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ASSESSMENT OF ANTIMICROBIAL, ANTIOXIDANT AND CYTOTOXIC PROPERTIES OF THE ETHANOLIC EXTRACT FROM *Dracontomelon dao* (BLANCO) Merr. & Rolfe

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ABSTRACT

The bark and wood of sapwood, *Dracontomelon dao* (Anacardiaceae) are used in traditional medicine to treat several health problems including infections, inflammations, and tumors. This study was therefore conducted to provide some information on the antimicrobial, antioxidant, and cellular metabolic activities of the ethanolic extract of the bark and wood of *D. dao*. The phytochemical components were assessed to evaluate whether the extract contains the presence of phytochemicals that are considered antioxidants. GCMS analysis was done to identify the compounds present and evaluate their functions through data mining. Antimicrobial activity evaluated using agar well diffusion method showed inhibition of the extract against the gram-positive and gram-negative bacteria *Salmonella typhimurium*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Bacillus subtilis*, and the two disease-causing species of fungi, *Candida albicans*, and *Aspergillus niger*. The antioxidant activity of the extract was evaluated using 1, 1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging method showed strong antioxidant activity with an IC_{50} <<<<5 ppm comparable to the antioxidant activity of Vitamin C. Cytotoxic activity of the extract evaluated using trypan blue exclusion test showed low survival of normal human lymphocytes with only 20.3% live cells remaining after and being subjected to 1 mg/ml of the extract. Phytochemical screening of the extract revealed the presence of alkaloids, anthraquinones, flavonoids, saponins, steroids, and tannins which are known as antimicrobial and antioxidants. Identification of the bioactive compounds in the extract using GCMS revealed a total of 54 compounds. Twenty-one of these compounds have antimicrobial properties while 15 of these compounds have antioxidant properties based on published literature. Other compounds also suggest biological activities other than antimicrobial and antioxidant that also indicate potential biomedical applications. The information from the results in this study supports the traditional medicinal claims of *D. dao* have scientific bases.

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Introduction

For many years, natural products have been used as medicinal agents all over the world. Still continuing to be widely used in most countries, traditional and complementary medicine are the primary source of healthcare for millions of people especially the use of ethnomedicinal plants like dao, *Dracontomelon dao* (Fig. 1), a Philippine native tree under family Anacardiaceae that is widely distributed throughout South and Southeast Asia [1, 2]. The tree is planted as an ornamental in roadside plantings and used for firewood. The flowers and leaves are cooked and eaten as a vegetable as well as for food flavoring. The bark of the tree is used in traditional medicine such as in dentistry, depurative, treatment for dysentery, skin

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infections, dermatitis, sore throat, and is given to parturient women as a general medicine [3-5]. Locals use a decoction of the trunk's wood as an anti-inflammatory and antitumor agent [pers. Comm.]. Several pharmacological studies conducted on *D. dao* reported antimicrobial, antioxidant, anti-inflammatory, anti-diabetic, and anti-trypanosomal activities [6-9] and the isolation of the chemical constituents of its several parts suggest the possession of medicinal and therapeutic properties [10, 11]. Since plants are complex and evolve based on many environmental factors, local varieties of the species may have evolved biochemical protection for herbivores and other disease-causing organisms which may have medicinal values to human. In this study, a local variety of *D. da*o is to be evaluated for its potential antimicrobial, antioxidant, cytotoxic properties which may have a basis for its use as a natural medicinal source. Phytochemical and GCMS analysis are also included to evaluate the presence of biochemical compounds that may have possible therapeutic applications.

Fig. 1. Trunk and tree of *Dracontomelon dao.*

Materials and Methods

Collection of Plant Material

The sapwood sample of *D. dao* was collected in Tagmamarkay, Santiago, Agusan del Norte. The tree and its parts were photographed for taxonomic keys and identification which were then verified by a botanist and systematist. The collected sample was washed with water, air dried, and was then pulverized into a fine powder using a blender. The powder was then stored in an airtight container for further use.

Fig. 2. The location where the tree, *D. dao* was sampled.

Preparation of Crude Ethanolic Extract

Two hundred fifty (250.0) grams of the powdered sapwood was soaked in 500.0 ml of 99% absolute ethanol and was stored in an area with no sunlight for 168 hours. The supernatant was filtered using Whatman No. 1 (Whatman, UK) filter paper and the filtrate was concentrated using a rotary evaporator at 45 °C temperature. The crude extract was collected and was

allowed to completely dry at room temperature. The collected crude extract was then stored in storage vials which were used later on in tests for antimicrobial, antioxidant and cytotoxic activities, phytochemical screening, and GC-MS analysis.

***In-vitro* Antimicrobial Assay**

Antimicrobial activity of *D. dao* ethanolic extract was assessed using agar well diffusion method against gram-negative bacteria *Salmonella typhimurium* UPCC 1368 and *Klebsiella pneumoniae* UPCC 1360, gram-positive bacteria *Staphylococcus aureus* UPCC 1143 and *Bacillus subtilis* UPCC 1295, and fungi *Candida albicans* UPCC 2168 and *Aspergillus niger* UPCC 4219. The antimicrobial assay was conducted at the Biological Research and Services Laboratory in Natural Sciences Research Institute, University of the Philippines in Diliman.

DPPH Radical Scavenging Method

Free radical scavenging activity of *D. dao* sapwood ethanolic extract was measured by 0.1 mM solution of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) in ethanol. The samples were screened for the presence of phytochemicals and antioxidant activity at the Department of Chemistry in MSU-IIT (Mindanao State University-Iligan Institute of Technology), Iligan City,

Cell Viability Assay

The cytotoxic activity of *D. dao* sapwood ethanolic to normal human lymphocytes was conducted using the trypan blue exclusion test. The cell viability test was done at the Biological Research and Services Laboratory of the Natural Sciences Research Institute, University of the Philippines, Diliman, Quezon City, Philippines.

Phytochemical Screening

The phytochemical screening of the *D. dao* sapwood ethanolic extract was aligned on the standard phytochemical methods described by Aguinaldo et al. (2005) which was further modified according to the laboratory analysis of Department of Chemistry, MSU-Iligan Institute of Technology. The extract was subjected to a qualitative evaluation of phytochemicals such as alkaloids, saponins, flavonoids, tannins, cyanogenic glycosides, steroids, and anthraquinones. The findings were recorded using a 3-point scale [+ turbid, ++ moderate and +++ heavy] in scoring based on the Handbook of Philippine Medicinal Plants by de Padua et al. [12].

Gas Chromatography-Mass Spectrometry [GC-MS] Analysis

The GC-MS analysis was conducted following the protocol of Chipiti *et al*. [13] with modifications to identify the compounds present in the *D. dao* sapwood ethanolic extract. Direct comparison of the mass spectrum of the analyte at a particular retention time to that of a reference standard found in the National Institute of Standards and Technology (NIST) library was used in the identification of compounds where at least 80% similarity index was considered significant [14]. The ethanolic extract was qualitatively analyzed using GC-MS at the Chemistry Analytical and Research Laboratory of the Ateneo de Davao University in Davao City,

Results and Discussions

***In-vitro* Antimicrobial Assay**

The results of the antimicrobial tests revealed that the extract possesses inhibitory effects against all test organisms- *S. typhimurium*, *K. pneumoniae*, *S. aureus*, *B. subtilis*, *C. albicans* and *A. niger* (Fig. 3, Table 1). The result of this assay is supported by earlier studies conducted using different plant parts (leaves, stem, root barks) of *D. dao* using various extraction solvents (petroleum ether, chloroform, dichloromethane, ethyl acetate, butanol) against several microbial strains, including *E. coli*, *P. aeruginosa*, *S. aureus*, methicillin-sensitive *S. aureus*, methicillin-resistant *S. aureus*, and *E. coli* multiple drug-resistant bacteria [6, 15-18].

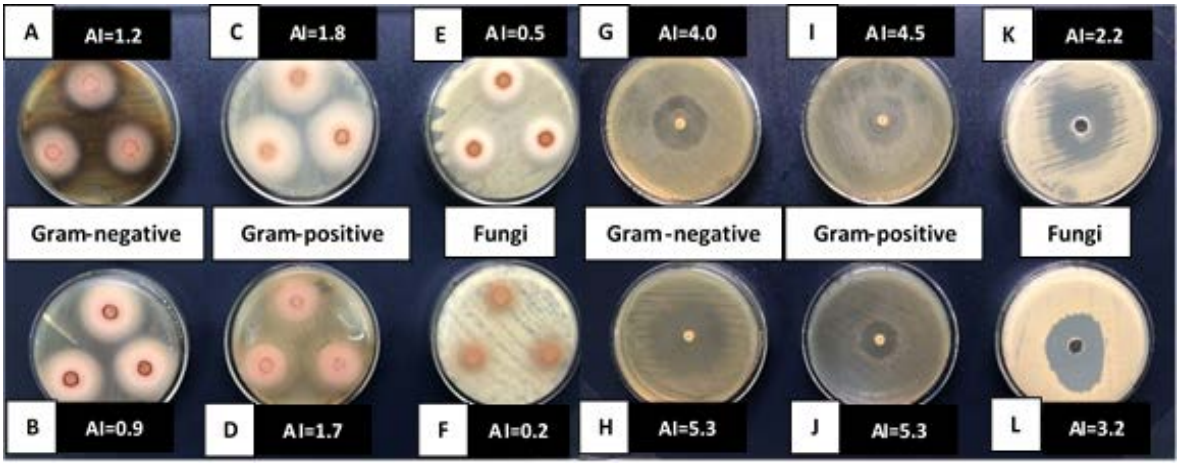

Figure 3. The antimicrobial indices (AIs) of gram-negative bacteria: *S. typhimurium* (A) and *K. pneumoniae* (B); gram-positive bacteria: *S. aureus* (C) and *B. subtilis* (D); Fungi: *C. albicans* (E) and *A. niger* (D); positive control:

Chloramphenicol used in Gram-negative bacteria [G & H] and Gram-positive bacteria [I & J]; and Canesten solution used in Fungi [K & L] using agar well diffusion method in triplicates of the *D. dao* sapwood ethanolic extract.

Table 1. Mean clearing zone (mm) and antimicrobial index (AI) from the test organisms in the *D. dao* sapwood ethanolic extract.

UPCC Test Organism	Sample	Mean Clearing Zone, mm	AI
Gram-negative Bacteria			
***S. typhimurium* UPCC 1368**	Dao (*Dracontomelon dao)*	22	1.2
	Chloramphenicol disc[a]	30	4.0
***K. pneumoniae* UPCC 1360**	Dao (*Dracontomelon dao)*	19	0.9
	Chloramphenicol disc	38	5.3
Gram-positive Bacteria			
***S. aureus* UPCC 1143**	Dao (*Dracontomelon dao)*	28	1.8
	Chloramphenicol disc	33	4.5
***B. subtilis* UPCC 1295**	Dao (*Dracontomelon dao)*	27	1.7
	Chloramphenicol disc	20	2.3
Fungi			
***C. albicans* UPCC 2168**	Dao (*Dracontomelon dao)*	14.67	0.5
	Canesten solution[b], 100 µl	32	2.2
***A. niger* UPCC 4219**	Dao (*Dracontomelon dao)*	12	0.2
	Canesten solution, 100 µl	42	3.2

[a] 6-mm diameter disc, contains 30 µg chloramphenicol
[b] Contains 1% clotrimazole

DPPH Radical Scavenging Method

The result of the antioxidant activity evaluation of *D. dao* sapwood ethanolic extract using DPPH (2, 2-diphenyl-1-picrilhidrazine) radical scavenging method is summarized in Table 2. Based on the result of the assay, the extract exhibited strong antioxidant activity since it obtained an IC_{50} value of <<<<5 ppm with a 95.14% inhibition at 5 ppm. Based on studies conducted by Mosmann (1983), compounds having a>80% inhibition is considered a good antioxidant, 50-80% inhibition a moderate antioxidant and <50% a weak antioxidant. An IC_{50} value of <50 ppm indicates that the substance is a highly active antioxidant Jun *et al.* (2003). The results of this study therefore clearly show that the extract has a strong antioxidant property. A related study conducted by Shimizu *et al.* (2002) have shown the antioxidant activity of *D. dao* heartwood methanolic extract has potent antioxidant activity thus also indicates that the plant has compounds that are strong antioxidants [19].

Table 2. Antioxidant activity of the *D. dao* sapwood ethanolic extract on different concentrations (ppm).

Extract Concentration (ppm)	Percent Inhibition (%)
Control	0
5	95.14
10	95.74
20	95.95
30	95.54
50	95.54
100	96.35
$IC_{50\ (ethanolic\ exctract)}$<<<<5ppm	
$IC_{50\ (Vitamin\ C)}$<<<<5ppm	

Phytochemical Screening

Phytochemical screening of *D. dao* sapwood ethanolic extract revealed the presence of alkaloids, anthraquinones, flavonoids, saponins, steroids and tannins as shown in table 3. These detected phytochemicals may explain the biological and pharmacological activities of the plant.

Table 3. Results of phytochemical screening of *D. dao* sapwood ethanolic extract.

Phytochemicals Screened	Method	Indication of Positive Results	Remarks
Alkaloids	Mayer's Test	White precipitate Brown precipitate	+
Saponins	Froth Test	Honeycomb froth	+++
Flavonoids	Bate-Smith & Metcaff Test	Shades of red	+++
Steroids	Keller-Kiliani Test	Reddish brown	+++
Tannins	Gelatin Test	Precipitate	+++
Cyanogenic Glycosides	Guignard Test	Shades of red	-
Anthraquinones	Brontrager's Test	Red color	+++

Alkaloids are considered as essential models in the design and development of antimicrobial agents [20]. Also, they often exhibit pharmacological effects and are used as anesthetics, stimulants, analgesics, and anti-malarial [21]. Anthraquinones cover a wide range of pharmacological activities including laxative, anticancer, anti-inflammatory, antiarthritic, antifungal, antibacterial, antiviral, antiplatelet, and neuroprotective effects [22]. Saponins possess a broad spectrum of biological and pharmacological activities as having been shown in many in vitro and in vivo bioassays which include anti-tumor, chemopreventive, antiphlogistic, immunomodulating, antihepatotoxic, antiviral, hypoglycemic, antifungal and molluscicidal activities [23]. The abundant presence of these compounds may attribute for the antimicrobial activities exhibited by the plant extract.

Flavonoids from plant sources have been linked to versatile health benefits reported in various epidemiological studies. They are shown to exhibit antioxidative, hepatoprotective, anti-inflammatory, anticancer and antiviral activities [24]. Steroids are associated with their potent anti-inflammatory and immune-modulating properties as well as antioxidative properties [25, 26]. Tannins have been found to have noticeable biological and pharmacological activities such as inhibition of carcinogenesis, host-mediated antitumor activity, antiviral activity, and inhibition of active oxygen [27]. The rich presence of these compounds may attribute the strong antioxidant activity of the extract.

Cyanogenic glycosides offer a potential source of cyanide, a highly toxic poison [28, 29]. Its toxicity may result in acute cyanide poisoning and several chronic diseases [30]. The absence of cyanogenic glycosides in the extract suggests that the probability of the plant to inflict serious toxic effects is decreased.

The results of the phytochemical screening of the extract support the antimicrobial, antioxidant, and cytotoxic activities shown by the extract brought by this study. Based on the data mentioned about the compounds' medicinal and pharmacological activities, it could be noticed that these compounds also possess anti-inflammatory and anticancer properties. These may support the claim of the traditional medicinal use of the plant as anti-inflammatory and antitumor agents, aside from being used in treating skin infections and other infections.

Cell Viability Assay

The result of the evaluation of the cytotoxic activity of *D. dao* sapwood ethanolic extract on normal human lymphocytes using tryphan blue exclusion test is presented in Table 3. The result revealed that for the administered concentration of 1 mg/ml of the extract, an average of 20.3% live cells remains. The results could be attributed on the high presence of anthraquinones in the sample (Table 3) since the quinone moiety that may be present in anthraquinone derivatives have long been associated with the toxicity to the blood and bone marrow [31]. Nevertheless, while the result suggests that the plant may potentially have cytotoxic effects, a high amount is needed to cause side effects [32].

Table 3. Cytotoxic activity of *D. dao* sapwood ethanolic extract to normal human lymphocytes.

Treatment	Average # Live Cells	Average # Dead Cells	Average # Total Cells	Average % Live Cells
Supplemented RPMI[a]	61.56	4.00	65.56	93.9
Triton X-100[b] 0.1%	0.00	56.56	56.56	0.0
DMSO[c] 2%	49.67	6.67	56.33	88.02
D. dao, 1 mg/ml	13.33	52.33	65.67	20.3

[a]Negative control
[b]Positive control
[c]Vehicle control

Gas Chromatography-Mass Spectrometry [GC-MS] Analysis

To identify the specific bioactive compounds responsible for the activities exhibited by *D. dao* sapwood ethanolic extract, GC-MS analysis was carried out. The chromatogram of the analysis is shown in Figure 4. The bioactive compounds with their base peak, mass peak, molecular weight, similarity index, formula, and biological activities are presented in Table 5. According to Kulathilaka and Senarath [33], a similarity index of at least 80% are considered significant. Out of the 54 compounds found in the extract, only 2 of these are below 80% although their values are not too deviated from the

considered significant value (79% for caryophyllene oxide and 76% for Bicyclo [3.1.1] hept-3-en-2-ol, 4,6,6-trimethyl-, [1S-(1.α,2.β,5.α)]-). This means that most of the compounds are closely similar to the reference standard found in the National Institute of Standards and Technology (NIST) library and thus, considered significant. Based on the results, a total of 54 compounds were present in the plant extract. Twenty-one of these compounds were found to have antimicrobial properties, 15 antioxidants, seven anti-inflammatories, two anticarcinogenic, and 2 with anticancer properties. It can be argued therefore that the traditional medicinal uses of *D. dao* as a treatment for infections as well as being used as anti-inflammatory and antitumor agents may have biochemical basis as shown by the results of the study.

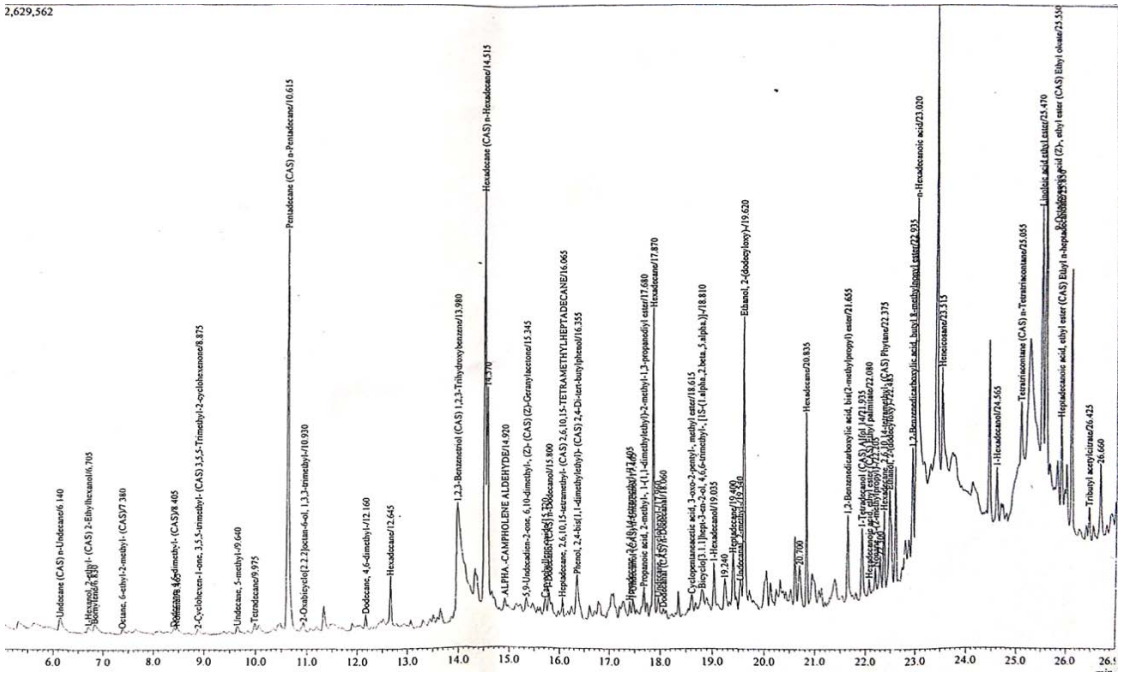

Figure 4. GC-MS chromatogram of *D. dao* sapwood ethanolic extract.

Table 5. Bioactive compounds isolated from the ethanolic extract of *D. dao* sapwood by GC-MS analysis.

	Compound Name	MW[a]	SI[b]	Formula	Biological Activities	Reference
1	Undecane	156	93	$C_{11}H_{24}$	No reported activity	-
2	1-Hexanol, 2-ethyl-	130	89	$C_8H_{18}O$	Antifungal, antibiotic, antioxidant, anticholereste-rolemic	Fernando *et al.* [34] Parama-nantham & Murugesan [35]
3	Bornylene	136	91	$C_{10}H_{16}$	No reported activity	-
4	Octane, 6-ethyl-2-methyl-	156	90	$C_{11}H_{24}$	No reported activity	-
5	Dodecane, 4,6-dimethyl-	198	85	$C_{14}H_{30}$	No reported activity	-
6	Nonanal	142	88	$C_9H_{18}O$	Antifungal, antidiarrheal, anti-inflammatory, antioxidant, antiviral (HIV), antitoxic, free radical scavenging, cardiopro-tective, hepatopro-tective, antitussive, antihemorr-hagic	Fernando *et al.* [34] Zavala-Sanchez *et al* [36]; Ramya *et al.*, [37]
7	2-Cyclohexen-1-one, 3,5,5-trimethyl-	138	80	$C_9H_{14}O$	No reported activity	
8	Undecane, 5-methyl-	170	91	$C_{12}H_{26}$	Antibacterial	Kushwaha *et al.* [14]
9	Tetradecane	198	88	$C_{14}H_{30}$	Antimicrobial	Arora *et al.* [38]
10	Pentadecane	212	97	$C_{15}H_{32}$	Antimicrobial, sugar-phosphatase inhibitor, chymosin inhibitor	Yogeswari *et al.* [39]; Arora *et al.* [38]

11	2-Oxabicyclo [2.2.2]octan-6-ol, 1,3,3-trimethyl-	170	91	$C_{10}H_{18}O_2$	No activity reported	-
12	Dodecane, 4,6-dimethyl-	198	92	$C_{14}H_{30}$	No activity reported	-
13	Hexadecane	226	96	$C_{16}H_{34}$	Antifungal, antibacterial, antioxidant	Abdul Kaffoor *et al.*[40]; Yogeswari *et al.* [39]
14	1,2,3-Benzenetriol	126	86	$C_6H_6O_3$	Antioxidant, antiseptic, antibacterial, antidermatitic, Fungicide, Pesticide, antimutagenic dye, candidicide	Kala *et al.* [41]
15	Hexadecane	226	96	$C_{16}H_{24}$	Antifungal, antibacterial, antioxidant	Abdul Kaffoor *et al.*[40]; Yogeswari *et al.* [39]
16	Unknown compound	-	-	-	-	-
17	α-campholene aldehyde	152	91	$C_{10}H_{16}O$	No reported activity	-
18	5,9-Undecadien-2-one, 6,10-dimethyl-, (Z)-	194	89	$C_{13}H_{22}O$	No reported activity	-
19	Caryophyllene oxide	220	79	$C_{15}H_{24}O$	Antimicrobial, anti-inflammatory, Anticarcinogenic	Ulubelen *et al.* [42]; Zheng *et al.* [43]
20	1-Dodecanol	186	94	$C_{12}H_{26}O$	Antibacterial	Bodoprost & Rosemeyer [44]
21	Heptadecane, 2,6,10,15-tetramethyl-	296	89	$C_{21}H_{44}$	Antibacterial, anti-inflammatory	Isaiah *et al.* [45]
22	Phenol, 2,4-bis(1,1-dimethylethyl)-	206	86	$C_{14}H_{22}O$	Antibacterial, antioxidant, anti-inflammatory, anticarcino-genic	Arora and Saini [46]; Amaral *et al.* [47]; Rajamani [48]
23	Hexadecane, 2,6,10,14-tetramethyl	282	93	$C_{20}H_{42}$	No reported activity	-
24	1-Undecanol	172	92	$C_{11}H_{24}O$	Bactericidal	Arora *et al.* [49]
25	Propanoic acid, 2-methyl-, 1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester	286	89	$C_{16}H_{30}O_4$	No reported activity	-
26	Hexadecane	226	96	$C_{16}H_{34}$	Antifungal, antibacterial, antioxidant	Abdul Kaffoor *et al.* [40]; Yogeswari *et al.* [39]
27	Undecane, 4-cyclohexyl-	238	88	$C_{17}H_{34}$	No reported activity	-
28	Dodecanal	184	89	$C_{12}H_{24}O$	No reported activity	-
29	Cyclopentane-acetic acid, 3-oxo-2-pentyl-, methyl ester	226	86	$C_{13}H_{22}O_3$	No reported activity	-
30	Bicyclo[3.1.1]hept-3-en-2-ol, 4,6,6-trimethyl-, [1S-(1.α,2.β,5.α)]-	152	76	$C_{10}H_{16}O$	Antioxidant, anti-inflammatory, anti-ischemic	Choi *et al.* [50]
31	1-Hexadecanol	242	95	$C_{16}H_{34}O$	Antioxidant	Kumarade-van *et al.*[51]
32	Unknown compound	-	-	-	-	-
33	Heptadecane	240	93	$C_{17}H_{36}$	Antioxidant	Arora *et al.* [49]
34	Undecanal, 2-methyl-	184	80	$C_{12}H_{24}O$	Pesticides, Flavors, Fragrance	Keerthiga & Anand

						[52]
35	Ethanol, 2-(dodecyloxy)-	230	95	$C_{14}H_{30}O_2$	No reported activity	-
36	Unknown compound	-	-	-	-	-
37	Hexadecane	226	96	$C_{16}H_{34}$	Antifungal, antibacterial, antioxidant	Abdul Kaffoor *et al.* [40]; Yogeswari *et al.* [39]
38	1,2-Benzenedicar-boxylic acid, bis(2-methylpropyl) ester	278	94	$C_{16}H_{22}O_4$	Antimicrobial, antioxiodant, antidiabetic	Arora *et al.* [49]; Govindappa *et al.*[53]
39	1-Tetradecanol	214	90	$C_{14}H_{30}O$	Antifungal	Rajamani [48]
40	Hexadecanoic acid, ethyl ester	284	90	$C_{18}H_{36}O_2$	Antimicrobial, antioxidant, hypocholesterolemic, antiandrogenic, 5-alpha-reductase inhibitor, phagocytosis inhibitor	Rouis-Soussi *et al.* [54]; Sheela & Uthayakumari [55]; Kanimozhi & Bai [56]
41	Nonane, 5-(2-methylpropyl)-	184	85	$C_{13}H_{28}$	No reported activity	-
42	Unknown compound	-	-	-	-	-
43	Hexadecane, 2,6,10,14-tetramethyl-	282	88	$C_{20}H_{42}$	No reported activity	-
44	Ethanol, 2-(dodecyloxy)-	230	93	$C_{14}H_{30}O_2$	No reported activity	-
45	1,2-Benzenedicar-boxylic acid, butyl 8-methylnonyl ester	362	85	$C_{22}H_{34}O_4$	Antimicrobial	Ogunlesi *et al.*[57]
46	n-Hexadecanoic acid	256	93	$C_{16}H_{32}O_2$	Antimicrobial, antioxidant, larvicidal, anti-inflammatory, anticancer, nematicide, pesticide, antiandrogenic, hypocholeste-rolemic, hemolytic, 5-alpha reductase inhibitor	Bodoprost & Rosemeyer [44]; Falodun *et al.*[58], 2009; Arora, and Pandey-Rai [38]; Pascual *et al.*[59]; Zayed *et al.* [60]
47	Heneicosane	296	95	$C_{21}H_{44}$	No reported activity	-
48	1-Hexadecanol	242	93	$C_{16}H_{34}O$	Antioxidant	Kumaradevan *et al.* [51]
49	Tetratriacon-tane	479	89	$C_{34}H_{70}$	Antibacterial, antifungal	Mahmood *et al.* [61]
50	Linoleic acid ethyl ester	308	93	$C_{20}H_{36}O_2$	Antisclerotic, anti-inflammatory, hypocholeste-rolemic, cancer preventive, hepatoprotec-tive, antihistaminic, antieczemic, antiacne, 5-alpha reductase inhibitor, antiandro-genic, antiarthritic	Novikova [62]; Herrera & Barbas [63]
51	9-Octadecenoic acid (Z)-, ethyl ester	310	88	$C_{20}H_{38}O_2$	Antibacterial, Anti-inflammatory, anticancer, antiandrogenic, dermatitigenic, hypocholeste-rolemic, 5-Alpha reductase inhibitor, Anemiagenic	Akin-Osanaiye *et al.* [64]; Manonmani & Catharin [65]; Selvin *et al* [66]
52	Heptadecanoic acid, ethyl ester	298	90	$C_{19}H_{38}O_2$	Antimicrobial	Zheng *et al.* [67]
53	Tributyl acetylcitrate	402	90	$C_{20}H_{34}O_8$	Antimicrobial	Khalil *et al.* [68]

54	Unknown compound	-	-	-	-	-

[a]Molecular weight
[b]Similarity index

Conclusion

The study showed that the ethanolic extract from the sapwood of *D. dao* exhibited antimicrobial activity against *S. typhimurium*, *K. pneumoniae*, *S. aureus*, *B. subtilis*, *C. albicans*, and *A. niger*. The extract also showed strong antioxidant activity. Phytochemical screening detected the presence of alkaloids, anthraquinones, flavonoids, saponins, steroids, and tannins which were known to have antimicrobial and antioxidant properties. The extract also demonstrated cytotoxic potential but require a higher dose. GC-MS analysis revealed that out of the 54 compounds found in the extract, 21 compounds have antimicrobial properties while 15 compounds have antioxidant properties. Other compounds also suggest biological activities other than being antimicrobial and antioxidant, such as anti-inflammatory, anticarcinogenic and anticancer. These results may add credence to the traditional medicinal claims on *D. dao* sapwood.

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References

1. WHO traditional medicine strategy 2014-2023. (2013). Geneva: World Health Organization.
2. Goeltenboth, F., & Goeltenboth, A. (2000). Agroecological comparison of „rainforestation “farming sites on Leyte, Philippines. Proceedings Dt. Tropentag 2000, 84-85.
3. Hoang Van Sam; Khamseng Nanthavong; Kessler P.J.A. Trees of Laos and Vietnam - A Field Guide to 100 species.
4. French, B., n.d. Food Plants of Papua New Guinea: a Compendium. Shellield, Published by the author.
5. http://www.worldagroforestry.org
6. Khan, M., & Omoloso, A. (2002). Antibacterial and antifungal activities of Dracontomelon dao. Fitoterapia, 73(4), 327-330. doi:10.1016/s0367-326x(02)00076-x.
7. Mazura, M. P., Siti Nur Aisyah, M. H., & Idayu, B. (2016). Assessing Malaysian Forest Species for Lipogenase Inhibitory Activity. In Healing Power from Nature: Current Trends & Perspectives (pp. 236-239).
8. Yusro, F., Ohtani, K., & Kubota, S. (2016). Inhibition of α-Glucosidase by Methanol Extracts from Wood Bark of Anacardiaceae, Fabaceae, Malvaceae and Phyllanthaceae Plants Family in West Kalimantan, Indonesia.
9. Norhayati, I., Getha, K., Nurhanan Murni, Y., Ling, S. K., Lili Sahira, H., Asiah, O., Mohd Hafidz Hadi, A., & Nor Azlianie, A. (2016). In Vitro Anti-trypanosomal Activity of Selected Forest Plant Species. In Healing Power from Nature: Current Trends & Perspectives (pp. 269-272).
10. Su, X. F., Liang, Z. Y., & Zhang, Y. X. (2008). Study on the chemical constituents of essential oil from the skins of stem of Dracontomelon dao (Blanco) Merr. et Rolfe. Lishizhen Med. Mat. Med. Res, 19, 1640-1641.
11. Ragasa, C. Y., Batarra, T. C., Vivar, J. L., Mariquit M. De Los Reyes, & Shen, C. (2017). Chemical Constituents of Dracontomelon Dao (Blanco) Merr. et Rolfe. Pharmacognosy Journal,9(5), 654-656. doi:10.5530/pj.2017.5.103
12. De Padua, L.S., Lugod, G.C., and Pancho J.V. (2005). Handbook of Philippine Medicinal Plants. University of the Philippines, Los Baños 1981; 1-4: 66.
13. Chipiti, T., Ibrahim, M. A., Koorbanally, N. A., & Islam, M. S. (2015). In vitro antioxidant activity and GC-MS analysis of the ethanol and aqueous extracts of Cissus cornifolia (Baker) Splanch (Vitaceae) parts. Acta Pol Pharm, 72, 119-27.
14. Kushwaha, P., Singh, D., & Bisht, G. (2015). Chemical Composition of Volatile Extract of Gnaphaliumluteo-Album l. and Its Antibacterial Activity. Journal of Drug Discovery and Therapeutics, 3(30), 23–28.
15. Liu, S., Zhao, Y., Zeng, N., Liu, T., Zhang, Y., Han, B., Li, J., Wang, L., Wang, R., Gong, M., & Xiao, X. (2013). Anti-bacterial effect of four extracts from leaves of Dracontomelon dao on Escherichia coli growth using microcalorimetry coupled with principal component analysis. Journal of Thermal Analysis and Calorimetry, 116(1), 491-497. doi:10.1007/s10973-013-3516-2
16. Xiaojun Ma, Yinghong Wu, Liuyi Li, Qian Xu, Bijie Hu, Yuxing Ni, Anhua Wu, Shumei Sun, Vincent Jarlier and Jérôme Robert. 2015. First multicenter study on multidrug resistant bacteria carriage in Chinese ICUs. BMC Infectious Diseases, 15:358.

17. Zhao J.-Y., Zhu Y.-Q., Li Y.-N., Mu X.-D., You L.-P., Xu C., Et Al. (2015). Coexistence Of Sfo-1 And Ndm-1 B-Lactamase Genes And Fosfomycin Resistance Gene Fosa3 In An Escherichia Coli Clinical Isolate. Fems Microbiol. Lett. 362 1–7
18. Yuniati,Y., Hasanah, N., 2 Ismail, S., Anitasari, S., Paramita, S. 2018. Antibacterial Activity Of Dracontomelon Dao Extracts On Methicillin-Resistant S. Aureus (Mrsa) And E. Colimultiple Drug Resistance (Mdr). Afr J Infect Dis. 12(1 Suppl): 62–67.
19. Shimizu, K., Kondo, R., & Sakai, K. (2002). Antioxidant activity of heartwood extracts of Papua New Guinean woods. Journal of Wood Science,48(5), 446-450. doi:10.1007/bf00770708
20. Meléndez-Gómez CM, & Kouznetsov VV (2013) Recent Developments on Antimicrobial Quinoline Chemistry. In: Mendez-Vilas A (ed.). Microbial pathogens and strategies for combating them: science, technology and education. Spain: FORMATEX Research Center 2013, pp. 666-677.
21. Thomson, R. H. (1994) The Chemistry of Natural Products. Chapman and Hall Publishers, 218-219.
22. Malik, E. M., & Müller, C. E. (2016). Anthraquinones As Pharmacological Tools and Drugs. Medicinal Research Reviews, 36(4), 705–748.doi:10.1002/med.21391
23. Lacaille-Dubois, M. A., & Wagner, H. (1996). A review of the biological and pharmacological activities of saponins. Phytomedicine, 2(4), 363–386. doi:10.1016/s0944-7113(96)80081-x
24. Sangeetha K.S.S., Umamaheswari S, Reddy C.U.M., & Kalkura S.N. (2016). Flavonoids: Therapeutic Potential of Natural Pharmacological Agents. International Journal of Pharmaceutical Sciences and Research, 7(10) 3924-30. doi: 10.13040/IJPSR.0975-8232.7(10).3924-30.
25. Ericson-Neilsen, W., & Kaye, A. D. (2014). Steroids: pharmacology, complications, and practice delivery issues. Ochsner Journal, 14(2), 203-207.
26. Mooradian, A. D. (1993). Antioxidant properties of steroids. The Journal of Steroid Biochemistry and Molecular Biology, 45(6), 509–511.doi:10.1016/0960-0760(93)90166-t
27. Okuda, T., Yoshida, T., & Hatano, T. (1992). Pharmacologically Active Tannins Isolated from Medicinal Plants. Plant Polyphenols, 539–569.doi:10.1007/978-1-4615-3476-1_31.
28. Williams, D. J., Pun, S., Chaliha, M., Scheelings, P., & O'Hare, T. (2013). An unusual combination in papaya (Carica papaya): The good (glucosinolates) and the bad (cyanogenic glycosides). Journal of Food Composition and Analysis, 29(1), 82–86.doi:10.1016/j.jfca.2012.06.007
29. Oke, O. L. (1980). Toxicity of cyanogenic glycosides. Food Chemistry, 6(2), 97–109.doi:10.1016/0308-8146(80)90025-4
30. Bolarinwa, I. F., Oke, M. O., Olaniyan, S. A., & Ajala, A. S. (2016). A Review of Cyanogenic Glycosides in Edible Plants. Toxicology - New Aspects to This Scientific Conundrum.doi:10.5772/64886
31. Irons, R. D. (1985). Quinones as toxic metabolites of benzene. Journal of Toxicology and Environmental Health, 16(5), 673–678.doi:10.1080/15287398509530777
32. Dunnill, C. W., & Parkin, I. P. (2012). Antimicrobial coatings for "self-sterilisation." Sterilisation of Biomaterials and Medical Devices, 240–260.doi:10.1533/9780857096265.240
33. Kulathilaka, P. S., & Senarath, W. T. P. S. K. (2014). Determination of cytotoxicity and chemical identities in natural plants and callus cultures of Spilanthe spaniculata Wall. Ex DC. International Journal of Herbal Medicine 2013; 1(3):135- 141.
34. Fernando, W. D., Ramarathnam, R., Krishnamoorthy, A. S., & Savchuk, S. C. (2005). Identification and use of potential bacterial organic antifungal volatiles in biocontrol. Soil Biology and Biochemistry,37(5), 955-964. doi:10.1016/j.soilbio.2004.10.021
35. Paramanantham, M., & Murugesan, A. (2014). GC-MS analysis of Holarrhena antidysentrica Wall flower. International Journal of Science Engineering and Technology Research, 3(3), 631-39.
36. Zavala-Sanchez, M. A., Pérez-Gutiérrez, S., Perez-González, C., Sanchez-Saldivar, D., & Arias-García, L. (2002). Antidiarrhoeal activity of nonanal, an aldehyde isolated from Artemisia ludoviciana. Pharmaceutical biology, 40(4), 263-268.
37. Ramya, B., Malarvili, T., & Velavan, S. (2015). GC-MS analysis of bioactive compounds in Bryonopsis laciniosa fruit extract. International Journal of Pharmaceutical Sciences and Research, 6(8), 3375.
38. Arora, N., & Pandey-Rai, S. (2014). GC–MS analysis of the essential oil of Celastrus paniculatus Willd. seeds and antioxidant, anti-inflammatory study of its various solvent extracts. Industrial Crops and Products,61, 345-351. doi:10.1016/j.indcrop.2014.07.025
39. Yogeswari, S., Ramalakshmi, S., Neelavathy, R., & Muthumary, J. (2012). Identification and comparative studies of different volatile fractions from Monochaetia kansensis by GCMS. Global Journal of Pharmacology, 6(2), 65-71.
40. Abdul Kaffoor, H., Arumugasamy, K., Jemimma, H. L. & Nantha Kumar, R. (2017). GC-MS Analysis of Root and Aerial Parts Ethanolic Extract of Phyllanthus vasukii Parthipan et al., Sp. Nov. (Phyllanthaceae). International Journal of Ayurvedicand Herbal Medicine, 2672-2684.
41. Kala, S. M. J., Balasubramanian, T., Soris, P. T., & Mohan, V. R. (2011). GC-MS determination of bioactive components of Eugenia singampattiana Bedd. International Journal of ChemTech Research, 3(3), 1534-1537.

42. Ulubelen, A., Topcu, G., Eri§, C., Sönmez, U., Kartal, M., Kurucu, S., & Bozok-Johansson, C. (1994). Terpenoids from Salvia sclarea. Phytochemistry,36(4), 971-974. doi:10.1016/s0031-9422(00)90474-6
43. Zheng, G.Q., Kenney, P. M., & Lam, L. K. T. (1992). Sesquiterpenes from Clove (Eugenia caryophyllata) as Potential Anticarcinogenic Agents. Journal of Natural Products, 55(7), 999–1003.doi:10.1021/np50085a029
44. Bodoprost, J., & Rosemeyer, H. (2007). Analysis of Phenacylester Derivatives of Fatty Acids from Human Skin Surface Sebum by Reversed-Phase HPLC: Chromatographic Mobility as a Function of Physico-Chemical Properties. International Journal of Molecular Sciences,8(11), 1111-1124. doi:10.3390/i8111111
45. Isaiah, S., Arun Kumar, C., & Senthamizh Selvan, N. (2016). Phytochemical screening, anti-microbial activity and GC-MS analysis of Corchorus tridens L. IJPR, 6(12), 353.
46. Arora, S., & Saini, M. (2017). "Gas Chromatography Mass Spectrometry Profiling in Methanolic and Ethyl-Acetate Root and Stem Extract of Corbichonia Decumbens (Forssk.) Exell from Thar Desert of Rajasthan, India. Pharmacognosy Research, 9(5), 48. doi:10.4103/pr.pr_62_17
47. Amaral, A. C. F., Gomes, L. A., Silva, J. R. de A., Ferreira, J. L. P., Ramos, A. de S., Rosa, M. do S. S., Vermelho, A., Rodrigues, I. A. (2014). Liposomal Formulation of Turmerone-Rich Hexane Fractions fromCurcuma longaEnhances Their Antileishmanial Activity. BioMed Research International, 2014, 1–8.doi:10.1155/2014/694934
48. Rajamani, L. (2018). Phytochemical and GC-MS Analysis of Brassica oleracea Var. Capitata F. Rubra. World Journal of Pharmaceutical Research, 7(3), 1392-1400.
49. Arora, S., Kumar, G., & Meena, S. (2017). Gas chromatography-mass spectroscopy analysis of root of an economically important plant, Cenchrus ciliaris L. from Thar desert, Rajasthan (India). Asian J. Pharm. Clin. Res, 10, 64-69.
50. Choi, I. Y., Lim, J. H., Hwang, S., Lee, J. C., Cho, G. S., & Kim, W. K. (2010). Anti-ischemic and anti-inflammatory activity of (S)-cis-verbenol. Free radical research, 44(5), 541-551.
51. Kumaradevan, G., Damodaran, R., Mani, P., Dineshkumar, G., & Jayaseelan, T. (2015). Phytochemical screening and GC-MS analysis of bioactive components of ethanol leaves extract of Clerodendrum phlomidis (L.). Am J Biol Pharm Res, 2(3), 142-8.
52. Keerthiga, M., & Anand, S. P. (2015). Bioactive compound evaluation of ethanol extract from Geodorum densiflorum (Lam.) Schltr. by GC-MS analysis. International Journal of Pharmaceutical Research, 5(6), 139-144.
53. Govindappa M., Prathap S., Vinay V., & Channabasava R. (2014). Chemical composition of methanol extract of endophytic fungi, Aiternariasp of Tebebuia argentea and their antimicrobial & antioxidanty activity. International Journal of biological & pharmaceutical research, 5(11), 861-869
54. Rouis-Soussi, L. S., Ayeb-Zakhama, A. E., Mahjoub, A., Flamini, G., Jannet, H. B., & Harzallah-Skhiri, F. (2014). Chemical composition and antibacterial activity of essential oils from the Tunisian Allium nigrum L. EXCLI journal, 13, 526–535.
55. Sheela, D., & Uthayakumari, F. (2013). GC-MS analysis of bioactive constituents from coastal sand dune taxon-Sesuvium portulacastrum (L.). Bioscience discovery, 4(1), 47-53.
56. Kanimozhi, D., & Bai, V. R. (2012). Analysis of Bioactive Components of Ethanolic Extract of Coriandrum Sativum L. International Journal of Research in Pharmacy & Science, 2(3).
57. Ogunlesi, M., Okiei, W., Ofor, E., & Osibote, A. E. (2009). Analysis of the essential oil from the dried leaves of Euphorbia hirta Linn (Euphorbiaceae), a potential medication for asthma. African Journal of Biotechnology, 8(24).
58. Falodun, A., Siraj, R., & Choudhary, M. (2009). GC-MS Analysis of Insecticidal Leaf Essential Oil of Pyrenacantha Staudtii Hutch and Dalz (Icacinaceae). Tropical Journal of Pharmaceutical Research,8(2). doi:10.4314/tjpr.v8i2.44522
59. Pascual, G., Avgustinova, A., Mejetta, S., Martín, M., Castellanos, A., Attolini, C. S., Berenguer, A., Prats, N., Toll A., Hueto, J. A., Bescós, C., Di Croce, L., & Benitah, S. A. (2016). Targeting metastasis-initiating cells through the fatty acid receptor CD36. Nature,541(7635), 41-45. doi:10.1038/nature20791
60. Zayed, M. Z., Ahmad, F. B., Ho, W. S., & Pang, S. L. (2014). GC-MS analysis of phytochemical constituents in leaf extracts of Neolamarckia cadamba (Rubiaceae) from Malaysia. Int J Pharm Pharm Sci, 6, 123-7.
61. Mahmood, A., Ahmed, R., & Kosar, S. (2009). Phytochemical screening and biological activities of the oil components of Prunus domestica Linn. Journal of Saudi Chemical Society, 13(3), 273–277.doi:10.1016/j.jscs.2009.10.008
62. Novikova, N. A. (1968). Effect of ethyl linoleate on regression of experimental atherosclerosis in rabbits. Bulletin of Experimental Biology and Medicine, 66(5), 1199-1200.
63. Herrera, E., & Barbas, C. (2001). Vitamin E: action, metabolism and perspectives. Journal of physiology and biochemistry, 57(2), 43-56.
64. Akin-Osanaiye, C. B., Gabriel, A. F., & Alebiosu, R. A. (2011). Characterization and antimicrobial screening of ethyl oleate isolated from Phyllanthus amarus (Schum and Thonn). Ann Biol Res, 2(2), 298-305.
65. Manonmani, R., & Catharin, S. S. (2015). GC-MS Analysis of bioactive components of an important medicinal fern Actiniopteris radiata (Swartz) link. World Journal of Pharmaceutical Researc, 4, 1860-1869.

66. Selvin, J., Shanmughapriya, S., Gandhimathi, R., Seghal Kiran, G., Rajeetha Ravji, T., Natarajaseenivasan, K., & Hema, T. A. (2009). Optimization and production of novel antimicrobial agents from sponge associated marine actinomycetes Nocardiopsis dassonvillei MAD08. Applied Microbiology and Biotechnology, 83(3), 435–445.doi:10.1007/s00253-009-1878-y
67. Zheng, C. J., Yoo, J.-S., Lee, T.-G., Cho, H.-Y., Kim, Y.-H., & Kim, W.-G. (2005). Fatty acid synthesis is a target for antibacterial activity of unsaturated fatty acids. FEBS Letters, 579(23), 5157–5162.doi:10.1016/j.febslet.2005.08.028
68. Khalil, N. M., Shalaby, E. A., Ali, D. M., Ali, E. M., & Aboul-Enein, A. M. (2014). Biological activities of secondary metabolites from Emericella nidulans EGCU 312. African Journal of Microbiology Research, 8(20), 2011-2021. doi:10.5897/ajmr2014.6827.