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Saphan Ochieng Anode
Department of Allied Sciences,
Hamelmalo Agricultural College,
Eritrea

Tesfamichael Abraha
Directorate of Postgraduate
Studies and Research,
Hamelmalo Agricultural College,
Eritrea

Samsom Araya Fisseha
Quality Control Laboratory, Azel
Pharmaceutical plc. Eritrea

Phytochemical analysis of *Dodonaea angustifolia* plant extracts

Saphan Ochieng Anode, Tesfamichael Abraha and Samsom Araya Fisseha

Abstract

This study was conducted to investigate the presence of potential bioactive phytochemicals such as flavonoids, alkaloids, triterpenoids, saponins, tannins, steroids, proteins and cardiac glycosides in the leaves, bark and stems extracts of *Dodonaea angustifolia* plant commonly found in Eritrea. Plant parts samples were collected from Habrengaka and Balwa areas in Anseba region, Eritrea. Ethanol, diethyl ether and methanol solvents were used in the extraction. Preliminary phytochemical analysis and Thin Layer Chromatography (TLC) was carried out on plant extracts. Elution was done by step gradient method in which polar and non-polar solvents in varying proportions were used as mobile phase. It was found that alkaloids, anthraquinones, glycosides, essential oils, phenolics, saponins and terpenoids were present in leaf, stem and bark extracts of all the three solvents used. Flavonoids was detected in leaf extracts of all the three solvents and in ethanolic stem extracts but was not detected in methanolic and diethyl ether stem extracts and in the bark extracts of all the three solvents. Steroids was present in ethanolic leaf extract and in bark extracts of all the three solvents while tannin was present only in methanolic leaf and bark extracts.

Keywords: Bioactive, *Dodonaea angustifolia*, phytochemicals, bark extracts, leaf extracts, stem extracts

1. Introduction

Knowledge of individual chemical constituents of a medicinal plant is essential for optimizing extraction procedures, understanding pharmacological activity as well as potential toxicity and interaction with pharmaceutical drugs [1]. *Dodonaea* plants have many medicinal properties and have been used by native peoples from all regions where it is found. It is a traditional medicine worldwide, administered orally or as poultice to treat a great variety of ailments. Stem or leaf infusions are used to treat sore throats; root infusions to treat colds [2]. The stems and leaves are used to treat fever, and seeds (in combination with those of other plants and coated in honey) to treat malaria. The stems are used as fumigants to treat rheumatism. The leaves are used to relieve itching, fevers, swellings, aches and can be used as antispasmodic agent leaves and roots as a painkiller to soothe toothaches and headaches and a lotion made from unspecified plant parts to treat sprains, bruises, burns and wounds. Recent phytochemical studies have confirmed a positive correlation between several groups of active phytoconstituents and the ethnopharmacological usage [2, 3]. A review of the chemistry and pharmacology of *Dodonaea* plant species especially *D. angustifolia* observed that many uses of the herb by indigenous people from various countries show remarkable similarities, which in turn appear to correlate with the known active phytoconstituents [4, 5]. The methanolic extract of *D. viscosa* was found to contain bioactive compounds like flavonoids, terpenoids, tannins, and volatile oil [6]. The ethanolic extract of *D. viscosa* leaf was found to contain alkaloids, flavonoids, saponins, steroids, triterpenoids and phytosterols [7]. Tannins, saponins, flavonoids and terpenoids were detected from the aqueous extract of all the plant parts of *D. viscosa* species [8, 9]. However little work has been conducted on *D. angustifolia* specimen found in Eritrean where it grows in a variety of habitats from riverine forest to rocky soils or arid marginal areas [10]. The plant is found in many parts of Eritrea where it's commonly known as *Tahses*. Eritreans used it commonly to brush their teeth and is therefore suspected to contain phytochemicals that may be active against some common oral and periodontal pathogens [10]. There is currently a large consumer demand for natural products that relieve pain and inflammation associated with chronic conditions, and this plant species has the potential to produce a natural extract possessing these characteristics. Knowledge of the chemical constituents of plants is desirable because such information will be of value for synthesis of complex chemical substances [8, 11].

Correspondence

Saphan Ochieng Anode
Department of Allied Sciences,
Hamelmalo Agricultural College,
Eritrea

2. Materials and Methods

2.1 Collection of samples

The medicinal plants used for the experiment were leaves, bark and stems of *Dodonaea angustifolia* collected from the hilly areas of Habrengaka and Balwa of towns in Anseba region, Eritrea.

2.2 Chemicals and Reagents

All the chemicals and reagents including were of Analytical Grade and purchased from Merck and Sigma-Aldrich. The TLC silica plates were purchased from Merck of HPTLC Grade.

2.3 Preparation of Plant Extract

The plant material samples of *D. angustifolia* were surface-rinsed with tap water then with distilled water to remove surface dust and other solid contaminants. They were then dried in the shade and milled to a fine powder. Extracts were prepared using a method described by Eloff, (1999) [12] with slight modifications. Three solvents were used for extraction: Diethyl ether, methanol (Merck Chemicals Pty. Ltd, SA), and ethanol (Sigma-Aldrich, SA). Ten gram of powder was mixed with 100 ml of the solvent, vortexed for 30 minutes using Genie 2 vortexer (Lasec, SA) and centrifuged at 10000 rpm for 20 minutes using a micro centrifuge 5424 (Merck Chemicals Pty. Ltd, SA). The supernatant was collected in a pre-weighed 500 ml beaker. The above procedure was repeated three times using the same powder. All three supernatants were pooled together in the same beaker and the solvent was allowed to evaporate under a cold air stream. The beaker was weighed again with the dried plant extract. A yield of dried extract was calculated by subtracting the weight of the empty beaker from the weight of the beaker with the plant extract. The crude extracts were then stored at 4°C for further analysis.

2.4 Preliminary phytochemicals analysis

The extracts prepared were analyzed for the presence of alkaloids, essential oils, saponins, tannins, steroids, flavonoids, anthraquinones, cardiac glycosides, phenolics and terpenoids based on the protocols available in the literature [13, 14, 15].

2.4.1 Test for alkaloids

1g of leaf, bark and stem dry extract powder of each solvent was evaporated to dryness in boiling water bath. The residues were dissolved in 100mls of 2M Hydrochloric acids. The mixture was filtered and the filtrate was divided into three equal portions of 30mls. One portion was treated with a few drops of Mayer's reagent, another portion was treated with equal amount of Dragondroff's reagent and the third portion was treated with equal amount of Wagner's reagent. The appearance of cream precipitate (for Myaer's test), the orange precipitate (for Dragondroff's test) and brown precipitate (in Wagner's test) indicates the presence of respective alkaloids [13].

2.4.2 Test for anthraquinones

1.0 g of plant extracts from each solvent was put in a dry test tube and 10 ml of chloroform added and shaken for 5 min. The extract solution was filtered and the filtrate shaken with equal volume of 10%v/v ammonia solution. A pink violet or red colour in the ammonical layer indicates the presence of anthraquinones [15].

2.4.3 Test for glycosides

1.0 g of plant extracts from each solvent was dissolved in 5 ml of glacial acetic acid containing 1 drop of ferric chloride solution. This was then under layered with 1ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of a deoxysugar characteristic of glycosides [14].

2.4.4 Test for Essential oils

Small quantity of different extracts was separately hard-pressed between two filter papers. Oil stain appears on the paper which indicates the presence of fixed oil.

2.4.5 Test for flavonoids

1.0 g of plant extracts from each solvent was treated with 5 ml of 50%v/v methanol solution. The solution was warmed and metal magnesium was added. To this solution 2-3 drops of concentrated Hydrochloric acid was added. Appearance of red colour indicates the presence of flavonoids [14].

2.4.6 Test for Phenolics

5mls of 10%w/v lead acetate was added to 0.5g of sample extract from each solvent. Appearance of white precipitate indicates the presence of phenolics.

2.4.7 Test for saponins

1.0 g of the plant extracts of each solvent were vigorously shaken with water in a test tube and then heated to boil. Production of froths from the test solution was treated as a preliminary evidence for the presence of the saponins.

2.4.8 Test for steroids

2 ml of acetic anhydride was added to 2 ml of plant extract of each solvent sample along with 2ml sulphuric acid. Colour change from violet to blue or green indicates the presence of steroids.

2.4.9 Test for tannins

1.0g of plant extracts from each solvent was added into 20 ml of water in a flask and filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or blue-black coloration [13].

2.4.10 Test for Tri-terpenoids

5ml of chloroform and 3ml of concentrated sulphuric acid was added to 1.0 g of plant extract from each solvent in the test tube. Appearance of monolayer of reddish brown colour indicates the presence of tri-terpenoids.

2.5 Phytochemicals separation and isolation by Thin Layer Chromatography

TLC plate (Merck No. 5554) of 20×20cm aluminum-backed Kieselgel 60 having 0.2mm thickness of silica sorbent was used. Fifty milligrams of the dry, powdered extracts were dissolved in 1 ml of solvent (acetone) and spotted on a line drawn by pencil at one end of the silica gel plate. The plates were prepared by adding silica gel adsorbent with inert binder (CaSO₄ and H₂O). Thick slurry was prepared by spreading the mixture on a sheet made up of thick aluminum foil. The resulting plate was then heated for thirty minutes at 110 °C for drying and activation. The combined extracts of leaf, stem and bark of ethanol, methanol and diethyl ether extracts were then subjected to Thin Layer Chromatography using different solvent systems and observed for characteristic spots under

UV light and Iodine chamber. The solvent systems used are described in Table 4 below.

The solutions used were of 0.5% strength. The Retention factor (R_f) values of each spot were then calculated by dividing the distance travelled by the product by the total distance travelled by the solvent [16].

$$R_f = \frac{\text{Compound distance from origin (b)}}{\text{Solvent front distance from origin (a)}}$$

2.6 Data Analysis

Each treatment was carried in triplicates. The results are

expressed as mean ($n = 3$), by using one-tail analysis of variance (ANOVA).

3. Results and Discussion

3.1 Plant crude extract yield

The crude extracts yield of leaves, bark and stem of *D. angustifolia* extracted with ethanol, methanol and diethyl ether solvents are shown in Table 1 below. The highest yield of 51.39% was obtained from leaves extracted with diethyl ether solvent while bark extracted with methanol solvent gave the lowest yield of 28.76%.

Table 1: Extract yield of leaf, bark and stem of *D. angustifolia* plant

Type of extract	No. of extractions	Mass of plant extracted (g)	Ethanol extract yield (g)	Methanol extract yield (g)	Diethyl ether extract yield (g)
Leaf extract	1	10.00	4.35	3.83	5.45
	2	10.00	3.57	4.24	4.82
	3	10.00	4.25	3.68	5.14
	Total	30.00	12.17	11.75	15.41
	% Yield	N/A	40.57	39.19	51.39
Stem extract	1	10.00	2.84	3.67	4.05
	2	10.00	3.28	3.24	4.57
	3	10.00	3.52	4.13	4.43
	Total	30.00	9.14	11.05	13.05
	% Yield	N/A	30.47	36.85	43.51
Bark extract	1	10.00	3.37	2.54	4.43
	2	10.00	3.85	2.85	4.18
	3	10.00	4.58	3.23	3.85
	Total	30.00	11.79	8.63	12.46
	% Yield	N/A	39.31	28.76	41.54

The graphical presentation of extract yield of leaf, bark and stem parts *D. angustifolia* is shown in Fig 1 below.

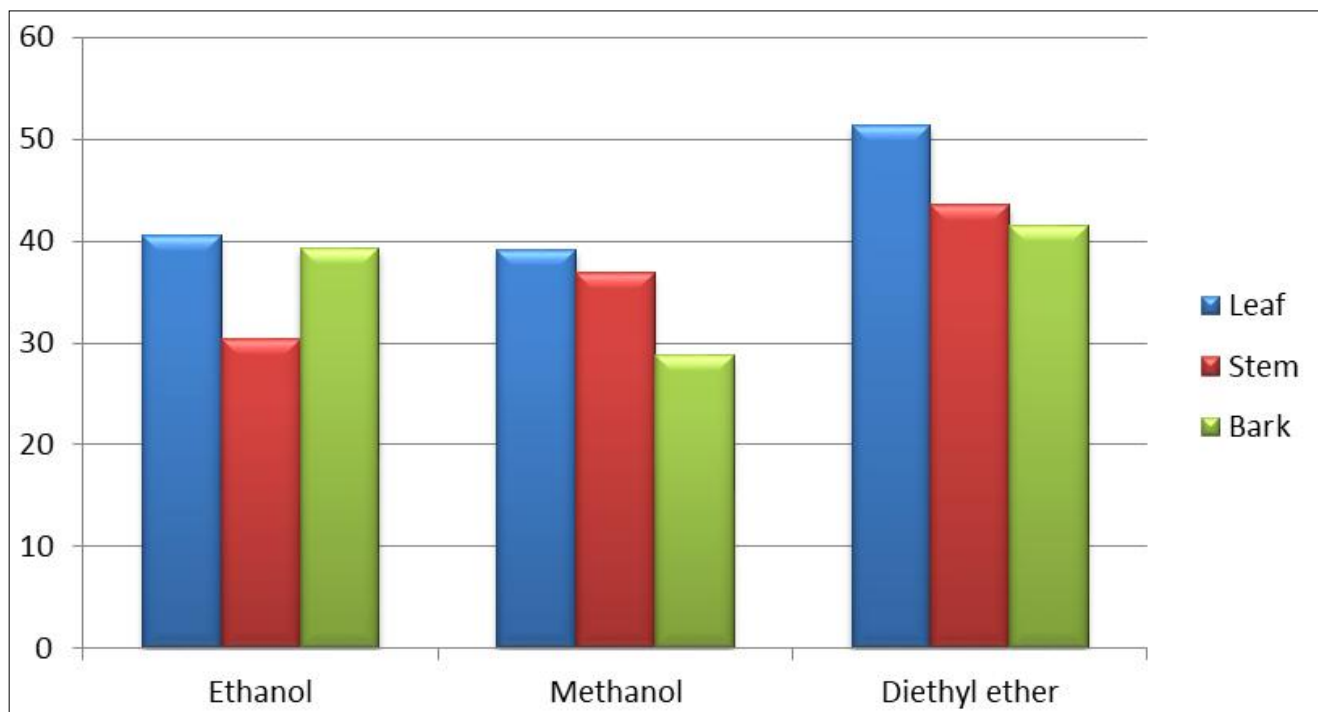


Fig 1: Percent (%) extract yield of leaf, stem and bark extracts of *D. angustifolia* plant

3.2 Phytochemical screening tests

Table 2 below summarizes the various phytochemical identification tests done on the *D. angustifolia* leaves, stems

and bark extracts of ethanol, methanol and diethyl ether solvents.

Table 2: Phytochemicals test results of crude extracts of leaves, stem and bark of *D. angustifolia* plant.

Phytochemical tested	EL	ML	DiL	ES	MS	DiS	EB	MB	DiB
Alkaloids	++	++	++	+	+	+	++	++	++
Anthraquinones	++	++	+	++	++	++	++	++	++
Glycosides	++	++	++	++	++	++	++	++	++
Essential oils	++	++	++	+	+	+	++	++	++
Flavonoids	++	++	++	+	-	-	-	-	-
Phenolic	++	++	++	++	++	++	++	++	++
Saponins	+	+	+	+	+	+	++	++	+
Steroids	++	-	-	-	-	-	+	+	++
Tannins	-	+	-	-	+	-	-	++	-
Tri-terpenoids	++	++	++	++	++	++	++	++	++

EL = Ethanol Leaf; ML = Methanol Leaf; DiL = Diethylether Leaf; ES = Ethanol Stem; MS = Methanol Stem; DiS = Diethylether Stem; EL = Ethanol Bark; MB = Methanol Bark; DiB = Diethylether Bark.

++ = Present in Appreciable quantity; + = Present in low quantity; - = Negative result

Alkaloids, anthraquinones, glycosides, essential oils, phenolics, saponins and terpenoids were present in leaf, stem and bark extracts of all the three solvents used. Flavonoids was detected in leaf extracts of all the three solvents and in ethanolic stem extracts but was not detected in methanolic and diethyl ether stem extracts and in the bark extracts of all the three solvents. Steroids was present in ethanolic leaf extract and in bark extracts of all the three solvents while tannin was present only in methanolic leaf and bark extracts. The high polarity of tannins may have reduced their extraction in the non-polar solvents ethanol and diethyl ether [6, 7]. Previous studies on other *Dodonaea* species especially *D. viscosa* had reported low concentration of steroids in the

ethanolic leaf extracts and absence in bark and stem of the plant extracts [8, 9].

3.3 Phytochemical separation and isolation by TLC

Ethanol, Methanol and diethyl ether extracts of leaf, stem and bark parts of *D. angustifolia* showed characteristic spots with the different solvent systems. Elution was done by step gradient method in which polar and non-polar solvents in varying proportions were used as mobile phase. Ten solvents systems were used as mobile phase. The results are shown in Table 3 below.

Figure 2 shows TLC profiles of the some six fractional elutes viewed at 366nm.

Table 3: TLC chromatogram profile for multiple mobile phase solvent for separation of different phytochemicals from *D. angustifolia* extracts

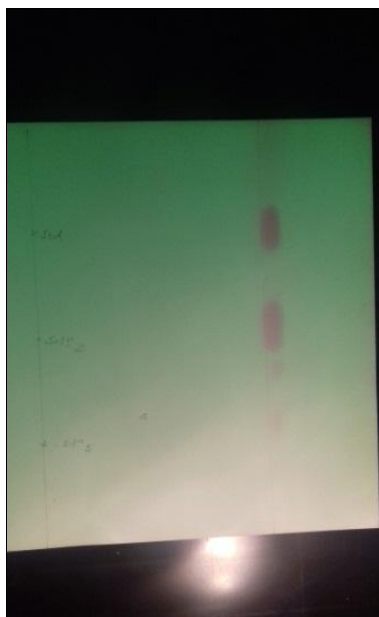
Phytochemical parameters	Mobile phase	No. of spots and colour	Rf values of samples	Rf values of standards
Alkaloids	Benzene : Ethanol (Be : Et) = 9:1	5 (black (3), blue, violet)	0.45, 0.65, 0.75, 0.25, 0.12	0.47, 0.51, 0.45 (Atropine)
Anthraquinones	Methanol : Distilled Water (Me : DW) = 8 : 2	3 (light blue, green, black)	0.50, 0.75, 0.44	0.54, 0.67, 0.60 (Salinosporamide)
Essential oils	Petroleum ether : Ethyl acetate (Pe : Ea) = 2:1	4 (dark blue (2), brown (2))	(0.65, 0.45, 0.24, 0.71)	0.77, 0.65, 0.78 (Eugenol)
Flavonoids	Ethyl acetate : Glacial acetic acid : Formic acid : Distilled Water (Ea : Gaa : Fa : DW) = 12.1 : 1.3 : 1.1 : 2.8	5 (Dark brown (2); yellow, violet (2)).	0.16, 0.22, 0.44, 0.34, 0.53	0.33, 0.44 (Flavonol)
Glycosides	Petroleum ether : Ethyl acetate (Pe : Ea) = 1 : 1	2 (Dark blue, brown)	0.71, 0.82	0.67, 0.81 (Glycerol)
Phenolics	Ethyl acetate : Methanol (Ea : Met : DW) = 20:5:4	4 (Yellow, light green, dark green brown)	0.61, 0.43, 0.22, 0.15	0.44 (Phenolic acid)
Saponins	Methanol : Distilled water (Met : DW) = 8 : 2	1 (Brown)	0.77	0.65 (Sodium palmate)
Steroids	Chloroform : Ethanol (CCl3 : Et) = 96 : 4	2 (black, dark green)	0.53, 0.60	0.42 (Diosgenin)
Tannins	Ethyl acetate : Methanol (Ea : Met) = 2 : 1	4 (green, light green, black)	0.47, 0.47, 0.73, 0.45	0.47, 0.71, 0.44 (Gallic acid)
Terpenoids	Ethyl acetate : Glacial acetic acid : Formic acid (Ea : Gaa : Fa) = 4.5 : 2 : 6.5	2 (Light blue, blue)	0.3, 0.44	0.22, 0.35 (Menthol)



a) Be: Et = 9:1



b) Me: E: Gaa: W (12.1:1.3:1.1:2.8)



c) Me: DW (8:2)



d) Me: Ea: DW (20:5:4)

Fig 2: TLC chromatograms obtained from varying proportion of mobile phase solvents viewed under Iodine chamber and UV light at 366nm

4. Conclusion

The findings of this research study confirm the presence of some pharmacologically important phytochemicals in the leaves, stem and bark of *D. angustifolia* plant found in Eritrea. Eritreans have for many years used the plant to clean their teeth as periodontal and oral disease management. The phytochemicals present in *D. angustifolia* have also been found to have pharmacological properties like antifungal, anti-inflammatory, antidiarrheal and antioxidant activities. Further studies are therefore recommended to assay qualitative and quantitative contents of this phytochemicals and to elucidate the chemical structure of those phytochemicals for Structure-Activity Relationship (SAR) analysis and for correct formulations of derived medicinal products.

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