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Antiproliferative and phytochemical analyses of leaf extracts of ten Apocynaceae species

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ABSTRACT

Background: The anticancer properties of Apocynaceae species are well known in barks and roots but less so in leaves. **Materials and Methods:** In this study, leaf extracts of 10 Apocynaceae species were assessed for antiproliferative (APF) activities using the sulforhodamine B assay. Their extracts were also analyzed for total alkaloid content (TAC), total phenolic content (TPC), and radical scavenging activity (RSA) using the Dragendorff precipitation, Folin–Ciocalteu, and 1,1-diphenyl-2-picrylhydrazyl (DPPH) assays, respectively. **Results:** Leaf extracts of *Alstonia angustiloba*, *Calotropis gigantea*, *Catharanthus roseus*, *Nerium oleander*, *Plumeria obtusa*, and *Vallaris glabra* displayed positive APF activities. Extracts of *Allamanda cathartica*, *Cerbera odollam*, *Dyera costulata*, and *Kopsia fruticosa* did not show any APF activity. Dichloromethane (DCM) extract of *C. gigantea*, and DCM and DCM:MeOH extracts of *V. glabra* showed strong APF activities against all six human cancer cell lines. Against breast cancer cells of MCF-7 and MDA-MB-231, DCM extracts of *C. gigantea* and *N. oleander* were stronger than or comparable to standard drugs of xanthorrhizol, curcumin, and tamoxifen. All four extracts of *N. oleander* were effective against MCF-7 cells. Extracts of *Kopsia fruticosa* had the highest TAC while those of *Dyera costulata* had the highest TPC and RSA. Extracts of *C. gigantea* and *V. glabra* inhibited the growth of all six cancer cell lines while all extracts of *N. oleander* were effective against MCF-7 cells. **Conclusion:** Extracts of *C. gigantea*, *V. glabra*, and *N. oleander* therefore showed great promise as potential candidates for anticancer drugs. The wide-spectrum APF activities of these three species are reported for the first time and their bioactive compounds warrant further investigation.

Key words: Antiproliferative, Apocynaceae, radical scavenging, total alkaloid content, total phenolic content

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INTRODUCTION

The family Apocynaceae consists of about 250 genera and 2000 species of tropical trees, shrubs, and vines.[1] With the inclusion of species of Asclepiadaceae, the family has now been enlarged from two to five subfamilies.[2] Characteristic features of the family are that almost all species produce milky sap; leaves are simple, opposite, or whorled; flowers are large, colorful, and slightly fragrant with five contorted lobes; and fruits are in pairs.[1,3]

In traditional medicine, Apocynaceae species are used to treat gastrointestinal ailments, fever, malaria, pain, and diabetes.[1] Of the 10 species studied, leaves of *Allamanda cathartica* are used as a purgative or emetic in Southeast Asia.[4] Leaves are also used as an antidote, and for relieving coughs and headaches. Stems, leaves, and latex of *Alstonia angustiloba* are used for gynecological problems and skin sores in Indonesia.[5] Leaves are externally applied to treat headache in Malaysia.[6] Roots and leaves of *Calotropis gigantea* are used to treat skin and liver diseases, leprosy, dysentery, worms, ulcers, tumours, and earache.[7] Its latex has wound-healing properties.[8] A decoction of all parts of *Catharanthus roseus* is used to treat malaria, diarrhea, diabetes, cancer, and skin diseases.[9] The species is also well known as an oral hypoglycemic agent. Extracts prepared from leaves have been used as an antiseptic agent for healing wounds and as a mouthwash to treat toothache. In Southeast Asia, leaves of *Cerbera odollam* are used in aromatic bath by women after childbirth.[10] Leaves, bark and latex are emetic and purgative, and seeds are toxic and

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strongly purgative. Leaves and bark of *Dyera costulata* have been used for treating fever, inflammation, and pain.[11] *Kopsia fruticosa* has cholinergic effects and is used to treat sore and syphilis.[12] *Nerium oleander* is highly poisonous with no reported benefits in traditional medicine. In Asia, a decoction of leaves of *Plumeria obtusa* is used for treating wounds and skin diseases.[13] Its latex and bark are known to have purgative and diuretic properties. *Vallaris glabra* is well known in Thailand because the scent of its flowers is similar to that of pandan leaves and aromatic rice.[14] Its use in traditional medicine is not known.

Apocynaceae species have been reported to possess anticancer properties. They include those of *Allamanda*,[15] *Alstonia*,[16,17] *Calotropis*,[18-20] *Catharanthus*,[21] *Cerbera*,[22,23] *Nerium*,[24,25] *Plumeria*,[26] and *Tabernaemontana*.[27]

Prompted by the anticancer properties found in many species of Apocynaceae, leaf extracts of 10 species were assessed for antiproliferative activity against six human cancer cell lines. Their extracts were also analyzed for total alkaloid content, total phenolic content, and radical scavenging activity.

MATERIALS AND METHODS

Plant materials

The 10 Apocynaceae species studied were *A. cathartica*, *Alstonia angustiloba*, *Calotropis gigantea*, *Catharanthus roseus*, *Cerbera odollam*, *Dyera costulata*, *Kopsia fruticosa*, *Nerium oleander*, *Plumeria obtusa*, and *Vallaris glabra* [Figure 1]. Their common or vernacular names and brief descriptions are given in [Table 1]. Leaves of the species were collected from Sunway, Puchong, or Kepong, all in the state of Selangor, Malaysia. Identification of species was based on documented descriptions and illustrations.[1,3] The voucher specimens of these species were deposited in the herbarium of Monash University Sunway Campus.

Extraction of leaves

For crude extraction, fresh leaves of each species (40 g) were cut into small pieces and freeze-dried overnight. Dried samples were blended and extracted with 250 ml of methanol (MeOH) three times for 1 h each time. Samples were filtered and the solvent was removed using a rotary evaporator (Eyela). The dried crude extracts were stored at –20°C for further analysis. For sequential extraction, fresh leaves of each species (40 g) were freeze-dried, ground, and extracted successively with hexane, dichloromethane (DCM), DCM:MeOH (1:1), and MeOH (HmbG Chemicals). For each solvent, the suspension of ground leaves in 250–300 ml of solvent was shaken for 1 h on the orbital shaker. After filtering, the samples were extracted two more times for each solvent. Solvents were removed with a rotary evaporator to obtain the dried extracts, which were stored at –20°C for further analysis.

Antiproliferative activity

Antiproliferative (APF) activity of extracts (25 μg/ml) was initially screened for growth inhibitory activity against three human cancer cell lines (MCF-7, MDA-MB-231, and HeLa) using the sulforhodamine B (SRB) assay.[28-30] Growth inhibitory activity with less than 50% cell growth was considered positive while that with more than 50% cell growth was considered negative. Extracts with positive growth inhibition were further tested against six human cancer lines (MCF-7, MDA-MB-231, HeLa, HT-29, SKOV-3, and HepG2) using six different extract concentrations. Human cancer cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). The

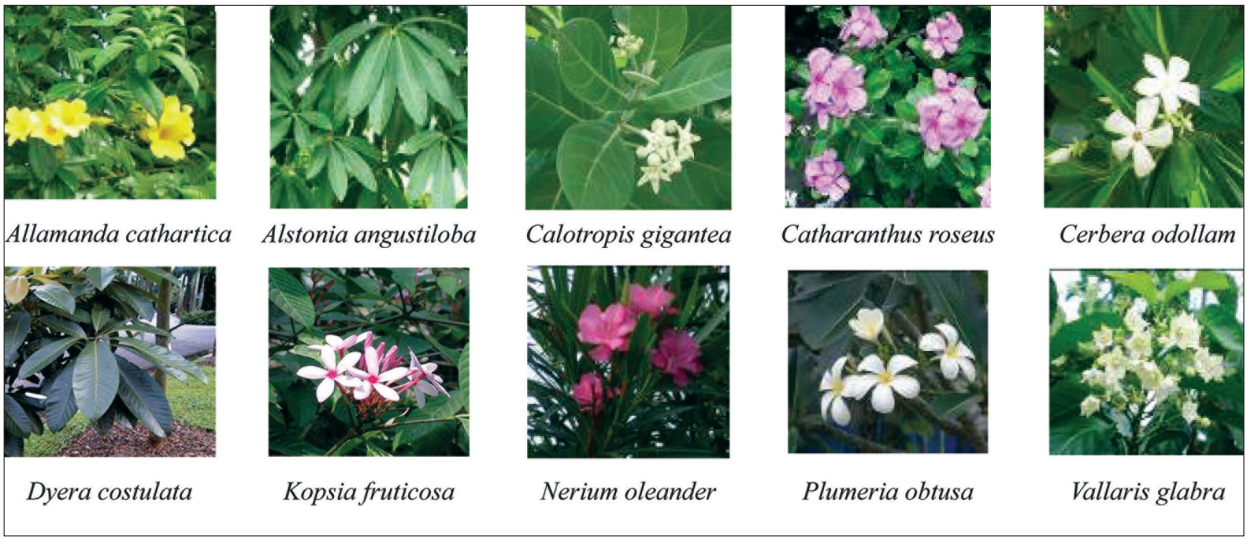

Figure 1: The ten Apocynaceae species studied

Table 1: Common or vernacular names and brief description of Apocynaceae species studied

Species (common/vernacular name)	Brief description
Allamanda cathartica L. (Common allamanda)	A shrub with trumpet-shaped yellow flowers similar in size as leaves which are in whorls
Alstonia angustiloba Miq. (Pulai)	A medium-sized tree with leaves in whorls and having fine secondary veins
Calotropis gigantea (L.) Aiton (Giant milkweed)	A shrub with pale green leaves and white or lilac flowers with a crown rising from the center
Catharanthus roseus (L.) G. Don (Madagascar periwinkle)	A common ornamental shrub with oval to oblong leaves and white, pink or purple flowers with a dark colored center
Cerbera odollam Gaertner (Pong-pong)	A tree bearing white flowers in clusters and rounded fruits that are green when young and red when mature
Dyera costulata Hook (Jelutong)	A tall timber tree with straight columnar bole, leaves in whorls and latex which was an important source of chewing gum
Kopsia fruticosa (Ker.) A. DC. (Pink kopsia)	A shrub with large glossy leaves and clusters of light pink flowers resembling those of Ixora
Nerium oleander L. (Oleander)	An ornamental shrub with thick narrow leaves in pairs or whorls and bearing clusters of pink, red, or purple flowers
Plumeria obtusa L. (Frangipanni)	A tree producing dark green, glossy and oval leaves and white fragrant flowers with a yellow center
Vallaris glabra Kuntze (Kesidang)	A woody climber producing clusters of white flowers with a scent characteristic of pandan leaves or fragrant rice

cells were seeded 24 h prior to treatment in 96-well plates at densities of 10,000–20,000 cells/well. Each cell line was designated one plate. Initial cell population of each cell lines prior to addition of extracts was determined by fixing with trichloroacetic acid (TCA) (Sigma). Extracts were dissolved in dimethyl sulfoxide (DMSO) (Sigma) and serially diluted from 8–25 μg/ml. Control cultures were treated with the same volume of DMSO. The concentration of DMSO was kept within 1% to avoid any interference with cell viability. After the addition of extracts, the plates were incubated for 48 h. After incubation, the cells were fixed with 50 μl of cold 50% TCA and incubated for 1 h at 4°C. The plates were then washed with tap water and air dried. Cells were stained with 100 μl of 0.4% SRB solution (Sigma) diluted with 1% acetic acid followed by incubation for 10 min at room temperature. Unbound dye was removed by washing with 1% acetic acid. Bound stain was then solubilized with 200 μl of 10 mM trizma base (Sigma). Absorbance of each well at 505 nm was obtained using a microplate reader. Dose–response curves were constructed to obtain GI_{50} or growth inhibition of cell lines by 50%. Activity is considered to be effective when GI_{50} value ≤20 μg/ml.[31]

Analysis of TAC, TPC, and RSA

Total alkaloid content (TAC) of extracts was determined using the Dragendorff precipitation assay.[32] For each species, extracts (15 mg) were dissolved in 1 ml of distilled water that was acidified to pH 2.0–2.5 with 0.01 M HCl. Analysis was conducted in triplicate. Alkaloids were then precipitated with 0.4 ml of Dragendorff reagent. Washed with 0.5 ml of distilled water to remove traces of the reagent, the precipitate was later treated with 0.4 ml of 1% sodium sulfide, resulting in a brownish-black precipitate. Precipitates formed at each stage were recovered by centrifugation at 14,000 rpm for 1 min. The resulting precipitate was dissolved in 0.2 ml of concentrated nitric acid and diluted to 1 ml with distilled water. Addition of 2.5 ml of 3% thiourea to 0.5 ml aliquots of this solution resulted in a yellow-colored complex. Absorbance was measured at 435 nm and TAC was expressed as boldine equivalent in milligram per gram of extract. The calibration equation for boldine (Sigma) was $y = 1.068x$ ($R^2 = 0.9959$) where y is absorbance and x is mg/ml of boldine. Dragendorff reagent was prepared by dissolving 0.8 g of bismuth nitrate (Sigma) in 40 ml of distilled water and 10 ml of glacial acetic acid. The resulting solution was mixed with 20 ml of 40% potassium iodide.

Total phenolic content (TPC) of extracts was determined using the Folin–Ciocalteu (FC) assay.[33] Extracts (300 μl in triplicate) were introduced into test tubes followed by 1.5 ml of FC reagent (Fluka) at 10 times dilution and 1.2 ml of sodium carbonate (Fluka) at 7.5% w/v. The tubes were allowed to stand for 30 min in the dark before absorbance was measured at 765 nm. TPC was expressed as gallic acid (GA) equivalent in milligram per gram of extract. The calibration equation for GA (Fluka) was $y = 0.0111x - 0.0148$ ($R^2 = 0.9998$) where y is absorbance and x is mg/ml of GA.

Radical scavenging activity (RSA) of extracts was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay.[33] Different dilutions of extracts (1 ml in triplicate) were added to 2 ml of DPPH (Sigma). The concentration of DPPH used was 5.9 mg in 100 ml of methanol. Absorbance was measured at 517 nm after 30 min. RSA was calculated as IC_{50}, the concentration of extract to scavenge 50% of the DPPH radical. RSA was then expressed as ascorbic acid equivalent antioxidant capacity (AEAC) using the equation of AEAC (mg ascorbic

acid/g of extract) = $IC_{50(ascorbate)}/IC_{50(sample)} \times 10^5$. IC_{50} of ascorbic acid used for calculation of AEAC was 0.00387 mg/ml.

RESULTS AND DISCUSSION

Initial screening of leaf extracts of 10 Apocynaceae species against three human cancer cell lines (MCF-7, MDA-MB-231, and HeLa) showed that extracts of *A. angustiloba*, *C. gigantea*, *C. roseus*, *N. oleander*, *P. obtusa*, and *V. glabra* displayed positive growth inhibitory activity, that is, inhibition with ≤50% cell growth [Table 2]. DCM extract of *C. gigantea*, and DCM and DCM:MeOH extracts of *N. oleander* and *V. glabra* inhibited all three cancer cell lines. All four extracts of *N. oleander* were effective against MCF-7 cells. Only DCM:MeOH extract of *C. roseus* was active against MCF-7 and HeLa cells. Hexane extract of *N. oleander* inhibited MCF-7 cells while hexane extract of *P. obtusa* inhibited MCF-7 and HeLa cells. MeOH extracts of *N. oleander* and *V. glabra* inhibited MCF-7 cells. In general, DCM and DCM:MeOH extracts of these species were the most effective. All extracts of *A. cathartica*, *C. odollam*, *D. costulata*, and *K. fruticosa* did not show any APF activity.

Contrary to findings of this study, cytotoxic activities have been reported in species of *Cerbera*, *Allamanda*, and *Kopsia*. Methanol extract of leaves of *C. odollam* strongly inhibited MCF-7 and T47D cells.[23] Cardenolides from seeds of *C. odollam* had cytotoxic activity against KB, BC, and NCI-H187 cells.[34] Potent cytotoxic activity was reported in ethanol extracts of fruits and leaves of *Cerbera manghas*.[35] Cardenolides from roots of *C. manghas* also showed APF activity.[22] Ethanol root extracts of *A. schottii* and *A. blanchetti* displayed stronger cytotoxicity against K-562 cells than leaf and stem extracts.[15] Valparicine from the stem bark of *Kopsia arborea* showed cytotoxic effects against KB and Jurkat cells[36] while kopsimaline from leaves and stem bark of *Kopsia singapurensis* was found to inhibit KB cells.[37]

Table 2: Leaf extracts of Apocynaceae species with positive growth inhibitory activity against three human cancer cell lines

Species	Leaf extract[3]	Growth inhibitory activity[1]		
		MCF-7[2]	MDA-MB-231[2]	HeLa[2]
Alstonia angustiloba	DCM	-	+	+
Calotropis gigantea	DCM	+	+	+
	DCM:MeOH	+	-	+
Catharanthus roseus	DCM:MeOH	+	-	+
Nerium oleander	HEX	+	-	-
	DCM	+	+	+
	DCM:MeOH	+	+	+
	MeOH	+	-	-
Plumeria obtusa	HEX	+	-	+
	DCM	-	-	+
Vallaris glabra	DCM	+	+	+
	DCM:MeOH	+	+	+
	MeOH	+	-	-

[1]Growth inhibitory activity with <50% cell growth is considered positive (+) while that with >50% cell growth is considered negative (-); [2]MCF-7 and MDA-MB-231 are human breast cancer cells, and HeLa are human cervical cancer cells; [3]HEX = Hexane, DCM = Dichloromethane, and MeOH = Methanol.

Extracts of the six species were further tested against six human cancer cell lines (MCF-7, MDA-MB-231, HeLa, HT-29, SKOV-3, and HepG2). Results showed that DCM extract of *A. angustiloba* inhibited only MDA-MB-231, HeLa, and SKOV-3 cells with GI_{50} values of 20, 20, and 16 μg/ml, respectively [Table 3]. DCM extract of *C. gigantea* strongly inhibited all cancer cell lines with GI_{50} values ranging from 1.3–3.3 μg/ml. Against MCF-7 and MDA-MB-231 cells, GI_{50} of DCM extract of *C. gigantea* (1.9 and 1.3 μg/ml) was much stronger than that of xanthorrhizol (11 and 8.7 μg/ml), curcumin (4.1 and 8.7 μg/ml), and tamoxifen (8.3 and 4.6 μg/ml), respectively.[30] DCM:MeOH extract of *C. roseus* strongly inhibited MCF-7 and HeLa cells with GI_{50} of 3.5 and 4.7 μg/ml, respectively. All four extracts of *N. oleander* were effective against MCF-7 cells with GI_{50} ranging from 3.7–12 μg/ml. DCM and DCM:MeOH extracts inhibited all cell lines except HepG2 cells. Hexane extract of *P. obtusa* was effective against MCF-7 and HeLa cells, while its DCM extract was effective against HeLa cells. DCM and DCM:MeOH extracts of *V. glabra* inhibited all cell lines with GI_{50} values ranging from 7.5–12 μg/ml and 5.8–13 μg/ml, respectively. In addition, MeOH extract of *V. glabra* also inhibited the growth of MCF-7 and HepG2 cells. Against MCF-7 cells, GI_{50} of DCM and DCM:MeOH extracts of *V. glabra* (7.7 and 7.0 μg/ml) was stronger than xanthorrhizol (11 μg/ml) and comparable to tamoxifen (8.3 μg/ml), respectively.[30]

To the best of our knowledge, this study represents the first report of APF activities from leaf extracts of *A. angustiloba*, *P. obtusa*, and *V. glabra*. Earlier studies have reported cytotoxic activity from the root bark of *Alstonia macrophylla*[16] and from the stem bark of *Alstonia scholaris*.[17] Iridoids isolated from the bark of *Plumeria rubra* were cytotoxic.[26] A recent study reported potent cell growth inhibition of cardenolide glycosides isolated from *Vallaris solanacea*.[38] The finding of strong APF activities from DCM and DCM:MeOH extracts of *C. gigantea* from this study is supported by earlier reports that leaf and root extracts of *C. gigantea* had strong inhibitory activity against cancer cells.[18-20] Isolated from leaves of *N. oleander*, pentacyclic triterpenoids were cytotoxic to KB cells.[24] Against HL60 and K562 cells, the stem extract of *N. oleander* displayed stronger cytotoxic activity than leaf and root extracts.[39]

Table 3: Antiproliferative activity of leaf extracts of Apocynaceae species with positive growth inhibitory activity against six human cancer cell lines

Species	Leaf extract[3]	Growth inhibition (GI_{50})[1]					
		MCF-7[2]	MDA-MB-231[2]	HeLa[2]	SKOV-3[2]	HT-29[2]	HepG2[2]
Alstonia angustiloba	DCM	-	20 ± 1.7	20 ± 1.1	16 ± 1.4	-	-
Calotropis gigantea	DCM	1.9 ± 0.2	1.3 ± 0.3	2.5 ± 0.5	2.5 ± 0.2	3.3 ± 0.2	1.8 ± 1.7
	DCM:MeOH	13 ± 0.3	-	15 ± 1.0	20 ± 2.3	24 ± 0.7	16 ± 3.5
Catharanthus roseus	DCM:MeOH	3.5 ± 0.1	-	4.7 ± 0.6	-	-	-
Nerium oleander	HEX	11 ± 0.9	-	-	12 ± 2.8	-	-
	DCM	3.7 ± 0.1	5.2 ± 0.7	5.1 ± 0.7	4.5 ± 1.0	6.1 ± 0.9	-
	DCM:MeOH	4.3 ± 0.2	18 ± 2.3	6.8 ± 0.9	7.6 ± 2.1	9.2 ± 2.2	-
	MeOH	12 ± 1.1	-	-	-	-	-
Plumeria obtusa	HEX	5.7 ± 0.8	-	10 ± 1.4	-	-	-
	DCM	-	-	19 ± 4.0	-	-	-
Vallaris glabra	DCM	7.7 ± 1.3	12 ± 2.0	9.8 ± 1.5	7.5 ± 4.5	9.3 ± 2.0	7.6 ± 0.2
	DCM:MeOH	7.0 ± 2.5	13 ± 6.3	8.5 ± 2.9	7.7 ± 2.4	12 ± 1.2	5.8 ± 1.2
	MeOH	16 ± 2.1	-	-	-	-	19 ± 0.9

[1]GI_{50} (μg/ml) is growth inhibition of cancer cell lines by 50% and inhibition is not effective (-) if values >20 μg/ml; [2]MCF-7 and MDA-MB-231, HeLa and SKOV-3, HT-29, and HepG2 are human breast, cervical, colon, and liver cancer cells, respectively; [3]HEX = Hexane, DCM = Dichloromethane, and MeOH = Methanol

It is interesting to note that out of four leaf extracts of *C. roseus* tested against six cell lines, only DCM:MeOH extract inhibited MCF-7 and HeLa cells. The species is well known for its indole alkaloids notably vinblastine and vincristine, which are used to treat Hodgkin's disease and acute leukemia in children, respectively.[1] It can be inferred that the APF activities of *C. roseus* may be cell line specific unlike those of *C. gigantea*, *N. oleander*, and *V. glabra* which are wide spectrum, inhibiting most or all cell lines tested.

Of the 10 species analyzed, MeOH crude and DCM extracts of *K. fruticosa* had the highest TAC (100 and 129 mg BE/g of extract), respectively [Table 4]. Other species with moderately high TAC were *D. costulata*, *C. roseus*, and *A. angustiloba* with MeOH crude and DCM:MeOH extracts having values ranging from 23–58 and 58–68 mg BE/g of extract, respectively. Based on TAC, the species can be ranked as high (*K. fruticosa*), moderate (*D. costulata*, *C. roseus*, and *A. angustiloba*), and low (*C. odollam*, *C. gigantea*, *V. glabra*, *P. obtusa*, *A. cathartica*, and *N. oleander*). Extracts of *D. costulata* had the highest TPC and strongest RSA. MeOH crude, DCM:MeOH, and MeOH extracts of *D. costulata* yielded TPC values of 319, 354, and 279 mg GAE/g of extract, and RSA values of 377, 349, and 278 mg AA/g of extract, respectively. Compared to *D. costulata*, extracts of other species can be categorized as moderate to low.

The high TAC of leaf extracts of *K. fruticosa* may be attributed to the presence of alkaloids identified as fruticosamine, fruticosine, and kopsine.[40] The presence of flavonols identified as 3'7-dimethoxyquercetin and quercetin-3-*O*-α-L-rhamnopyranoside[11] may contribute to the high TPC and RSA of leaf extracts of *D. costulata*.

Overall, there is a strong correlation between TPC and RSA of extracts (R^2 = 0.992) but not with TAC (R^2 = 0.135). The strong correlation between TPC and RSA of extracts affirms that Apocynaceae species with higher concentration of phenolic compounds in the leaves also have stronger radical scavenging capacity. This would mean that the phenolic compounds are the main contributors to the antioxidant potential of leaves. Similar findings have been reported in medicinal plants and herbs,[41-43] plants of industrial interest,[44] wild edible fruits,[45] and mushrooms.[46] The correlation of results of phytochemical analysis with APF activities remains unclear. Extracts of *C. gigantea*, *N. oleander*, and *V. glabra* which showed strong APF activities had low TAC, and moderate to low TPC and RSA.

CONCLUSION

Out of 10 species of Apocynaceae, leaf extracts of *A. angustiloba*, *C. gigantea*, *C. roseus*, *N. oleander*, *P. obtusa*, and *V. glabra* displayed positive APF activities. DCM extract of *C. gigantea*, and DCM and DCM:MeOH extracts of *V. glabra* inhibited the growth of all six human cancer cell lines. Against MCF-7 and MDA-MB-231 breast cancer cells, DCM extracts of *C. gigantea* and *N. oleander* were stronger than or comparable to standard drugs of xanthorrhizol, curcumin, and tamoxifen. All four extracts of *N. oleander* were effective against MCF-7 cells. With wide-spectrum APF activities, leaves of these three species are therefore promising candidates as alternative resources for anticancer drugs. Their wide-spectrum APF activities are reported for the first time and this warrants further investigation into their bioactive compounds.

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Table 4: Total alkaloid content, total phenolic content, and radical scavenging activity of leaf extracts of Apocynaceae species

Species	MeOH crude extract[1]	Sequential extract[1]			
		HEX	DCM	DCM:MeOH	MeOH
TAC (BE mg/g extract)[2]					
Kopsia fruticosa	100 ± 4.2	63 ± 1.1	129 ± 4.0	99 ± 2.5	46 ± 1.6
Dyera costulata	58 ± 2.5	2.4 ± 0.6	11 ± 1.8	68 ± 2.7	41 ± 2.4
Catharanthus roseus	37 ± 2.8	18 ± 0.9	9.1 ± 0.8	76 ± 5.5	35 ± 3.3
Alstonia angustiloba	23 ± 0.3	13 ± 2.3	27 ± 3.1	58 ± 1.2	27 ± 1.0
Cerbera odollam	2.8 ± 0.9	3.6 ± 0.5	3.0 ± 0.5	5.0 ± 1.5	3.2 ± 0.4
Calotropis gigantea	2.7 ± 0.3	3.7 ± 1.0	8.6 ± 1.2	9.6 ± 1.9	9.2 ± 2.7
Vallaris glabra	2.7 ± 0.4	4.4 ± 0.8	8.9 ± 0.6	9.2 ± 0.2	8.7 ± 2.2
Plumeria obtusa	2.3 ± 0.3	2.3 ± 0.4	2.5 ± 0.1	4.3 ± 1.3	5.8 ± 1.7
Allamanda cathartica	2.0 ± 0.1	4.4 ± 1.3	8.7 ± 0.9	12 ± 1.0	8.7 ± 2.1
Nerium oleander	1.4 ± 0.7	1.6 ± 0.0	2.8 ± 0.8	3.9 ± 0.7	8.3 ± 0.5
TPC (GAE mg/g extract)[2]					
Dyera costulata	319 ± 5.5	21 ± 0.2	23 ± 0.1	354 ± 6.0	279 ± 3.0
Vallaris glabra	99 ± 3.8	15 ± 0.9	24 ± 0.5	134 ± 1.0	164 ± 13
Plumeria obtusa	85 ± 0.9	21 ± 1.7	52 ± 1.3	104 ± 4.0	134 ± 3.0
Kopsia fruticosa	83 ± 1.1	39 ± 0.5	20 ± 0.5	129 ± 1.0	84 ± 1.0
Alstonia angustiloba	68 ± 2.0	17 ± 0.6	24 ± 0.6	96 ± 1.1	94 ± 1.1
Nerium oleander	56 ± 3.8	18 ± 0.3	23 ± 0.1	57 ± 0.3	29 ± 0.3
Catharanthus roseus	53 ± 2.5	24 ± 0.5	46 ± 1.4	75 ± 0.7	61 ± 1.1
Allamanda cathartica	37 ± 0.8	11 ± 0.4	27 ± 0.2	39 ± 0.9	42 ± 0.5
Cerbera odollam	30 ± 4.1	19 ± 0.7	26 ± 0.5	30 ± 0.6	40 ± 0.9
Calotropis gigantea	28 ± 0.8	14 ± 0.6	44 ± 1.7	42 ± 0.8	33 ± 0.5
RSA (AA mg/g extract)[2]					
Dyera costulata	377 ± 25	15 ± 0.3	9.3 ± 0.5	349 ± 21	278 ± 5.4
Vallaris glabra	84 ± 0.5	6.0 ± 0.3	8.4 ± 0.4	77 ± 2.2	119 ± 6.8
Plumeria obtusa	66 ± 3.5	4.9 ± 0.2	10 ± 0.9	58 ± 0.1	115 ± 5.3
Kopsia fruticosa	63 ± 3.2	12 ± 1.4	7.5 ± 0.3	70 ± 2.0	48 ± 1.5
Nerium oleander	42 ± 1.3	6.4 ± 0.1	8.5 ± 0.6	48 ± 1.2	33 ± 0.2
Alstonia angustiloba	29 ± 0.9	10 ± 0.2	5.7 ± 0.7	50 ± 1.2	46 ± 2.2
Catharanthus roseus	24 ± 0.8	13 ± 0.5	21 ± 0.5	33 ± 1.1	31 ± 0.3
Allamanda cathartica	17 ± 1.1	11 ± 0.1	7.9 ± 0.8	18 ± 1.4	24 ± 2.0
Cerbera odollam	15 ± 1.0	16 ± 0.4	5.5 ± 0.1	14 ± 1.3	25 ± 0.7
Calotropis gigantea	7.6 ± 0.4	5.6 ± 0.3	6.1 ± 0.4	8.0 ± 0.6	14 ± 1.3

[1]HEX = Hexane, DCM = Dichloromethane, and MeOH = Methanol; [2]BE = Boldine equivalent, GAE = Gallic acid equivalent, AA = Ascorbic acid, TAC = Total alkaloid content, TPC = Total phenolic content, and RSA = Radical scavenging activity

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