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In vitro Screening of Phytochemicals, Antioxidant and Anticancer Activities of *Derris indica* Extracts

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ABSTRACT

Derris indica (Leguminosae) has been used in folk medicine to treat ulcers, bronchitis, coughing, whooping cough, and diabetes and has also been used pharmacologically for its anti-inflammatory, antimicrobial, antidiabetic, and anticancer activities. Fifteen extracts from leaf, flower, fruit, twig, and bark of *D. indica* were investigated by standard phytochemical screening tests, and the activity was confirmed by quantitative analysis based on the determination of total flavonoid contents. Free radical scavenging activities were evaluated *in vitro* by two methods: DPPH and ABTS, and the anticancer activity was measured by cell growth inhibition determined using a resazurin microplate assay (REMA). Phytochemical screening revealed the phenolics, flavonoids, terpenoids, alkaloids, saponins, and anthraquinones, and quantitative analysis showed that hexane and ethyl acetate extracts showed significantly higher TFC than methanol extracts. A positive correlation was established by the Pearson correlation test between DPPH and ABTS measurements (r = 0.831). Interestingly, the free radical scavenging activity depended on the structure- function relationship of flavonoids, with a negative correlation (r = 0.758, 0.846, 0.972, 0.601) for DPPH and (r = 0.946, 0.480, 0.965, 0.686) for ABTS which was observed in leaf, flower, fruit, and twig methanolic extracts, respectively. Anticancer activity was tested against the MCF7 breast cancer cell line and the NCI-H187 small cell lung cancer line. Hexane extract of the fruit, and ethyl acetate extract of the twig and bark were active against NCI-H187 cancer cells.

Keywords: ABTS; Anticancer; Antioxidant; *Derris indica*; DPPH; Phytochemical

1. Introduction

Cancer kills more people worldwide than any other illness and the number of deaths from cancer increases every year. The World Health Organization reported that in 2018 there were 18.1 million new cases of

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cancer and 9.6 million deaths from cancer. The five most common cancers in the world are lung cancer, breast cancer, colon cancer, prostate cancer, and stomach cancer. The risk factors of cancer may be environmental, dietary, or genetic. In Thailand, cancer has been the number one cause of death for over 20 years. According to statistics, liver cancer is the most serious type of cancer, lung and breast cancer are the second and third most serious, respectively. Even though it is a terrible disease, it is possible to prevent and reduce the risk of cancer. At present, there is growing interest in the preventive and therapeutic use of natural products. Phytochemicals from leaf, flower, fruit, seed, stem, and bark of plants [1] contain active components that may help to reduce chronic disease risks, including the risk of cancer [2-4]. Active components may be compounds such as terpenoids, glycosides, alkaloids, tannins, and flavonoids, which scavenge free radicals that are important mediators of several diseases. These compounds have been reported to possess antioxidant [5], antibacterial [6, 7], and anticancer activities [8].

Derris indica (Lam.) Bennet [synonyms: *Derris pinnata* (Lour), *Pongamia pinnata* (L.) Pierre, *Pongamia pinnata* (L.) Merr., *Pongamia glabra* Vent., and *Cytisus pinnaus* (L.)] is a medicinal plant belonging to the Leguminosae family. Its phytochemicals are produced under the extreme environmental conditions of strong winds, high temperatures, and strong salinity prevalent in mangrove areas. Various parts of *D. indica* have been used in folk medicine as antimicrobials, antiseptics, as liniment for rheumatism and diabetes, or even to cure tumors, skin ailments, bronchitis, and whooping cough [9]. In traditional Thai medicine, the leaf, stem, seed, root, bark, and fruit are often used for their anti-inflammatory, anti-plasmodial, antioxidant, and anti-diarrheal activities [9, 10]. An ethanolic extract of the flower has been used for its anti-hyperglycemic and anti-lipid peroxidation effects and to enhance the antioxidant defense system in alloxan-induced diabetic rats [11]. An ethanolic bark extract exhibited anti-inflammatory activity in a rat model [12] and the isolated pure compounds showed antioxidant activity [13]. In addition, compounds from a hexane extract of the fruit of this plant showed cytotoxicity against cholangiocarcinoma and HepG2 cell lines [14]. Previous studies have established that plants containing these and similar bioactive compounds have been used in traditional medicine to treat cancer.

However, the growth of plants is affected by location, climate, rainfall, and altitude. Variations in these conditions produce major variations in bioactive ingredients, even in plants grown in the same country [15]. Therefore, qualitative phytochemical screening must be the first step in identifying interesting bioactive compounds in medicinal plants for extraction, isolation, purification, and further investigation of pharmacological activities. Some data have been obtained from the phytochemical screening of various parts of *D. indica.* An aqueous bark extract showed the presence of alkaloids, terpenoids, and saponins [16]. The leaf, root bark, and root heart-wood extracted with various solvents (petrol, dichloromethane, ethyl acetate, butanol, and methanol) contains alkaloids, steroids, triterpenoids, flavonoids, saponins, and tannins, and exhibits antimicrobial activity, especially as a methanolic extract of leaf and root heart-wood. Petrol, butanol, and methanol extracts of the root bark showed good antibacterial activity [17]. The results of these studies suggest that the solvent systems selected for extraction play a significant role in the recovery of desired biomolecules.

The chemical investigation of various parts of this plant has identified several bioactive compounds (flavonoids, flavone, chalcone derivatives, and furanoflavonoid glycosides) that exhibit different biological activities. For instance, candidone, a flavone derivative isolated from a hexane extract of

D. indica fruit, had an antitumor effect on a model cell line and could be a treatment for cholangiocarcinoma [8]. The compounds derrivanone and derrischalcone showed strong activity against human hepatoma HepG2 cells [14]. Karanjin, pongamol, pongagalabrone, pongapin, pinnatin, kanjone, glabrachalcone, and isopongachromene were isolated from the seeds of *D. indica* [9]. The leaf and stem of the plant yielded several flavone and chalcone derivatives, such as galbone, pongone, pongalabol and pongagallone A and B [18] and flavonoids were isolated from the root bark [19]. *D. indica* fruit yielded the new compounds, furanoflavonoid glycoside, and pongamoside A-C, as well as the new flavonols glycoside, and pongamoside D [20]. Scientists have continued to study the pharmacological activity of extracts of the leaf, fruit, and flower of this plant but very little information exists about the activity of the twig and bark.

Therefore, the main objective of this work was to study the different phytochemical constituents of various parts of *D. indica* such as leaf, flower, fruit, twig, and bark, to investigate the correlation between total flavonoid content and free radical scavenging activity by DPPH and ABTS methods, and to study the anticancer activities of these extracts.

2. Materials and Methods

2.1 Collection and preparation of extracts

Fresh leaf, flower, fruit, twig, and bark samples of *D. indica* were collected in April 2017 from Rajamangala Mangrove Forest, Rajamangala University of Technology Srivijaya, Trang Province and identified by Assistant Professor Sittichoke Junyong. The specimen voucher CMUB. 39893 was deposited in the Department of Biology, Faculty of Science, Chiang Mai University. The dried powder of each part was extracted for a week with sequentially polar organic solvents hexane, ethyl acetate, and methanol in the ratio of 1:5 (w/v). The resulting extracts were evaporated to dry residue using a rotary evaporator at 45°C and refrigerated until further use. The yields of leaf, flower, fruit, twig, and bark extracts were recorded and the dried extracts were screened for phytochemicals and biological activities.

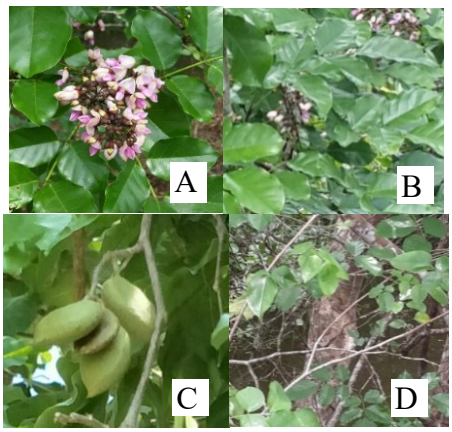

Fig. 1. *Derris indica* (A) flower, (B) leaf, (C) fruit and (D) twig and bark.

2.2 Phytochemical screening test

The analysis of the phytochemical constituents of the various extracts of *D. indica* followed established standard techniques [5]. The detection of phenolics, flavonoids, terpenoids, alkaloids, saponins, and anthraquinone was carried out as previously described [21]. In the qualitative analysis, absence was expressed as a negative (-) and presence as a positive (+), and the intensity of characteristic color or solidity was expressed as (+), (++), or (+++).

2.3 Determination of total flavonoid contents

The flavonoid contents (mg/mL) of *D. indica* extracts were determined as previously described [22]. For each extract, a reaction mixture was composed consisting of 0.1 mL of the extract at a concentration of 1 mg/mL and 0.5 mL of 5% $NaNO_2$. The mixture was left to stand for 6 minutes at room temperature before the addition of 0.2 ml of 10% $AlCl_3$. The mixture was then vortexed for 5 min, after which 0.5 mL of 1 M NaOH was added and the volume was

made up to 1.5 mL with distilled water. The solution was mixed well again and absorbance was measured against a blank at 510 nm using a UV-Visible spectrophotometer. The total flavonoid content of each sample was calibrated alongside the standard curve of rutin at concentrations of 50-500 µg/mL and is expressed in terms of rutin equivalents per gram of dried crude extract (mg RU/g CE).

2.4 Free radical scavenging activity

2.4.1 DPPH scavenging assay

The free radical scavenging effect on DPPH radicals was determined by slightly modifying the method of Vittaya et al. [5]. Briefly, 0.5 mL of 0.15 mM methanolic DPPH solution was mixed with 0.5 mL of each sample (1 mg/mL) and standard. The reaction mixture was shaken vigorously and allowed to stand at room temperature in darkness for 30 min. The control was prepared as above without the addition of the extract sample. Ascorbic acid and butylated hydroxytoluene (BHT) were used as positive controls. The absorbance of the solution was measured at 517 nm against the blank. The scavenging ability of each plant extract was calculated using the following equation: DPPH Scavenging activity

$$(\%) = \left(1 - \frac{\left(Abs_{sample} - Abs_{sample\ blank}\right)}{Abs_{control}}\right) \times 100,$$

where Abs_{sample} is the absorbance of the test sample with DPPH solution, $Abs_{sample\ blank}$ is the absorbance of the test sample only, and $Abs_{control}$ is the absorbance of DPPH solution. All measurements were performed in triplicate and are expressed as average values.

2.4.2 ABTS scavenging assay

The 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulphonate radical cation ($ABTS^+$) decoloration assay was performed as described by Vittaya et al. [21]. The $ABTS^+$ working solution was prepared by mixing 5 mL of 7 mM ABTS with 880 µL of 140 mM $K_2S_2O_8$ (potassium persulfate). The mixture was allowed to stand in darkness for 16 h at room temperature and then diluted with methanol to give an absorbance of 0.700 ± 0.25 units using the spectrophotometer at an excitation wavelength of 734 nm. A sample extract aliquot of 0.1 mL was added to 0.9 mL of diluted $ABTS^+$ solution. The reaction mixture was shaken and left to stand for 6 min in darkness. After incubation, absorbance was measured at 734 nm. The percentage of scavenging inhibition of $ABTS^+$ was determined in triplicate and was calculated using the following equation:

$$\%\ \text{inhibition} = \frac{\left(Abs_{control} - Abs_{sample}\right)}{Abs_{control}} \times 100,$$

where $Abs_{control}$ is the absorbance of the extract without $ABTS^+$ solution and Abs_{sample} is the absorbance of the extract with $ABTS^+$ solution.

2.5 Anticancer screening

The anticancer activity of each extract was evaluated from cell growth inhibition determined using a resazurin microplate assay (REMA) in a 2-cell line panel consisting of MCF7 breast cancer cells and NCI-178 small cell lung cancer cells. Results were compared with positive controls of Ellipticin, Doxorubicin, and Tamoxifen. The extracts were tested in a range of concentrations (0.21-50.00 µg/mL) and the positive controls were dissolved in DMSO. The primary anticancer assay was performed at the National Center for Genetic Engineering and Biotechnology (BIOTEC) of the National Science and Technology Development Agency (NSTDA). Anticancer activity was determined by % cytotoxicity: < 50% was considered non-cytotoxic and > 50% was considered cytotoxic (IC_{50} included) [3].

2.6 Statistical analysis

The results are expressed as mean ± standard error of triplicate determination. Statistical analysis was carried out using a one-way analysis of variance (ANOVA) followed by Duncan multiple comparison. A value of $p < 0.05$ was considered significant. Two-way ANOVA was used to determine the interaction between plant parts and the three organic solvents. Correlation between the flavonoid contents and free radical scavenging activity was carried out using Person correlation coefficients.

3. Results and Discussion

3.1 Phytochemical screening

Various parts of *D. indica* were analyzed to determine their phytochemical composition, which showed the presence of several secondary metabolites such as anthraquinones, saponins, alkaloids, phenolics, flavonoids, and terpenoids. The results are summarized in Table 1. All studied parts contained flavonoids and phenolic compounds, which are among the most important groups of plant metabolites [23]. Phenolics were found in the fractions extracted with ethyl acetate and methanol (medium to high polarity solvents). In contrast to phenolics, flavonoids were found in the fractions extracted with hexane and ethyl acetate. Both metabolites have been reported to possess biological properties such as anticandidal activity [24] and antioxidant activity [25-29]. Terpenoids were found in leaf, flower, and twig extracted with hexane, ethyl acetate, and methanol but only in fruit and bark extracted with hexane. The therapeutic properties of terpenoids have generally been reported in use against bacteria, fungi, and cancers [24, 30, 31]. Alkaloids and saponins were detected only in methanolic extracts and several works have reported their anti-inflammatory [33], antimicrobial [33], and antimalarial [34] activities. Saponins were found to exercise antimicrobial activity against a wide range of microorganisms in vitro [35]. However, anthraquinone was found only in leaf samples extracted with ethyl acetate and methanol.

Table 1. Qualitative phytochemical screening of extracts of *D. indica*.

Plant constituent	Procedure	Observations	*D. indica* extracts													
			Leaf			Flower			Fruit			Twig			Bark		
			H	E	M	H	E	M	H	E	M	H	E	M	H	E	M
Phenolics	Extract+$FeCl_3$	Dark green precipitate	-	+	+	-	+	+	-	-	+	-	-	+	-	-	+
Flavonoids	Extract+Mg+HCl	Formation of a cherry color	+	+	-	+	+	-	+	+	+	+	+	-	+	+	-
Terpenoids	Extract+chloroform+ conc.H_2SO_4	Reddish brown ring	+	+	+	+	+	+	+	-	-	+	+	+	+	-	-
Alkaloids	Extract+Dragendroff's reagent	Orange-yellow precipitate	-	-	+	-	-	+	-	-	-	-	-	+	-	-	+
Saponins	Extract+H_2O+shaking	Formation of a stable form	-	-	+	-	-	-	-	-	+	-	-	+	-	-	+
Anthraquinones	Extract+dil. H_2SO_4+ chloroform+dil.NH_3	Reddish color	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-

Note: + means present and - means absent; H=hexane; E=ethyl acetate; M=methanol.

3.2 Determination of flavonoid contents

Total flavonoid contents (TFC) were analyzed using the equation:

$$y = 0.0016x - 0.0033 \quad \left(R^2 = 0.9995\right),$$

as rutin equivalents (mg rutin equivalents/g crude extract), and are presented in Table 2. The TFC was in the range 0.12 to 1.76 mg RU/g CE as shown in Table 2. Two-way ANOVA revealed that the main effects (plant parts and solvents) have a significant effect on the TFC ($F = 63.745$, $p < 0.001$ for plant parts; $F = 339.194$, $p < 0.001$ for solvents). Interaction between plant parts and solvents also had significant effects on the TFC ($F =$

45.214; $p < 0.001$). Based on a two-way ANOVA, TFC was affected by plant parts and solvents ($p < 0.001$). Considering the effects of solvents (averaged across plant parts), TFC was found highest in hexane (1.30±0.31 mg RU/g CE) and ethyl acetate (1.24±0.52 mg RU/g CE), as shown in Fig. 2. When the effect of plant parts was analyzed, the TFC was found to be highest in the leaf (1.31±0.60 mg Ru/g CE) followed by bark (1.20±0.87 mg RU/g CE). Fruit (1.00±0.27 mg Ru/g CE) and twig (0.97±0.37 mg RU/g CE) were similar and the lowest was found in flower (0.64±0.20 mg RU/g CE).

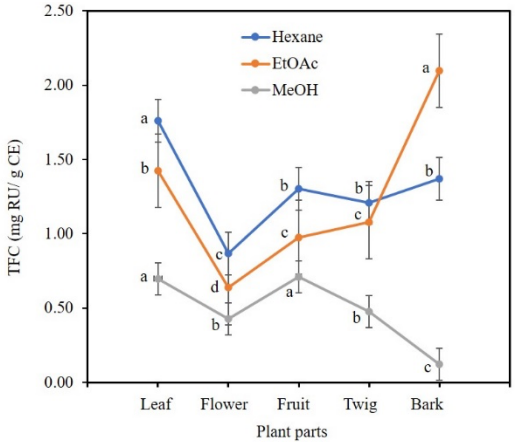

Fig. 2. Interaction effect of plant parts and solvents on the quantity of TFC in *Derris indica* extracts, where different lowercase letters indicate significant ($p < 0.05$) differences among means and error bars represent ± SD.

Table 2. Percentage of extract yield, concentration of total flavonoid contents and free radical scavenging activity of *D. indica* extracts.

Part used	Sol	Extract yield (%)	Total flavonoid Content (mg RU/g CE)	Free radical scavenging percentage (%)	
				DPPH assay	ABTS assay
Leaf	H	1.11	1.76 ± 0.17 ^{b}	55.52 ± 1.45 ^{d}	22.44 ± 0.72 ^{i}
	E	1.33	1.43 ± 0.02 ^{c}	92.56 ±0.17 ^{a}	48.31 ± 0.84 ^{fg}
	M	10.56	0.70 ± 0.06 ^{i}	94.12 ± 0.89 ^{a}	67.82 ± 1.61 ^{b}
Flower	H	3.00	0.87 ± 0.14 ^{gh}	11.99 ± 7.31 ^{g}	9.70 ± 0.23 ^{j}
	E	5.40	0.64 ± 0.03 ^{i}	95.11 ± 0.61 ^{a}	99.55 ± 0.78 ^{a}
	M	54.60	0.43 ± 0.02 ^{j}	95.02 ± 1.19 ^{a}	53.81 ± 1.96 ^{e}
Fruit	H	2.70	1.31 ± 0.14 ^{cd}	65.95 ± 2.39 ^{c}	50.76 ± 3.12 ^{f}
	E	7.40	0.98 ± 0.07 ^{fg}	82.38 ± 3.71 ^{b}	70.46 ± 2.50 ^{b}
	M	23.20	0.72 ± 0.01 ^{hi}	94.25 ± 1.29 ^{a}	99.09 ± 0.69 ^{a}
Twig	H	0.62	1.21 ± 0.02 ^{de}	50.31 ± 3.16 ^{e}	32.34 ± 0.12 ^{h}
	E	2.31	1.08 ± 0.22 ^{ef}	92.39 ± 1.72 ^{a}	63.92 ± 0.08 ^{c}
	M	4.93	0.48 ± 0.02 ^{j}	91.33 ± 1.21 ^{a}	68.38 ± 1.26 ^{b}
Bark	H	1.10	1.37 ± 0.03 ^{cd}	28.23 ± 3.01 ^{f}	22.55 ± 2.22 ^{i}
	E	5.75	2.10 ± 0.10 ^{a}	65.55 ± 1.94 ^{c}	47.69 ± 0.49 ^{g}
	M	10.50	0.12 ± 0.01 ^{k}	70.05 ± 3.75 ^{c}	57.13 ± 3.87 ^{d}
BHT				94.12 ± 0.06 ^{a}	98.90 ± 0.57 ^{a}
Ascorbic acid				96.22 ± 0.17 ^{a}	99.74 ± 0.18 ^{a}

Two-way ANOVA

Variable	df	*F*	*P*	*F*	*P*	*F*	*P*
Plant Parts (PP)	4	63.745	< 0.001	143.973	< 0.001	415.528	< 0.001
Solvents (S)	2	339.194	< 0.001	1258.003	< 0.001	2601.350	< 0.001
PP x S	8	45.214	< 0.001	66.459	< 0.001	277.032	< 0.001
Error	30						

Note: Data shown as mean ± SD values from triplicate analysis. Different lower case letters (a-k) in each sample denote significant differences ($p < 0.05$). H=hexane; E=ethyl acetate; M=methanol.

3.3 Free radical scavenging activity

Due to the complex nature of phytochemical substances, the antioxidant activity of a plant extract cannot be evaluated by any single method. It is also important to use generally accepted assays to determine antioxidant activity. DPPH and ABTS were selected to determine the free radical scavenging activity. The DPPH method was used to test the oxidation reaction of crude

extracts with stable, purple DPPH at room temperature. Taking all extracts of all parts of *D. indica* into consideration, scavenging of the DPPH free radical ranged from 11% to 95% (Table 2). The $ABTS^+$ method was also chosen as it is effective in determining antioxidant activity. The decolorization of the $ABTS^+$ radical indicates the capacity of an antioxidant species to inactivate radical species by donating electrons or hydrogen atoms. The observed reductions in absorbance of the samples indicated moderate scavenging activity that ranged from 9% to 99% when all fifteen *D. indica* extracts were taken into consideration. It was found that the ethyl acetate and methanol extracts from all studied parts of *Derris indica* possessed higher free radical scavenging activity than the hexane extracts by both DPPH and ABTS assays, despite their lower flavonoid contents. This is caused by the presence of an active flavonoid structure obtained through high polar solvent extraction. Although the flavonoid contents from methanol crude extract were lower than other solvents, there might exist some specific flavonoid derivatives which are potentially active to free-radical scavengers, that provided activity against the DPPH and ABTS radicals. In this work, we did not isolate each derivative of flavonoid. However, based on a previous literature review, it is possible that the presence of a diphenylpropane (C6-C3-C6) skeleton, substitution on the flavonoid skeleton, gallate and galactouronate moieties [36] could give rise to free radical scavenging activity (the structure-function relationship). Such compounds acting as antioxidants due to their conjugated π-electron systems allow donation of electrons or hydrogen atoms from the hydroxyl moieties to free radicals [37]. Two-way ANOVA revealed the main effects (plant parts and solvents) showing significant effect on antioxidant capacity. The highly significant effect, plant parts × solvents, on DPPH ($F = 66.459, p < 0.001$), and on ABTS ($F = 277.032, p < 0.001$) are shown in Table 3.

Fig. 3 and Fig. 4 show that antioxidant activity increases as the concentration of sample extracts increases. Extracts of fruit and twig, especially, showed potent antioxidant activities. The antioxidant activity of fruit and twig extracts was influenced by secondary metabolites in the TFC (Table 2). This detected compound could be responsible for antioxidant activity. Flavonoids are oxidized by free radicals of DPPH and the ABTS ion, resulting in a more stable and less reactive radical [33]. On the other hand, hydroxyl groups of flavonoids stabilize the reactive oxygen species by reacting with the radicals. Both these reactions produce inactive radicals. TFC was higher in the hexane and ethyl acetate extracts than in the methanolic extracts which correlated with the results of free radical scavenging with various concentrations of each extract. This negative correlation of flavonoids with antioxidant capacity was observed in previous research [38], that reported that flavonoids could be related to other antioxidant compounds contained in extracts. The results of the DPPH and ABTS assays were also well in agreement, with a correlation coefficient of 0.831 at a statistically significant level ($p < 0.01$) (Table 3).

Table 3. Correlation coefficient between variables of total flavonoid and free radical scavenging activity in organic extract of *D. indica*.

Variables	TFC	DPPH	ABTS
TFC	1		
DPPH	-0.345*	1	
ABTS	-0.455**	0.831**	1

Note: TFC = total flavonoid content; DPPH = 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity; ABTS = 2,2′-azinobis-3 ethylbenzothiazoline-6-sulfonic acid; * Correlation is significant at the 0.05 level; ** Correlation is significant at the 0.01 level.

3.3 Correlation between TFC and the free radical scavenging in all parts extracts

Since the strength of free radical scavenging activity of extracts depended on the presence of considerable quantities of flavonoids (Table 1), we investigated the correlation between TFC in *D. indica* extracts and free radical scavenging activity as measured by the DPPH and ABTS assays. The results are presented in Table 4. In this work, the correlation between TFC and the DPPH/ABTS assay results was satisfactory for all five parts of the plant. Good correlations were found between TFC and ABTS scavenging for leaf, fruit, and twig extracts and between TFC and DPPH scavenging for leaf, flower, and fruit extracts. In the case of fruit extracts, TFC correlated significantly with the results of the DPPH and ABTS assays. Bark extracts showed a poor correlation and no significance. Table 4 shows the negative correlation between each sample of *D. indica* and free radical scavenging, indicating that the activity did not depend on TFC but rather on the structure-function relationship of flavonoids. This result was used to explain the results of all extracts except bark. In addition, ethyl acetate and methanol are more effective organic solvents for extracting bioactive flavonoid compounds than hexane. These correlations confirm that the flavonoids are the main active component contributing to the antioxidant activities in extracts of leaf, flower, fruit, and twig but not bark. It is possible that other secondary metabolic compounds such as phenolics, terpenoids, alkaloids, and saponins have an effect on activity in the bark. These compounds are certain to be phenolics and alkaloids [39] which were only found in methanolic extracts of *D. indica*. This result supports the use of this plant in traditional medicines. Its bioactive compounds can scavenge the free radicals implicated in several disease conditions.

Table 4. Correlation coefficient (r) between variables of total flavonoid followed by each of parts and free radical activity analyzed by different methods.

Methods	TFC				
	Leaf	**Flower**	**Fruit**	**Twig**	**Bark**
DPPH	-0.758 (p = 0.018)	-0.846 (p = 0.004)	-0.972 (p = 0.000)	-0.601 (p = 0.087)	-0.246 (p = 0.523)
ABTS	-0.946 (p = 0.000)	-0.480 (p = 0.191)	-0.965 (p = 0.000)	-0.686 (p = 0.041)	-0.399 (p = 0.288)

Note: TFC = total flavonoid content; DPPH = 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity; ABTS = 2,2′-azinobis-3 ethylbenzothiazoline-6-sulfonic acid; * Correlation is significant at the 0.05 level; ** Correlation is significant at the 0.01 level.

3.4 Anticancer activity

Cancer is one of the most serious diseases to affect humans. Plants are bioactive sources of drugs for the treatment of cancer and can help the development of novel anticancer agents. This research aimed to find new potential anticancer agents from a natural source. In this work, the anticancer activity of *D. indica* extracts is shown in Table 5. The hexane extract of fruit, and the ethyl acetate extracts of twig and bark showed anticancer activity against the NCI-H187 small cell lung cancer cells. The IC_{50} values were 46.23±3.37 for the hexane extract of fruit, 47.77±1.13 the ethyl acetate extract of twig, and 46.87±0.57 µg/mL for the ethyl acetate extract of bark. The anticancer activity of these extracts could be attributed to the presence of flavonoids and terpenoids in the hexane and ethyl acetate extracts. Previous studies have shown that flavonoids exhibit cytotoxic activity against human cholangiocarcinoma cells [8, 20, 40, 41]. Reports also mentioned that hexane extract of *D. indica* fruit contained flavone derivatives [8], derrivanone and derrischalcone compounds [13], furanoflavonoid glycoside, pongamoside, and flavonol glycoside [19]. Other extracts appeared to be inactive against this cell line (IC_{50} > 50 µg/mL). None of the extracts inhibited the growth of MCF7 breast cancer cells. These results support the potential benefits of the reported use of this plant for the treatment of cancer in traditional medicine [8, 9, 19, 38].

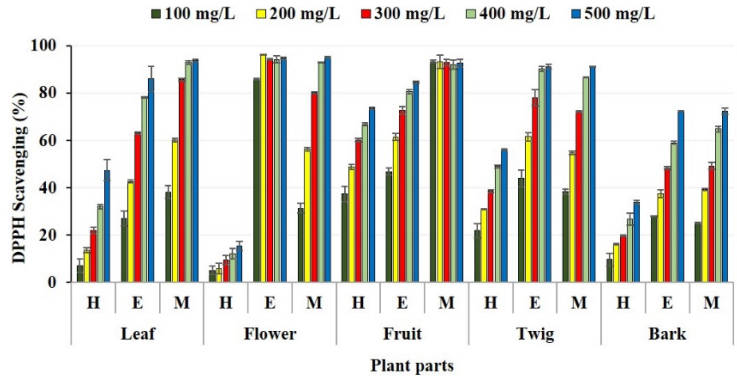

Fig. 3. The DPPH free radical scavenging activity (%) of *D. indica* leaf, flower, fruit, twig, and bark extracts at different concentrations, where H, E, and M are hexane, ethyl acetate, and methanol, respectively, and error bars indicate ± SD from triplicate analysis.

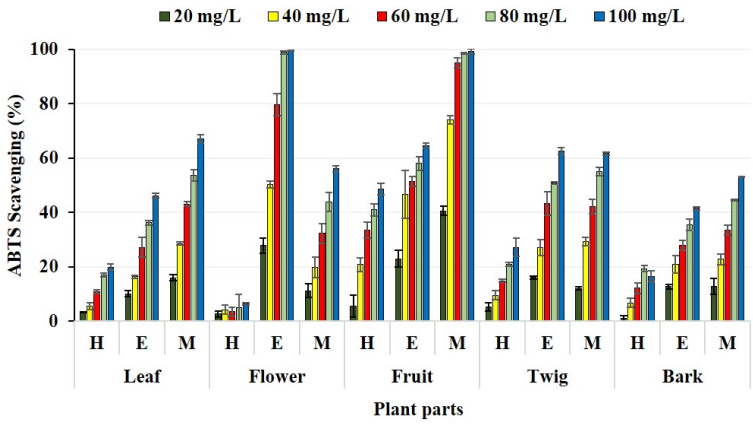

Fig. 4. The ABTS free radical scavenging activity (%) of *D. indica* leaf, flower, fruit, twig, and bark extracts at different concentrations, where H, E, and M are hexane, ethyl acetate, and methanol, respectively, and error bars indicate ± SD from analysis of triplicate analysis.

Table 5. IC_{50} values of the *D. indicia* extracts against cancer cell lines.

Part used	Solvent	Cell lines IC_{50} (µg/mL)	
		MCF7	NCI-H187
Leaf	H	> 50	> 50
	*E	> 50	> 50
	M	> 50	> 50
Flower	H	> 50	> 50
	E	> 50	> 50
	M	> 50	> 50
Fruit	H	> 50	46.23 ± 3.37
	E	> 50	> 50
	M	> 50	> 50
Twig	*H	> 50	> 50
	*E	> 50	47.77 ± 1.13
	M	> 50	> 50
Bark	H	> 50	> 50
	E	> 50	46.87 ± 0.57
	M	> 50	> 50
Ellipticine		-	2.02 ± 0.57
Doxorubicin		-	0.10 ± 0.03
Tamoxifen		7.92 ± 0.84	-

Note: The extracts with an IC_{50} value > 50 µg/mL were considered inactive. Data shown as mean ± SD values from analysis of triplicate analysis. H=hexane, E=ethyl acetate, M=methanol; * = partially soluble 100 % DMSO. H=hexane; E=ethyl acetate; M=methanol.

4. Conclusion

The bioactive compounds in leaf, flower, fruit, and twig of *D. indica* showed good free radical scavenging activity arising from the structure-function relationship of the flavonoids, rather than TFC. In addition, ethyl acetate and methanol solvents were good organic solvents for extracting bioactive flavonoids. The activities of hexane and ethyl acetate extracts of fruit and twig against small cell lung cancer cells were comparable with the ethyl acetate bark extract. The results of this study showed that extracts of *D. indica* could be promising sources of antioxidants for pharmaceutical applications. To fully understand the structure-function relationship of flavonoids, further study is needed to purify, characterize, and identify bioactive compounds from the plant extracts.

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