.54	22.39	0.18	6.68	0.14	nd	—	37.32	0.19
1410	Aromadendrene	0.16	0.01	2.91	0.08	3.21	0.03	11.60	0.08	nd	—	1.85	0.07
1413	Sesquiterpene	0.22	0.01	nd	—	nd	—	nd	—	7.73	0.24	nd	—
1424	Sesquiterpene	0.41		nd	—	1.07	0.02	1.40	0.03	0.96		nd	—
1435	Sesquiterpene	0.16		nd	—	nd	—	nd	—	nd	—	2.12	0.03
1440	Calarene	1.15	0.03	nd	—	0.45	0.01	0.76	0.01	2.19	0.02	nd	—
1452	α-caryophyllene	nd	—	8.70	0.25	12.00	0.07	7.12	0.17	nd	—	7.16	0.08
1454	Sesquiterpene	4.26	0.02	nd	—	0.55	0.01	nd	—	nd	—	nd	—
1462	Isoledene	1.80	0.04	nd	—	nd	—	0.43	0.01	3.41	0.19	nd	—
1469	Sesquiterpene	nd	—	nd	—	nd	—	nd	—	3.88	0.21	nd	—
1471	γ-muurolene	0.99	0.01	2.95	0.07	1.55	0.02	1.96	0.02	2.74	0.13	1.92	0.07
1476	β-cadinene	1.80	0.03	2.10	0.08	2.01	0.02	1.80	0.01	2.41	0.08	2.18	0.12
1486	D-germacrene	27.83	0.17	7.11	0.16	10.09	0.09	21.13	0.31	7.29	0.15	1.96	0.03
1487	β-selinene	nd	—	nd	—	0.83	0.02	0.66	0.02	nd	—	1.24	0.03
1496	α-selinene	nd	—	nd	—	0.51	0.03	0.53	0.01	1.81	0.08	nd	—
1499	γ-elemene	3.69	0.04	5.76	0.31	12.45	0.19	11.18	0.12	3.28	0.09	1.13	0.07
1516	Sesquiterpene	1.15	0.07	nd	—	nd	—	nd	—	nd	—	nd	—
1519	δ-cadinene	2.14	0.07	5.75	0.43	4.70	0.05	5.17	0.11	8.89	0.13	5.78	0.15
1527	Sesquiterpene	18.07	0.16	nd	—	1.47	0.03	2.73	0.09	0.23	0.01	nd	—
1531	1,2,3,4,4a,7-naphthalene hexahydro-1,6-dimethyl-4-(1-methylethyl)	0.10	—	nd	—	0.19	0.01	0.24	0.01	0.76	0.02	0.26	0.02
1537	α-muurolene	0.29	—	nd	—	0.22	0.01	0.24	0.02	1.54	0.09	0.46	0.02
1557	Sesquiterpene	0.82	0.02	nd	—	0.32	0.02	0.25	0.02	1.96	0.04	0.44	0.03
1563	Calamenene	nd	—	nd	—	0.05	—	0.07	0.01	0.19	0.01	0.99	0.02
1612	α-calacorene	nd	—	nd	—	0.02	—	0.03	0.01	0.08	0.01	0.64	0.01
1642	Sesquiterpene	nd	—	nd	—	nd	—	0.05	0.01	0.25	0.02	2.08	0.07
1663	Humulene oxide	nd	—	nd	—	0.05	—	0.03		nd	—	nd	—
1667	Nerolidol	nd	—	nd	—	0.15	0.01	nd	—	nd	—	nd	—
1691	Spathulenol	nd	—	nd	—	0.26	0.02	0.16		nd	—	0.84	0.02
1728	α-cadinol	0.01	—	nd	—	nd	—	nd	—	0.11	0.02	0.17	0.02
	Total	**94.3**		**61.59**		**95.58**		**93.95**		**72.24**		**79.3**	

[a]The Kovats retention index calculated for Rtx-Wax capillary column (25 m × 0.25 μm × 0.25 mm i.d.).
[b]Normalized amount of volatile compounds (percentage); (peak of volatile compound/total peak area of all volatile compounds) of *B. orellana* ($n = 3$) obtained from different parts.
[c]Normalized amount of volatile compounds (percentage); (peak of volatile compound/total peak area of all volatile compounds) of *B. orellana* living plants using *in vivo* sampling ($n = 3$).
s.d.: ± standard deviation.
nd: not detected.

Sesquiterpenes were significantly higher than mono-diterpenes in all plant organs analyzed. Leaves bark and seeds showed higher sesquiterpene concentration than wood and fruits dried or *in vivo* sampled. The total percentage of VOCs identified in the wood was rather low, only 61.59%, as compared to the other plant samples. Also, Zollo et al., found low levels of volatile compounds extracted from leaves and wood of *B. orellana*. The authors identified a great percentage of ishwarane, a sesquiterpene hydrocarbon not found in the present research [36].

Elemene and δ-cadinene have been found in all samples at different percentages. Minor sesquiterpenes found in *B. orellana* extracts that have distinctive aromas include cubebene, β-, and δ-cadinene. Cubebene has been described as having a fruity, sweet, citrus-like smell. β- and δ-cadinene are compounds occasionally used as fixatives in candy

flavours and have a dry-woody, slightly medicinal-tarry odour with some similarity to spices in the cumin-thyme family [37]. Some of the monoterpenes and sesquiterpenes found in our extracts have been previously described as having antimicrobial properties or as being associated with pharmacological properties [38]. D-germacrene, mainly found in dried seeds and leaves samples (27.83% and 21.13%, resp.), showed antimicrobial activity [39].

β-caryophyllene, mainly found in *in vivo* samples (37.32%), has been proved to have an anti-inflammatory effect by selectively binding cannabinoid receptors type 2 (CB_2) [37].

Cis-ocimene, mainly found in dried fruits samples (19.99%), showed antibacterial activity against Gram+ and Gram− (*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa,* and *Klebsiella pneumoniae*) [40].

4. Conclusions

This study has renewed the state of knowledge about *B. orellana* bringing together the existing literature information and novelties such as VOCs profiling and indications about mosquito repellent capacity of seed extracts. At present, this research represents the first investigation on volatile compounds conducted on different *B. orellana* organs by means of a fast sample preparation method. In addition, SPME was confirmed to be a good technique to analyze volatile compounds emitted by *in vivo* plants, sampled *in situ,* in a nondisruptive way, with consequent potential big advantages for phytochemical and ecophysiological studies, particularly regarding rare and/or protected plants. General screening confirmed the literature data about phenol compounds content and radical scavenging capacity of *B. orellana* seed extracts.

The preliminary results on mosquito repellent activity confirmed the potential of the plant to protect against *A. aegypti* representing another theme of future investigation, fundamental in linking traditional value of a well-known plant with scientifically obtained proof of efficacy.

Conflict of Interests

The authors declare that they have no conflict of interests.

Acknowledgment

The authors are grateful to the Itapury Cooperation Project for supporting the mission costs.

References

[1] C. Antweiler, "Local knowledge and local knowing: an anthropological analysis of contested "cultural products" in the context of development," *Anthropos*, vol. 93, no. 4–6, pp. 469–494, 1998.

[2] M. Coelho-Ferreira, "Medicinal knowledge and plant utilization in an Amazonian coastal community of Marudà, Parà State (Brazil)," *Journal of Ethnopharmacology*, vol. 126, no. 1, pp. 159–175, 2009.

[3] Brasil, *Política nacional de plantas medicinais e fitoterápicos, Ministério da Saúde, Secretaria de Ciência Tecnologia e Insumos Estratégicos*, Departamento de Assistência Farmacêutica, Brasília, Brazil, 2007.

[4] R. Rajendran, *Achiote:* Bixa Orellana *L. A Natural Food, Colour and Dye*, AES, College of Agricultural Science, Mangilao, Guam, 1st edition, 1990.

[5] P. R. N. Carvalho and M. Hein, "Urucum: uma fonte de corante natural," *Coletânea do ITAL*, vol. 19, pp. 25–33, 1989.

[6] A. T. Selvi, R. Aravindhan, B. Madhan et al., "Studies on the application of natural dye extract from *Bixa orellana* seeds for dyeing and finishing of leather," *Industrial Crops and Products*, vol. 43, pp. 84–86, 2013.

[7] M. G. Parra and L. M. Fidalgo, "Bixa orellana: properties and applications," in *Drug Plants IV*, V. K. Singh and J. N. Govil, Eds., pp. 25–35, 2010.

[8] M. P. Corrêa, *Dicionário das Plantas Úteis Do Brasil e das Exóticas Cultivadas*, vol. 4, Ministério da Agricultura/IBDF, Rio de Janeiro, Brazil, 1978.

[9] R. A. Voeks and A. Leony, "Forgetting the forest: assessing medicinal plant erosion in Eastern Brasil," *Economic Botany*, vol. 58, supplement 1, pp. S294–S306, 2004.

[10] M. T. L. A. Camargo, *Medicina Popular: Aspectos Metodologicos Para Pesquisa, Garrafada, Objeto de Pesquisa, Componentes Medicinais de Origem Vegetal, Animal e Mineral*, ALMED Editora e Livraria Ltda, Sao Paulo, Brasil, 1985.

[11] L. C. di Stasi, E. M. G. Santos, C. M. Dos-Santos, and C. A. Hiruma, *Plantas Medicinas da Amazonia*, UNESP Editora, Sao Paolo, Brasil, 1989.

[12] S. Patnaik, S. R. Mishra, G. B. Choudhury et al., "Phytochemical investigation and simultaneously study on anticonvulsant, antidiabetic activity of different leafy extracts of *Bixa orellana* Linn," *International Journal of Pharmaceutical and Biological Archives*, vol. 2, no. 5, pp. 1497–1501, 2011.

[13] K. Asokkumar, P. Jagannath, M. Umamaheswari et al., "Cardioprotective activity of *Bixa orellana* L. on isoproterenol induced myocardial necrosis in rats," *Journal of Pharmacy Research*, vol. 5, no. 4, pp. 1930–1934, 2012.

[14] A. T. Selvi, M. G. Dinesh, R. S. Satyanet et al., "Leaf and seed extracts of *Bixa orellana* L. exert anti-microbial activity against bacterial pathogens," *Journal of Applied Pharmaceutical Science*, vol. 1, no. 9, pp. 116–120, 2011.

[15] T. C. Fleischer, E. P. K. Ameade, M. L. K. Mensah, and I. K. Sawer, "Antimicrobial activity of the leaves and seeds of ,*Bixa orellana*," *Fitoterapia*, vol. 74, no. 1-2, pp. 136–138, 2003.

[16] A. G. Barrio, M. M. M. Grueiro, D. Montero et al., "*In vitro* antiparasitic activity of plant extracts from panama," *Pharmaceutical Biology*, vol. 42, no. 4-5, pp. 332–337, 2004.

[17] M. Viuda-Martos, G. L. Ciro-Gomez, Y. Ruiz-Navajas et al., "*In vitro* antioxidant and antibacterial activities of extracts from annatto (*Bixa orellana* L.) leaves and seeds," *Journal of Food Safety*, vol. 32, no. 4, pp. 399–406, 2012.

[18] B. Radhika, N. Begum, and K. Srisailam, "Pharmacognostic and preliminary phytochemical evaluation of the leaves of *Bixa orellana*," *Pharmacognosy Journal*, vol. 2, no. 7, pp. 132–136, 2010.

[19] M. L. T. Nguyen, "Cultivated plant collections from market places," *Ethnobotany Research & Applications*, vol. 3, pp. 5–15, 2005.

[20] U. P. Albuquerque, *Folhas Sagradas: as Plantas Liturgicas e Medicinais nos Cultos Afro-Bresileiros*, Nupeea, Recife, Brazil, 2nd edition, 2006.

[21] A. Giorgi, S. Panseri, M. S. Mattara, C. Andreis, and L. M. Chiesa, "Secondary metabolites and antioxidant capacities of *Waldheimia glabra* (Decne). Regel from Nepal," *Journal of the Science of Food and Agriculture*, vol. 93, no. 5, pp. 1026–1034, 2013.

[22] WHO, "Report of the WHO informal consultation on the evaluation and testing insecticides," Tech. Rep. CTD/WHO PES/IC/96.1, Control of Tropical Diseases Division, World Health Organization, Geneva, Switzerland, 1996.

[23] A. Giorgi, M. Madeo, G. Speranza, and M. Cocucci, "Influence of environmental factors on composition of phenolic antioxidants of *Achillea collina* Becker ex Rchb," *Natural Product Research*, vol. 24, no. 16, pp. 1546–1559, 2010.

[24] L. S. Anker, P. C. Jurs, and P. A. Edwards, "Quantitative structure-retention relationship studies of odor-active aliphatic compounds with oxygen-containing functional groups," *Analytical Chemistry*, vol. 62, no. 24, pp. 2676–2684, 1990.

[25] J. K. Moon and T. Shibamoto, "Antioxidant assays for plant and food components," *Journal of Agricultural and Food Chemistry*, vol. 57, no. 5, pp. 1655–1666, 2009.

[26] C. R. Cardarelli, M. D. T. Benassi, and A. Z. Mercadante, "Characterization of different annatto extracts based on antioxidant and colour properties," *LWT—Food Science and Technology*, vol. 41, no. 9, pp. 1689–1693, 2008.

[27] R. C. Chisté, A. Z. Mercadante, A. Gomes, E. Fernandes, J. L. F. D. C. Lima, and N. Bragagnolo, "*In vitro* scavenging capacity of annatto seed extracts against reactive oxygen and nitrogen species," *Food Chemistry*, vol. 127, no. 2, pp. 419–426, 2011.

[28] M. C. Castello, A. Phatak, N. Chandra, and M. Sharon, "Antimicrobial activity of crude extracts from plant parts and corresponding calli of *Bixa orellana* L.," *Indian Journal of Experimental Biology*, vol. 40, no. 12, pp. 1378–1381, 2002.

[29] A. Abdul Rahuman, "Efficacies of medicinal plant extracts against blood-sucking parasites," in *Nature Helps, Parasitology Research Monographs*, H. Mehlhorn, Ed., Springer, Berlin, Germany, 2011.

[30] J. Fang, "Ecology: a world without mosquitoes," *Nature*, vol. 466, pp. 432–434, 2010.

[31] A. Seyoum, K. Pålsson, S. Kung'a et al., "Traditional use of mosquito-repellent plants in western Kenya and their evaluation in semi-field experimental huts against *Anopheles gambiae*: ethnobotanical studies and application by thermal expulsion and direct burning," *Transactions of the Royal Society of Tropical Medicine and Hygiene*, vol. 96, no. 3, pp. 225–231, 2002.

[32] K. Karunamoorthi, K. Ilango, and A. Endale, "Ethnobotanical survey of knowledge and usage custom of traditional insect/mosquito repellent plants among the Ethiopian Oromo ethnic group," *Journal of Ethnopharmacology*, vol. 125, no. 2, pp. 224–229, 2009.

[33] A. Giorgi, S. Panseri, N. N. M. C. Nanayakkara, and L. M. Chiesa, "HS-SPME-GC/MS analysis of the volatile compounds of *Achillea collina*: evaluation of the emissions fingerprint induced by *Myzus persicae* infestation," *Journal of Plant Biology*, vol. 55, no. 3, pp. 251–260, 2012.

[34] S. Terashima, M. Shimizu, S. Horie, and N. Morita, "Studies on aldose reductase inhibitors from natural products. IV. Constituents and aldose reductase inhibitory effect of Chrysanthemum morifolium, *Bixa orellana* and *Ipomoea batatas*," *Chemical and Pharmaceutical Bulletin*, vol. 39, no. 12, pp. 3346–3347, 1991.

[35] V. Galindo-Cuspinera, M. B. Lubran, and S. A. Rankin, "Comparison of volatile compounds in water- and oil-soluble annatto (*Bixa orellana L.*) extracts," *Journal of Agricultural and Food Chemistry*, vol. 50, no. 7, pp. 2010–2015, 2002.

[36] P. H. A. Zollo, L. Biyiti, F. Tchoumbougnang, C. Menut, G. Lamaty, and P. Bouchet, "Aromatic plants of tropical Central Africa. Part II. Chemical composition and antifungal activity of thirteen essential oils from aromatic plants of Cameroon," *Flavour and Fragrance Journal*, vol. 13, pp. 107–114, 1998.

[37] J. Gertsch, M. Leonti, S. Raduner et al., "Beta-caryophyllene is a dietary cannabinoid," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 26, pp. 9099–9104, 2008.

[38] J. A. Shilpi, M. Taufiq-Ur-Rahman, S. J. Uddin, M. S. Alam, S. K. Sadhu, and V. Seidel, "Preliminary pharmacological screening of Bixa orellana L. leaves," *Journal of Ethnopharmacology*, vol. 108, no. 2, pp. 264–271, 2006.

[39] X. S. Wang, W. Yang, S. J. Tao et al., "The effect of δ-elemene on hela cell lines by apoptosis induction," *Yakugaku Zasshi*, vol. 126, no. 10, pp. 979–990, 2006.

[40] T. Jovanovic, D. Kitic, R. Palic, G. Stojanovic, and M. Ristic, "Chemical composition and antimicrobial activity of the essential oil of *Acinos arvensis* (Lam.) Dandy from Serbia," *Flavour and Fragrance Journal*, vol. 20, no. 3, pp. 288–290, 2005.