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BIOASSAY-GUIDED FRACTIONATION IN *Anacardium excelsum* (Bert. & Balb. Ex Kunth) SKEELS (ANACARDIACEAE)

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Abstract

Anacardium excelsum is a tree between 15 and 50 meters high, with a thick trunk and is present in some departments of Colombia. Its nut-shaped fruits were used in the old Upar Valley by natives (Valledupar) to make Caracolí bread. Most research has been done at the forest level, but there are few phytochemical and biological activity studies. This bioguided work shows the application of antioxidant methods ($ABTS^{+\bullet}$, $DPPH^{\bullet}$ and $DMPD^{+\bullet}$) in the search for active compounds present in the fraction and subfractions of greater activity. The antioxidant capacity was measured to the crude extract in ethanol and the antioxidant activity of the fractions in petroleum ether, dichloromethane, and ethanol-butanol of testa, tegument, flower, fruit, seed, bark, and leaf. Column Chromatography, Thin Layer Chromatography and Gas Chromatography coupled to Mass Spectrometry were performed on the petroleum ether fraction of testa with greater antioxidant capacity, finding the following compounds: 3-pentadec(en)il-phenol, 3-pentadecyl-phenol, 3-heptadeca(dien)il-phenol, 3-heptadec(en)il-phenol, 3-heptadecyl-phenol, 3-nonadec(en)il-phenol, ethyl ester of hexadecanoic acid, ethyl ester of heptadecanoic acid , ethyl ester of linolenic acid, hexadecanoic acid butyl ester, ethyl ether of octadecanoic acid, ethyl ester of (Z)-9-octadecenoic acid, ethyl ester of 9,12-octadecadienoic acid and ethyl ester of eicosanoic acid. The results indicate that *Anacardium excelsum* is a potential source of bioactive compounds.

Keywords: *Antioxidant, Anacardium excelsum, phenolic lipids, 3-pentadecyl-phenol, 3-heptadecyl-phenol*

Introduction

The Anacardiaceae family includes 73 genera and 850 species of trees, shrubs and lianas distributed worldwide grouped into five tribes: Anacardieae, Spondiadeae, Semecarpeae, Rhoeae and Dobineae, approximately 41% of its genera are native to America [1, 2]. The Anacardiaceae family includes trees and shrubs with alternate pinnate-compound or simple leaves. The flowers are regular and can be bisexual or unisexual, composed of 5 joined sepals, 5 free petals and 5 to 10 stamens inserted into a fleshy disc [3]. The fruit can be a drupe, a walnut, or a samara, also, some species exude irritating resins for the skin [4, 5]. This family is distributed mainly in tropical and subtropical zones, some of them present in temperate zones, with economic importance to produce edible fruits, gums, resins, tannins, dyes, and woods of commercial importance [5-9]. Among the most valuable species of fruit trees are *Anacardium occidentale* L. [10, 11], *Mangifera indica* L. [12, 13] and *Pistacia vera* L. [14, 15], and among those for timber use are *Anacardium excelsum* (Bert. & Balb. ex Kunth) Skeels [16-19], *Astronium graveolens* Jacq. *[20, 21]* and *Spondias mombin* L. [22, 23]. *Anacardium occidentale* L. shows antioxidant activity due to the content of phenolic compounds [24, 25], and the antitumor activity to long-chain derivatives of salicylic acid commonly called anacardic acids, such as, 6-[8(Z)-pentadecenyl] salicylic acid [26, 27]. *Lannea coromandelica* (Houtt.) Merr. shows sporicidal activity against zoospores of *Aphanomyces cochlioides* attributed to the content of poliflavonoid tannins and antihelminth activity against *Caenorhabditis elegans* [28, 29]. From *Pistacia lentiscus* L., known as lentiscus, essential oils are extracted for different use; antioxidant [30], antitumoral [31] and antibacterial against *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* whose action derives in the terpenes content such as □-pineno (40%), □-pineno (1.5%), □-myrcene (9.0%), limonene (1.0%) and □□caryophyllene (5%) [32]. *Pistacia vera* L. commonly known as pistachio is highly appreciated for its drupes that are used to make sweets and sausages [33, 34], shows antibacterial activity against *Corynebacterium xerosis*, *Bacillus brevis*, *Bacillus megaterium*, *Bacillus cereus*, *Mycobacterium smegmatis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Klebsiella oxytocica A*, *Enterococcus faecalis*, *Micrococcus luteus*, *Escherichia coli*, *Yersinia enterocolitica*, and also against pathogenic yeasts like *Kluvyeromyces fragilis*, *Rhodotorula rubra* and *Candida albicans*, all this spectrum of bactericidal action is due to the content of □□pinene (75.6%), □-pinene (9.5%), trans-verbenol (3.0%), camphene (1.4%), trans-pinocarveol (1.2%) and limonene (1.0%) [35, 36]. *Magnifera indica* L. commonly known as mango has antioxidant activity attributed to the metabolites; mangiferin, isomangiferin and quercetin, all quercetin 3-O-rhac derivatives [37, 38]. The mango seed is associated with functional properties when used as a prebiotic in feed of *Labeo rohita* fingerlings, increasing the immune response and resistance to diseases caused by *Aeromonas hydrophila* infections [37, 39]. *Rhus coriaria* L. recognized as Sumac is grown to obtain tannins for industrial usage and presents antioxidant activity, finding the gallic and protocatechuic acids as responsibles for this activity [40, 41]. *Rhus toxicodendron* L. is characterized by producing skin dermatitis on contact with urushiol resin, this type of pathology (dermatitis) is detected on contact with poison ivy [42], however, homeopathic medicine used this plant for the treatment against herpes and in recent years studies were conducted to evaluate anti-inflammatory activity of different extracts (tincture in alcohol) [43-45]. *Schinus mole* L. demonstrates antidepressant activity when testing for tail immobilization and forced swimming in mice, two predictive models of depression [46], and repellent activity of ovocidal and adulticidal type on *Triatoma infestans* and *Blattella germanica* related to the content of tannins, alkaloids, terpenes, and flavonoids, among others [47, 48]. *Sclerocarya birrea* (A. Rich.) Hochst. shows antiproliferative and apoptotic activity against breast cancer cells (MCF-7) and has antioxidant activity due to its content of glucosidated derivatives of quercetin and kaempferol such as quercetin 3-O-□-D-(6''-alloyl) glucopyranoside and kaempferol 3-O-□-D-(6''-galloyl) glucopiranoside [49, 50]. *Spondias mombin* L. grows in the rainforest and coastal area of Africa, is used in traditional medicine as a diuretic, against diarrhea, dysentery, fevers, hemorrhoids, gonorrhea and leucorrhoea, as an expectorant, stomach pain and to expel the tapeworm. Faced with a spectrum of

broad action in medicine, researchers like Ayoka conducted studies for the treatment of psychiatric disorders evaluating anxiolytic, antiepileptic and antisychotic activity with excellent results in rats and mice. In those extracts' tannins, saponins, anthraquinones, flavonoids and cardatonic glycosides were found [51, 52]. Likewise, anthelminthic activity was evidenced when extracts were evaluated on earthworms of the genus *Eudrilus eugeniae*, this activity is attributed to the content of phenolic acids and ellagitannins [53].

In Colombia we find species as *Anacardium excelsum* (Bert. & Balb. ex Kunth) Skeels, a tree between 15 and 50 m high, with a thick trunk, sympodial ramification, kidney-shaped fruits and leaves simple, alternate, obovate with entire margin and a size between 10-60 cm long 6-12 cm wide. Inflorescences are clusters (panicles) 23-45 cm long with white-yellow, pink-creamy, white, or yellow flowers, see **Figure 1** [54, 55]. The fruits are about 3 cm in diameter, reniform nuts of floury consistency called noses due their pear shape. This nuts where used in the old Upar Valley to make a kind of edible bread called snail bread and consumed cooked in syrup or cakes [56]. The pendulums of *Anarcardium excelsum* (Bert. & Balb. ex Kunth) Skeels are curved and fleshy and serve as food for monkeys and bats. The exudate serves as food for *Sanguinus oedipus*, the white-headed titi monkey. Nutritional analysis reveals that the exudate is a rich source of calcium, proteins, carbohydrates, and water. It is currently cultivated as a shade tree and its edible fruit has not been economically exploited [57]. Its wood was formerly used to build canoes and some kitchen tools such as rafts, bongos, trays, and plates [17]. Also has been used for veneres, floors of modern constructions and some expensive furniture [16, 55]. *Anacardium excelsum* (Bert. & Balb. ex Kunth) Skeels has few studies in the field of Pharmacognosy & Phytochemistry [18, 58-61], however, it is widely studied in the field of reforestation as an alternative for the exchange of carbon dioxide-oxygen and for the acquisition and fixation of phosphorus and nitrogen in soils (biomass) [19, 62]. Therefore, this bioguided research focused on the evaluation of the antioxidant capacity of the total extracts and fractions of the *Anacardium excelsum* (Bert. & Balb. ex Kunth) Skeels plant using $ABTS^{+\bullet}$, $DPPH^{\bullet}$, $DMPD^{+\bullet}$ methods and subsequent identifying the compounds present in the most active fraction using Gas Chromatography coupled to Mass Spectrometry (GC-MS)

Methods

Collecting botanical material

Sample of plant material for leaves, flowers, fruits, seed coat, seed, integument, and bark, were collected during the flowering season in municipality of Bucaramanga (department of Santander, Colombia), under the following coordinates 7°06'32.51" N and 73°06'15.19" W at elevation of 986m. The National Herbarium of Colombia identified the sample as *Anacardium excelsum* (Bert. & Balb. ex Kunth) Skeels, under classification number COL 520397.

Extraction and fractionation

The material was dried to the environment for 15 days, until humidity less than 8%. Between 50–800 g was soxhlet extracted with 4 liters of ethanol. After filtration, the extracts were concentrated using vacuum at 40°C. Partial liquid-liquid extractions was made in continuously using solvents in different polarities obtaining fractions in petroleum ether (PE), dichloromethane (CH_2Cl_2) and ethanol-butanol (EtOH-BuOH), as shown in Table 1. The extract bioactive was fractionated by Column Chromatography (4 x 60 cm) using SiliaFlash Irregular Silica Gel G60, (60 - 200 µm, 60 Å, SiliCycle) eluted with mixtures of chloroform ($CHCl_3$) and methanol (MeOH) in a relationship [95:5], [70:30] and [5:95]. The fractionation of the sub-fractions was performed using a discontinuous gradient with n-Hexane, ethyl acetate (EtOAc) and acetic acid (AcOH) in the proportion of [90:10:1], [80:20:1] and [50:50:1]. The fractions and subfractions were monitored and regroupded by Thin Layer Chromatography (TLC) using SiliaPlate F254 (20 x 20 cm, SiliCycle) plates, and mixtures of n-hexane, EtOAc, AcOH and MeOH as mobile phase (Plates and Solvents of Merck). Vainillin was used as developer. The extracts, fractions and sub-fractions using antioxidant capacity and activity, were bioassay-guided study [63].

Methods of antioxidant capacity and activity

The measurement of the antioxidant capacity of the crude ethanol extract from each part of the plant was preparaded using several methodologies ($ABTS^{+\bullet}$, $DPPH^{\bullet}$ and $DMPD^{+\bullet}$), while the antioxidant activity of the fraction and group of subfractions was carried out using the most sensitive method [64]. All crude extracts were prepared in the range of 10 to 10,000 µg/mL and the fractions in PE, CH_2Cl_2, and EtOH-BuOH between 10 and 1,000 µg/mL. Results were expressed as extract concentration µg/mL or fractions capable of inhibiting 50% radical absorbance (IC50). The pooled fractions and subfractions from the PE fraction of testa were prepared at one single concentration (100 µg/mL) and the antioxidant capacity was determined as percentage of radical inhibition.

ABTS method of antioxidant activity

The antioxidant capacity of whole extracts, fractions and subfractions was determined by the method described by Re et al. [65, 66]. The stock solution of $ABTS^{+\bullet}$ (2,2'-Azinobis-(ethyl-benzothiazoline-6-sulfonic acid) was prepared per solubilizing 20mg of ABTS in 10mL of deionized water and 2.45 mg of potassium persulfate ($K_2S_2O_8$). The solution could react at room temperature for 16 hours in the dark. After working solutions were prepared in MeOH, obtaining an absorbance at 0.75 ± 0.02 to 744nm in a spectrophotometer Sanyo SP50 and quartz cuvettes using a 10x10x45 mm. The solution of $ABTS^{+\bullet}$ loses its color blue-green reaching colorless when added an antioxidant compound, reducing the absorbance of the solution, which is measured at 744 nm, which is expressed as percentage inhibition. The percentage of inhibition (turning blue-green to transparent color) was determined by using the I = [(Ao - Ae) / Ao] x 100 equation, where Ao is absorbance without extract, and Ae is the absorbance with extracts. To get IC50 of extracts turning to transparent color that indicate antioxidant capacity, was calculated by plotting % of inhibition vs. extract concentration; then linear regression equation was calculated (y = mx + b), and 50% inhibitory concentration by the equation IC50 = (50 - b) / m. As antioxidant standards, 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (trolox), butylhydroxytoluene (BHT), L-ascorbic acid (vitamine C), and α-tocopherol (vitamin E) were used [54, 66, 67].

DPPH method of antioxidant activity

We used the methodology proposed by Brand-Williams et al (1995) [68, 69], where 10 mg of $DPPH^{\bullet}$ (α,α-diphenyl-β-picrylhydrazyl radical) were solubilized in 10 mL of MeOH (4.34 mM), then working solutions were prepared to obtain an absorbance of 0.75 ± 0.05 for all cases to a wavelength of 514 nm and read in a spectrophotometer Sanyo SP50, quartz cells using 1.0 cm. When an antioxidant compound is added to the solution of $DPPH^{\bullet}$ radical, it loses its violet, causing the decrease of the initial absorbance causing an inhibition. The conditions for assessing the antioxidant capacity of the extracts were the same of ABTS assay [54, 68, 70, 71].

DMPD method of antioxidant activity

The method developed by Fogliano et al. (1999) was implemented to determine the antioxidant activity [72]. 41.8 mg of DMPD (N,N-dimethyl-p-phenylenediamine dihydrochloride) were solubilized in 2 mL of distilled water, a 0.25 mL aliquot was diluted to 25 mL with acetate buffer solution pH 5.25. The cation radical $DMPD^{+\bullet}$ was formed by adding 50 mL of 0.5 M $FeCl_3$ solution and monitored at 505 nm [54, 72]. The conditions for assessing the antioxidant capacity of the extracts were the same of ABTS assay.

Antioxidant method selection and statistical analysis

The antioxidant method that delivers greater sensitivity and moderate cost will be selected, to perform the bioguided study to find the fraction or subfractions with high antioxidant activity, then, a tentative identification of the compounds responsible for the activity will be made using Gas Chromatography coupled to Mass Spectrometry (GC-MS). All experiments were done by triplicate (n = 3) and results were expressed as means ± standard deviations [73].

Gas Chromatography-Mass Spectrometry (GC-MS)

Analyses were performed in a gas chromatograph Agilent 6850 Series II Network System equipped with mass selective detector Agilent 5975B VL (Electron impact ionization, EI, 70 eV), a split/splitless injector (1:100 split ratio) and Enhanced ChemStation MSD D.03.00.52 data system, that included the spectral libraries Wiley and Nist. A fused-silica capillary column is a HP-1MS (30 m x 0.20 mm i.d, 0.33 µm film thickness) was used. Chromatographic conditions were: The GC oven temperature was programmed from 100 °C (2 min) to 285 °C (10 min) at 25 °C/min slope and post run to 320 °C (3 min). The temperatures the injection port, ionization chamber and the transfer line were set at 300, 185 and 285 °C, respectively. Helium (99.999%, AGA-Fano) was used as carrier gas, with 85 kPa column head pressure and linear velocity at constant flow (1 mL/min). Mass spectra and reconstructed (total) ion chromatograms were obtained by scanning in the mass range m/z 30-500 Da at 2.2 scan/s. Chromatographic peaks were checked for homogeneity with the aid of the mass chromatograms for the characteristic fragment ions and with the help of the peak purity program. Tentative identification criteria of compounds were based on coincidence percentage (≥ 85%) of obtained compounds compared to Wiley 7n.1 and Nist 05a.L mass spectra libraries [71].

Results

Obtaining extracts and fractions

Extraction and fraction yields for each plant organ are found in **Table 1**. Extract values between 19.8 and 45.6% were observed for flowers and tegument, respectively. Fractions highest yield corresponds to petroleum ether and EtOH-BuOH of fruits and tegument, 57.7 and 54.0%, respectively. The lower efficiency corresponds to leaves and testa (0.4 and 0.9%, respectively) where mixture of EtOH-BuOH solvents was used. The highest accumulated fraction (PE, CH_2Cl_2, EtOH-BuOH) corresponds to tegument (86.2%).

Antioxidant method selection and the best antioxidant fraction

The antioxidant capacity (AOC) for crude extracts (**Figure 2A**) and fractions (**Figure 2B**) was measured by different methods. Comparison of all methods using 50% inhibition (IC50) as criteria, indicates that all EtOH extract from testa exhibit high values of IC50, 164.3 ± 7.0, 239.5 ± 54.8 and 2929.0 ± 100.4 µg/mL by $ABTS^{+\bullet}$, $DPPH^{\bullet}$ and $DMPD^{+\bullet}$, respectively. Besides, the highest sensitivity method corresponds to $ABTS^{+\bullet}$ whose value 164.3 ± 7.0 µg/mL is statistically comparable with the values of standart antioxidants used as: Trolox (146.0 ± 4.2 µg/mL), vitamin C (135.6 ± 6.4 µg/mL), vitamin E (153.3 ± 9.3 µg/mL) and HBT (250.2 ± 13.9 µg/mL) (**Table 2A**). The comparison of antioxidant methods is shown in **Table 2B**. It was observed that $DMPD^{+\bullet}$ method is the least suitable to perform the AOC measurements because it is not stable under working conditions, adittionally, presents interferences and it is necessary to adjust the pH of the working solution precisely (pH 5.25). Moreover, between the $DPPH^{\bullet}$ and $ABTS^{+\bullet}$ methods were observed differences in the price per milligram, U$ 0.3 for $DPPH^{\bullet}$ against U$ 0.02 for $ABTS^{+\bullet}$. In the other hand, some antioxidants such as carotenoids present UV-Vis spectra like DPPH, which can lead to erroneous interpretations of AOA measurement, so this method could present interferences. Another difference between methods is solubility, $DPPH^{\bullet}$ is soluble in polar medium and $ABTS^{+\bullet}$ presents solubility in polar and non-polar medium, offering a great advantage to evaluate hydrophilic and liposoluble antioxidants [74].

$ABTS^{+\bullet}$ method was selected as the one with the highest sensitivity, the shortest work time, the lowest cost, the best solubility, and the highest stability to measure the antioxidant activity in the fractions and subfractions. Testa extract was selected with the purpose of looking for fractions and subfractions with potential antioxidant activity. The subfractions with the highest activity were analyzed using GC-MS.

Fractionation

The PE testa fraction with the highest antioxidant activity (IC50 = 164.3 ± 7.0 µg/mL, by $ABTS^{+\bullet}$) was subjected to fractionation and sub-fractionation, results that are recorded in **Table 3**. The fractionation of 2.49 g of the active fraction was performed on silica gel (146 g) using as eluent a

discontinuous gradient of 1,440 mL $CHCl_3$-MeOH [100: 0], followed by 2,880 mL [95:5], then 1,440 mL [70:30], and ending with 1,320 mL [5:95]. In total 59 fractions were collected (120 mL each) and monitored by TLC (normal phase) using $CHCl_3$-PE [80:20] and $CHCl_3$-MeOH [95: 5] as the mobile phase. According to the results of TLC, the fractions were regrouped into 10 groups registered as G1 (Fr.1-3), G2 (Fr.4-11), G3 (Fr.12-20), G4 (Fr.21-24), G5 (Fr.25-35), G6 (Fr.36-41), G7 (Fr.42-45), G8 (Fr.46-51), G9 (Fr.52-54) and G10 (Fr. Fr.55-59), which were evaluated by the $ABTS^{+\bullet}$. The bioguided work found the following groups of fractions with greater antioxidant activity: G2 (Fr.4-11), G3 (Fr.12-20) and G8 (Fr.46-51) with inhibition values of 49.3 ± 2.0, 45.9 ± 1.5 and 41.6 ± 1.2%, respectively (**Table 3**). For subfractionation of the groups with the highest activity fractions (G1, G2 and G8), a solvent gradient of Hexane-OEtAc-AcOH in the ratio of [90:10:1], [80:20:1] and [50:50:1] was used. The subfractions groups with higher antioxidant activity were: G1S1, G2S2 and G3S2 with 44.2 ± 3.1, 38.1 ± 3.9 and 41.6 ± 4.0% percentage of inhibition values of, respectively. GC-MS analyzed these sub-fractions to perform the tentative identification of the compounds responsible for the antioxidant activity.

Tentative identification of compounds by Gas Chromatography-Mass Spectrometry (GC-MS)

The tentative identification of compounds, with percentages of coincidence (> 85%) and area (1.0%) in the active subfractions is shown in **Table 4**.

Discussion

Table 4 shows the compounds identified in each of the active subfractions (G1S1, G2S2 and G3S2) analyzed by GC-MS. Identified compounds in subfractions G1S1 and G2S2 correspond to the family of saturated and unsaturated fatty acids in their ethyl ester form, such as ethyl oleate and ethyl ester of 9,12-octadecadienoic acid, among others. 3-pentadecylphenol compound was identified in G3S2 subfraction, with an 8.01 minutes t_R and molecular peak $[M]^+$ in m/z 304, corresponding to the phenolic lipids family also called long chain phenols (alkylphenols). These compounds are characteristic of the Anacardiaceae family [75-80]. However, only one compound (3-pentadecylphenol) from this fraction was identified by comparison with Wiley and Nist database spectra, the other five compounds are not found in the GC-MS equipment data library. However, the relative intensities of fragments in the mass spectra were collected to identify base and diagnostic ions of each family of compounds and with it the most probable structure for the 5 unidentified compounds in G3S2 fractions, see **Table 5**.

Analysis of fragmentation patterns of unidentified compounds

Compounds 1, 3, 4, 5 and 6 of G3S2 subfraction with retention times 7.96, 8.55, 8.64, 8.66 and 9.25 minutes, presented molecular ions $[M]^+$ in m/z 302, 328, 446, 332 and 358, respectively; a base ion in m/z 108 appears in all mass spectra which is typical for cardanols (**Table 5**). This fragment (m/z 108) is formed by a McLafferty type rearrangement in the phenolic ring and the fragment in m/z 107 of lower intensity corresponding to the hydroxypropyl ion formed by a direct beta break of the aromatic ring. The fragments in m/z 91 and 77 indicate the presence of a monosubstituted aromatic ring to an aliphatic chain [76, 81], see **Figure 3A**. Tyman, identified by Gas-Liquid Chromatography (GLC) and thin-layer chromatography (TLC), both coupled to mass spectrometry, homologs corresponding to lipid phenols of the family of cardanols. Length of the saturated alkyl chain was found in the order of C13, C15 and C17, whose molecular masses were 276, 304 and 332 uma's, for 3-tridecyl-phenol, 3-pentadecyl-phenol, and 3-heptadecyl-phenol, respectively [77]. Liu and Abreu (2006) identified 41 compounds belonging to the alkyl- and alkenyl-phenols (cardanols) family, characterized by GC-MS, NMR-^{13}C and NMR-1H techniques, by alpha, beta-thiomethylated derivatives. Researchers determined that, 3-pentadec(en)il-phenol and 3-nonadec(en)il-phenol compounds have 302 and 358 uma's, respectively [76].

Given the above and based on the pattern of fragmentation in the mass spectrum by electron impact (**Table 5**), it is possible to assign a structure to the unidentified compounds of the active fraction G3S2.

Compound 1, with t_R 7.96 minutes, $[M^{+\bullet}]$ 302, base peak at m/z 108 for cardanols, mono substituted aromatic group evidenced by ions in m/z

91, 77 and 65, unsaturated aliphatic chain due to ions CnH2n+1, CnH2n-1, and CnH2n-3 series in m/z 53, 55, 57, 67, 69, 79, 81 and 83, correlate for compound 3-pentadec(en)il-phenol [76, 82, 83]. **Compound 3**, with t_R 8.55 minutes, [$M^{+\bullet}$] 328, base peak at m/z 108 for cardanols, mono substituted aromatic group evidenced by ions in m/z 91, 77 and 65, unsaturated C17 aliphatic chain due to ions CnH2n+1, CnH2n-1 and CnH2n-3 series in m/z 53, 55, 57, 67, 69, 79, 81 and 83, correlate for the compound 3-heptadeca(dien)il-phenol [76, 77, 82, 83]. **Compound 4**, with t_R 8.64 minutes, [$M^{+\bullet}$] 330, base peak at m/z 108 for cardanols, mono substituted aromatic group evidenced by ions in m/z 91, 77 and 65, unsaturated aliphatic chain due to ions series CnH2n+1, CnH2n-1 and CnH2n-3 in m/z 53, 55, 57, 67, 69, 79, 81 and 83, correlate for the compound 3-heptadec(en)il-phenol [76, 77, 82, 83]. **Compound 5**, with t_R 8.66 minutes, [$M^{+\bullet}$] 332, base peak at m/z 108 for cardanols, mono substituted aromatic group evidenced by ions in m/z 91, 77 and 65, unsaturated aliphatic chain due to ions series CnH2n+1, CnH2n-1, and CnH2n-3 in m/z 53, 55, 57, 67, 69, 79, 81 and 83, correlate for the compound 3-heptadecyl-phenol (69, 71, 73, 74). Compound 6, with t_R 9.25 minutes, [$M^{+\bullet}$] 358, base peak at m / z 108 for cardanols, mono substituted aromatic group evidenced by the ions in m/z 91, 77 and 65, unsaturated aliphatic chain due to the series of ions CnH2n+1, CnH2n-1 and CnH2n-3 in m/z 53, 55, 57, 67, 69, 79, 81 and 83, correlate for the compound 3-nonadec(en)il-phenol [76, 77, 82, 83]. The active compounds present in the subfraction G3S2 tentatively identified by comparison with the spectra of the databases (Nist and Wiley), by fragmentation patterns and by bibliographic review, are part of the family of phenolic lipids corresponding to the alkyl phenols (3-pentadecyl-phenol, 3-heptadecyl-phenol) and alkenyl-phenols (3-pentadec(en)yl-phenol, 3-heptadeca(dien)il-phenol, 3-heptadec(en)yl-phenol, and 3-nonadec(en)il-phenol) [78-80], however for the latter it was not possible to assign the position of the double bonds .

Analysis of fragmentation patterns of identified compounds

In the subfractions G2S1 and G2S2 mass spectra (**Table 5**), typical fragmentation patterns are observed for ethyl esters of fatty acids (FAEE's), characterized by a base peak in m/z 88 that corresponds to an McLafferty arrangement, also the ions in m/z 269, 239, 73, 29 and 15 are from an alpha rupture [81, 84-87]. In **Figure 3B** the fragmentation pattern for hexadecanoic acid as ethyl ester is shown.

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References

1. Mabberley DJ (2017). Mabberley's Plant-book: A Portable Dictionary of Plants, Their Classification and Uses: Cambridge University Press.
2. Mitchell JD, Mori SA (1987). The Cashew and its Relatives (Anacardium: Anacardiaceae). *Memoirs of the New York Botanical Garden*, 7(42), 1-76.
3. Anacardiaceae (2017). Familia Anacardiaceae. Available from: http://www.arbolesornamentales.es/Anacardiaceae.htm.
4. ElSohly MA, Adawadkar PD, Ma C-Y, Turner CE (1982). Separation and Characterization of Poison Ivy and Poison Oak Urushiol Components. *Journal of Natural Products*, 45(5), 532-538.
5. Pell SP (2017). Anacardiaceae. Cashew family Brooklyn, New York, USA: Brooklyn Botanic Garden. Available from: http://tolweb.org/Anacardiaceae/21262.
6. De Pinto GL, Martinez M, Mendoza JA, Ocando E, Rivas C (1195). Comparison of Three Anacardiaceae Gum Exudates. *Biochemical Systematics and Ecology*, 23(2), 151-156.
7. Dong Z, Baas P (1993). Wood Anatomy of Trees and Shrubs from China. V. Anacardiaceae. *IAWA Journal*, 14(1), 87-102.
8. Salehi B, Gültekin-Özgüven M, Kırkın C, Özçelik B, Morais-Braga MFB, Carneiro JNP,

et al. (2019). Anacardium Plants: Chemical, Nutritional Composition and Biotechnological Applications. *Biomolecules*, 9(465).
9. Salehi B, Gultekin-Ozguven M, Kirkin C, Ozcelik B, Morais-Braga MFB, Carneiro JNP, et al. (2020). Antioxidant, Antimicrobial, and Anticancer Effects of Anacardium Plants: An Ethnopharmacological Perspective. *Frontiers in Endocrinology (Lausanne)*, 11, 295.
10. Duarte FND, Rodrigues JB, da Costa Lima M, Lima MdS, Pacheco MTB, Pintado MME, et al. (2017). Potential prebiotic properties of cashew apple (*Anacardium occidentale* L.) agro-industrial byproduct on Lactobacillus species. *Journal of the Science of Food and Agriculture*, 97(11), 3712-3719.
11. Moreira RF, Trugo LC, Pietroluongo M, De Maria CA (202). Flavor Composition of Cashew (*Anacardium occidentale*) and Marmeleiro (Croton species) Honeys. *Journal of Agricultural and Food Chemistry*, 50(26), 7616-7621.
12. Berardini N, Fezer R, Conrad J, Beifuss U, Carle R, Schieber A (2005). Screening of Mango (*Mangifera indica* L.) Cultivars for Their Contents of Flavonol O- and Xanthone C-glycosides, Anthocyanins, and Pectin. *Journal of Agricultural and Food Chemistry*, 53(5), 1563-1570.
13. Jincy M, Djanaguiraman M, Jeyakumar P, Subramanian KS, Jayasankar S, Paliyath G (2017). Inhibition of Phospholipase D Enzyme Activity Through Hexanal Leads to Delayed Mango (*Mangifera indica* L.) Fruit Ripening Through Changes in Oxidants and Antioxidant Enzymes Activity. *Scientia Horticulturae*, 218(Suppl. C), 316-325.
14. Flamini G, Bader A, Cioni PL, Katbeh-Bader A, Morelli I (2004). Composition of the Essential Oil of Leaves, Galls, and Ripe and Unripe Fruits of Jordanian *Pistacia palaestina* Boiss. *Journal of Agricultural and Food Chemistry*, 52(3), 572-576.
15. Sciubba F, Avanzato D, Vaccaro A, Capuani G, Spagnoli M, Di Cocco ME, et al. (2017). Monitoring of Pistachio (*Pistacia vera*) Ripening by High Field Nuclear Magnetic Resonance Spectroscopy. *Natural Product Research*, 31(7), 765-772.
16. Araujo NC (2008). Árboles Nativos Bogotá, Colombia. Available from: http://www.lablaa.org.
17. Salcedo ML, Pérez PY, Millán RE (2017). Asexual, Sexual Reproduction and Seed Vigor of Caracolí *Anacaridum excelsum* (Bertero ex Kunth) Skeels in the Sucre Department. *Revista Colombiana de Ciencia Animal*, 9(Supl 2), 336-344.
18. Salisbury CL, Potvin C (2015). Does Tree Species Composition Affect Productivity in a Tropical Planted Forest. *Biotropica*, 47(5), 559-568.
19. Losi CJ, Siccama TG, Condit R, Morales JE (2003). Analysis of Alternative Methods for Estimating Carbon Stock in Young Tropical Plantations. *Forest Ecology and Management*, 184(1), 355-368.
20. Longui EL, Gondo CCS, de Lima IL, Freitas MLM, Florsheim SMB, Zanatto ACS, et al. (2016). Some Properties of *Astronium graveolens* Wood Along the Stem. *Floresta e Ambiente*, 23(1), 142-149.
21. Piotto D, Víquez E, Montagnini F, Kanninen M (2004). Pure and Mixed Forest Plantations with Native Species of the Dry Tropics of Costa Rica: A Comparison of Growth and Productivity. *Forest Ecology and Management*, 190(2), 359-372.
22. Duvall CS (2006). On the Origin of the Tree *Spondias mombin* in Africa. *Journal of Historical Geography*, 32(2), 249-266.
23. Jiménez-Ferrer G, Velasco-Pérez R, Gómez MU, Soto-Pinto L (2008). Livestock and Local Knowledge of Fodder Trees and Shrubs in Lacandon Rainforest, Chiapas, Mexico. *Zootecnia Tropical*, 26(3), 333-337.
24. Chotphruethipong L, Benjakul S, Kijroongrojana K (2017). Optimization of Extraction of Antioxidative Phenolic Compounds from Cashew (*Anacardium occidentale* L.) Leaves Using Response Surface Methodology. *Journal of Food Biochemistry*, 41(4), e12379.
25. Razali N, Razab R, Junit SM, Aziz AA (2008). Radical Scavenging and Reducing Properties

of Extracts of Cashew Shoots (*Anacardium occidentale*). *Food Chemistry*, 111(1), 38-44.
26. Barcelos GRM, Shimabukuro F, Mori MP, Maciel MAM, Cólus ImdS (2007). Evaluation of Mutagenicity and Antimutagenicity of Cashew Stem Bark Methanolic Extract in vitro. *Journal of Ethnopharmacology*, 114(2), 268-273. doi: 10.1016/j.jep.2007.08.006
27. Kubo I, Ochi M, Vieira PC, Komatsu S (1993). Antitumor Agents From the Cashew (*Anacardium occidentale*) Apple Juice. *Journal of Agricultural and Food Chemistry*, 41(6), 1012-1015. doi: 10.1021/jf00030a035.
28. Islam MT, Ito T, Sakasai M, Tahara S (2002). Zoosporicidal Activity of Polyflavonoid Tannin Identified in Lannea coromandelica Stem Bark against Phytopathogenic Oomycete Aphanomyces cochlioides. *Journal of Agricultural and Food Chemistry*, 50(23), 6697-6703. doi: 10.1021/jf020554g.
29. Panda SK, Padhi L, Leyssen P, Liu M, Neyts J, Luyten W (2017). Antimicrobial, Anthelmintic, and Antiviral Activity of Plants Traditionally Used for Treating Infectious Disease in the Similipal Biosphere Reserve, Odisha, India. *Frontiers in Pharmacology*, 8:658. doi: 10.3389/fphar.2017.00658.
30. Barra A, Coroneo V, Dessi S, Cabras P, Angioni A (2007). Characterization of the Volatile Constituents in the Essential Oil of *Pistacia lentiscus* L. from Different Origins and its Antifungal and Antioxidant Activity. *Journal of Agricultural and Food Chemistry*, 55(17), 7093-7098. doi: 10.1021/jf071129w.
31. Catalani S, Palma F, Battistelli S, Benedetti S (2017). Oxidative Stress and Apoptosis Induction in Human Thyroid Carcinoma Cells Exposed to the Essential Oil from *Pistacia lentiscus* Aerial Parts. *PloS One*. 12(2), e0172138. doi: 10.1371/journal.pone.0172138.
32. Koutsoudaki C, Krsek M, Rodger A (2005). Chemical Composition and Antibacterial Activity of the Essential Oil and the Gum of *Pistacia lentiscus* Var. Chia. *Journal of Agricultural and Food Chemistry*, 53(20), 7681-7685. doi: 10.1021/jf050639s.
33. Mozaffari Nejada AS (2012). Global Pistachio Production and Marketing Challenges in Iran. *Acta Horticulturae*, 133-141. doi: 10.17660/ActaHortic.2012.963.23.
34. Tsokou A, Georgopoulou K, Melliou E, Magiatis P, Tsitsa E (2007). Composition and Enantiomeric Analysis of the Essential Oil of the Fruits and the Leaves of *Pistacia vera* from Greece. *Molecules*, 12(6), 1233-1239. doi: 10.3390/12061233.
35. Alma MH, Nitz S, Kollmannsberger H, Digrak M, Efe FT, Yilmaz N (2004). Chemical Composition and Antimicrobial Activity of the Essential Oils from the Gum of Turkish Pistachio (*Pistacia vera* L.). *Journal of Agricultural and Food Chemistry*, 52(12), 3911-3914. doi: 10.1021/jf040014e.
36. Smeriglio A, Denaro M, Barreca D, Calderaro A, Bisignano C, Ginestra G, et al. (2017). In Vitro Evaluation of the Antioxidant, Cytoprotective, and Antimicrobial Properties of Essential Oil from *Pistacia vera* L. Variety Bronte Hull. International Journal of Molecular Sciences, 18(6), 1212. doi: 10.3390/ijms18061212.
37. Nadeem M, Imran M, Khalique A (2016). Promising Features of Mango (*Mangifera indica* L.) Kernel Oil: A Review. *Journal of Food Science and Technology*, 53(5), 2185-2195. doi: 10.1007/s13197-015-2166-8.
38. Ribeiro SMR, Barbosa LCA, Queiroz JH, Knödler M, Schieber A (2008). Phenolic Compounds and Antioxidant Capacity of Brazilian Mango (*Mangifera indica* L.) Varieties. *Food Chemistry*, 110(3), 620-626. doi: 10.1016/j.foodchem.2008.02.067.
39. Sahu S, Das BK, Pradhan J, Mohapatra BC, Mishra BK, Sarangi N (2007). Effect of *Mangifera indica* Kernel as a Feed Additive on Immunity and Resistance to *Aeromonas hydrophila* in *Labeo rohita* Fingerlings. *Fish & Shellfish Immunology*, 23(1), 109-118. doi: 10.1016/j.fsi.2006.09.009.
40. Abu-Reidah IM, Jamous RM, Ali-Shtayeh MS (2014). Phytochemistry, Pharmacological Properties and Industrial Applications of *Rhus coriaria* L. (Sumac). *Jordan Journal of Biological Sciences*, 7(4), 233-244. doi: 10.12816/0008245.
41. Kosar M, Bozan B, Temelli F, Baser KHC (2007). Antioxidant Activity and Phenolic

Composition of Sumac (*Rhus coriaria* L.) Extracts. *Food Chemistry*, 103(3):952-959. doi: 10.1016/j.foodchem.2006.09.049.
42. Kurlan JG, Lucky AW (2001). Black Spot Poison Ivy: A Report of 5 Cases and a Review of the Literature. *Journal of the American Academy of Dermatology*, 45(2), 246-249. doi: 10.1067/mjd.2001.114295.
43. Berg C (2017). Homeopathy: Innovations in *Rhus toxicodendron*. Pharm Ztg, 162(20).
44. Cardinali C, Francalanci S, Giomi B, Caproni M, Sertoli A, Fabbri P (2004). Contact Dermatitis from *Rhus toxicodendron* in a Homeopathic Remedy. *Journal of the American Academy of Dermatology*, 50(1), 150-151. doi: 10.1016/s0190-9622(03)01476-2.
45. dos Santos AL, Perazzo FF, Cardoso LG, Carvalho JC (2007). In Vivo Study of the Anti-inflammatory Effect of *Rhus toxicodendron*. *Homeopathy: The Journal of the Faculty of Homeopathy*. 96(2), 95-101. doi: 10.1016/j.homp.2007.03.001.
46. Machado DG, Bettio LE, Cunha MP, Santos AR, Pizzolatti MG, Brighente IM, et al. (2008). Antidepressant-like Effect of Rutin Isolated from the Ethanolic Extract from *Schinus molle* L. in Mice: Evidence for the Involvement of the Serotonergic and Noradrenergic Systems. *European Journal of Pharmacology*, 587(1-3), 163-168. Doi: 10.1016/j.ejphar.2008.03.021.
47. Ferrero AA, Chopa CS, Gonzalez JO, Alzogaray RA (2007). Repellence and Toxicity of *Schinus molle* Extracts on *Blattella germanica*. *Fitoterapia*, 78(4), 311-314. doi: 10.1016/j.fitote.2006.11.021.
48. Ferrero AA, Werdin Gonzalez JO, Sanchez Chopa C (2006). Biological Activity of *Schinus molle* on *Triatoma infestans*. *Fitoterapia*, 77(5), 381-383. doi: 10.1016/j.fitote.2006.03.004.
49. Braca A, Politi M, Sanogo R, Sanou H, Morelli I, Pizza C, et al. (2003). Chemical Composition and Antioxidant Activity of Phenolic Compounds from Wild and Cultivated *Sclerocarya birrea* (Anacardiaceae) Leaves. *Journal of Agricultural and Food Chemistry*, 51(23), 6689-6895. doi: 10.1021/jf030374m.
50. Tanih NF, Ndip RN (2013). The Acetone Extract of *Sclerocarya birrea* (Anacardiaceae) Possesses Antiproliferative and Apoptotic Potential against Human Breast Cancer Cell Lines (MCF-7). *The Scientific World Journal*, 2013, 956206. doi: 10.1155/2013/956206
51. Ayoka AO, Akomolafe RO, Iwalewa EO, Akammu MA, Ukponmwan OE (2005). Studies on the Anxiolitic Effect of *Spondias mombin* L. (Anacardiaceae) Extracts. *African Journal of Traditional, Complementary and Alternative Medicines*, 2(2), 153-165.
52. Ayoka AO, Akomolafe RO, Iwalewa EO, Akanmu MA, Ukponmwan OE (2006). Sedative, Antiepileptic and Antipsychotic Effects of *Spondias mombin* L. (Anacardiaceae) in Mice and Rats. *Journal of Ethnopharmacology*, 103(2), 166-175. doi: 10.1016/j.jep.2005.07.019.
53. Gbolade AA, Adeyemi AA (2008). Anthelmintic Activities of Three Medicinal Plants from Nigeria. *Fitoterapia*, 79(3), 223-225. doi: 10.1016/j.fitote.2007.11.023
54. Sequeda-Castañeda LG (2008). Antioxidant Activity of Total Extracts and Fractions of the Plant Species *Anacardium excelsum* (Bert. & Balb. Ex Kunth) Skeel by spectrophotometric methods and composition of the active fraction by GC-MS. Bogotá, Colombia: Pontificia Universidad Javeriana.
55. STRI (2008). Smithsonian Tropical Research Institute. Avalaible from: https://stri.si.edu/
56. Urrea-Victoria V, Sequeda-Castañeda LG (2012). Evaluation of the Extracts of *Anacardium excelsum* (Anacardiaceae) as an Alternative to Food Preservation. *Vitae*, 19(1), S394-S396.
57. Correa JE, Bernal HY (1989). Especies Vegetales Promisorias de los Países del Convenio Andrés Bello: Secretaria Ejecutiva del Convenio Andres Bello.
58. Aguilar-Ortigoza C, Sosa V, Aguilar-Ortigoza M (2003). Toxic Phenols in Various Anacardiaceae Species. *Economic Botany*, 57(3), 354-356.
59. Celis C, García A, Sequeda G, Mendez G, Torrenegra R (2011). Antimicrobial Activity of Extracts Obtained from *Anacardium*

excelsum Againts Some Pathogenic Microorganisms. *Emirates Journal of Food and Agriculture*, 23(3), 249-257.
60. Diaz T, Villanueva M (1998). Preliminary Phytochemistry Investigation of the *Anacardium excelsum*: Ethanol Extract. Natura (Panamá), 3, 21-23.
61. Lizcano LJ, Bakkali F, Begoña Ruiz-Larrea M, Ignacio Ruiz-Sanz J (2010). Antioxidant Activity and Polyphenol Content of Aqueous Extracts from Colombian Amazonian Plants with Medicinal Use. *Food Chemistry*, 119(4), 1566-1570. doi: 10.1016/j.foodchem.2009.09.043.
62. Zeugin F, Potvin C, Jansa J, Scherer-Lorenzen M (2010). Is Tree Diversity an Important Driver for Phosphorus and Nitrogen Acquisition of a Young Tropical Plantation. *Forest Ecology and Management*, 260(9), 1424-1433. doi: 10.1016/j.foreco.2010.07.020.
63. Thangaraj P (2016). Pharmacological Assays of Plant-Based Natural Products: Springer International Publishing.
64. Pisoschi AM, Pop A, Cimpeanu C, Predoi G (2016). Antioxidant Capacity Determination in Plants and Plant-Derived Products: A Review. *Oxidative Medicine and Cellular Longevity*, 2016, 9130976. doi: 10.1155/2016/9130976.
65. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1997). Antioxidant Activity Applying an Improved ABTS Radical Cation Decolorization Assay. Free radical biology & medicine. , 26(9-10), 1231-12317. doi: 10.1016/S0891-5849(98)00315-3.
66. Dong JW, Cai L, Xing Y, Yu J, Ding ZT (2015). Re-evaluation of ABTS*+ Assay for Total Antioxidant Capacity of Natural Products. *Natural Product Communications*, 10(12), 2169-2172.
67. Rajurkar NS, Hande SM (2011). Estimation of Phytochemical Content and Antioxidant Activity of Some Selected Traditional Indian Medicinal Plants. *Indian Journal of Pharmaceutical Sciences*, 73(2), 146-151.
68. Brand-Williams W, Cuvelier ME, Berset C (1995). Use of a Free Radical Method to Evaluate Antioxidant Activity. *LWT - Food Science and Technology*, 28(1), 25-30. doi: 10.1016/S0023-6438(95)80008-5
69. Xie J, Schaich KM (2014). Re-Evaluation of the 2,2-diphenyl-1-picrylhydrazyl Free Radical (DPPH) Assay for Antioxidant Activity. *Journal of Agricultural and Food Chemistry*, 62(19), 4251-4260.
70. Asadujjaman M, Hossain A, Kumar-Karmakar U (2013). Assessment of DPPH free radical scavenging activity of some medicinal plants. *Pharmacologyonline*, 1, 161-165.
71. Sequeda-Castañeda LG, Celis CA, Uribe-Holguín C, Ortíz-Ardila AE, Romero-Arias AC (2016). Biossay-Guided Study in Leaves of *Pentacalia nitida* (Baskin) and *Pentacalia corymbosa* (Romerillo). *Pharmacologyonline*, 2, 28-37.
72. Fogliano V, Verde V, Randazzo G, Ritieni A (1999). Method for Measuring Antioxidant Activity and its Application to Monitoring the Antioxidant Capacity of Wines. *Journal of Agricultural and Food Chemistry*, 47(3), 1035-1040. doi: 10.1021/jf980496s.
73. Sabo R, Boone E (2013). Statistical Research Methods: A Guide for Non-Statisticians: Springer New York.
74. Karadag A, Ozcelik B, Saner S (2009). Review of Methods to Determine Antioxidant Capacities. *Food Analytical Methods*, 2(1), 41-60.
75. Kozubek A, Tyman JH (1999). Resorcinolic Lipids, the Natural Non-isoprenoid Phenolic Amphiphiles and Their Biological Activity. *Chemical Reviews*, 99(1), 1-26.
76. Liu Y, Abreu PJM (2006). Long Chain Alkyl and Alkenyl Phenols from the Roots of *Ozoroa insignis*. *Journal of the Brazilian Chemical Society*, 17, 527-532.
77. Tyman JHP (1977). Long-Chain Phenols: VIII. Quantitative Analysis of the Unsaturated Constituents of Phenolic Lipids by Thin-Layer Chromatography-Mass Spectrometry. *Journal of Chromatography A*, 136(2), 289-300.
78. Kozubek A, Tyman JHP (2005). Bioactive Phenolic Lipids. In: Atta ur R, editor. Studies in Natural Products Chemistry. 30: Elsevier; p. 111-90.

79. Stasiuk M, Kozubek A (2010). Biological Activity of Phenolic Lipids. *Cellular and Molecular Life Sciences*, 67(6), 841-860.
80. Hadacek F (2017). Phenolic Lipids in Plants: Functional Diversity of. In: Wenk MR, editor. Encyclopedia of Lipidomics. Dordrecht: Springer Netherlands, p. 1-4.
81. Stashenko E, Martínez JR (2010). Separate, Fragment and Integrate: The Routine of a Analysis by GC-MS. Fragmentation Patterns of Organic Molecules. *Scientia Chromatographica*, 2(2), 23-46.
82. Ramos F, Osorio C, Duque C, Cordero C, Aristizábal F, Garzón C, et al (2004). Estudio Químico de la Nez del Marañón Gigante (*Anacardium giganteum*). Revista de la Academia Colombiana de Ciencias Exactas, Físicas y Naturales, 28(109), 565-575.
83. Rodríguez W (2002). Composición de Ácidos Grasos Ligados a Fosfolípidos en Esponjas Marinas del Orden Halichondrida (Porifera, Demoespongiae), del Caribe Colombiano y su Significancia como Carácter Químico de Clasificación [Thesis]. Bogotá, Colombia: Universidad Nacional de Colombia.
84. Budzikiewicz H, Djerassi C, Williams DH (1965). Interpretation of Mass Spectra of Organic Compounds: Holden-Day.
85. Downard K (2007). Mass Spectrometry: A Foundation Course: Royal Society of Chemistry.
86. Kulig CC, Beresford TP, Everson GT (2006). Rapid, Accurate, and Sensitive Fatty Acid Ethyl Ester Determination by Gas Chromatography-Mass Spectrometry. *Journal of Laboratory and Clinical Medicine*, 147(3), 133-138.
87. McLafferty FW, Turecek F (1993). Interpretation Of Mass Spectra: University Science Books.
88. Smith RM (2004). Understanding Mass Spectra: A Basic Approach: Wiley.
89. Ross AB, Åman P, Andersson R, Kamal-Eldin A (2004). Chromatographic Analysis of Alkylresorcinols and Their Metabolites. *Journal of Chromatography A*, 1054(1), 157-64.

Table 1. Mass [g] of total extracts and fractions from *Anacardium excelsum*.

Part Plant	Dry weight	Extract (*)	Fraction (*)		
			PE	CH_2Cl_2	EtOH-BuOH
Integument	68.2	31.1 **(45.6)**	4.12 (20.6)	2.32 **(11.6)**	10.81 **(54.0)**
Seed	785.8	183.4 (23.3)	2.02 (4.1)	1.31 (2.6)	13.65 (27.3)
Fruit	50.2	19.4 (38.6)	8.65 **(57.7)**	1.01 (6.7)	2.01 (13.4)
Flower	336.4	66.7 **(19.8)**	6.15 (12.3)	5.45 (10.9)	2.31 (4.6)
Seed coat	309.1	65.4 (21.2)	8.52 (17.0)	8.95 (17.9)	0.45 **(0.9)**
Bark	349.4	95.6 (27.4)	1.61 **(3.2)**	1.21 **(2.4)**	12.35 (24.7)
Leave	416.8	97.5 (23.4)	2.71 (5.4)	6.62 (13.2)	0.21 **(0.4)**

* Percent yield (%Y). Amount to start the fractionation: 15 g (fruit), 20 g (integument), and 50 g (seed, flower, seed coat, bark, and leave). Bold number corresponds the higher and minor percent yield. PE: Petroleum ether. CH_2Cl_2: Dichloromethane. EtOH-BuOH: Ethanol-Butanol.

Table 2A. Antioxidant methods comparation: IC_{50} (µg/mL) *

Part plant	$ABTS^{+\bullet}$	$DPPH^{\bullet}$	$DMPD^{+\bullet}$
Integument	221.5 ± 8.8	271.6 ± 20.8	5728.6 ± 176.2
Seed	179.7 ± 6.1	257.3 ± 17.7	4082.9 ± 144.7
Fruit	228.0 ± 9.0	1288.9 ± 79.3	3324.7 ± 69.0
Flower	1599.0 ± 51.8	1497.1 ± 78.8	7995.1 ± 182.3
Seed coat	**164.3 ± 7.0**	**239.5 ± 54.8**	**2929.0 ± 100.4**
Bark	852.3 ± 22.8	1137.1 ± 74.3	3223.7 ± 114.2
Leave	927.1 ± 33.1	1114.1 ± 84.8	5241.4 ± 135.6
Trolox	146.0 ± 4.2	158.3 ± 2.4	319.8 ± 4.1
Vitamin E	153.3 ± 9.3	164.5 ± 4.0	347.3 ± 12.0
Vitamin C	135.6 ± 6.4	152.4 ± 5.3	337.5 ± 24.6
BHT	250.2 ± 13.9	270.1 ± 5.9	429.4 ± 25.1

*Average of four determinations in crude extracts. Bold number corresponds the best capacity antioxidant.

Table 2B. Antioxidant methods comparation: Variables

Variable	$ABTS^{+\bullet}$	$DPPH^{\bullet}$	$DMPD^{+\bullet}$
Stable	Yes	Yes	No
Radical	No	Yes	No
Time Ao [min]	6	20	10
Interference	No	Yes	Si
Solubility	Polar/Apolar	Apolar	Polar
Wavelength [nm]	744	517	505
pH adjustment	No	No	5.25
U$/mg	0.02	0.3	0.003

Table 3. % Inhibition for fractions and subfractions groups from the fraction in petroleum ether of Seed coad

Groups (Fractions)	**Mass [mg] [1]**	**% Inhibition [2]**
G1 (Fr.01 to Fr.03)	16.9	18.6 ± 1.4
G2 (Fr. 04 to Fr.11)	120.5	**49.3 ± 2.0 ***
G3 (Fr. 12 to Fr.20)	78.5	**45.9 ± 1.5 ***
G4 (Fr.21 to Fr.24)	1307.1	15.3 ± 1.1
G5 (Fr.25 to Fr.35)	115.2	20.4 ± 1.1
G6 (Fr.36 to Fr.41)	51.4	24.4 ± 1.2
G7 (Fr.42 to fr.45)	106.5	16.1 ± 1.5
G8 (Fr.46 to Fr.51)	75.9	**41.6 ± 1.2**
G9 (Fr.52 to Fr.54)	50.0	21.1 ± 1.5
G10 (Fr.55 to Fr.59)	21.9	7.1 ± 1.2
Group G2 (Subfractions)	**Mass [mg] [1]**	**% Inhibition [2]**
G2S1 (Subfr. 01)	53.1	**44.2 ± 3.1 ***
G2S2 (Subfr. 02)	9.4	**38.1 ± 3.9 ***
G2S3 (Subfr. 03 to Subfr.12)	5.6	17.6 ± 2.3
Group G3 (Subfractions)	**Mass [mg] [1]**	**% Inhibition [2]**
G3S1 (Subfr. 01)	21	4.9 ± 0.7
G3S2 (Subfr. 02)	38.7	**41.6 ± 4.0 ***
G3S3 (Subfr. 03)	14.7	19.0 ± 1.6
G3S4 (Subfr. 04 to Subfr.12)	4.1	3.7 ± 0.4
Group G8 (Subfractions)	**Mass [mg] [1]**	**% Inhibition [2]**
G8S1 (Subfr. 01 to subfr.09)	2.1	4.4 ± 1.0
G8S2 (Subfr. 10 to Subfr.16)	4.3	8.7 ± 1.6
G8S3 (Subfr. 17 to Subfr.24)	12.4	17.5 ± 1.6
G8S4 (Subfr. 25 to Subfr.30)	52.5	11.9 ± 1.6

[1] Mass obtained by grouping of fractions or subfractions.

[2] Average for four determinations (100 µg/mL).

***** In bold number corresponds the best capacity antioxidant.

Table 4. Identification tentative of compounds in active subfractions [1] from *Anacardium excelsum* by GC-MS (Databases Wiley 7n.1 and Nist 05a.L.) [2]

Subfraction G2S1				Subfraction G2S2			
No.	**t_R [min]**	**[$M^{+\bullet}$]**	**Compound**	**No.**	**t_R [min]**	**[$M^{+\bullet}$]**	**Compound**
1	6.31	284	Hexadecanoic acid, ethyl ester	1	6.31	284	Hexadecanoic acid, ethyl ester
2	6.68	298	Heptadecanoic acid, ethyl ester	2	6.91	308	9,12-Octadecadienoic acid, ethyl ester
3	6.91	308	Linoleic acid ethyl ester	3	6.95	310	Ethyl Oleate
4	6.96	310	Ethyl Oleate	4	7.05	312	Octadecanoic acid, ethyl ester
5	7.02	312	Hexadecanoic acid, butyl ester	**Subfraction G3S2**			
6	7.05	312	Octadecanoic acid, ethyl ester	1	7.96	302	[3] Phenol, 3-pentadec(en)yl-
7	7.61	338	(Z)-9-Octadecenoic acid butyl ester	2	8.01	304	Phenol, 3-pentadecyl-
8	7.70	340	Octadecanoic acid, 2-methylpropyl ester	3	8.55	328	[3] Phenol, 3-heptadeca(dien)yl-
9	7.73	340	Eicosanoic acid, ethyl ester	4	8.64	446	[3] Phenol, 3-heptadec(en)yl-
10	8.38	368	Docosanoic acid, ethyl ester	5	8.66	332	[3] Phenol, 3-heptadecyl-
11	8.98	396	Ethyl tetracosanoate	6	9.25	358	[3] Phenol, 3-nonadec(en)yl-

[1] Active subfractions of petroleum ether fraction of Seed Coat.

[2] Matching percentage >85%. Percentage of area >1.0%.

[3] Assignment given by the fragmentation pattern (**see Table 5, Figure 3**).

Table 5. Relative intensities (%) of characteristic fragments in the mass spectra of the active subfractions*

Subfraction G2S1

No.	t_R [min]	[$M^{+\bullet}$]	Base peak	Diagnostic ions, m/z (%)	Other ions, mz (%)
1	6.31	284	88(100)	101(63), 157(23), 55(21), 73(16)	57(16), 284(15), 70(15), 239(14), 69(13), 83(10), 143(9), 61(9), 199(8)
2	6.68	298	88(100)	101(65), 55(49), 69(33), 83(26)	97(24), 57(24), 74(21), 157(20), 70(20), 87(18), 84(18), 98(17), 67(17)
3	6.92	308	67(100)	81(94), 95(73), 79(57), 55(57)	82(42), 96(37), 68(37), 109(36), 69(32), 54(29), 80(29), 93(25), 263(22)
4	6.96	310	55(100)	69(75), 83(67), 88(67), 97(63)	96(60), 101(57), 264(56), 84(56), 265(54), 98(50), 67(47), 81(46), 222(37)
5	7.02	312	56(100)	257(77), 57(54), 239(38), 73(36)	55(36), 129(30), 60(25), 69(22), 71(20), 83(19), 61(18), 101(17), 97(15)
6	7.05	312	88(100)	101(65), 157(23), 55(23), 89(20)	57(18), 312(17), 269(16), 73(15), 70(14), 69(14), 83(11), 267(10), 213(9)
7	7.61	338	55(100)	69(80), 83(78), 97(74), 57(68)	265(66), 98(52), 56(49), 96(48), 84(48), 81(48), 67(48), 264(44), 111(40)
8	7.70	340	56(100)	55(62), 57(61), 285(41), 69(39)	73(36), 83(33), 97(30), 129(28), 101(25), 71(25), 60(25), 98(17), 61(17)
9	7.73	340	88(100)	101(66), 55(24), 89(23), 57(23)	157(21), 69(16), 340(14), 70(14), 73(14), 83(13), 97(11), 71(11), 143(8)
10	8.38	368	88(100)	101(72), 89(27), 57(27), 55(24)	69(18), 368(17), 83(14), 70(14), 73(13), 71(13), 97(12), 143(8), 85(8)
11	8.98	396	88(100)	57(32), 89(29), 55(26), 157(25)	69(20), 396(19), 83(16), 71(16), 97(14), 73(14), 70(14), 353(9), 85(9)

Subfraction G2S2

No.	t_R [min]	[$M^{+\bullet}$]	Base peak	Diagnostic ions, m/z (%)	Other ions, mz (%)
1	6.31	284	88(100)	101(62), 157(21), 55(21), 241(16)	89(16), 73(16), 70(15), 57(15), 284(13), 69(13), 239(12), 83(10) , 61(9)
2	6.91	308	67(100)	81(94), 95(74), 55(58), 79(54)	82(43), 96(39), 68(38), 109(36), 69(33), 54(29), 80(28), 93(24), 110(22)
3	6.95	310	55(100)	69(78), 88(66), 97(64), 96(60)	101(55), 84(55), 98(49), 67(47), 264(46), 81(46), 265(45), 95(38), 70(35)
4	7.05	312	88(100)	101(62), 55(22), 157(21), 89(19)	57(18), 73(15), 70(14), 69(14), 269(13), 312 (11), 83(11), 97(9), 213 (8)

Subfraction G3S2

No.	t_R [min]	[$M^{+\bullet}$]	Base peak	Diagnostic ions, m/z (%)	Other ions, mz (%)
1	7.96	302	108(100)	107(38), 120(18), 55(13), 121(10)	109(8), 302(6), 147(6), 77(6), 133(5), 69(5), 161(3), 149(3), 79(3)
2	8.01	304	108(100)	107(38), 304(10), 121(9), 109(8)	120(5), 149(4), 77(4), 55(4), 305(2), 150(2), 91(2), 79(2), 57(2)
3	8.55	328	108(100)	107(83), 120(47), 147(39), 67(37)	81(28), 95(23), 133(21), 79(19), 109(18), 55(18), 77(17), 328 (16), 121(16)
4	8.64	330	108(100)	107(38), 55(12), 330(10), 121(10)	109(8), 147(6), 133(5), 77(5), 69(5), 331(3), 161(3), 149(3), 67(3)
5	8.66	332	108(100)	107(33), 121(9), 109(8), 332(7)	120(7), 55(6), 149(4), 77(4), 133(3), 333(2), 330(2), 150(2), 147(2), 95(2)
6	9.25	358	108(100)	107(38), 120(16), 55(13), 121(10)	358(9), 109(8), 147(6), 69(6), 133(4), 77(4), 359(3), 149(3), 83(3)

* Active subfractions of petroleum ether fraction of Seed Coat from *Anarcadium excelsum*.

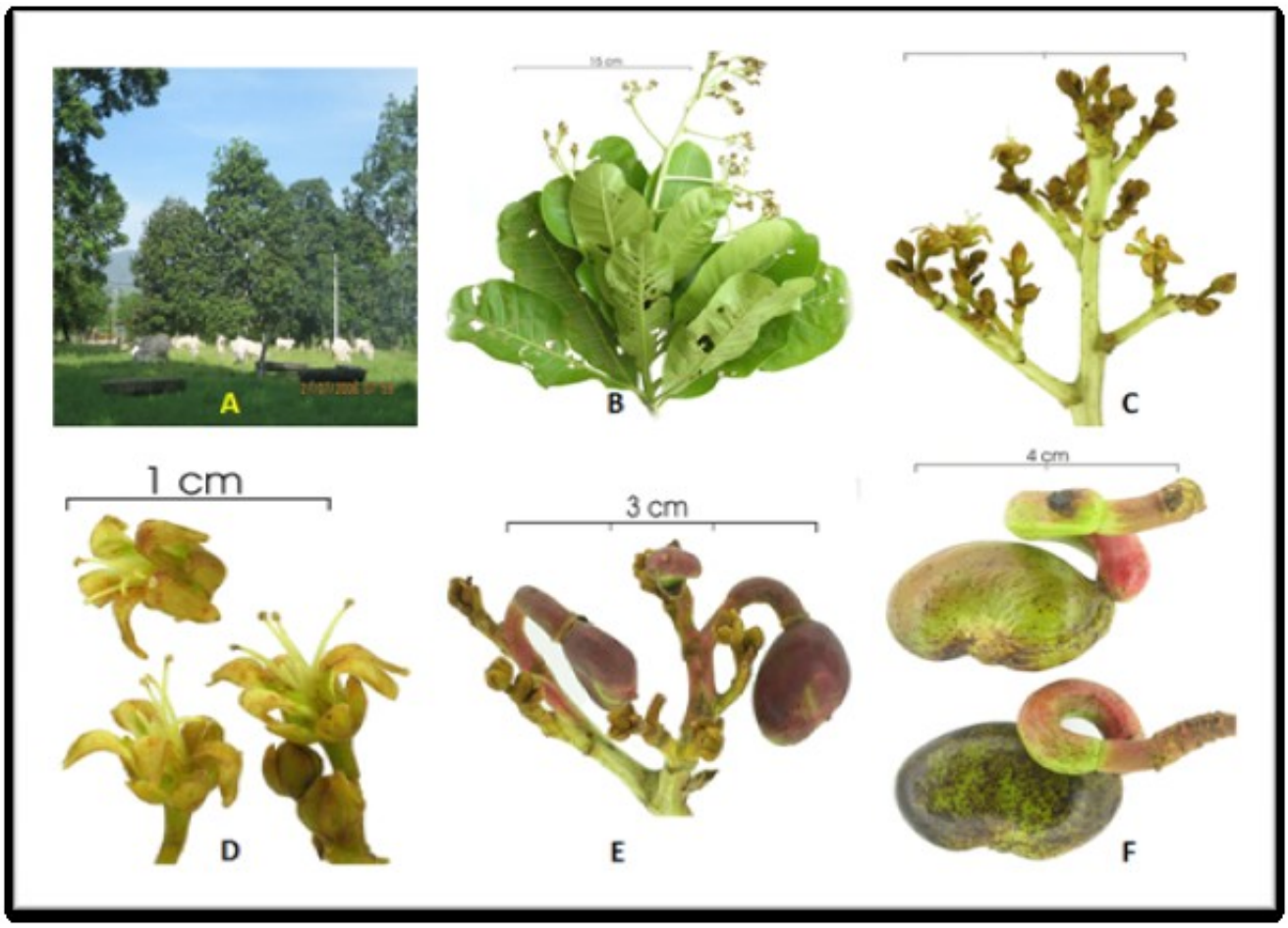

Figure 1. *Anacardium excelsum*: Tree (A). Leaves and flower-bud (B). Inflorescences (C). Flowers (D). Infrutescences (E). Fruits and seed-wet (F). Taken from Smithsonian Tropical Research Institute (B-F, 2016)

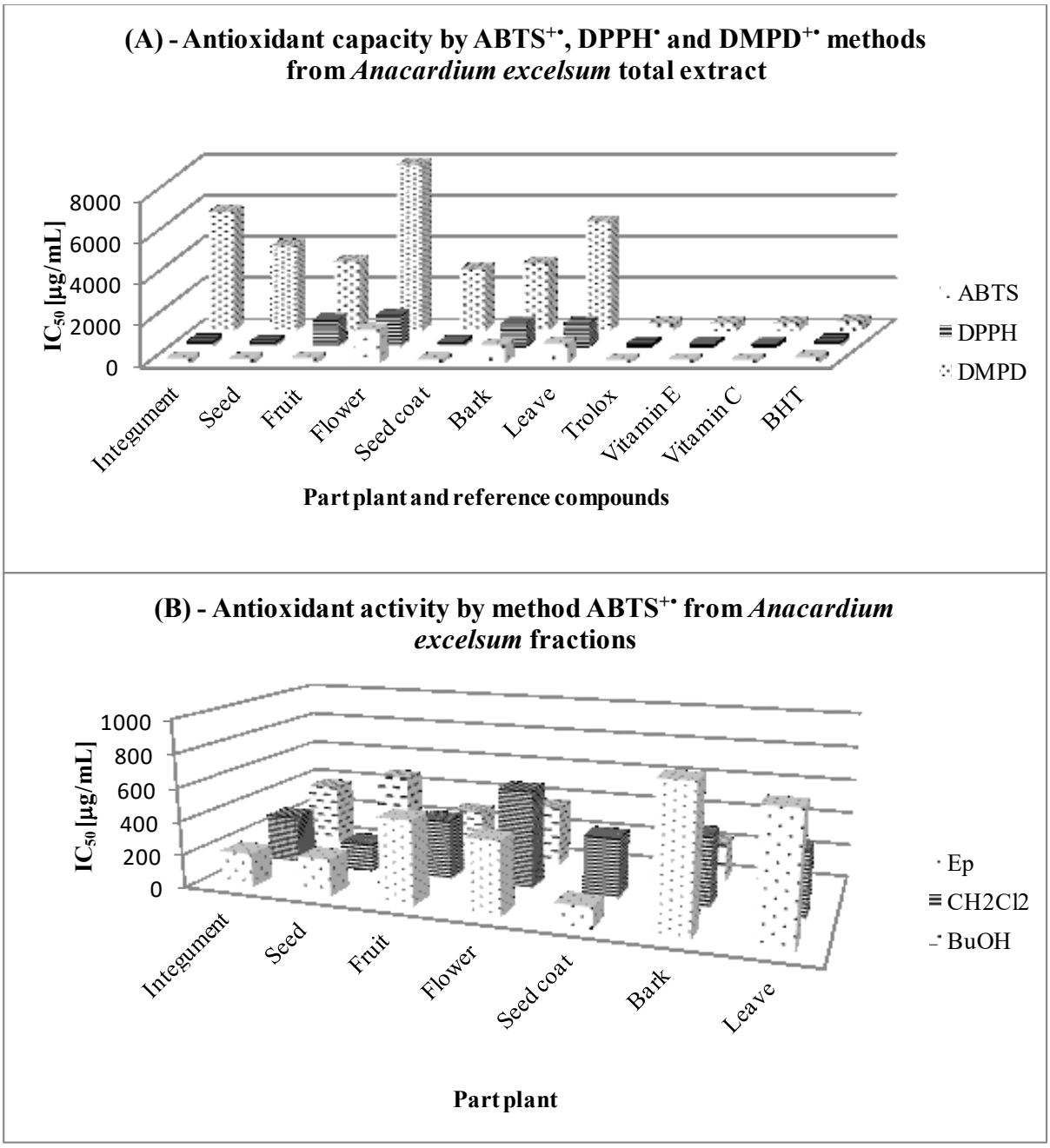

Figure 2. Capacity antioxidant by ABTS$^{+\bullet}$, DPPH$^{\bullet}$ and DMPD$^{+\bullet}$ methods from *Anacardium excelsum* total extract **(A).** Activity antioxidant by method ABTS$^{+\bullet}$ from *Anacardium excelsum* fractions **(B)**

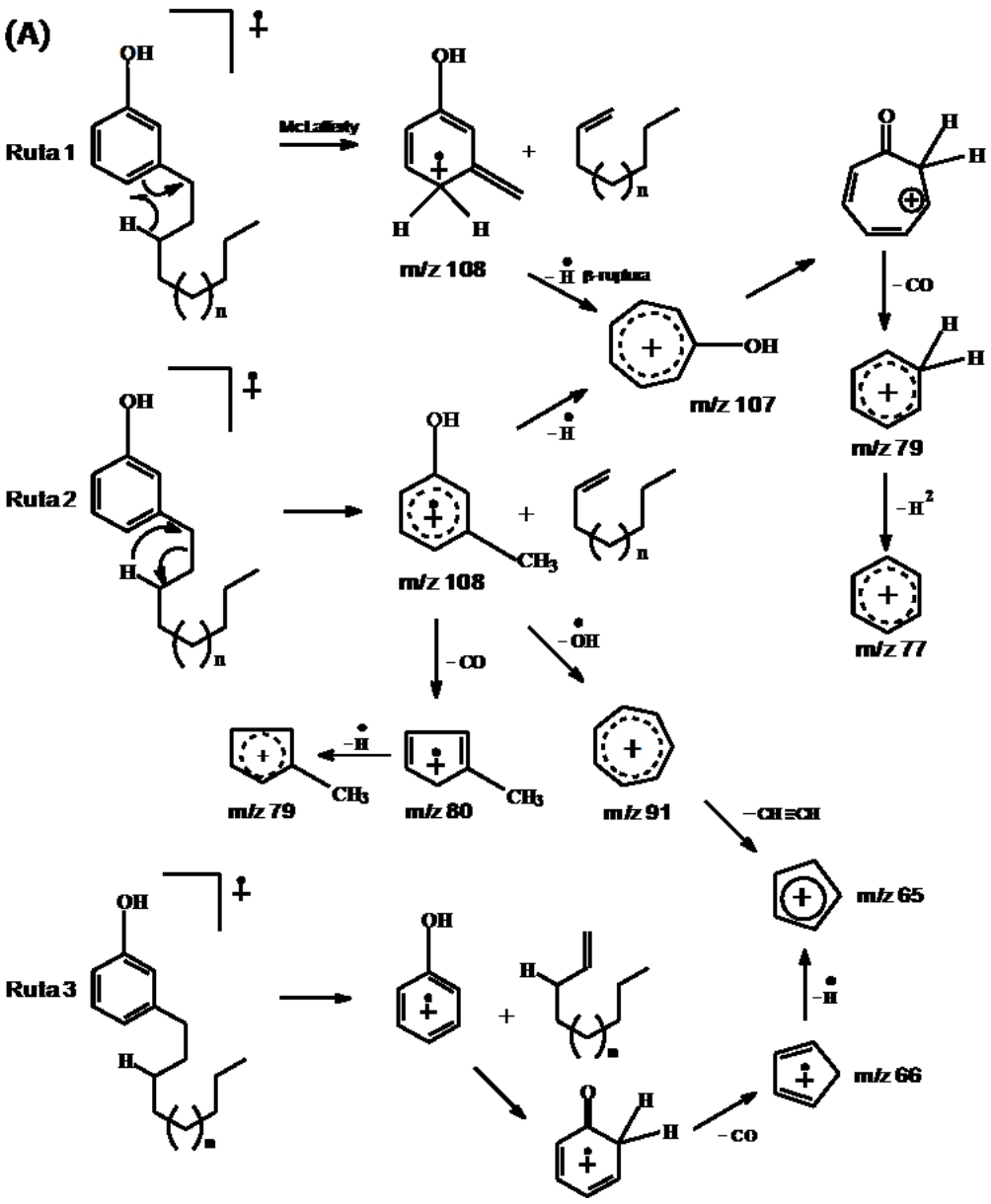

Figure 3 A. Fragmentation pattern for phenolic lipids [76, 81, 84, 85, 87-89].

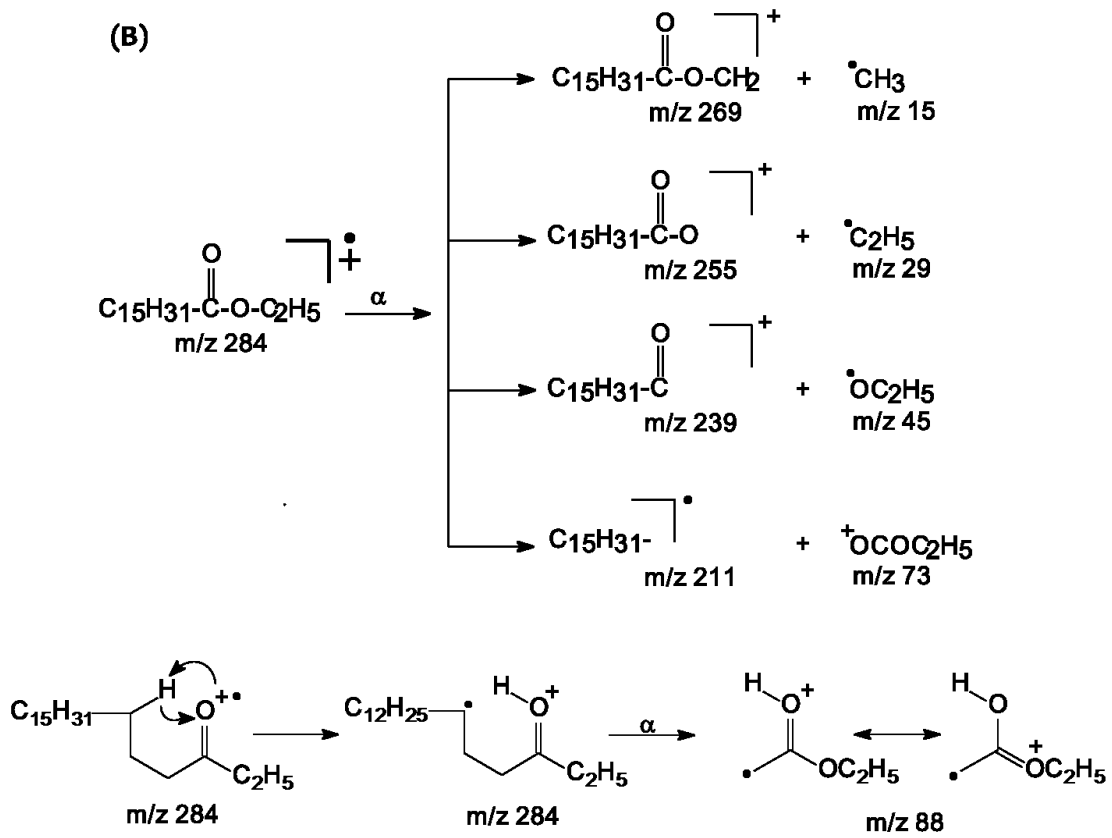

Figure 3 B. Fragmentation pattern for ethyl esters of fatty acids (hexadecanoic acid) [76, 81, 84, 85, 87-89].