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Research Article

Antioxidant Activity of Flavonoids and Phenolic Acids from *Dodonaea angustifolia* Flower: HPLC Profile and PASS Prediction

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Background. *Dodonaea angustifolia* is a known medicinal plant across East Arica. The flower of *D. angustifolia* is not well investigated in terms of phytochemistry and biological activities. This study aims to investigate the presence of flavonoids and phenolic acids in the flower of *D. angustifolia* and its antioxidant activity. *Methods*. Preliminary phytochemical screening was carried out using the standard protocols. Antioxidant activity evaluation using DPPH assay and total phenol content (TPC) and total flavonoid content (TFC) determinations in the flower extract were compared with the values of the leaf extract. UHPLC-DAD analysis was managed to develop the profile of the flower extract. Prediction of biological activity spectra for substances (PASS) was done using an online server for antioxidant and related activities. *Results*. Preliminary phytochemical screening and TPC and TFC values confirmed the presence of flavonoids and phenolic acids. From the HPLC analysis of flavonoids, quercetin, myricetin, rutin, and phenolic acids such as chlorogenic acid, gallic acid, and syringic acid were detected and quantified. The biological activity spectrum was predicted for the detected and quantified polyphenols. *Conclusions*. *D. angustifolia* flower is a rich source of flavonoids and phenolic acids, which are extractable and can be checked for further biological activity. It was possible to identify and quantify phenolic compounds through HPLC analysis in the methanol extract of *D. angustifolia* flower. The PASS biological activity prediction results showed that there were stronger antioxidant activities for the identified flavonoids. Future work will emphasize the isolation and characterization of active principles responsible for bioactivity.

1. Introduction

Dodonaea angustifolia Lf (syn*: Dodonaea viscosa*) is a species belonging to the family Sapindaceae and known for many therapeutic purposes [1]. Locally, in Ethiopia, it is known by the names such as Karkare (Agew), Kitkitta (Amh, Gur), Termien (Geez), Ettecca (Oro), Intanca (Sid), Tahses (Tre, Tya), and Den (Som) [2, 3]. The plant is an erect bushy shrub 5–8 m high, with simple and alternate leaves, yellowish-green small flowers stacked without petals, yellowish-green capsule fruits, and flowering after the rainy season from August to September [3]. It occurs in most parts of Ethiopia and is pantropically known for fast-growing and used for soil stabilizing and reforestation activities [1].

Ethno-medicinal reports showed that parts of this plant are known to cure different human and cattle ailments. The roots are used for toothache and wound healing [4], parasitic worms [5], and tapeworms [6, 7]. Roots with leaves have

been used for trachoma [8]. Leaf decoctions, juice, and extracts of *D. angustifolia* are used for the treatment of taeniasis [9], liver ailment [10], wound healing [8, 11, 12], eye infection, herps and fire burn [13], malaria [12, 14], cancer [15], and skin infection and wound [16–18]. The leaves are also used externally for itchy skin and as a remedy for skin rashes [19]. It is also reported that the leaf extract is known for mild purgative and soared throat [17] and hemorrhoid [20]. As an ointment for head swelling, bursting, and hair fungus, dried, powdered leaves and a paste made of oil have been employed [12].

A large group of phytochemicals have been reported from *Dodonaea* species. Melaku et al. isolated pinocembrin (flavanone), santin (flavanol), and clerodane diterpenes using bioassay-guided extraction and chromatographic separation [21] from *D. angustifolia*. Similarly, Omosa et al. reported flavonoids (3,4′,5,7-tetrahydroxy-6-ethoxyflavone, 5-hydroxy-3,4′,7-trimethoxyflavone, isokaempferide, kumatakenin, rhamnocitrin, and diterpenoids ((ent-3β,8α)-15,16-epoxy-13(16),14-labdadiene-3,8-diol, 2β-hydroxyhardwickiic acid, and dodonic acid) from the leaf extract of same species using chromatographic separation [22]. Methoxymkapwanin and Mkapwanin were also isolated from the leaf surface exudate using serial extraction and column chromatography fractionation [23].

From earlier investigations, both crude methanol extract [24] and quercetin derivatives [22, 25, 26] isolated from *D. angustifolia* showed antibactericidal efficacy against gram-positive and gram-negative bacteria. The aqueous extract demonstrated analgesic and antipyretic potential in mice and rats [27]. Ethanol extract displayed a combination of antioxidant and antiproliferative properties with little to no damage to normal cells [28, 29]. Nonpolar extracts of the leaves of *D. angustifolia* inhibited the viral growth at subtoxic concentrations [30]. Crude extract of both the leave [31, 32] and root [33] showed the strong antiplasmodial activity against *P. berghei* infected mice. This is in agreement with its medicinal use traditionally to treat malaria [34]. The antihelminthic property of *D. angustifolia* was reasoned out for the polyphenols present, including kaempferol, quercetin, and myricetin-based flavanol [35].

D. angustifolia is a medicinal plant frequently used to treat toothache, microbial infections, and fever [36]. It showed antifungal activities and was found to be nontoxic. It is also used with other medicinal plants for musculoskeletal ailments [37] and bone fracture [6] with a higher informant agreement ratio.

The biological activity spectrum predicts many different compounds' biological activity types. Since it is solely dependent on the compound's structure, it is regarded as an intrinsic property of the substance [38]. The multilevel neighborhoods of atoms (MNA), which are original descriptors, are used in PASS (prediction of activity spectra for substances) to characterize chemical structure [39]. A MOL or SDF (structure data file formats) with the structural details of the molecules being studied serves as the input data for PASS. MNA descriptors are generated automatically using the data from the input files. Using data from MNA descriptors for both active and inactive compounds, for each activity, two probabilities are computed: Pa is the probability that a compound is active, while Pi is the probability that a compound is inactive. Pa and Pi have values that range from 0.000 to 1.000 since they represent probabilities (with 3 appropriate decimals determined) and Pa + Pi < 1 since these probabilities are computed independently. Pa and Pi can be thought of as measurements of the substance being studied that fall within the categories of active and inactive substances, respectively [38].

The antioxidant action of flavonoids and phenolic acids includes suppressing the formation of reactive oxygen species by inhibiting the respective enzymes, scavenging free radicals, and triggering antioxidant defence [40]. These phytochemicals also protect the disintegration of the lipid of biomembrane by preventing or hindering lipid peroxidation [41]. Flavonoids are synthesized by plants following a microbial infection and are known to possess antioxidant and antibacterial activities. The mechanism and activity level depends on the polyphenols' specific structural variety [41]. Flavonoids have a more significant number of physiological activities promoting human health and minimizing the risk of being infected by a broad spectrum of pathogens.

Flowers of *D. angustifolia* are ideal for bee forage and are considered a major agricultural value of the plant [3]. The seasonal flower of this plant is not well investigated in terms of phytochemistry and biological activities. Other parts including leaves, seeds, stem bark, and roots were investigated for their phytochemical constituents [22, 23, 26] and biological activities [21, 22, 25, 27, 31, 33, 34].

This study aimed to investigate the profile and antioxidant activity of the flower of *D. angustifolia* and compare it to the plant's leave. For this purpose, HPLC analysis and PASS online prediction of biological activities were used.

2. Materials and Methods

2.1. Chemicals and Reagents. All the extraction chemicals and reagents used for the total content of phenol and flavonoid determination were of AR grade. While for HPLC analysis and the antioxidant activity evaluation, HPLC grade solvents and reagents were used. Water was distilled and purified by MQ (18.2) at 21°C in a water purification system (Purelab flex 4 Elga). Phenolic acid standards: syringic acid, chlorogenic acid, and gallic acid; flavonoid standards: myricetin, quercetin, rutin, and kaempferol references purchased from Sigma (>99.9%, Sigma, China). 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) for antioxidant test and ascorbic acid are obtained from Sigma (>99.9%, Sigma, China).

2.2. Plant Material Collection and Pretreatment. The flower part of *D. angustifolia* was collected from Addis Ababa Science and Technology University campus. Ato Melaku Wondafrash identified the plant and a herbarium sample was deposited at the national herbarium (voucher number: FB-001/11) in the College of Science, Addis Ababa University, Ethiopia. Following collection, the samples were

cleaned with tap water and then distilled water to get rid of dirt and other debris. Then, the samples were chopped into smaller sizes and spread onto clean polyethylene plastic sheets at room temperature (23 ± 3°C). The air-dried samples were ground using a sample grinder (stainless steel 700 g electric grains, spices, herbs, cereals, and dry food grinding mill, China).

2.3. Ultrasonic Assisted Extraction (UAE). The extracts of air-dried and blended leaves and flower samples of *D. angustifolia* (5 g of each) were obtained from an intelligent ultrasonic processor (SJIA-950W, probe Φ 6) sonicator in 25 mL methanol. The method optimization of UAE followed a method reported by Zakaria et al. [42], with minor modifications considering the bioactive components we are dealing with. Briefly, the settings for sonication were temperature 35°C, time 15 min, and power rate 50%. Up on the optimized method suggested by Zakaria et al. (2021), the nature of the solvent (methanol) was expected to compromise the polarity difference between the phenolic acid and flavonoid with varied polarity. The temperature is also reasonable for the extraction of bioactive components. After 2x successive sonication for each aliquot extract, the extracts were centrifuged using proanalytical @ 600 (10x) for 20 min. Whatman no. 1 filter paper was then used to filter the supernatant, adjusted to a volume of 50 ml, and kept in an amber vial for further analysis.

2.4. Preliminary Phytochemical Screening. Using standard procedures [43], the presence of alkaloids, flavonoids, phenolics, tannins, steroids, triterpenoids, saponins, glycosides, carboxylic acids, anthraquinones, and essential oil was assessed. Briefly, to check for the presence of alkaloids, Wagner's reagent was used. Triterpenoids were checked by using Salkowski's reaction. The presence of flavonoids was confirmed by the lead acetate test showing yellow precipitate. Acetic anhydride test was carried out to check the presence of steroids. The presence of tannins was checked by adding 10% of NaOH and shaking well for an emulsion formation. Saponins were checked by foam test, where plant extract was mixed with water and shaken vigorously to see persistent foam for 10 min. Glycosides were checked using an aqueous NaOH test from the methanol extracts. Borntrager's test determined the presence of anthraquinones. An effervescence test using a sodium bicarbonate solution was used to check carboxylic acid's presence. To check for the presence of volatile oils, fluorescence tests were conducted. Details of the screening tests are shown in Table 1. The phytochemicals present in the methanol extracts of the leaves and flowers of *D. angustifolia* were compared.

2.5. Evaluation of Antioxidant Activity. We used the DPPH radical assay because it is easily available, has better radical scavenging potential, and is a commonly used free radical to evaluate the antioxidant potential of plant extracts [44, 45]. Modified protocol for the effect of free-radical scavenging on the DPPH assay from Banothu et al. [46] was followed. More briefly, to 0.25 mL of sample solution, 0.75 mL of DPPH was added and the reaction was left in the dark for 30 minutes. As a control, 0.25 mL of methanol and 0.75 mL of DPPH solution were combined. The standard utilized was ascorbic acid. In a 50 mL brown volumetric flask, 1.9716 mg of DPPH was dissolved in methanol to prepare DPPH solution and then adjusted to nearly 1.000 absorbance. A standard solution of ascorbic acid was prepared at a 1000 ppm concentration by dissolving 0.10 g in 100 ml of methanol for the calibration curve. From this stock solution, 25, 50, 100, 150, 200, 250, 300, 350, 400, 450, and 500 mg/L concentrations were prepared. The flower sample extract (0.1 g/mL) was diluted with methanol using 5, 10, 25, 50, 100, 150, and 200 dilution factors. The absorbance was measured using a JASCO V-770 spectrophotometer (Jasco, USA) at 517 nm with a 1 mm path length in a rectangular cell holder (500 μL cuvette). The percentage of inhibition is used to measure radical scavenging activity. The following formula was used to determine the DPPH radical scavenging capacity:

$$\text{scavenging activity (\%)} = \left(\frac{\text{absorbance}_{\text{control}} - \text{absorbance}_{\text{sample}}}{\text{absorbance}_{\text{control}}}\right) x\ 100 \quad (1)$$

IC_{50} values were computed from the relation log (sample) vs absorbance (normalized) using graph pad prism 8 software as suggested for better IC_{50} estimation [47].

2.6. Total Phenol and Total Flavonoid Content Determination. The total phenol content (TPC) of the flower sample extract was determined by the Folin–Ciocalteu colorimetric method as described by McDonald et al. [48] with some modifications. More briefly, 0.2 mL of 2% Na_2CO_3 was added to the mixture after 0.4 mL of the extract and 0.4 mL of Folin–Ciocalteu reagent (10x diluted) were combined. Various concentrations of sample extracts were checked, and the one with dilution factor 5 was used for the determination as the absorbance was between 0.1 to 1 (within Beer's Law) following consistent color changes. For control, a reagent without the methanol extract was used. The V-770 UV-Vis spectrophotometer (Jasco, USA) was used to measure the absorbance at 765 nm in triplicate after the mixture was incubated at room temperature for 35 minutes. The standard gallic acid was prepared for calibration at 1000 ppm and was serially diluted and 6.25, 12.5, 25, 50, 100, 150, 200, 250, and 500 mg/L standard solutions were prepared. Equation (2) was used to calculate TPC as the milligrams of gallic acid equivalent (GAE) per gram of extract (dry weight).

Table 1: Qualitative tests for phytochemical screening [43].

SN	Phytochemical groups	Test	Procedure	Observations (indicating positive test)
1	Alkaloids	Wagner's test	Few mL filtrates (50 gm solvent-free extract is mixed with a few mL dil. HCl and then filtered) + 1-2 drops of Wagner's reagent (along the sides of the test tube)	A brown/reddish precipitate
2	Glycosides	Aqueous NaOH test	Alcoholic extract + dissolved in 1 mL of water + a few drops of aqueous NaOH solution	A yellow color
3	Flavonoids	Lead acetate test	1 mL of plant extract + few drops of 10% lead acetate solution	A yellow precipitate
4	Phenolic compounds	Ferric chloride test	Extract aqueous solution + few drops 5% ferric chloride sol	Dark green/bluish black color
5	Tannins	10% NaOH test	0.4 mL of plant extract + 4 mL of 10% NaOH + shaken well	Formation of emulsion (hydrolysable tannins)
6	Saponins	Foam test	0.5 gm of plant extract + 2 mL of water (vigorously shaken)	Persistent foam for 10 min
7	Steroids	Acetic anhydride test	0.5 mL of plant extract + 2 mL of acetic anhydride + 2 mL of conc. H_2SO_4	Change in color from violet to blue/green
8	Triterpenoids	Salkowski's test	Filtrate (equal quantity of chloroform is treated with plant extract and filtered) + a few drops of conc. H_2SO_4 (shaken well and allowed to stand)	Golden yellow layer (at the bottom)
9	Anthraquinones	Borntrager's test	10 mL of 10% ammonia sol. + Few ml filtrating (shaken vigorously for 30 sec.)	A pink, violet, or red-colored solution
10	Carboxylic acid	Effervescence test	1 mL of plant extract + 1 mL of sodium bicarbonate solution	Appearance of effervescence
11	Volatile oils	Fluorescence test	10 mL of extract, filtered till saturation, exposed to UV light	Bright pinkish fluorescence

$$\text{Concentration } (mg/100\ g) = \frac{C\ x\ V\ x\ DF}{m}\ x\ 100, \quad (2)$$

where C is the concentration obtained from the calibration curve in mg/L, V is the final volume of the sample in L, m is the mass of the sample powder taken for extraction, and DF is the dilution factor.

Total flavonoid content (TFC) was determined using Chang et al.'s aluminum chloride colorimetric method [49] with slight modifications. More briefly, the mixture of 0.3 mL extract and 0.3 mL of 2% $AlCl_3$, 0.3 mL of 1% $NaNO_2$, and 0.3 mL of 5% NaOH were mixed and incubated at room temperature for a total of 30 min. Sample extracts were prepared in a similar way as for the TPC determination mentioned above. Methanol was used as a control. Absorbance was recorded in triplicate at λ 314 nm using a V-770 UV-Vis spectrophotometer (Jasco, USA). A standard stock solution of quercetin (1000 ppm) (mg/L) was prepared. The calibration standards were also prepared similarly. TFC was calculated as milligrams of quercetin equivalent (QE) per gram of the flower extract (dry weight).

2.7. HPLC Analysis. Stock solutions for standards (1 mg/mL) of both phenolic acids and flavonoids were prepared by dissolving an appropriate amount in methanol. Calibration standard solutions at 6 concentrations ranging from 2.5 to50 mg/mL were prepared and obtained by appropriate dilutions from the stock solutions in the selected mobile phase. The selected mobile phase was a binary isocratic elution consisting of (A) methanol and (B) acidified (1% acetic acid) ultra-pure water (60/40, v/v). The 10 μL injection volume was used at a flow rate of 0.8 mL/min.

Ultrahigh-performance liquid chromatography coupled with a diode array detector (Ultimate 3000 UHPLC-DAD, Thermo Scientific Dionex, USA) was used to separate the analytes via chromatography. The UHPLC system was equipped with a pump (model: LPG-3400SD), autosampler (model: WPS-3000TSL), and temperature-controlled column compartment (model: TCC-3100). Monitoring and quantitation were performed at 254 nm, 272 nm, 360 nm, and 372 nm. Chromeleon (c) Dionex version 7.2.4.8179 was used for instrument control and data acquisition. Chromatographic separation was performed on reverse phase column (Acclaim (TM) 102 with Fortis 5 μm, C18 (column dimension: 4.6 × 250 mm) Thermo Scientific Technologies, USA) operated at 30°C. Using these conditions, flavonoids and phenolic acid with external standards were separated within 25 min per sample. By comparing the retention times of the various compounds to standards, the individual compounds were identified and quantified. The amounts of the flavonoids and phenolic acids in the methanol extract of *D. angustifolia* flower were calculated using equation (2) [50].

2.8. PASS (Prediction of Activity Spectra for Substances) Test. PASS prediction for the flavonoids, phenolic acids, and reference antioxidant (ascorbic acid) was performed using the PASS online web server (http://www.pharmaexpert.ru/passonline). The PASS prediction uses canonical smiles to determine the probability of being active (Pa) and probability of being inactive (Pi) values. Pa and Pi values indicate a compound's biological activity. The biological activities selected were those activities related in one way or another to antioxidant activity [51]. Interpretation of the result as recommended by Langunin et al. [39] and Maharani et al. [52] was as follows:

(i) If Pa > 0.7, the compound is very active and is likely to display the aforementioned activity in trials conducted in a wet lab.

(ii) If 0.5 < Pa < 0.7 is likely to exhibit the activity and there is a lower chance than in the first case, then the compound will demonstrate the activity in a wet lab experiment.

(iii) If Pa < 0.5, the compound is unlikely to show the respective activity in the wet lab.

The 2.0 version of PASS online was used to perform the PASS test [53]. First, SMILES were retrieved for the candidate's compounds from PubChem (http://pubchem.ncbi.nlm.nih.gov), then the MOL or SDF file for the compounds was given as input to the PASS software and activity prediction was performed (get prediction). Before conducting lab testing, it was crucial to confirm the results of the PASS biological activity test. The probability activity score would display the findings and indicate the likelihood of success if lab tests were conducted.

3. Results

3.1. Phytochemical Screening. The preliminary phytochemical screening revealed that the methanol extracts of the flower and the leaves were almost comparable qualitatively for the tested secondary metabolites. The glycoside, saponin, triterpenoid, and anthraquinone tests indicated more concentration in the leaves than in the flowers. Alkaloids and tannins were not detected in the flower extract (Table 2).

3.2. Antioxidant Activity Evaluation. The IC_{50} values and % radical scavenging activity are almost identical for the leaf and flower extracts.

3.3. Total Phenol and Total Flavonoid Determination. Equation of calibration curve for TPC determination was $y = 0.0023x - 0.0693$, where $R^2 = 0.9981$, and for TFC determination was $y = 0.002x + 0.0399$, where $R^2 = 0.9971$.

3.4. HPLC Analysis. The number of components identified by HPLC analysis was 15 using a polar solvent system (Figure 1). Among these 6 were determined by using an external standard method on UHPLC-DAD (Table 3). The concentration (mg/100 g) was computed using $V = 0.050$ L and $m = 5.0046$ g on the equation given above in the method section.

Among the investigated flavonoids, myricetin took the lead with a concentration of 219.35 mg/100 g. Quercetin was

Table 2: Test result for qualitative phytochemical screening for leaves and flowers.

SN	Phytochemical groups	Test	Methanol extract of leaves of *D. angustifolia*	Methanol extract of flowers of *D. angustifolia*
1	Alkaloids	Wagner's test	+	−
2	Glycosides	Aqueous NaOH test	++	+
3	Flavonoids	Lead acetate test	++	++
4	Phenolic compounds	Ferric chloride test	++	++
5	Tannins	10% NaOH test	+	−
6	Saponins	Foam test	++	+
7	Steroids	Acetic anhydride test	+	+
8	Triterpenoids	Salkowski's test	++	+
9	Carboxylic acid	Effervescence test	++	+
10	Anthraquinones	Borntrager's test	++	+
11	Volatile oils	Fluorescence test	+	+

Result indications: ++ = present in appreciable amount; + = present in low amount; − = negative result.

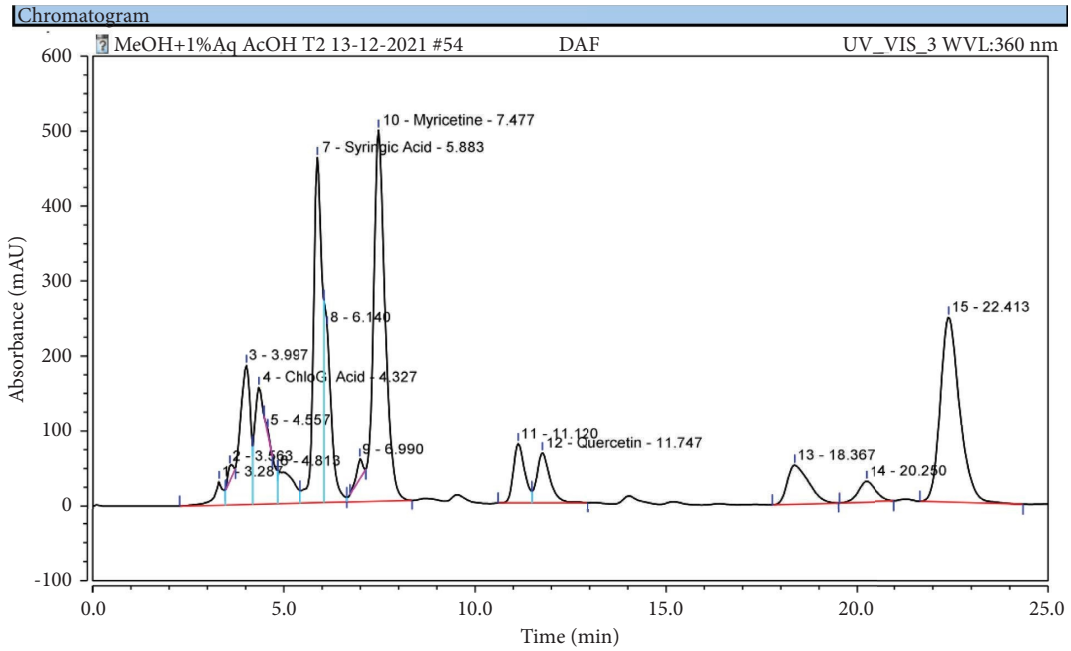

Figure 1: UHPLC-DAD chromatogram for the methanol extract of *D. angustifolia* flower.

Table 3: HPLC data table for the identified components of *D. angustifolia* flower.

Compound	Retention time (min)	Amount (mg/L)	Concentration (mg/100 g)	Remark
Chlorogenic acid	4.327	24.58 ± 1.76	24.56 ± 1.75	Phenolic acid
Syringic acid	5.883	81.42 ± 1.79	81.35 ± 1.79	Phenolic acid
Myricetin	7.477	219.55 ± 9.61	219.35 ± 9.6	Flavonoid
Quercetin	11.747	11.7 ± 1.13	11.69 ± 1.13	Flavonoid
Rutin	20.110	5.8 ± 0.74	5.8 ± 0.74	Flavonoid
Gallic acid	3.971	7.51 ± 0.23	7.51 ± 0.23	Phenolic acid
Kaempferol	12.767	No data	No data	Flavonoid

20x less than myricetin. The concentrations of rutin and gallic acid were found to be less than 10 mg/100 g. The concentration of syringic acid was 4x larger than chlorogenic acid. The data for kaempferol could not appear on the chromatogram and data table for unknown reasons after initially being tracked in the standard mixture. This might be due to precipitation and/or degradation of kaempferol at high temperatures (analysis temperature at 35°C). The rutin and gallic acid are not shown on the chromatogram due to the smaller amounts compared to the others.

3.5. PASS Prediction of Biological Activity. Biological activities used to describe the mechanism of antioxidant activity are considered in the PASS test. Other activities such as antimutagenic, cardioprotection, anticarcinogenic,

chemopreventive, proliferative disease treatment, antibacterial, antiprotozoal, antifungal, anti-inflammatory, and antiviral activities were also considered. The PASS results (Supplement 1) are summarized for the compounds considered in Table 4.

4. Discussion

From the preliminary phytochemical screening test result (shown in Table 2), the leaves and flowers of *D. angustifolia* were similar in the constituency of the major phytochemical groups. Moreover, the results obtained for the leaf extract are in close agreement with the literature report [51]. Generally, the screening study indicated that flavonoids and phenolic compounds are the major constituents of the leaf and flower extracts of *D. angustifolia* [54].

The radical scavenging activity of both the leaves and flowers of *D. angustifolia* was assessed using the DPPH method. The percentage of inhibition used to measure the DPPH radical scavenging activity using the formula is as follows: percentage effect (E %) = $(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}) \times 100/\text{Abs}_{\text{control}}$. As one can see from the structures of the flavonoids and phenolic acids, the presence of a more significant number of hydroxyl groups can result in higher antioxidant activity. As shown in Tables 5–7 and Figure 2, there is no significant difference in the antioxidant activity and TPC and TFC values between the leaves and flower parts of *D. angustifolia*.

Mobile phase selection for HPLC–DAD method for both extract samples was one of the big challenges to get better separation as the polarity of phenolic acids and flavonoids are closer. To come up with a solution for such problems, we have considered the advantage of the gradient method [55] for HPLC analysis. However, we successfully used isocratic elution for our flower sample. As shown on the chromatogram (Figure 1), the peak shape and separation were good for the selected polar solvent system. Methanol extract of *D. angustifolia* leaves was not showing separate peaks for the identified polyphenols in the case of the flower. Additional methods like hyphenation with mass spectroscopy should be considered for further component identifications.

The flavonoids (flavanols) considered in our study are sourced similarly following closely related biosynthesis in most cases. They are even common in dietary sources [56]. Flavonoids occur in most plant parts, specifically photosynthesizing plant cells as major coloring components of flowering plants [41]. For flowers, the matrix's complexity may be lower compared to the leaves where chlorophyll and related components are more concentrated.

Investigations of phenolic acids and flavonoids among the phytochemicals from natural products are usually conducted using HPLC analysis with a DAD detector [57]. Such analysis has been attempted by Mizzi et al. (2020) [55]. Due to the complexity of the matrix analysis, it is not simple to do such an investigation for medicinal plants. Alam et al. [58] and Thomas et al. [59] mention an HPLC method using a gradient of acetonitrile and methanol to estimate some of these phenolics in *Moringa oleifera*. As the retention times of these compounds are closer to each other, making simultaneous measurements of both the groups was not possible in our case. Less than 1 mg/100 g of concentration was reported for rutin, myricetin, and gallic acid for *D. viscosa* flower [60]. Tong et al. reported gallic acid and quercetin 19.68 and 5.95 mg/100 g, respectively, from the flower of the same *Dodonaea* species [61]. In this study, HPLC analysis results are 5 to 220 mg/100 g for both flavonoids and phenolic acids. Myricetin was nearly 220 mg/100 g dry weight basis. Because of the few attempts on the synonym *Dodonaea* species, it is impossible to compare the results from this current investigation.

Biological activities predicted by PASS include main pharmacological effects, mechanisms of action, specific toxicities, interactions with antitargets, metabolic actions, influence in gene expression, and action on transporters [38, 53]. Biological activities related to antioxidant activity were selected for discussion from the activity spectrum of the individual flavonoids and phenolic acids under consideration. The Pi values were not significant for decision-making of inactivity for most of the compounds considered. On average, each of the considered flavonoids was predicted to have 90 pharmacological activities with Pa value greater than 0.700, which was doubled that of the standard reference compound. The gallic acid and syringic acid predicted pharmacological activities were 375 and 220, respectively. As a result, the flower of *D. angustifoli*a can be considered a potential pharmacological agent [62], which is consistent with the therapeutic use of the plant's leaves.

The antioxidant and other related activities of the four flavonoids, quercetin, rutin, myricetin, and kaempferol, have been predicted. Similarly, three phenolic acids, namely, gallic acid, syringic acid, and chlorogenic acid have been predicted using PASS. The PASS results were compared with ascorbic acid as a reference standard (Table 4). The Pa values for the flavonoids and chlorogenic acid were greater than 0.700. This indicates that the antioxidant activities of these compounds are very likely to be positive if attempted via wet lab experiments. These results agreed with the DPPH assay results of the extracts. The Pa value for ascorbic acid, a standard reference for *in vitro* antioxidant activity, was comparable with the flavonoids, more specifically myricetin and rutin.

Activities such as free radical scavengers, peroxidase inhibitors, membrane integrity agonists, dioxygenase inhibiter, and NADPH oxidase inhibitors refer to the mechanisms of action for the antioxidant activity by the respective compounds [51]. The Pa values of these activities were also greater than 0.700, indicating all to be among the possible mechanisms of action for the antioxidant activities of the compounds considered in this investigation. Considering the activation of internal antioxidants with Pa values less than 0.300, the polyphenols' role in the activation of internal antioxidant enzymes was not expected.

The Pa values related to the antioxidant activity such as antimutagenic, cardioprotective, anticarcinogenic, chemopreventive, and proliferative disease treatment were also greater than 0.700. Except for minor inconsistencies in the treatment of proliferative illnesses, the compounds under

Table 4: Probability of activity (Pa) summary for the flavonoids and phenolic acids.

SN	Activity	Standard	Polyphenols					
		AA	F1	F2	F3	F4	PA1	PA2	PA3
1	Antioxidant	0.928	0.872	0.923	0.856	0.924	0.520	0.403	0.785
2	Free radical scavenger	0.564	0.811	0.988	0.771	0.832	0.570	0.619	0.856
3	Peroxidase inhibitor	0.252	0.962	0.987	0.956	0.966	0.891	0.846	0.855
4	Membrane integrity agonist	0.815	0.973	0.984	0.974	0.968	0.890	0.837	0.940
5	Membrane integrity antagonist	0.561	0.454	0.758	0.530	0.410	0.543	0.627	0.304
6	Quercetin 2,3-dioxygenase inhibitor	0.206	0.934	0.371	0.951	0.917	0.422	nd	nd
7	NADPH oxidase inhibitor	nd	0.928	0.850	0.889	0.939	0.509	0.520	
8	Antimutagenic	nd	0.940	0.503	0.948	0.963	0.597	0.821	0.409
9	Cardioprotection	0.229	0.833	0.988	0.814	0.886	0.468	0.463	nd
10	Anticarcinogenic	0.332	0.757	0.983	0.715	0.784	0.395	0.413	0.846
11	Chemopreventive	0.382	0.717	0.968	0.669	0.734	0.406	0.452	0.833
12	Proliferative disease treatment	nd	0.614	0.952	0.602	0.645	0.324	0.317	0.769
13	Antibacterial	0.377	0.387	0.677	0.395	0.421	0.418	0.395	0.537
14	Antiprotozoal (Leishmania)	0.205	0.575	0.907	0.554	0.521	0.329	0.347	0.655
15	Antifungal	0.332	0.490	0.784	0.495	0.508	0.398	0.366	0.638
16	Anti-inflammatory	0.779	0.689	0.728	0.676	0.720	0.548	0.498	0.598
17	Antiviral (herps)	0.418	0.484	0.526	0.483	0.500	0.404	0.377	0.411
18	Antiviral (influenza)	0.459	0.403	0.743	0.400	0.444	0.654	0.607	0.537
19	Antiviral (hepatitis B)	0.180	0.498	0.451	0.496	0.519	nd	nd	0.528

Pa, probability to be active and no data; codes for compounds: AA, ascorbic acid; F1, quercetin; F2, rutin; F3, kaempferol; F4, myricetin; PA1, gallic acid; PA2, syringic acid; PA3, chlorogenic acid.

Table 5: Result summary of IC_{50} and R^2 values.

	Log (inhibitor) vs. normalized response: variable slope			
Sample	LogIC50	HillSlope	IC_{50}	R squared
DAF	−0.162 ± 0.003	−5.547 ± 0.218	0.689 ± 0.005	0.997 ± 001
DAL	−0.156 ± 0.001	−5.121 ± 0.036	0.698 ± 0.002	0.995 ± 002
Ascorbic acid	−0.9075 ± 0.01	−3.439 ± 0.02	0.1237 ± 0.01	0.991 ± 0.02

DAF: *D. angustifolia* flower part; DAL: *D. angustifolia* leaf part.

Table 6: % radical scavenging activity.

Concentrations (mg/mL)	% RSA of DAL	% RSA of DAF
5	22.98	22.60
10	34.46	34.99
25	55.72	54.67
50	82.72	85.11
100	93.74	94.46
150	93.12	94.40
200	92.11	94.78
250	91.32	94.31

Table 7: Total phenol and total flavonoid content for the flower and leaf extracts.

Sample	TPC (mg/100 g)	TFC (mg/100 g)
DAF	502.71 ± 7.56	488.23 ± 23
DAL	765.85 ± 16.95	700.66 ± 39.14

consideration were also most likely projected to be similarly potent. Other activities such as antibacterials, antiprotozoal, antifungal, anti-inflammatory, and antiviral activities were predicted to show activities less likely in the wet lab experiments. Here, the probability will be less than the first case as Pa values are between 0.500 and 0.700, with few irregularities among the compounds and the activities. As flavonoids display varied cellular effects, they affect the overall process of carcinogenesis by varied mechanisms [63].

Phenolic acids: syringic acid, chlorogenic acid, gallic acid, and flavonoids: quercetin, myricetin, rutin, and kaempferol are common in food substances, including alcoholic drinks and fruits [56]. Phenolic compounds are known to impart beneficial properties such as antimicrobial,

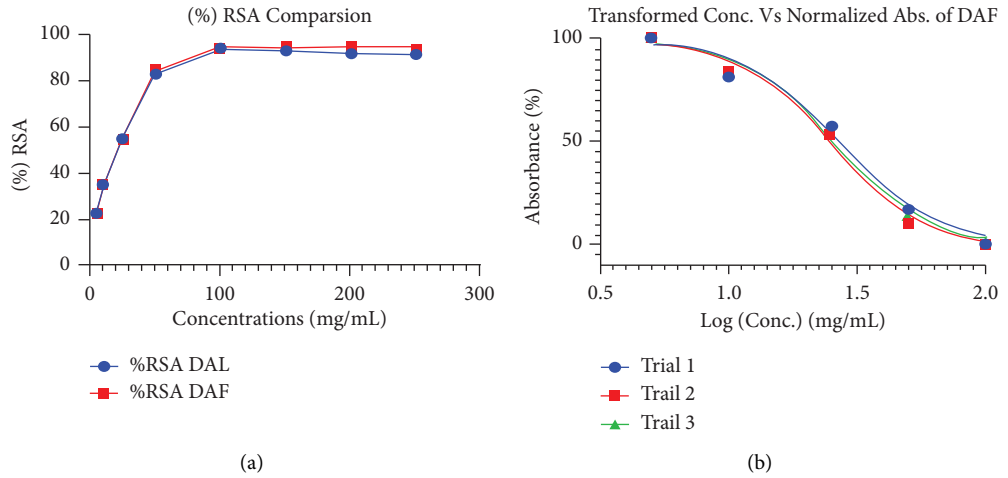

Figure 2: (a) Graph comparisons for % RSA and (b) log (conc.) vs. normalized absorbance.

preservatives, antioxidants [55], and other varied physiological properties [64]. Antioxidant properties dictate so many pharmacological activities such as cardioprotective, anticarcinogenic, gastroprotective, anti-inflammation, and antimicrobial effects in the human body, and hence they are considered nutraceuticals [65]. As an example, rutin and quercetin showed the gastroprotective effects due to their antioxidant properties [66]. This is also shown on the biological activity spectrum for PASS online prediction. The mechanisms of such pharmacological activities include enzyme inhibition, disruption of cell membranes, blocking viral attachments and cell penetration, and activating the host cell's self-defense mechanism [67].

5. Conclusions

D. angustifolia flower is a rich source of phytochemicals, which are extractable and can be checked for further biological activities depending on the phytochemical screening. A preliminary phytochemical study, the DPPH radical scavenging activity, and TPC, and TFC determinations all confirmed that the flowers and leaves of *D. angustifolia* are nearly similar in terms of phytoconstituency. From HPLC analysis, phenolic compounds identified clearly in the methanol extract of *D. angustifolia* flower include flavonoids: quercetin, myricetin, rutin, and phenolic acids: chlorogenic acid, syringic acid, and gallic acid. PASS biological activity prediction results show the stronger antioxidant activity of the identified flavonoids. Future work will emphasize the isolation and characterization of active principles responsible for bioactivity.

Data Availability

The data used to support this study are available in this article and the supplementary materials.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Supplementary Materials

Supplementary file 1. PASS Data. (*Supplementary Materials*)

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