GC-MS ANALYSIS OF BIO-ACTIVE COMPOUNDS IN METHANOL EXTRACTS OF *PRUNUS DULCIS* (ALMOND) LEAVES AND HULL.

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ABSTRACT

Almonds are tree nuts recognized as a healthy snack and known to be a good source of protein, monosaturated fatty acids and dietary fiber. The purpose of the study is to identify some bio-active substances in methanol extracts of Prunus dulcis leaves and hull. The leaves and hull of Prunus dulcis were collected at Abraham Adesanya Polytechnic, Abusi Campus, of Abraham Adesanya Polytechnic, Ijebu Igbo, Ogun state. They were sun dried for seven days, dried in an oven at 103 ^{0}C. The dried leaves and hull samples were pulverized into fine powders with mortar and pestle while 2 mm mesh sieve was used to filter the sample. Twenty grams of the samples were extracted with 250 mL of methanol at 60 ^{o}C for 8 hours using soxhlet extractor. The methanol extracts of Prunus dulcis leaves and hull were filtered through Whatmann No. 1 filter paper respectively. The compounds identified from GC-MS analysis of the leaves extract were 2-Pyrrolidinone (3.273%), 1-methyl, Phorone (5.603%), Cyclohexanecarboxlyic acid, (23.995%), Diethyl Phthalate (15.456%), Ar-Tumerone (2.758%), Cyclopentadecanone (2.295%), Oleic Acid (2.408%), Cis-7, cis-11-hexadecadien-1-yl acetate (1.268%), 3-Eicosene (2.581%), 2-Propionyloxytetradecane (4.903%). Eucalypo (1.320%), Caryophyllene (2.616%), Humullene (0.514%), Ar-Tumerone (0.318%), 9, 12-octadecadienoic acid (0.730%), and tricyclo[2.2.1.0,(2,6)] heptane 1,3 were confirmed in methanol extract of the hull. Ar-Tumerone which is present in Prunus dulcis leaves and hull can be used as dietary supplement for variety of conditions like arthritis, digestive disorder and respiratory infections.

Key words: GC-MS, *Prunus dulcis*, Methanol, Leaves and Extracts.

INTRODUCTION

Prunus dulcis (Almond) belongs to the family *Rosaceae*, Traditionally, almonds were placed in *Prunoideae* (sub-family), but most of the time, they are kept in their own family (*Amygdalaceae* or *Prunaceae*) Ayesha et al (2015). Commercially, two major kinds of almonds are cultivated, which can be classified as bitter almonds (*Prunus amygdalus amara*) and sweet almonds (*Prunus amygdalus* dulcis). Bitter and sweet almond producing plants can be identified on the basis of their flower color. Bitter almonds produce flowers of pinkish color, while flowers of sweet almonds are white Hari (2012). *Prunus dulcis* (Almond) leaves are lanceolate, 3 to 5cm wide, 4-13cm long, having serrated tips. Pale pink or white flowers having diameter (3-5cm), are produced singly or in form of clusters, in early spring. Almond hulls are anatomically similar to the fleshy portion of peach that humans consume. Almonds are a good source of nutrients which are associated with the health of the heart, such as vitamin E, mono unsaturated fatty acids, poly-unsaturated fatty acids (PUFA), arginine, and potassium. Almonds are among the richest food sources of vitamin E, as RRR-α-

tocopherol. Almonds also contain a variety of phenolic compounds which are localized principally in their skin, including flavonols (isorhamnetin, kaempferol, quercetin, catechin and epicatechin), flavanones (naringenin), anthocyanins (cyanidins and delohinidin), procyanidins, and phenolic acids (caffeic acid, ferulic acid, P-coumaric acid and Vanillic acid) Frison & Sporns (2002). Almonds can be eaten whole (fresh or roasted) and in spreads like almond butter or they can be used in awide range of food products and recipes. Almond leaves and hull are basically considered as waste until Aguilar et a1 (1984) reported the importance of almond hull in feedstuff for dairy cattle. This preliminary study is aimed at determining some active compounds in the leaves and hull of *Prunus dulcis* (Almond) with the use of Gas chromatography Mass Spectrophotomer (GC-MS).

METHODOLOGY

Prunus dulcis leaves and hull were identified with the help of a botanist in Department of Science Laboratory Technology, Abraham Adesanya Polytechnic, Ijebu igbo. The leaves and the hull were collected at Abraham Adesanya Polytechnic, Abusi Campus, of Abraham Adesanya Polytechnic, Ijebu Igbo, Ogun state. The collected samples were sun dried for seven days and oven dried for 103 ^{o}C. The dried leaves and hull samples were pulverized into fine powder with mortar and pestle while 2 mm mesh sieve was used to filter the samples to obtain fine powdery samples.

EXTRACTION OF PLANT MATERIALS

Plant samples of 20 g were extracted with 250 ml of methanol at 60 ^{0}C for 8 hrs in soxhlet extractor. The methanol extracts were filtered through whatmann NO. 1 filter paper. The filter was evaporated to dryness at 80 ^{0}C and sorted for further analysis. The extracts were reconstituted in methanol.

GAS CHROMATOGRAPHY – MASS SPECTROMETRY

The methanol extracts of the leaves and hull of *Prunus dulcis* were subjected to GC-Ms analysis on a GC-MS clarus 500 perkin Elmer system comprising a AOC-20i autosampler and gas chromatography interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: column Elite-1 fused silica capillary column (30mm x 0.25 ID x 1 mdf, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70 ev; helium (99.999%) was used as carrier gas at a constant flow of 1ml/min and an injection volume of 0.51 was employed (Split ratio of 10:1); injector temperature 250 ^{0}C. The oven temperature oven was programmed from 110C (isothermal for 2min), with an increase of 10c/min to 200 ^{0}C. 5c/min to 280 ^{o}C, ending with a 9 min isothermal at 280 ^{o}C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 40 to 550 Da. The mass spectra of the separated components were compared with those stored in the NIST data base (NIST version 2.1).

RESULTS

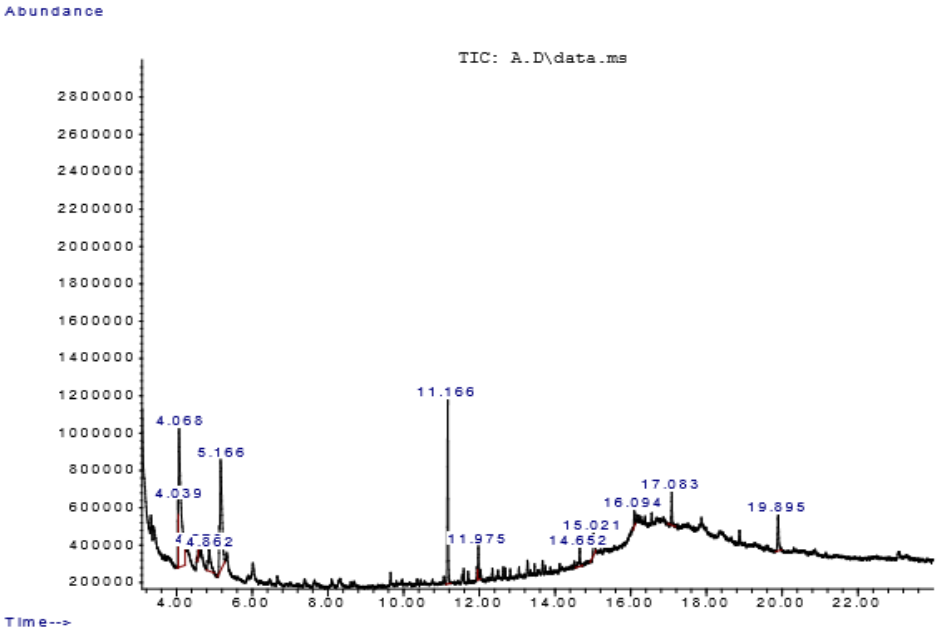

Figure 1: GC-MS spectra of methanol extract of *Prunus dulcis* Leaves.

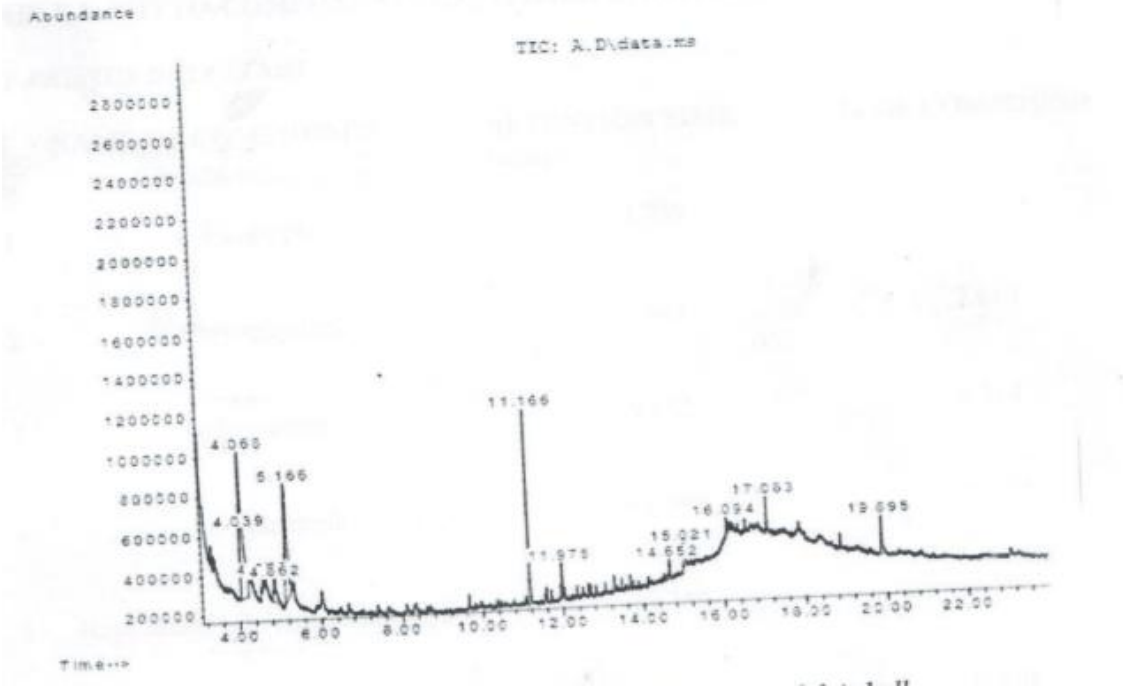

Figure 2: GC-MS spectra of methanol extract of *Prunus dulcis* Hull.

TABLE 1: BIO – ACTIVE COMPOUNDS IDENTIFIED IN METHANOL EXTRACT OF *PRUNUS DULCIS* LEAVES.

S/N	NAME OF COMPOUNDS	RETENTION TIME (mins)	% CONCENTRATION
1	2-Pyrrolidinone, 1-methyl	4.039	3.273
2	Phorone	4.862	5.603
3	Cyclohexanecarboxlyic acid, 1-ethyl- methyl ester	5.166	23.995
4	Diethyl Phthalate	11.166	15.456
5	Ar-Tumerone	11.975	2.758
6	Cyclopentadecanone	14.652	2.295
7	Oleic Acid	15.021	2.408
8	Cis-7, cis-11-hexadecadien-1-yl acetate	16.094	1.268
9	3-Eicosene	17.083	2.581
10	2-Propionyloxytetradecane	19.895	4.903

TABLE 2: BIO – ACTIVE COMPOUNDS IDENTIFIED IN METHANOL EXTRACT OF

S/N	NAME OF COMPOUNDS	RETENTION TIME (mins)	% CONCERNTRATION
1	Eucalyptol	3.755	1.320
2	Caryophyllene	9.081	2.616
3	Humulene	9.512	0.514
4	Ar-Tumerone	11.990	0.318
5	9, 12-Octadecadienoic acid	16.400	0.730
6	Tricyclo [2.2.1.0,(2,6)] heptane 1,3	4.634	0.730

***PRUNUS DULCIS* HULL**

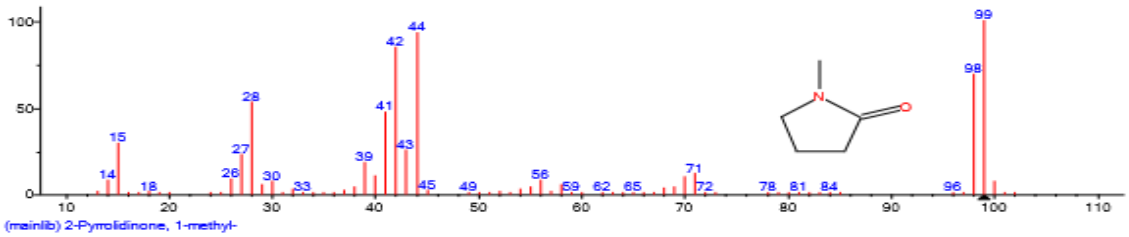

Figure 3: Mass spectra of Pyrrolidinone, 1-methyl

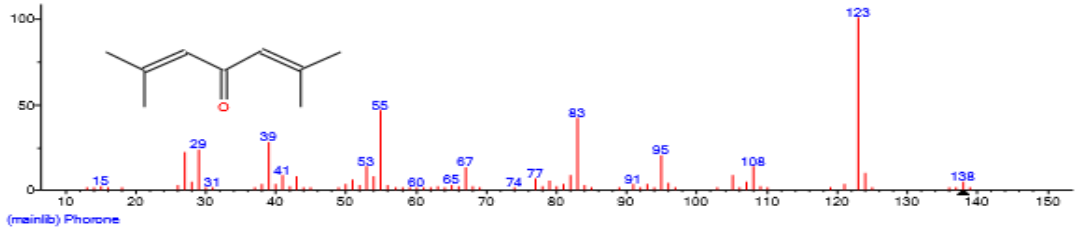

Figure 4: Mass spectra of Phorone

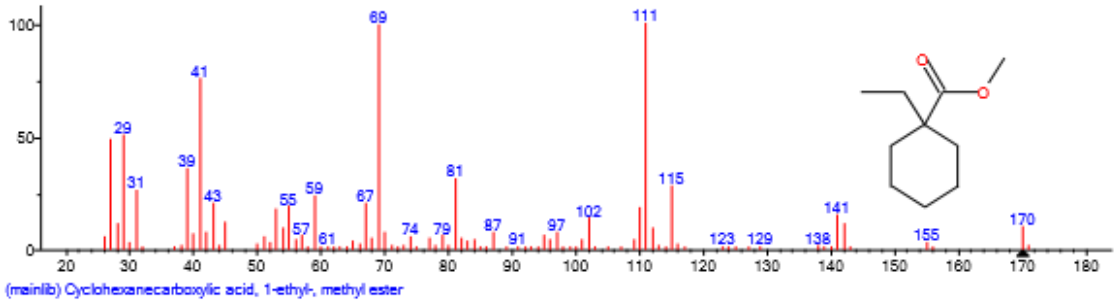

Figure 5: Mass spectra of Cyclohexanecarboxlyic acid, 1-ethyl, methyl ester

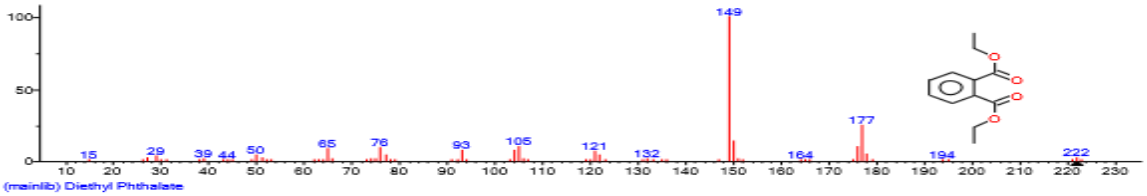

Figure 6: Mass spectra of Diethyl Phthalate

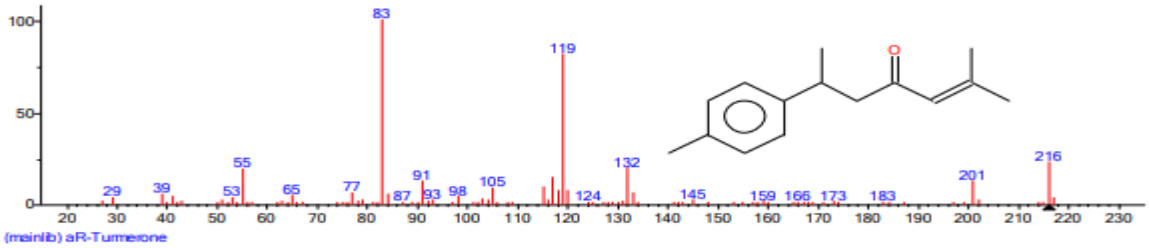

Figure 7: Mass spectra of Ar-Tumerone

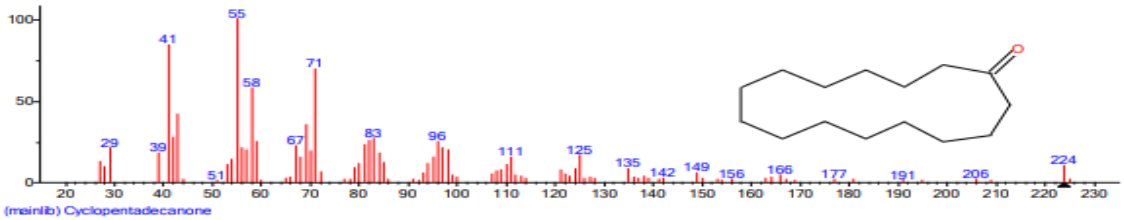

Figure 8: Mass spectra of Cyclopentadecanone

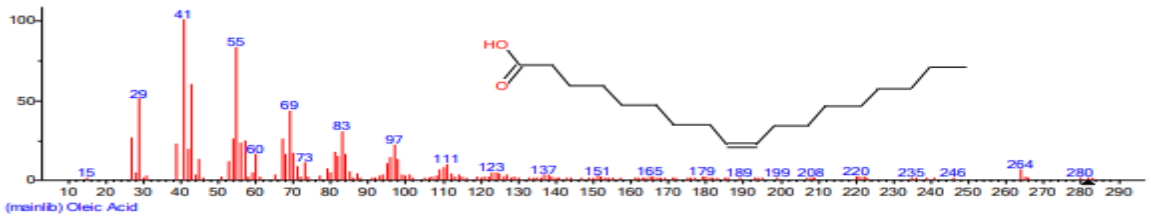

Figure 9: Mass spectra of Oleic Acid

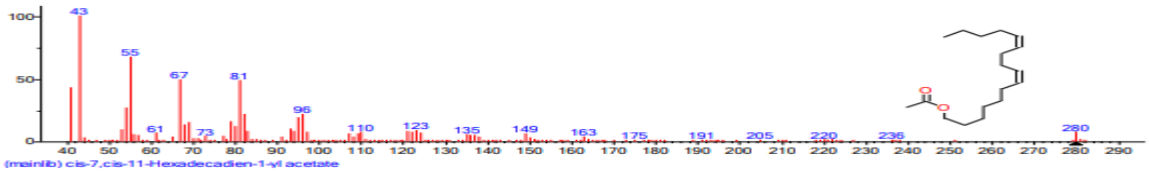

Figure 10: Mass spectra of Cis-7, cis-11-hexadecadien-1-yl acetate

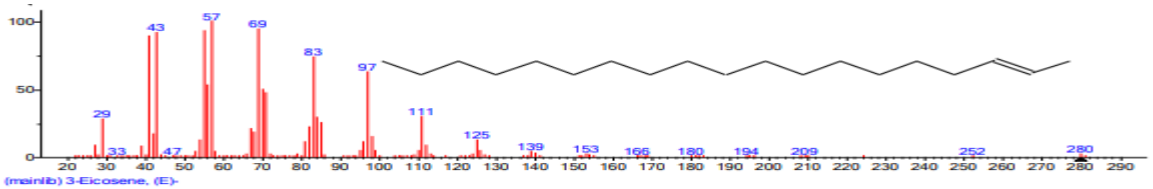

Figure 11: Mass spectra of 3-Eicosene

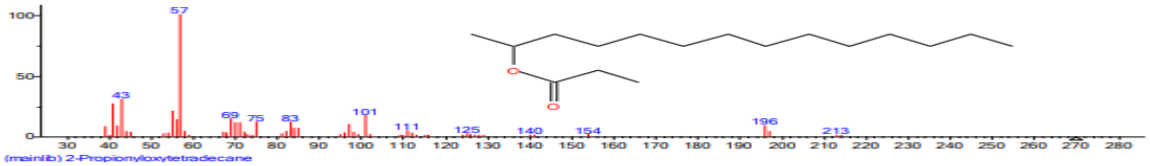

Figure 12: Mass spectra of 2-Propionyloxytetradecane

DISCUSSION

Bio-active compounds present in the methanol extract of *Prunus dulcis* leaves and hull were identified by gas chromatography-mass spectrometry (GC-MS) as shown in the chromatogram in Figure 1 and 2 respectively.. These compounds with their retention time and concentration were presented in Table 1. The major components present in P*runus dulcis* Leaves were 2-Pyrrolidinone, 1-methyl (3.273%), 1-methyl, Phorone (5.603%), Cyclohexanecarboxlyic acid, 1-ethyl- methyl ester (23.995%), Diethyl Phthalate (15.456%), Ar-Tumerone (2.758%), Cyclopentadecanone (2.295%), Oleic Acid (2.408%), Cis-7, cis-11-hexadecadien-1-yl acetate (1.268%), 3-Eicosene (2.581%), 2-Propionyloxytetradecane (4.903%). Oleic acid induced hepatocellular lipotoxicity Zeng, et al (2020). It can act as a carrier for anti-cancer drugs. Oleic acid regulates female fertility Fayezi, et al (2018) and it helps in germ cell growth and development. Diethyl Phthalate is used in industries to produce plastics, insecticides, cosmetics, aspirin Caldeirão, et al (2021). 2-Pyrrolidinone, 1-methyl is a solvent used in variety of industries and applications such as paint and coating removal, petrol chemical processing, engineering plastic coating, and agricultural chemicals (https://www.ataman chemicals.com). Cyclohexanecarboxlyic acid, 1-ethyl- methyl ester is an anti-cancer according to McGinty, et al (2011) reported that cyclopentadecanone is used as a fragrance ingredient. Eucalyptol (1.320%), Caryophyllene (2.616%), Humulene (0.514%), Ar-tumerone (0.318%), 9, 12-Octadecadienoic acid (0.730%), Tricyclo [2.2.2.0 (2,6)] heptane, 1, 3 (2.228%) were the compounds found in *Prunus dulcis* Hull as shown in Table 2. Eucalyptol has diverse biological activities such as anti-inflammatory, decongestant, anti-nociceptive and insect repellent Juergen et al (2004). Caryophyllene pocess anti-inflammatory, anti-bacterial and anti-oxidant properties. Dahham et al (2015). Humulene provides some defense to plants and their products, as this compound can inhibit fruit fly mating Shelly and Nishimoto (2015). Muthukumaran, et al (2019) reported that 9, 12-octadecadienoic acid is used as hypocholesterolemic, nematicide antiarthritic hepatoprotective anti-androgenic 5-alpha reductase inhibitor, antihistaminic. Ar-tumerone was confirmed present in the leaves and hull extracts of *Prunus dulcis.* According to Pakkirisamy, et al (2017), Ar-tumerone is an anti-tumor, analgesic, anti-bacterial and anti-inflammatory. Some of the bio-active compounds confirmed from this study such as Ar-tumerone and oleic acid were confirmed present in methanol extract of *Rhazya stricta* reported by Baeshen et al (2023).

CONCLUSION

This study investigated bio-active compounds in *Prunus dulcis* leaves and hull. The extracted bio-active compounds revealed the medicinal potential of *Prunus dulcis* leaves and hull for female fertility, decongestant, anti-nociceptive, antihistaminic, nematicide, fungicide, anti-HIV and anti-cancer. Future research will need to focus on isolating and identifying a few of the bio-active chemicals from crude extracts of *Prunus dulcis* leaves and hulls, then testing them in living creatures to better understand their actions.

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