DODONAEA ANGUSTIFOLIA EXTRACTS: A PHYTOCHEMICAL STUDY

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Abstract

In order to determine whether the leaves, bark, and stem extracts of the Dodonaea angustifolia plant, which is commonly found in Eritrea, contain any potentially bioactive substances like flavonoids, alkaloids, triterpenoids, saponins, tannins, steroids, proteins, and cardiac glycosides, this study was carried out. Samples of plant parts were gathered in the Eritrean regions of Habrengaka and Balwa. The extraction process employed methanol, diethyl ether, and ethanol as solvents. Plant extracts were subjected to preliminary phytochemical examination and Thin Layer Chromatography (TLC). Elution was carried out using the step gradient technique, where the mobile phase consisted of polar and non-polar solvents in various ratios. The leaf, stem, and bark extracts of all three solvents utilized included alkaloids, anthraquinones, glycosides, essential oils, phenolics, saponins, and terpenoids. The ethanolic stem extracts and leaf extracts of all three solvents contained flavonoids, whereas the methanolic and diethyl ether stem extracts as well as the bark extracts of all three solvents did not. All three solvents' ethanolic leaf and bark extracts included steroids, but only the methanolic leaf and bark extracts contained tannin.

Keywords: *Dodonaea angustifolia, bioactive, phytochemicals, leaf, and stem extracts*

Introduction

Understanding the specific chemical components of a medicinal plant is crucial for optimizing extraction processes, comprehending pharmacological activity, and identifying possible toxicity and medication interactions. Native Americans have employed dodonaea plants across their range because they offer several medical benefits. It is a widely used traditional medication that may be used topically or taken internally to treat a broad range of diseases. Root infusions are used to cure colds, whereas stem or leaf infusions are used to heal sore throats. Fever may be treated with the stems and leaves, and malaria can be treated with the seeds (when combined with those of other plants and coated in honey). To alleviate rheumatism, the stems are utilized as fumigants. The roots and leaves are used as a painkiller to relieve toothaches and headaches, and a lotion made from unidentified plant parts is used to treat sprains, bruises, burns, and wounds. The leaves are used to relieve itching, fevers, swellings, and aches. They can also be used as antispasmodic agents. A favorable link between numerous groups of active phytoconstituents and the ethnopharmacological use has been verified by recent phytochemical research [2, 3]. An analysis of the chemistry and pharmacology of Dodonaea plant species, particularly D. angustifolia, found that many of the herb's applications by native people in different regions exhibit significant similarities, which in turn seem to correspond with the known active phytoconstituents. It was shown that bioactive substances such flavonoids, terpenoids, tannins, and volatile oil were present in the methanolic extract of D. viscosa. Alkaloids, flavonoids, saponins,

steroids, triterpenoids, and phytosterols were discovered in the ethanolic extract of D. viscosa leaf. All plant components of the D. viscosa species' aqueous extract included tannins, saponins, flavonoids, and terpenoids. Although it thrives in a range of environments, including riverine forests, rocky soils, and dry marginal regions, little research has been done on D. angustifolia specimens discovered in Eritrea. The plant, which is also known as Tahses, may be found across Eritrea. It is believed to include phytochemicals that may be effective against several common oral and periodontal infections since Eritreans often used it to wash their teeth. This plant species has the potential to create a natural extract with the properties that are now in high demand among consumers for products that naturally reduce pain and inflammation linked to chronic diseases. The ability to synthesize complicated chemical compounds will benefit from knowledge of the chemical components of plants.

Materials and Methods

Collection of samples

Dodonaea angustifolia leaves, bark, and stems were employed as the experimental medicinal plants; they were gathered from the mountainous regions of the cities of Habrengaka and Balwa in the Anseba region of Eritrea.

Chemicals and Reagents

Each and every one of the chemicals and reagents was of the analytical grade and was obtained from Merck and Sigma-Aldrich. The HPTLC Grade TLC silica plates were bought from Merck.

Preparation of Plant Extract

To get rid of surface dust and other solid pollutants, the plant material samples of D. angustifolia were surface-rinsed with tap water and subsequently with distilled water. They were then ground into a fine powder and allowed to dry in the shade. With a few minor adjustments, the Eloff, (1999) technique was used to produce the extracts. Diethyl ether, methanol (Merck Chemicals Pty. Ltd, SA), and ethanol were the three solvents utilized for extraction (Sigma-Aldrich, SA). Using a Genie 2 vortexer (Lasec, SA) and a micro centrifuge 5424, 10 grams of powder were combined with 100 ml of the solvent. The mixture was vortexed for 30 minutes (Merck Chemicals Pty. Ltd, SA). In a 500 ml beaker that was already weighted, the supernatant was collected. The aforementioned process was carried out three times using the same powder. The solvent was allowed to evaporate while being blasted with cold air, pooling all three supernatants in one beaker. The dried plant extract was added, and the beaker was once again weighed. The weight of the empty beaker was subtracted from the weight of the beaker containing the plant extract to determine the yield of dried extract. The uncooked extracts were then kept at 4°C for further investigation.

Preliminary phytochemicals analysis

Based on techniques described in the literature, the produced extracts were examined for the presence of alkaloids, essential oils, saponins, tannins, steroids, flavonoids, anthraquinones, cardiac glycosides, phenolics, and terpenoids.

Test for alkaloids

In a boiling water bath, 1g of dried leaf, bark, and stem extract powder from each solvent was evaporated to dryness. 100 cc of 2M hydrochloric acid were used to dissolve the leftovers. After filtering the mixture, the filtrate was split into three equal 30 ml parts. A few drops of Mayer's reagent were added to one section, the

same quantity of Dragondroff's reagent was added to another, and the same amount of Wagner's reagent was added to the third. The presence of the corresponding alkaloids is indicated by the appearance of the cream precipitate in Myaer's test, the orange precipitate in Dragondroff's test, and the brown precipitate in Wagner's test.

Test for anthraquinones

10 ml of chloroform was added to 1.0 g of plant extracts from each solvent, which was then agitated vigorously for 5 minutes. After filtering the extract solution, an equal amount of 10% v/v ammonia solution was added to the filtrate and shaken. The presence of anthraquinones is indicated by a pink, violet, or red color in the ammonical layer.

Test for glycosides

Each solvent included 1.0 g of plant extracts that were dissolved in 5 ml of glacial acetic acid with a drop of ferric chloride solution. After that, 1ml of pure sulfuric acid was applied below. The existence of a deoxysugar, a glycoside feature, was suggested by the presence of a brown ring at the interface.

Test for Essential oils

A little amount of each extract was squeezed firmly between two filter sheets. The paper develops an oil stain, indicating the presence of fixed oil.

Test for flavonoids

5 ml of a 50% v/v methanol solution were used to treat 1.0 g of plant extracts from each solvent. Metal magnesium was added after the solution had been warmed. A few drops of strong hydrochloric acid were added to this solution. The presence of flavonoids is indicated by the color red.

Test for Phenolics

To 0.5g of the sample extract from each solvent, 5mls of 10%w/v lead acetate was added. The presence of phenolics is indicated by the presence of white precipitate.

Test for saponins

A test tube containing 1.0 g of each solvent's plant extracts was rapidly agitated before being heated till boiling. The test solution's ability to produce froths was seen as a preliminary indicator that saponins were present.

Test for steroids

Each solvent sample's 2 ml of plant extract received 2 ml of acetic anhydride, 2 ml of sulphuric acid, and 2 ml. Steroids are present when the color of violet changes to blue or green.

Test for tannins

20 ml of water were added to 1.0g of plant extracts from each solvent, and the mixture was then filtered. A few drops of 0.1% ferric chloride were added, and the color of the mixture was checked for brownish green or blue-black hues.

Test for Tri-terpenoids

In the test tube, 1.0 g of plant extract from each solvent was mixed with 5 ml of chloroform and 3 ml of strong sulfuric acid. Tri-terpenoids are present when a monolayer of reddish brown color is present.

Phytochemicals separation and isolation by Thin Layer Chromatography

The TLC plate (Merck No. 5554) was made of 20 by 20 cm aluminum-backed Kieselgel 60 with a 0.2 mm silica sorbent layer. One milliliter of the solvent (acetone) was used to dissolve fifty milligrams of the dry, powdered extracts before dotting them on a pencil-drawn line at one end of the silica gel plate. In order to produce the plates, silica gel adsorbent with inert binder was added (CaSO4 and H2O). Spreading the liquid over a piece of

thick aluminum foil produced a thick slurry. The resultant plate was then dried and activated by heating it for 30 minutes at 110 °C. The combined ethanol, methanol, and diethyl ether extracts of leaf, stem, and bark were then exposed to thin layer chromatography using various solvent systems, and distinctive spots were looked for under UV light and in an iodine chamber. Table 4 below lists the various solvent systems that were used.

The utilized solutions had a 0.5% strength. The distance traveled by the product was then divided by the total distance traveled by the solvent to get the Retention factor (Rf) values for each site.

Compound distance from the origin is Rf (b)

Solvent front separation from the source (a)

Data Analysis

Triplicates of each treatment were transported. Utilizing one-tail analysis of variance, the outcomes are presented as the mean (n = 3).

Results and Discussion

Plant crude extract yield

Table 1 below displays the crude extract yield of D. angustifolia leaves, bark, and stem after being extracted with ethanol, methanol, and diethyl ether solvents. Bark extracted with methanol yielded the lowest yield of 28.76%, while leaves extracted with diethyl ether gave the maximum yield of 51.39%.

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

In Fig. 1 below, the extract yield of the leaf, bark, and stem sections of D. angustifolia is shown graphically.

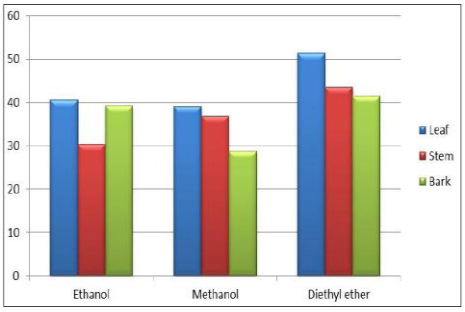

Fig : Percent (%) extract yield of leaf, stem and bark extracts of D. angustifolia plant

Phytochemical screening tests

The D. angustifolia leaves, stems, and bark extracts were tested for different phytochemical identification methods

using ethanol, methanol, and diethyl ether solvents. The results are summarized in Table 2 below.

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 stands for "Ethanol Leaf," ML for "Methanol Leaf," DiL for "Diethylether Leaf," ES for "Ethanol Stem," MS for "Methanol Stem," and DiB for "Diethylether Bark."+ = Present in Low Quantity; + = Present in Appreciable Amount; - = Negative Outcome

All three of the solvents employed have alkaloids, anthraquinones, glycosides, essential oils, phenolics, saponins, and terpenoids in their leaf, stem, and bark extracts. The ethanolic stem extracts and leaf extracts of all three solvents contained flavonoids, whereas the methanolic and diethyl ether stem extracts as well as the bark extracts of all three solvents did not. All three solvents' ethanolic leaf and bark extracts included steroids, but only the methanolic leaf and bark extracts contained tannin. The strong polarity of tannins may have hindered their extraction in ethanol and diethyl ether, which are non-polar solvents [6, 7]. Previous research on other Dodonaea species, particularly D. viscosa, had shown modest levels of steroids in ethanolic leaf extracts and none at all in the bark and stem extracts.

Phytochemical separation and isolation by TLC

D. angustifolia leaf, stem, and bark extracts in ethanol, methanol, and diethyl ether displayed distinctive markings with the various solvent systems. Elution was carried out using the step gradient technique, where the mobile phase consisted of polar and non-polar solvents in various ratios. Ten different solvent solutions used as the mobile phase. Table 3 below displays the findings.

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 :	3 (light blue, green, black)	0.50, 0.75, 0.44	0.54, 0.67, 0.60 (Salinosporamide)

	2			
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	1 (Brown)	0.77	0.65 (Sodium palmate
	(Met : DW) = 8 : 2			)
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)

Fig 2: TLC chromatograms obtained from varying proportion of mobile phase solvents viewed under Iodine chamber and UV light at366nm

Conclusion

The results of this investigation demonstrate that the leaves, stem, and bark of the Eritrean plant D. angustifolia contain several compounds that are significant from a pharmacological perspective. The herb has long been utilized by Eritreans to treat periodontal and dental diseases by cleaning their teeth. Additionally discovered to have pharmacological qualities including

antifungal, anti-inflammatory, antidiarrheal, and antioxidant activity are the phytochemicals identified in D. angustifolia. Therefore, more research is advised to determine the qualitative and quantitative concentrations of these phytochemicals as well as to clarify their chemical structure for use in Structure-Activity Relationship (SAR) analysis and the proper formulation of derived pharmaceuticals.

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