Sains Malaysiana 52(8)(2023): 2287-2296
http://doi.org/10.17576/jsm-2023-5208-09

Solvent Effect on the Phenolic Compounds and Biological Activity of Difference *Morinda citrifolia* Root Extract

(Kesan Pelarut pada Sebatian Fenol dan Perbezaan Aktiviti Biologi Ekstrak Akar *Morinda citrifolia*)

IDRIS ADEWALE AHMED[1], FASIHUDDIN BADRUDDIN AHMAD[2], GWENDOLINE CHENG LIAN EE[3], SIAU HUI MAH[4] & NOR HISAM ZAMAKSHSHARI[2,*]

[1]Centre for Natural Products Research and Drug Discovery (CENAR), Universiti Malaya, 50603 Kuala Lumpur, Malaysia
[2]Department of Chemistry, Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300 Kota Semarahan, Sarawak, Malaysia
[3]Department of Chemistry, Faculty of Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[4]School of Biosciences, Taylor's University, Lakeside Campus, 47500 Subang Jaya, Selangor, Malaysia

Received: 3 February 2023/Accepted: 26 July 2023

ABSTRACT

Cancer and antimicrobial resistance have become a threat to global health and development. This work aimed to identify the biological activities and phenolic compounds content of different *Morinda citrifolia* L. root extracts. The relationship between biological activities and phenolic content was also discussed. All extracts were screened for antioxidant activity using anti-oxidant assays (FRAP, DPPH, TAOC, ABTS, and BCB) and quantitative phytochemical analyses (TPC). Antimicrobial activity against four bacterial and two fungal strains as well as cytotoxic activities on stomach cancer (SNU-1), colon cancer (LS-174T and HT29), leukemia (K562), and breast cancer (MDA-MB-361) cell lines were also performed. With a value of 122.789 g of gallic acid equivalent/mg extract, the dichloromethane extract had the highest total phenolic content (TPC). The extract also showed high antioxidant activities in all the antioxidant assays and antimicrobial activity. The FRAP (r2 = 0.962) as well as antimicrobial activities against *Staphylococcus aureus* (r2 = 0.708), *Bacillus subtilis* (r2 = 0.890) and *Pseudomonas aeruginosa* (r2 = 0.870) were strongly correlated with the total phenolic content. The LS-174T, K562, HT-29, and MDA-MB-361 cytotoxic activities were also strongly correlated with the total phenolic content with r2 = -0.899, -0.845, -0.981, and -0.978, respectively. The results obtained suggested that the dichloromethane extract of *Morinda citrifolia* has high biological activity compared to other extracts.

Keywords: Antimicrobial; antioxidant; cytotoxic activity; *Morinda citrifolia;* root

ABSTRAK

Kanser dan perintang antibiotik telah menjadi ancaman kepada kesihatan manusia dan pembangunan global. Penyelidikan ini dilakukan adalah bertujuan untuk mengenal pasti aktiviti biologi dan kuantiti kandungan sebatian fenol pada ekstrak akar kayu *Morinda citrifolia* L. yang berbeza. Hubungan antara aktiviti biologi dan kandungan fenol turut dibincangkan dalam kajian ini. Kesemua ekstrak akan disaring untuk aktiviti anti-oksidan menggunakan asai anti-oksidan (FRAP, DPPH, TAOC, ABTS dan BCB) dan analisis fitokimia kuantitatif (TPC). Penyaringan aktiviti antimikrob terhadap empat strain bakteria dan dua strain kulat serta aktiviti sitotoksik pada sel kancer perut (SNU-1), sel kanser kolon (LS-174T dan HT29), leukemia (K562) dan karsinoma payudara (MDA-MB-361) juga turut di jalankan. Ekstrak diklorometana menunjukkan jumlah kandungan fenol (TPC) yang tinggi iaitu 122.789 g asid galik setara/ekstrak mg. Ekstrak diklorometana juga menunjukkan aktiviti antioksidan yang tinggi dalam semua asai antioksidan dan juga perencatan antimibrob aktiviti yang tinggi. Jumlah kandungan fenol menunjukkan korelasi yang tinggi dengan FRAP (r2 = 0.962) serta aktiviti antimikrob terhadap *Staphylococcus aureus* (r2 = 0.708), *Bacillus subtilis* (r2 = 0.890) dan *Pseudomonas aeruginosa* (r2 = 0.870). Aktiviti sitotoksik, LS-174T (r2 = -0.899), K562 (r2 = -0.845), HT-29 (r2 = -0.981) dan MDA-MB-361 (r2 = -0.978) juga menunjukkan korelasi yang tinggi dengan jumlah kandungan fenol. Hasil uji kaji yang diperoleh menunjukkan ekstrak diklorometana *Morinda citrifolia* mempunyai aktiviti biologi yang tinggi berbanding dengan ekstrak lain.

Kata kunci: Akar kayu; antimikrob; anti-oksidan; *Morinda citrifolia*; sitotoksik

Introduction

The genus Morinda belongs to the Rubiaceae family. There are roughly 80 species in this genus, mainly from the Old World (Oladeji, Oluyori & Dada 2022). In Malaysia, Morinda comprises nine species which include three species of trees and six species of climbers, including *Morinda citrifolia* L. Due to the outstanding nutritional value of *M. citrifolia*, a few commercial goods, including juice drinks, leaf powder, a powder made from dried fruits, and oil made from seeds have been developed (Almeida, de Oliveira & Hotza 2019; Nascimento et al. 2018). *M. citrifolia* is also used in conventional folk medicine to cure various illnesses, such as diabetes, high blood pressure, and cancer (Konsue, Yimthiang & Kwanhian 2018; Torres et al. 2017). According to scientific research, *M. citrifolia* has antibacterial, antiviral, antifungal, and anticancer properties (Assi et al. 2017). The biological activity is due to the secondary metabolite contained in this species. The metabolites include acid, phenol, saccharides, anthraquinones, carotenoids, terpenes, flavonoids, glycosides, ketones, terpenoids, and sterols (Almeida, de Oliveira & Hotza 2019; Sina et al. 2021; Zamakshshari et al. 2017). There is an intensive study on the phytochemistry and biological activity of the fruit, leaf, and bark of *M. citrifolia.* However, few studies have been conducted on the root of this species.

Globally, cancer is becoming the leading cause of mortality. In 2020, the World Health Organization predicted that nearly 10 million cancer-related deaths would occur. The most prominent causes of cancer death in 2020 were colon cancer, with 916,000 death, followed by stomach cancer (769,000 death) and breast cancer (685,000 deaths) (WHO 2022). In addition to cancer, infectious diseases rank among the world's major causes of death. The emergence and global spread of bacteria with medication resistance make it challenging to treat common infectious diseases. The current state of affairs results in protracted illness, disability, and death (WHO 2021). This condition necessitates a continuous search for novel anticancer and antibacterial drugs to combat infection, limit antibiotic resistance, and lower cancer incidence while reducing disease and mortality rates. The human body produces reactive oxygen and reactive nitrogen through enzymatic reactions and energy transfer activities (Kapoor et al. 2019). Reactive oxygen species cause various conditions and illnesses, including cancer, aging, atherosclerosis, and neurological diseases (Apea-Bah et al. 2016). By preventing reactive oxygen species and radical molecules, many problems and illnesses might be lessened.

This research aims to identify the biological properties including anticancer, antimicrobial, and antioxidant properties of different *M. citrifolia* root extracts extracted with various polarity solvents. It also aims to seek the relationship between total phenolic compounds and biological activity.

Materials and Methods

Chemicals

The analytical grade solvent such as hexane, dichloromrthane, eathyl acetate, methanol, and dimethyl sulfoxide were purchase from Merk (Darmstadt, Germany). Meanwhile, standard compounds and chemical such as Streptomycin sulfate, ketoconazole, gallic acid, ethylenediaminetetraacetic acid, Tamoxifen, paclitaxel, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and butylated hydroxytoluene were purchased from Thermo Scientific Chemicals.

Plant Collection

Morinda citrifolia's root was procured from Negeri Sembilan, Malaysia, and identified by Professor Dr. Rusea Go of the biology department at the Universiti Putra Malaysia. A voucher specimen with the voucher number RG2103 was placed at the herbarium of the Biology Department, Faculty of Science, Universiti Putra Malaysia.

Preparation of Plant Extraction

The gathered plant sample was dried outside and processed into a fine powder. *Morinda citrifolia* root powder was three times macerated in four different solvents. Hexane, dichloromethane, ethyl acetate, and methanol are the solvents used for 72 hours. The macerated sample was filtered and evaporated under low pressure to obtain dry extracts.

Total Phenolic Content

The Folin-Ciocalteu assay established by Zhen et al. (2016) was used to assess the total phenolic content. Gallic acid equivalence GAE (g of gallic acid/mg of oil extract) was used to express the total phenolic content of the crude extracts. The experiment was conducted three

times. The experiment was performed with only one concentration of 500 g/mg for the other extracts.

ANTIOXIDANT ASSAY

The antioxidant activity was assessed for each extract. Nitric oxide scavenging activity (NO), ferric reducing power (FRAP), β-carotene bleaching (BCB), and ferrous ion chelating (FIC) were the four antioxidant assays used. All antioxidant protocols were adapted from Zamakshshari et al. (2019). BHT was a standard in the BCB assay. Gallic acid is the benchmark for FRAP and NO. Last but not least, EDTA was used for FIC. All assays were performed in triplicate.

CYTOTOXIC ASSAY

The MTT assays reported by Kar Wei et al. (2011) were used to conduct the cytotoxic assay. Breast cancer (MDA-MB-361) and leukemia (K562) cells line, as well as stomach cancer (SNU-1) and colon cancer (LS-174T and HT29) cells line, were obtained from ATCC (American Type Culture Collection, USA) for this study. Each extract's IC_{50} value is determined. Tamoxifen and CISPLATIN were employed as positive controls.

ANTIMICROBIAL ACTIVITY

Stock cultures of bacteria and fungi were grown on potato dextrose agar and Muller-Hinton Agar, respectively, and stored at 4 °C. Two Gram-positive strains of bacteria, *Staphylococcus aureus* (ATCC 25923) and *Bacillus subtilis* (B145), and two Gram-negative strains, *Pseudomonas aeruginosa* (ATCC 27853) and *Escherichia coli* (a clinical isolate), were used to study the antibacterial activity. *Aspergillus niger* and *Candida albicans* (C2213) were the two fungi used in this investigation (A121). The Institute for Medical Research (IMR), Kuala Lumpur, provided all of the bacterial strains, while the Microbiology Laboratory, Medical Faculty, Universiti Putra Malaysia, provided all of the fungal strains. The antibacterial and antifungal experiments were conducted using the diffusion method, and each extract's concentration was 10 mg/mL (Zamakshshari et al. 2022). The antibacterial assay's positive control was streptomycin sulfate (100 g/mL). Ketoconazole served as a positive control for the antifungal test in the interim. As a benchmark, dimethyl sulfoxide (DMSO) was employed. The broth microdilution assay was used to calculate each plant extract's minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) (Zamakshshari et al. 2022).

STATISTICAL ANALYSIS

Data from the antioxidant test and phytochemical analysis were reported as a mean standard deviation and were subjected to triplicate independent analyses. In the meantime, the cytotoxicity test findings were expressed as mean ± standard error of three independent experiments performed in triplicate. Microsoft Excel software was used to generate the graphs (Version 2010). Turkey post hoc test (SPSS 14.0) was used to examine the data using one-way ANOVA to identify significant sample differences. A significant difference between samples and standard medications was found using the independent sample T-test (SPSS 14.0). To ascertain the connection between phytochemical analyses and biological activities, Pearson correlation (SPSS 14.0) was performed. At p 0.05, the significance level was established.

RESULTS AND DISCUSSION

EXTRACTION AND PHYTOCHEMICAL SCREENING (TOTAL PHENOLIC CONTENT)

The collected *Morinda citrifolia* root bark (1.5 kg) was dried under open air until consistence weight achieved. The dried root was ground into fine powder sample (900 g). The *M. citrifolia* dry root fine powder produced 35 g of methanol extract, 19 g of ethyl acetate extract, 18 g of dichloromethane extract, and 11 g of hexane extract. The quantification of phenolic content was obtained using a total phenolic content assay. The principle of the total phenolic content assay is an oxidation-reduction reaction based on the redox properties of an antioxidant compound, which can react with the Folin-Ciocalteau reagent to enhance the measurement of the phenolic concentrations (Norshazila et al. 2010). The calibration equation $y = 0.00458x + 0.0457 (R2 = 0.9997)$, where y is the absorbance and x is the weight of the gallic acid present, was used to generate the standard curve for gallic acid. The outcomes are displayed in Table 1. The dichloromethane extract gave the highest phenolic content, which is 122.789 GAE. Meanwhile, the methanol extract had the lowest total phenolic content value, 21.431 GAE. Isolation and extraction work

by Zamakshshari et al. (2017) shows that semi-polar extract is rich in anthraquinones. The anthraquinones are sorendidiol, rubiadin, alizarin, 1,4-dihydroxy-2-methoxy anthraquinone, and damnacanthol. On the other hand, the hexane extract contains large amounts of morindone, 1,6-dihydroxy-5-methoxy-2-methyl anthraquinone, damnathal, 1,3,5-trihydoxy-2-methoxy-2-methyl anthraquinone, and nordamnacanthal. Two anthraquinones, including 1,3-dihydroxy-2-methoxy anthraquinone and lucidin-ω-methyl ether, are present in the methanol extract.

ANTIOXIDANT ASSAY

The high-antioxidant diet is popular nowadays due to its health-promoting effect, including its role in cancer chemoprevention (Chaves, Santiago & Alías 2020). The value of this species as a new source of antioxidants may be discovered by the examination of the antioxidant activity of each extract. The antioxidant capabilities of each extract were measured using four different antioxidant assays based on various mechanisms. The BCB assay was aimed to quantify an antioxidant's capacity by reducing lipid peroxidation. The decrease of β-carotene will be hampered by the antioxidant chemicals present in the extracts. In the presence of antioxidant compounds, the yellow color of β-carotene will remain, but it will undergo rapid discoloration in its absence (Xiao et al. 2020). The extract with the highest percentage of β-Carotene bleaching was dichloromethane extract which was 13.58 % at 100 µg/mL. All the other extracts showed weak activities in inhibiting lipid peroxidation compared to the standard drug.

The theory behind ferric's antioxidant properties is based on reducing a Fe^{3+} complex to a brightly blue Fe^{2+} complex by an acidic substance in an acidic solution (Gülçin et al. 2011). Higher FRAP readings indicate a test compound's increased reducing power, indicating a high antioxidant activity level. The methanol extract gave the lowest reducing power activity compared to other extracts. Ferrozine can quantitatively bind Fe^{2+} to create a purple-colored complex in the FIC assay. Other chelating chemicals will inhibit the process, which will cause the ferrozine-Fe^{2+} complex's purple to fade (Oriola et al. 2020). The measurement of color decrease demonstrates the ferrous ion competition between the chelating agent and ferrozine. The extract's antioxidant components have chelating action, which prevents the transport of electrons by forming coordinated complexes with the metal ions (Eslami, Ebrahimzadeh & Biparva 2018). The oxidation reaction is stopped as a result, and no free radicals are generated. The control, EDTA, exhibited 50% inhibition at a 46.43 ± 2.39 µg/mL concentration for FIC assay. The ethyl acetate extract showed the lowest chelating effect among all the extracts at 4.77%. Due to the high concentration of phenolic chemicals that hindered the formation of the ferrozine-Fe2+ complex, the dichloromethane extract had the highest chelating impact (32.15%) among all the extracts. Even though most of these extracts showed chelating effects, compared to EDTA, all the extracts had weak chelating effects.

The excessive production of nitric oxide will damage the cellular DNA and protein. This situation will lead to apoptosis, mutagenesis, or carcinogenesis (Rana 2022). The principle of NO scavenging assay is based on the interaction between nitric oxide and oxygen to produce nitrite ions. The amount of nitrite ions is determined using the Griess reagent. The nitric oxide source was sodium nitroprusside (Kumar & Sharma 2018). The dichloromethane extract of *M. citrifolia* was the most potent as it removed the nitric oxide at a lower concentration than the other extracts. Table 1 shows all the antioxidant activities. The high phenolic content of each extract accounts for the significant antioxidant action (Siti Azima, Norriham & Manshoor 2022). Antioxidant capabilities of phenolic compounds shield cells against reactive oxygen and reactive nitrogen species. Most of the time, these phenolic compounds exhibit oxidative activity through various methods. The methods include a metal ion chelation, an antioxidant that donates hydrogen, a free radical scavenger, and a singlet oxygen quencher (Ruhomally et al. 2015). The phenolic components in the extracts may be responsible for the activities of the extracts (Akinwumi Bordun & Anderson 2018).

CYTOTOXIC ASSAY

The extracts were tested for their ability to inhibit the SNU-1, LS-174T, HT29, K562, and MDA-MB-361 cancer cells. In Table 2, the IC_{50} values for these cell lines are displayed. The hexane extract showed the most potent cytotoxic effect against SNU-1 with a 16.87 g/mL concentration compared to the other crude extracts. The high phenolic content of hexane extract is thought to be responsible for the significant inhibition action. The synergistic effects of anthraquinone in hexane extract are caused by inducing apoptosis, inhibiting cell growth, increasing the buildup of reactive oxygen species

(ROS), and inducing autophagy in stomach cancer cells (Zhang et al. 2022). Even though nordamnacanthal and damnacanthal show excellent anticancer properties toward T-lymphoblastic leukemia cells, the extract showed moderate cytotoxic activities against K562 (Yazan 2003). This characteristic is due to the antagonistic effect of the compound in hexane extract. Other antagonistic effects were also seen in cytotoxic activity between hexane extract, colon, and breast cancer cells. It is because morindone contained in hexane extract is known to have good inhibition activity against colon and breast cancer (Chee et al. 2022; Haryoto & Firdaus 2022).

The dichloromethane extract, however, demonstrated substantial cytotoxic effects against LS-174T and K562 with IC_{50} values of 16.87 g/mL and 17.14 g/mL, respectively. The large concentration of active phenolic compounds causes an intense inhibition activity. The semi-polar contains several anthraquinones, according to the earlier study. The anthraquinone, specifically rubiadin, in this extract may cause inhibition against colon cancer. *In silico* studies have shown that rubiadin has a similar affinity for various colon cancer targets, including -catenin, MDM2-p53, and KRAS (Chee et al. 2022). In the leukemia cell line, anthraquinone is found to be a potent inducer of MDM2 breakdown, which triggers the overexpression of p53 and apoptosis (Draganov et al. 2019). Besides that, the dichloromethane extract also shows the highest cytotoxic activity against HT-29 and MDA-MB-361 compared to other extracts. The significant cytotoxic effects may be due to the synergistic interaction of the various phenolic components, particularly anthraquinone in the dichloromethane extract. However, this species's methanol extract showed weak activity in all the cancer cell lines due to the low phenolic compound in the extract. From a previous study, two anthraquinone isolates from these extracts show weak and moderate activity against colon, stomach, and leukemia cell lines, leading to weak activity in the extract (Zamakshshari et al. 2017). The cytotoxic activities show that each extract consists of different compounds leading to various cytotoxic activities. The synergistic and antagonistic effects also play an essential role in the cytotoxic activity of each extract.

TABLE 1. Total phenolic content and antioxidant activities of extracts and standard drugs

Extract/ standard drugs	Nitric oxide radical scavenging activity IC_{50} (µg/mL)	Percentage ferrous ions chelation at concentration 500 µg/mL	FRAP (µM ferrous sulphate/mg dry extract)	Beta carotene bleaching (% of β-carotene bleaching of extract at 100µg/mL)	Total phenolic content (GAE, µg of gallic acid/mg of crude extract)
Hexane	>1000	26.81 ± 0.54^{c}	1.39 ± 0.03^{b}	9.38 ± 0.27*	42.24 ± 1.38^{b}
Dichloromethane	165.59 ± 1.37*	32.15 ± 1.68^{d}	3.031 ± 0.05^{d}	13.58 ± 0.52*	122.78 ± 0.55^{d}
Ethyl Acetate	172.36 ± 1.08*	4.77 ± 0.15^{a}	2.31 ± 0.01^{c}	9.33 ± 0.30*	88.58 ± 0.51^{c}
Methanol	>1000	13.01 ± 0.52^{b}	0.03 ± 0.01^{a}	12.48 ± 0.37*	21.43 ± 0.33^{a}
BHT	NA	NA	NA	83.01 ± 2.91	NA
Gallic Acid	26.6 ± 1.38	NA	NA	NA	NA
EDTA (IC_{50})	NA	46.43 ± 2.39	NA	NA	NA

ND= not detected; NA= not available; (*a-d*) denote significant difference between sample; (*) denote a significant difference between a sample and standard drugs using Tukey's post hoc test (SPSS 14.0) at $p < 0.05$

TABLE 2. Cytotoxic activities of plant extracts towards SNU-1, LS-174T, K562, HT-29 and MDA-MB-361 cell lines

Plant sample	Extract	IC_{50} (µg/mL)				
		SNU-1	LS174-T	K562	HT-29	MDA
Morinda citrifolia	Hexane	16.83 ± 0.56*	48.60 ± 0.23*	40.08 ± 1.11*	>100	>100
	Dichloromethane	19.36 ± 0.51*	16.87 ± 0.4*	17.14 ±0.20*	31.93 ± 2.97	46.20 ± 8.53
	Ethyl acetate	19.72 ± 0.18*	23.51 ± 1.4*	27.24 ± 0.21*	53.80 ± 2.89	61.16 ± 2.35
	Methanol	>100	>100	>100	>100	>100
Standard drugs	Cis-Diammineplatinum (II) chloride	9.64 ± 0.59	1.32 ± 0.03	4.08 ± 0.09	4.27 ± 0.50	ND
	Tamoxifen	ND	ND	ND	ND	3.15 ± 0.04

ND= not detected; The (*) symbol denote a significant difference between sample and standard drug ($p<0.05$)

ANTIMICROBIAL ASSAY

All four extracts were tested at 10 mg/mL concentrations for their preliminary antibacterial activity against two fungus strains and four bacterial strains. The two fungi used in this study, *Candida albicans* and *Aspergillus niger*, were chosen because they are opportunist pathogens that dwell inside people's bodies, induce fungal infection, and trigger the host immune response (Subroto et al. 2022). Meanwhile, the bacteria were chosen due to their frequent occurrence in wounds and involvement in bacterial diseases, such as diarrhea, urinary tract infection, and respiratory tract infection associated with coughing (Lagutchik 2020). The preliminary antimicrobial activity was categorized as weak antibacterials and antifungals if the inhibition zone extract was less than 10 mm. On the other hand, the extracts were categorized as powerful antibacterial and antifungals when their inhibitory zone was greater than 15 mm (Fu et al. 2007). Dichloromethane and ethyl acetate extracts show good inhibition activity towards all bacteria except *S. aureus*. While the polar methanol extract strongly inhibited both Gram-negative bacteria (Table 3). However, all the extracts showed weak inhibition with both fungal strains. A minimum inhibitory concentration and a minimum bactericidal concentration were further examined for the extracts that showed strong inhibition. Table 4 presents the MIC and MBC results. Both semi-polar extracts exhibited antibacterial activities against *P. aeruginosa* and *B. subtilis* with a low MIC value range of 31.25 µg/mL and 3.91 µg/mL, respectively. As a result, it can be concluded that ethyl acetate and dichloromethane, two semi-polar extracts, have strong antibacterial properties. This situation might be due to the anthraquinone in these extracts (Zamakshshari et al. 2017). Rubiadin has good antibacterial activity against several microbes by increasing the level of superoxide anion and/or singlet molecular oxygen (Watroly et al. 2021).

CORRELATION STUDY OF PHYTOCHEMICAL ANALYSIS WITH BIOLOGICAL ACTIVITIES

The role and impact of the phenolic components in the extracts on cytotoxic, antibacterial, and antioxidant properties were examined by correlation studies. Table 5 displays the correlation coefficients between the total phenolic content and each antioxidant and cytotoxic activity. The biological activity and the total phenolic content appeared to be strongly correlated, according to the correlation coefficients, which varied from 0.8 to 0.9. Correlation coefficients between total phenolic content and their biological activity ranged from 0.1 to 0.4, showing weak correlations, while those between 0.5 and 0.7 suggested a moderate correlation (Lim et al. 2007).

The total phenolic content and ferric-reducing power were strongly correlated. Most of the phenolic compounds in these extracts have the ability to convert the Fe^{3+} complex to the Fe^{2+} complex. Similar to this, it was demonstrated that the phenolic compounds play a significant role in the suppression of *S. aureus, B. subtilis,* and *P. aeruginosa* by the high correlations between the total phenolic content and antimicrobial activity. The extract produced strong antibacterial activity due to the interaction of various phenolic components. A strong negative correlation between the total phenolic content and the cytotoxic activity of extracts on four cancer cell lines was also identified (K562, LS-174T, HT-29, and MDA-MB-361). These results showed that high phenolic compounds in the extracts required less concentration to inhibit all four cancer cell lines, thus contributing to the low IC_{50} value. Therefore, phenolic compounds from both extracts could be selected as bioactive chemical marker compounds for antibacterial and cytotoxic activities. From this study, semi-polar solvents were more effective in extracting phenolic compounds from other solvents since both extracts gave higher TPC values. The extract's high levels of phenolic compounds were also the cause of its high biological activity, making it crucial in the fight against diseases like cancer, and cardiovascular disease (Elisa et al. 2005). Therefore, the extraction process is vital to the biological activity of plants. Each antioxidant assay evaluates antioxidant activity based on various reaction pathways, generating various antioxidant outcomes. Similarly, each compound's cytotoxic and microbial activity has other mechanisms of action toward specific types of cancer and bacteria. Additionally, because each extract comprises complex mixes of compounds, the changes in biological activities found through the phytochemical study were caused by the characteristics of the individual compounds in the mixture. It may contain various chemicals with antioxidant and pro-oxidant characteristics, causing changes in activity brought on by synergistic and antagonistic interactions between or among these molecules (Hincapie et al. 2011).

TABLE 3. Inhibition diameters of extracts of *Morinda citrifolia* and controls against selected microbes

	Fungi strains tested					
Extract	S.A	E.C	P.A	B. S	A. N	C.A
Hexane	13.67 ± 1.53	13.33 ± 1.53	15.33 ± 0.58*	9.67 ± 0.57	10.00 ± 0.00	9.67 ± 1.52
Dichloromethane	14.00 ± 1.73	15.00 ± 0.00*	16.33 ± 0.58*	17.33 ± 1.53*	10.67 ± 1.15	11.00 ± 1.73
Ethyl Acetate	13.00 ± 1.00	16.00 ± 0.00*	16.00 ± 0.00*	16.67 ± 1.53*	9.00 ± 0.00	11.67 ± 0.58
Methanol	11.33 ±0 .57	15.00 ± 0.00*	15.67 ± 0.58*	11.67 ± 0.57	9.00 ± 0.00	14.33 ± 1.15
Streptomycin (1 mg/mL)	24.33 ± 0.58	28.67 ± 0.57	26.00 ± 1.73	29.67 ± 0.58	ND	ND
Ketoconazole (1 mg/mL)	ND	ND	ND	ND	25.00 ± 1.00	26.33 ± 1.53
DMSO	NA	NA	NA	NA	NA	NA

NA= Not Active, ND= Not Detected. SA= *Staphylococcus aureus* (ATCC 25923); BS= *Bacillus subtilis* (B145); PA= *Pseudomonas aeruginosa* (ATCC27853); EC= *Escherichia coli*; CA= *Candida albicans* (C2213) and AN= *Aspergillus niger* (A121)

TABLE 4. Minimum inhibitory concentration of difference extracts of *Morinda citrifolia* and controls against selected microbes

Extract	Concentration (µg/mL)					
	Escherichia coli		*Pseudomonas aeruginos*a		*Bacillus subtilis*	
	MIC	MBC	MIC	MBC	MIC	MBC
Hexane	ND	ND	125.00	500.00	ND	ND
Dichloromethane	31.25	500.00	31.25	500.00	3.91	125.00
Ethyl Acetate	125.00	500.00	31.25	500.00	3.91	125.00
Methanol	62.50	500.00	125.00	500.00	ND	ND
Streptomycin	1.95	7.81	31.25	250.00	3.91	15.63

ND= not detected

TABLE 5. Correlation studies between phytochemical analysis and antioxidant and cytotoxic activity of extracts

No	Assay	Correlation studies Total phenolic content
1	Ferric reducing power	$r^2(4) = .962$, $p = 0.038$
2	Beta-Carotene bleaching assays	$r^2(4) = .254$, $p = 0.746$
3	Ferrous ion chelating	$r^2(4) = .346$, $p = 0.654$
4	IC_{50} of SNU-1	$r^2(4) = -.686$, $p = 0.314$
5	IC_{50} of K562	$r^2(4) = -.845$, $p = 0.155$
6	IC_{50} of LS-174T	$r^2(4) = -.899$, $p = 0.101$
7	IC_{50} of HT-29	$r^2(4) = -.981$, $p = 0.019$
8	IC_{50} of MDA-MB-361	$r^2(4) = -.978$, $p = 0.022$
9	*Staphylococcus aureus*	$r^2(4) = .708$, $p = 0.292$
10	*Bacillus subtilis*	$r^2(4) = .890$, $p = 0.292$
11	*Pseudomonas aeruginosa*	$r^2(4) = .870$, $p = 0.130$
12	*Escherichia coli*	$r^2(4) = .423$, $p = 0.577$
13	*Candida albicans*	$r^2(4) = -.406$, $p = 0.594$
14	*Aspergillus niger*	$r^2(4) = .569$, $p = 0.431$

Conclusions

The semi-polar extract of *M. citrifolia* has good biological activities compared to others. As a result of synergistic and antagonistic interactions, this study's findings show that different extracts can have various biological effects, including antibacterial, antioxidant, and cytotoxic effects. The total phenolic compounds in each extract strongly influence the biological activity of each extract. The identification of extract activities is vital to herba product development. Meanwhile, The identification of secondary metabolites of this species is essential to determine the bioactive marker or fingerprint for herbal standardization.

ACKNOWLEDGMENTS

The authors express our utmost gratitude to Universiti Malaysia Sarawak (UNIMAS) for providing financial funding under the PILOT research grant scheme (UNI/F07/ PILOT/85386/2022) grant as well as research facilities and technical support. The authors also would like to acknowledge Universiti Putra Malaysia, Universiti Malaya, and Taylor University for the research facilities. Special thanks go to Dr. Rusea Go for identifying the plant species.

REFERENCES

Almeida, É.S., de Oliveira, D. & Hotza, D. 2019. Properties and applications of *Morinda citrifolia* (noni): A review. *Comprehensive Reviews in Food Science and Food Safety* 18(4): 883-909.

Assi, R.A., Darwis, Y., Abdulbaqi, I.M., Khan, A.A., Vuanghao, L. & Laghari, M.H. 2017. *Morinda citrifolia* (Noni): A comprehensive review on its industrial uses, pharmacological activities, and clinical trials. *Arabian Journal of Chemistry* 10(5): 691-707.

Apea-Bah, F.B., Minnaar, A., Bester, M.J. & Duodu, K.G. 2016. Sorghum–cowpea composite porridge as a functional food, Part II: Antioxidant properties as affected by simulated *in vitro* gastrointestinal digestion. *Food Chemistry* 197: 307-315.

Akinwumi, B.C., Bordun, K.A.M. & Anderson, H.D. 2018. Biological activities of stilbenoids. *International Journal of Molecular Sciences* 19(3): 792.

Chaves, N., Santiago, A. & Alías, J.C. 2020. Quantification of the antioxidant activity of plant extracts: Analysis of sensitivity and hierarchization based on the method used. *Antioxidants* 9(1): 76.

Chee, C.W., Zamakshshari, N.H., Lee, V.S., Abdullah, I., Othman, R. & Lee, Y.K. 2022. Morindone from *Morinda citrifolia* as a potential antiproliferative agent against colorectal cancer cell lines. *PLoS ONE* 17(7): e0270970.

Draganov, A.B., Yang, X., Anifowose, A., De La Cruz, L.K.C., Dai, C., Ni, N. & Wang, B. 2019. Upregulation of p53 through induction of MDM2 degradation: Anthraquinone analogs. *Bioorganic and Medicinal Chemistry* 27(17): 3860-3865.

Elisa, T., Marco, G., Garden, T., Danila, D.M., Santo, G. & Maurizio, L.G. 2005. The phenolic compounds of olive oil: structure, biological activity and beneficial effects on human health. *Nutrition Research Reviews* 18: 98-112.

Eslami, S., Ebrahimzadeh, M.A. & Biparva, P. 2018. Green synthesis of safe zero-valent iron nanoparticles by *Myrtus communis* leaf extract as an effective agent for reducing excessive iron in iron-overloaded mice, a thalassemia model. *RSC Advances* 8(46): 26144-26155.

Fu, Y.J., Zu, Y.G., Shi, X.G., Wang, Z., Sun, S. & Efferth, T. 2007. Antimicrobial activity of clove and rosemary essential oils alone and in combination. *Phytotherapy Research* 21: 989-994.

Gülçin, İ., Topal, F., Sarikaya, S.B.Ö., Bursal, E., Bilsel, G. & Gören, A.C. 2011. Polyphenol contents and antioxidant properties of medlar (*Mespilus germanica* L.) *Records of Natural Products* 5(3): 158.

Haryoto, H. & Firdaus, G. 2022. Review artikel: Sitotoksisitas ekstrak etanol tumbuhan mengkudu (*Morinda Citrifolia* L) terhadap sel kanker. *Proceeding of the URECOL.* pp. 400-409.

Hincapie, C.A., Monsalve, Z., Seigler, D.S., Alarcon, J. & Cespedes, C.L. 2011. Antioxidant activity of *Blechnum chilense (Kaulf.) Mett*., *Curcuma domestica* Valeton and *Targetes verticillata* Lag. and Rodriquez. *Buletin Latinoamericano y del Caribe de Plantas Medicinales y Aromaticas* 10(4): 315-324.

Kar Wei, L., Zamakshshari, N.H., Ee, G.C.L., Mah, S.H. & Mohd Nor, S.M. 2018. Isolation and structural modifications of ananixanthone from *Calophyllum teysmannii* and their cytotoxic activities. *Natural Product Research* 32(18): 2147-2151.

Kapoor, D., Singh, S., Kumar, V., Romero, R., Prasad, R. & Singh, J. 2019. Antioxidant enzymes regulation in plants in reference to reactive oxygen species (ROS) and reactive nitrogen species (RNS). *Plant Gene* 19: 100182.

Kumar, R. & Sharma, S. 2018. *In-vitro* free radical scavenging potential of flavonoids with synergistic effect of their combination. *Journal of Drug Delivery and Therapeutics* 8(5): 200-203.

Konsue, N., Yimthiang, S. & Kwanhian, W. 2018. Effect of fermentation conditions of noni (*Morinda citrifolia* L.) juice on glutathione content and lipid oxidation in Vero cells. *International Food Research Journal* 25(4): 1534-1540.

Lim, H.W., Yoon, J.H., Kim, Y.S., Lee, M.W., Park S.Y. & Choi, H.K. 2007. Free radical-scavenging and inhibition of nitric oxide production by four grades of pine mushroom (*Tricholoma matsutake Sing*.). *Food Chemistry* 103: 1337-1342.

Lagutchik, M.S. 2020. Fever in the ICU patient. In *The Veterinary ICU Book.* Boca Raton: CRC Press. pp. 671-684.

Nascimento, L.C.S., da R. Rodrigues, N., Alves, M.P.C., Srur, S.A., Junior, J.B. & Barbosa, M.I.M.J. 2018. Chemical characterization, nutritional aspects and antioxidant capacity of noni (*Morinda citrifolia* L) produced in northeastern Brazil. *International Food Research Journal* 25(2): 870-875.

Norshazila, S., Syed Zahir, I., Mustapha Suleiman, K., Aisyah, M.R. & Kamarul Rahim, K. 2010. Antioxidant level and activity of selected seed of Malaysia tropical fruits. *Malaysian Journal of Nutrition* 16(1): 149-159.

Oladeji, O.S., Oluyori, A.P. & Dada, A.O. 2022. Genus *Morinda*: An insight to its ethnopharmacology, phytochemistry, pharmacology and industrial applications. *Arabian Journal of Chemistry* 15(9): 104024.

Oriola, A.O., Aladesanmi, A.J., Akinkunmi, E.O. & Olawuni, I.J. 2020. Antioxidant and antimicrobial studies of some hemiparasitic West African plants. *Eur. J. Med. Plants* 31: 17-26.

Rana, T. 2022. Influence and implications of the molecular paradigm of nitric oxide underlying inflammatory reactions of the gastrointestinal tract of dog: A major hallmark of inflammatory bowel disease. *Inflammatory Bowel Diseases* 28(8): 1280-1288.

Ruhomally, Z., Somanah, J., Bahorun, T. & Neerghee-bhujun, V.S. 2015. *Morinda citrifolia* L. fruit extract modulates H_2O_2 - induced oxidative stress in human liposarcoma SW872cell. *Journal of Traditional and Complementary Medicine* 6(3): 299-304.

Sina, H., Dramane, G., Tchekounou, P., Assogba, M.F., Chabi-Sika, K., Boya, B. & Baba-Moussa, L. 2021. Phytochemical composition and *in vitro* biological activities of *Morinda citrifolia* fruit juice. *Saudi Journal of Biological Sciences* 28(2): 1331-1335.

Siti Azima, A.M., Norriham, A. & Manshoor, N. 2022. Synergistic effects of *Garcinia mangostana* and *Clitoria ternatea* extract mixture on antioxidant activities, colour, and anthocyanin stabilities. *International Food Research Journal* 29(3): 631-645.

Subroto, E., van Neer, J., Valdes, I. & de Cock, H. 2022. Growth of *Aspergillus fumigatus* in biofilms in comparison to *Candida albicans. Journal of Fungi* 8(1): 48.

Torres, M.A.O., de Fátima Braga Magalhães, I., Mondêgo-Oliveira, R., de Sá, J.C., Rocha, A.L. & Abreu-Silva, A.L. 2017. One plant, many uses: A review of the pharmacological applications of *Morinda citrifolia*. *Phytotherapy Research* 31(7): 971-979.

Watroly, M.N., Sekar, M., Fuloria, S., Gan, S.H., Jeyabalan, S., Wu, Y.S. & Fuloria, N.K. 2021. Chemistry, biosynthesis, physicochemical and biological properties of rubiadin: A promising natural anthraquinone for new drug discovery and development. *Drug Design, Development and Therapy* 15: 4527.

World Health Organization (WHO). 2022. *Cancer, Fact Sheet*. Accessed on 12 July 2022. https://www.who.int/news-room/fact-sheets/detail/cancer

World Health Organization (WHO). 2021. *Antimicrobial Resistance, Fact Sheet.* Accessed on 12 July 2022. https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance

Xiao, F., Xu, T., Lu, B. & Liu, R. 2020. Guidelines for antioxidant assays for food components. *Food Frontiers* 1(1): 60-69.

Yazan, L.S. 2003. Cytotoxic properties of anthraquinones (nordamnacanthal and damnacanthal) from roots of *Morinda elliptica*. PhD dissertation. Universiti Putra Malaysia (Unpublished).

Zhang, Q., Chen, W.W., Sun, X., Qian, D., Tang, D.D., Zhang, L.L., Li, M.Y., Wang, L.Y., Wu, C.J. & Peng, W. 2022. The versatile emodin: A natural easily acquired anthraquinone possesses promising anticancer properties against a variety of cancers. *International Journal of Biological Sciences* 18(8): 3498-3527.

Zamakshshari, N.H., Mah, S.H., Ee, G.C.L., Ibrahim, Z., Teh, S.S. & Daud Shaari. 2017. Cytotoxic activities of anthraquinones from *Morinda citrifolia* towards SNU-1, LS-174T, and K562 cell lines. *Archives of Natural and Medicinal Chemistry* 2: 1-27.

Zamakshshari, N.H., Ee, G.C.L., Ismail, I.S., Ibrahim, Z. & Mah, S.H. 2019. Cytotoxic xanthones isolated from *Calophyllum depressinervosum* and *Calophyllum buxifolium* with antioxidant and cytotoxic activities. *Food and Chemical Toxicology* 133: 110800.

Zamakshshari, N.H., Ahmed, I.A., Didik, N.A.M., Nasharuddin, M.N.A., Hashim, N.M. & Abdullah, R. 2022. Chemical profile and antimicrobial activity of essential oil and methanol extract from peels of four *Durio zibethinus* L. varieties. *Biomass Conversion and Biorefinery* 2022: 1-9.

Zhen, J., Villani, T.S., Guo, Y., Qi, Y., Chin, K., Pan, M-H., Ho, C.T., Simon, J.E. & Wu, Q. 2016. Phytochemistry, antioxidant capacity, total phenolic content and anti-inflammatory activity of *Hibiscus sabdariffa* leaves. *Food Chemistry* 190: 673-690.

*Corresponding author; email: znhisam@unimas.my