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Fatty Acid Profile, Antibacterial and Antioxidant Activities of the Seeds Extracts of *Dodonaea angustifolia*

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Abstract:
Dodonaea angustifolia (Sapindaceae) is an ever-green shrub reported to have analgesic, antiviral, anti-inflammatory, antiulcer and antioxidant activities. With the objective to find out the active extracts, the powdered seeds of D. angustifolia were successively extracted with petroleum ether, EtOAc and methanol on maceration each for 72 hours to afford 13.2% petrol, 3.4% EtOAc and 6.9% methanol extracts. Phytochemical screening of the methanol extract of the seeds was shown to have flavonoid, phenolics, saponin, steroids and anthraquinones while the EtOAc extract contains tannins, saponins, flavonoids, steroids and phenolics. The ground seeds were also Soxhlet extracted to furnish 16.3% yellowish oil, which was analyzed using GC-MS after trans esterifcation with MeOH-BF_3. The result revealed that the seeds are rich in oil with diverse fatty acids profile including tetradecanoic, palmitoleic, palmitic, oleic, and stearic acids. The methanol extract of the seeds of D. angustifolia was assessed for its antibacterial activities using various bacterial pathogens. It was found active against S. aureus, E. coli and P. eruginosa which exhibited zone of bacterial inhibition by 11, 12 and 11 mm, respectively. The activity displayed was modest compared to ciprofloxacin. The antibacterial activity observed from the present study supports the traditional uses of this plant against diseases caused by bacteria. The methanol extract was also evaluated for its antioxidant activity using DPPH radical scavenging and ferric thiocyanate methods. The extract inhibited the DPPH radical and lipid peroxide formation by 90% and 74%, respectively. The result demonstrated strong antioxidant activities of the seeds of D. angustifolia

Keywords: *Dodonaea, D. angustifoila, seeds, fatty acids, antibacterial, antioxidant*

1. Introduction

Dodonaea angustifolia L.f„ locally called *Etacha* in Afan Oromo and *Kitkita* in Amharic (Ethiopia), is a shrub belonging to the family Sapindaceae and genus *Dodonaea* [1]. It grows at the altitudes between 800 and 2650 m above sea level. Although found in various parts of the world, it is a popular hedge plant in East Africa including Ethiopia, Kenya and Uganda. The wood is extremely hard and used in Ethiopia as a fuel wood and for construction materials [2]. In folk medicine, the leaves or roots of *D. angustifolia* is used against rheumatism, inflammations, swelling, pain, skin infections, aches, and gastro intestinal disorders including diarrhea, indigestion, ulcers, and constipation [3,4]. The leaves are scientifically reported to have analgesics and antipyretic [5], antiretroviral [6], antimalarial [7] and antioxidant [8] activities. The essential oil, the alcoholic and aqueous extracts from the leaves of *D. angustifolia* has been found to exhibit antibacterial activity [9].

D. angustifolia were reported to have essential oils, flavonoids, terpenoids, phenolics, coumarins, sterols and alcohols [10]. The leaves had many flavonoids including isorhamnentin-3-O-rutinoside, isoquercetin-3-O-rutinoside, quercetin, kaempferol, kaempferol-3,4'-trimethylether, kaempferol-7,4'-dimethylether, kaempferol-3-methylether, pinocembrin, acacetin-7-methylether, 5-hydroxy-3,6,7,4'-tetramethoxyflavone, and 5,7-triihydroxy-3'-(3-hydroxymethylbutyl)-3,6,4'-trimethoxyflavone [11,12]. Also, two acidic compounds are reported from the plant, namely, shikmic and chlorognic acid. The leaves were also reported to be rich in terpenoids, namely, hautriwaic acid, dodonic acid, hautriwaic acid etc. Oleic and linolenic acid are fatty acids reported from the leaves of *D. angustifolia* [13].

In spite of the various scientific reports available on the leaves and root extracts of *D. angustifolia*, the anti-bacterial and antioxidant activities of the seeds have not been reported. Hence, the present study was aimed to evaluate the antibacterial and antioxidant activities of the seeds extracts of *D. angustifolia* which may contribute in the long-run to the improvement of health care. Furthermore, this paper also includes the fatty acid profile of the seeds of *D. angustifolia* which has not been reported before from this source.

2. Materials and Method

2.1. Plant Material

The seeds of *D. angustifolia* were collected in the month of January, 2016 from Goro kebele, Meta Robi woreda, West Shoa, Oromia, which is 102 km from west of Addis Ababa, Ethiopia, the plant was authenticated by Prof. Legessa Nagesh and specimen stored (Voucher no: CB01/2016) in the National Herbarium of Addis Ababa University, Addis Ababa, Ethiopia.

2.2. Instrument

Gas Chromatography-Mass Spectrometry(GC-MS) analysis were performed using Agilent Technologies 7820A gas chromatograph system equipped with HP-5 capillary column (30mx0.25; coating thickness, 0.25µm) and Agilent technologies 5977 E mass spectroscopy ion trap detector. Analytical conditions were as follows: Injector and transfer line temperature are 220 and 260^{o}C, respectively; oven temperature programmed from 60^{o}C to 240^{o}C at 3^{o}C/min; carrier gas, helium at 1 mL/min; injection 5 µL;
split ratio, 1:30. Identification of the constituents were based on search through mass\hunter\library\NIST11.L
and mass\hunter\library\W9N11.L

2.3. Extraction of the Seeds of D. angustifolia

The washed, air dried and powdered seeds of *D. angustifolia* (250 g) were successively extracted with petroleum ether (1 L), EtOAc (1 L) and methanol (1 L) each for 72 hours with occasional shaking. Each were then filtered and concentrated under reduced pressure using rotary evaporator to afford 32.9 g (13.1%) of hexane, 8.6 g (3.4%) of EtOAc and 17.4 g (6.9%) of MeOH extracts.

2.4. Extraction of oil from the Seeds of D. angustifolia

The air dried and powdered seeds of *D. angustifolia* (100 g) were Soxhlet extracted with petroleum ether (400 mL) as a solvent for 6 hrs. It was filtered and concentrated to afford 16.5 g (16.3%) yellowish oil.

2.5. Preparation of Fatty Acid Methyl Esters (FAME)

D. angustifolia oil (2 g) was placed in round bottom flask which contained 10 mL hexane to which 5 mL BF_3 in MeOH solution was added. To the mixture boiling chips was added and refluxed for 30 min. To the cooled mixture, each 10 mL of water and hexane were added with vigorous shaking and two layers were formed. The upper layer was separated by using separatory funnel, dried over anhydrous sodium sulphate, filtered and concentrated to afford 500 mg (25%). A small portion of the methylated fatty acids was dissolved in hexane and analyzed using GC-MS. The fatty acid identifications were made by comparing the spectra of the components with the database of the spectrum of known components stored in the GC-MS library.

2.6. Phytochemical Screening

The ethyl acetate and methanol extracts of the seeds of *D. angustifolia* were screened for the presence or absence of secondary metabolites using previously developed protocols reported in the literature [14-17]

2.7. Evaluating Antibacterial Activities of the Seeds of D. angustifolia

The antibacterial activities of the crude methanol extracts of the seeds of *D. angustifolia* were tested against one Gram positive and three Gram negative bacterial pathogens using the well diffusion method following previously developed procedure with slight modifications [18]. *Escherichia coli, Staphylococcus aurous, Proteus miabilis* and *Pseudomonas aeruginosa* used in this study were obtained from Oromia public health research, capacity building and quality assurance. Ciprofloxacin was used as standard antibacterial drug. From inhibition zone data, the antimicrobial activities extracts were critically examined by comparing the inhibition diameters at two concentrations and relating them to the control. The test bacterial species were transferred from the stock cultures and streaked on Mueller Hinton plates and incubated for 24 hrs at 37^{o}C. Well-separated bacterial colonies were then used as inoculums. Bacteria were transferred using bacteriological loop to autoclaved Mueller Hinton agar that were cooled to about 45^{o}C in a water bath and mixed by gently swirling the flasks. The medium was then poured to sterile Petri dishes, allowed to solidify and used for the bio test. Dried test discs were placed gently on the agar surface and plated with the forceps so that it sticks. The Petri dish was incubated upside down at 37^{o}C for 24 h. Resulting zones of inhibition were observed and measured in millimeters. Tests were repeated in triplicate and were performed to insure reliability of the results.

2.8. Antioxidant Activities

2.8.1. DPPH radical-scavenging activity

The stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) was used for determination of free radical-scavenging activity of the extracts [19]. In this regards, different concentrations of the methanol extracts (500, 250, 125 and 62.5 µg/mL) were added to 4 mL methanolic solution of DPPH (100 µM) to furnish 100, 50, 25 and 12.5 µg/mL. After incubating at 37^{o}C in an oven for 30 min, the absorbance was recorded at 517 nm. The experiment was repeated for three times and an average was taken. Ascorbic acid was used as standard controls. The percent of DPPH discoloration of the samples was calculated according to the formula [20]:

$$(\%)\ \text{inhibition} = \frac{(\text{A control} - \text{A sample})}{\text{A control}} \times 100$$

Where A control was the absorbance of the DPPH solution and A sample was the absorbance in the presence of plant extract. Samples were analyzed in triplicate. Ascorbic acid was used as positive control.

2.8.2. Thiocyanate Method

The antioxidant potential of *M. stenopetala* and *M. oleifera* leaves were done according to the method of Nagatsu, A [21]. Each 0.1 mg EtOH extract of *M. oleifera* and *M. stenopetala*, 100 µL of linoleic acid, EtOH (5 mL) and phosphate buffer (5 mL, 0.05 M, pH = 7) in water were separately added in to a vial and incubated at 40°C in an oven. After 24 h, 0.1 mL from each were taken and added in to a vial containing 75% aqueous EtOH (7 mL), 30% of NH_4SCN (0.15 mL) and 0.15 mL of 0.02M $FeCl_2$ in 3.5% HCl. Each was then subjected to UV-Vis spectrophotometery to record the absorbance at 500 nm. The percentage inhibition using ferric thiocyante method was calculated according to the following formula.

$$\text{Percentage inhibition} = 100 - \left(\frac{As}{Ab} \times 100\right)\%,$$

where As is absorbance of the sample and Ab is absorbance of the blank [22].

Absorbance of the blank and ascorbic acid were done in the same fashion.

3. Results and Discussion

3.1. Phytochemical Screening

Phytochemical analysis of the seed extract revealed the presence of bioactive substances (Table 1). Results showed that the methanol extract of the seeds were found to have flavonoid, phenolics, saponins, steroids and anthraquinones (Table 1). On the other hand, the ethyl acetate extract of the seeds of *D. angustifolia* revealed that it contains tannins, saponins, flavonoids, steroids and phenol. The presence of pharmacologically useful substances such as tannins, flavonoids, saponins, phenolics, anthraquinones and steroids in the seeds of *D. angustifolia* as revealed by phytochemical screening test confirms the diverse claims and application of the seeds of the plant in treatment of various ailments

Phytochemicals	Tests/methods	MeOH Extract	EtOAc extract
Tanins	Ferric chloride test	-	+
	Gelatin test	-	+
	Potassium hydroxide test	-	+
Saponins	Froth test	+	+
Alkaloid	Wagner's test	-	-
Steroids	Salkowski Test	+	+
Flavonoids	Sodium Hydroxide Test	+	+
Anthraquinones	Bontrager's test	+	-
Phenols	10% $FeCl_3$	+	+

Table 1: Results of qualitative test for phytochemical screening tests
Where -ve: absence, +ve: presence

3.2. Antibacterial activities of the seeds of D. angustifolia

The antibacterial activity of the methanol extract was quantitatively assessed by measuring the diameter of the inhibition zone around the disks with the results presented in Table 2. The methanol extract showed significant antibacterial activity against *E. coli, S. aureus* and *P. aeruginosa* while *P. mirabilis* was found resistant. The extract inhibited the growth of *E. coli, S. aureus,* and *P. aeruginosa* by 12, 11 and 11 mm at 1.5 mg/mL with the results significant compared with the negative control. The result was found in agreement with the antibacterial results reported for the aerial parts of the same plant against *E. coli, S. aureus,* and *P. aeruginosa* [23]. As compared to standard drug the antibiotic activity of the methanol extract of the seeds of this species on *S. aureus, E. coli,* and *P. aeruginosa* is moderate. This shows the plant may be used as a good candidate as a source of antibacterial drugs. The activity displayed is likely attributed to the presence of phytoconstituents such as steroids, anthraquinones, flavonoids and phenolics. Hence the results obtained in the present study may partly accounts for the traditional uses of this plant as anti-infection and wound healing by peoples where it is grown.

Sample	Conc in mg/mL	Zone of bacterial growth inhibition (mm)			
		Gram-positive	Gram-negative bacteria		
		S. aureus	*P. mirabilis*	*E. coli*	*P. eruginosa*
MeOH extract	1.5	11	6	12	11
	0.5	6	6	6	6
Ciprofloxacin		23	21	19	24

Table 2: Zone of bacterial growth inhibition (mm) for methanol extract of the seeds of D. angustifolia

3.3. Antioxidant Activities

3.3.1. DPPH Radical Scavenging Activities

The antioxidant activity of the methanol extract of the seeds of *D. angustifolia* were measured by bleaching of the purple-colored solution of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH). The DPPH assay indicated that the methanol extract of *D. angustifolia* seeds displayed pronounceable free radical scavenging activity with an average percent inhibition of 90, 48, 25, and 14% at 100, 50, 25 and 12.5 µg mL^{-1}, respectively. The activity was further visualized from the observed immediate discoloration of the purple DPPH solution to yellow. The radical scavenging activity of the methanol extract of the seeds of *D. angustifolia* is accounted to the presence of phenolics and flavonoids in the extracts. This is most likely due to the strong ability of donating an electron and hydrogen to DPPH radical by phenolics and flavonoids. The result is significant compared to ascorbic acid which inhibited the radical by 94% at 100 µg mL^{-1}

3.3.2. Ferric Thiocyanate Method

Lipids having many unsaturation sites undergo deterioration producing a number of toxic metabolites [24] which can interact with biological materials thereby causing cellular damage [25]. The degree of lipid peroxidation can be used to estimate the antioxidant potential of compounds or extracts. This can be done using ferric thiocyanate method. The MeOH extract of the seeds of *D. angustifolia* inhibited peroxide formation by 74%, demonstrating its potential in preventing the formation of lipid peroxides. The anti-lipid per-oxidation displayed by the MeOH extract was comparable with ascorbic acid (80%) used as positive control (Table 3). This shows the potential of the seeds of *D. angustifolia* as natural antioxidants.

Sample name	Absorbance at 500 nm	% inhibition
Blank	0.56	-
Ascorbic acid	0.11	80
D. angustifolia MeOH extract	0.14	74

Table 3: Anti-lipid peroxidation activities of the MeOH extracts of D. angustifolia
Ascorbic acid was used as positive control; the experiments was done in triplicates and average was taken

3.4. Fatty Acids Profile of the Seeds of D. angustifolia.

The oil content of the seeds of *D. angustifolia* was determined by employing Soxhlet extraction with petrol as a solvent. The oil content was found to be 16.3%. The fatty acid compositions of *D. angustifolia* oil were determined using gas chromatography-mass spectrometry (GC-MS). This was done after the oil was converted to fatty acid methyl esters using BF_3-MeOH. The results obtained from GC-MS (Appendix 1) showed that *D. angustifolia* oil contains a large proportion of saturated fatty acids. The area percent of tetradecanoic (**1**), palmitoleic (**2**), palmitic (**3**), oleic (**4**), and stearic acids (**5**) were 8, 4, 41, 31 and 16%, respectively. The dominant saturated fatty acid was found to be palmitic acid while oleic acid being is the major fatty acid in the seeds of *D. angustifolia*. This was not yet reported from this source.

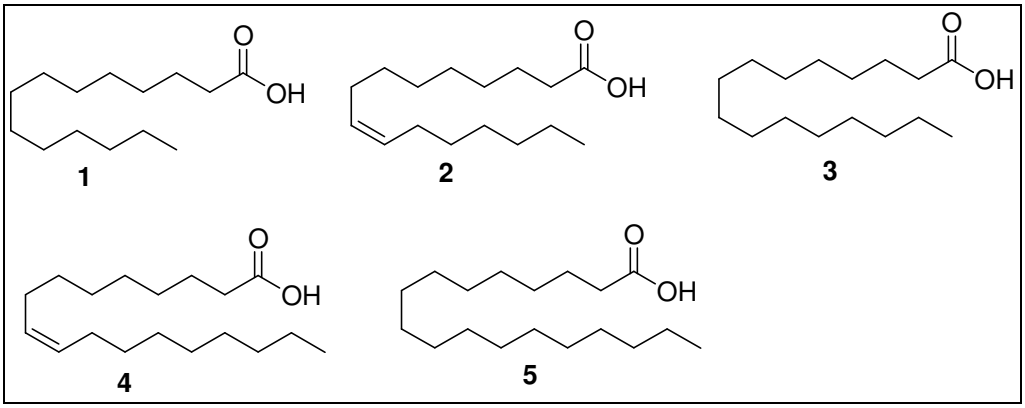

Figure 1: Fatty acids profile of the seeds of D. angustifolia

5. Conclusions

In conclusion, the methanolic extracts of the seeds of *D. angustifolia* were shown to contain secondary metabolites with wide-spectrum antibacterial activity, capable of inhibiting the growth of gram-positive and negative bacteria. The extract also displayed strong radical scavenging activity and anti-lipid peroxidation potential comparable to ascorbic acid which was used as positive control. The antiradical and anti-lipid peroxidation activities displayed by the extract of *M. stenopetala* seeds are accounted to the presence of flavonoids and phenolics. The oil obtained through Soxhlet extraction was found to be rich in oils with various fatty acids profile including tetradecanoic, palmitoleic, palmitic, oleic, and stearic acids.

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7. References

i. Avato, P., Rosito, I., Papadia, P. & Fanizzi, F. P**.** (2006). Characterization of seed oil components from Nephelium lappaceum L. J. Natural Product Communications. 1(9), 751-755
ii. Al-Asmari, A.K., Al Otaibi, K.E., Al-Omani, S., Athar, M.T. (2013). An Updated Phyto-pharmacological Review on Medicinal Plant of Saudi Arabia-Dodonaea viscosa Linn, American Journal of Research Communication, 1(12), 519–531
iii. Castillo, L., Gonzalez-Coloma, A., Gonzalez, A., Diaz, M., Alonso-Paz, E., Bassagoda, M. J. & Rossini, C. (2009). Screening of Uruguayan plants for deterrent activity against insects. J. Industrial Crops and Products. 29(1), 235-240
iv. Zhao, H., Qin, X. & Yang, M. (2006). Preliminary study contact toxicity of extracts from Dodonaea viscosa seed aganist Sitophilus oryzae. J. Yunnan Agricultural University. 22(6), 942-944
v. Amabeoku, P., Eagles, G., Scott, E. P., Springfield, Mayeng, I. (2001). "Analgesic and antipyretic effects of Dodonaea angustifolia and Salvia africana-lutea", J. Ethnopharmacology. 75, 117-124
vi. Muthukumran, P., Begumand, V. H. & Kalaiarasan, P. (2011). Antidiabetic activity of Dodonaea viscosa leaf extracts. International Journal of PharmTech Research, 3 (1), 136-139
vii. Teffo, L.S.,Aderogba, M.A., Eloff, J.N**.** (2010)."Antibacterial and antioxidant activities of four kaempferol methyl ethers isolated from Dodonaea viscose Jacq. var. angustifolia leaf extracts". J. South African Botany. 76, 2529
viii. Lemordant, D. (1971). Contribution of ethnobotany. J. Agric. Trop. Bot. appl. 18(35), 142-179.
ix. Sachdev, K. and Kulshreshtha, D. K. (1983). Flavoniods from Dodonaea Viscosa. J. Phytochemistry. 22(5), 1253-1256.
x. Siddiqui, A.A. (1998). Chemical and pharmacological evaluation of Dodonaea viscosa J. Asian Chemistry**,** 10, 14-16
xi. Siddiqui, A.A. (1998). Chemical and pharmacological evaluation of Dodonaea viscosa J. Asian Chemistry**,** 10, 14-16
xii. Ahmadi, L.; Mirza, M. & Shahmir, F. (2002). The volatile constituents of Artemisia marschaliana Sprengel and its secretory elements. J. Flavour Fragr. 17, 141-143.
xiii. Trease, G.E and Evans, W.C. (1989). Pharmacognosy: Thirteenth Edition. Bailliere Tindall. London; pp. 882.
xiv. Obadoni, B.O., and Ochuko, P.O. (2001). Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria. J. Global Pure and Applied Science. 8(2), 203 – 208.
xv. Pourmorad, F., Hosseinimehr, S.J. and Shahabimajid, N. (2006). Antioxidant Activity, phenol and flavonoid contents of some Iranian medicinal plants. J. African Biotecnology. 5 (11), 1142-1145.
xvi. Lim, Y.Y., Lim, T.T. and Jing, J. (2006). Antioxidant Properties of Guava Fruit. Comparison with Some Local Fruits. J. Sunway Academic. 9–20.
xvii. Savitree, M., Isara, P., Nittaya, S.L. and Worapan, S. (2004). Radical Scavenging Activity and Total Phenolic Content of Medicinal Plants Used in Primary Health Care. J. Pharm. Science, 9(1), 32-35.
xviii. Vinoth, B., Maniv, R.S., Balamurugan, S. (2012). Phytochemical Analysis and Antibacterial Activity of Moringa Oleifera Lam, International Journal of Research in Biological Sciences 2(3), 98-102
xix. Ghasemi, K., Ghasemi, Y., Ebrahimzadeh, M.L. (2009). Antioxidant Activity, Phenol and Flavonoid Contents of 13 Citrus Species Peels and Tissues, Pak. J. Pharm. Sci., 2 (3), 277-281
xx. Qusti SY, Abo-Khatwa AN and Lahwa MAB. (2010). Screening of Antioxidant Activity and Phenolic Content of Selected Food Items Cited in the Holly Quran, EJBS, 2(1), 40–52
xxi. Nagatsu, A. (2004). Investigation of Anti-oxidative Compounds from Oil Plant Seed, FABAD J. Pharm. Sci., 29, 203–210
xxii. Gulcin, I., Huyut, Z., Elmastas, M., Aboul-Enein, H.Y. (2010). Radical Scavenging and Antioxidant Activity of Tannic Acid, Arabian Journal of Chemistry, 3, 43–53
xxiii. Khurram, M., Khan, M.A., Hameed, A., Abbas, N., Qayum, A., Inayat, H. (2009). Antibacterial Activities of Dodonaea viscosa using Contact Bioautography Technique, Molecules, 14, 1332-1341
xxiv. Fang, Y., Yang, S., Wu, G. (2002). Free Radicals, Antioxidants, and Nutrition, Nutrition, 18, 872– 879
xxv. Frankel, E.N. (1984). Lipid Oxidation: Mechanisms, Products and Biological Significance**,** JAOCS, 61(12), 1908–2008

Appendix 1: GC-MS chromatogram of the fatty acid methyl esters of the seeds of *D. angustifolia*