Phytochemical Screening, antimicrobial and antioxidant efficacy of some plant extracts and their mixtures

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Abstract. Various phytochemicals and bioactive compounds are sourced from medicinal plants. This study focused on finding the most potent plant extracts whose combination could exhibit better antioxidant and antimicrobial activities compared to the efficacy of the individual extracts. The combined plant extracts were *O. stamineus, E. longifolia, A. bilimbi, P. granatum,* and *M. nigra.* Ultrasound extraction technique was used for the extraction of the plant material before screening the extracts for the presence of several phytochemical groups. After the screening for the presence of phytochemicals, the extracts were evaluated for antimicrobial activity against five microorganisms (*S. aureus, B. subtilis, P. vulgaris, E. coli, and C. albicans*) at 3 different concentrations (600, 400 and 200 mg/mL) using disc diffusion method. The aim of this antimicrobial screening was to identify and select the most potent extracts for the combination study. From the screening result, *P. ganatum* and *M. nigra* had the highest antimicrobial activity against all the tested organisms, as well as the highest DPPH radical scavenging activity of 95.40 % (IC_{50} = 120.2 µg/mL) and 90.20 % (IC_{50} = 330.0 µg/mL), respectively. Hence, both extracts were combined and screened for antimicrobial and antioxidant activities at the same concentration range but at different combination ratios of 1:1, 1:2 and 2:1 (v/v). The results showed the extract mixtures at different combination ratios of 1:1, 1:2 and 2:1 (v/v) to exhibit good antioxidant and antimicrobial activities. However, the combination ratio of 1:1 gave the best antioxidant activity as it achieved an IC_{50} value of 82.0 µg/mL. Therefore, it is concluded that the mixture of the selected extracts exhibited strong antioxidant and antimicrobial activities, coupled with a significant level of antifungal activity.

1. Introduction

One of the ancient forms of healthcare known to man is herbal medicine. More than 50% of the modern drugs used in healthcare are sourced from natural sources; hence, the role of natural products in the development of the pharmaceutical sector is paramount. Phytochemicals are plant chemicals which are secreted by plants in response to environmental constraints or diseases. They are not required for plant metabolism but are beneficial to man in managing several diseases. To encourage the use of plant materials as the possible sources of antimicrobial agents, there must be a careful evaluation of their composition and biological activity prior to use [1]. The emergence of antibiotic-resistant microorganisms has propelled the discovery of novel antibacterial drugs. The increase in the rate of multiple drug-resistant microorganisms is attributable to the rashness use or malmanagement of antibiotics which has resulted in many antibiotics losing their effectiveness against certain microorganisms [2].

The unbalanced generation of free radicals creates abnormal physiological conditions that lead oxidative damage to cells through lipids, proteins and nucleic acid biomolecules degradation [3]. Oxidative damage

caused by reactive oxygen species is repaired by the protective action of antioxidant which continuously converts the generated free radicals into less harmful molecules by intercepting radical chained reactions [4]. Plant-derived bioactive compounds have a protective role in minimizing oxidative stress. Several crude plant extracts possess high oxidative capacity and a remarkable concentration of phenolic compounds [5]. This study was designed to examine the phytochemicals content, antimicrobial, and antioxidant activities of selected medicinal plant extracts. The biological activity of the plant extracts was screened individually before combining the most potent extracts for the combination studies.

2. Methodology

2.1. Plant materials

Five plants (*Averrhoa bilimbi*, *Orthosiphon stamineus, Punica granatum, Morus nigra,* and *Eurycoma longifolia*) were collected from a herbal market in Kuantan, Pahang, Malaysia and transported to the laboratory unit of the Faculty of Industrial Sciences & Technology (FIST), Universiti Malaysia Pahang, Gambang campus for further preparation and investigations. The plant materials were manually cut into small pieces to increase the surface area during the extraction process. For the drying process, the plants were placed in a conventional oven at 40-45°C for 3 days [6]. Table 1 presents the plant materials used in this study.

Table 1: The English name, scientific name, code, family, and part of all plants used in this study.

NO.	English name	Scientific name	Code	Family	Part used
1	Tree sorrel	*Averrhoa bilimbi*	Ab	Oxalidaceae	Leaves
2	King of bitters	*Andrographis paniculata*	Ap	Acanthaceae	Whole plant
3	Cat's whiskers	*Orthosiphon stamineus*	Os	Lamiaceae	Leaves
4	Longjack	*Eurycoma longifolia*	El	Simaroubaceae	Roots
5	Pomegranate	*Punica granatum*	Pg	Lythraceae	Peels

2.2. Sample preparation

After drying the plant materials for 3 days at 40-50°C [6], they were pulverized in a dry mill blender (Panasonic MX-GM1011 H) and stored in airtight plastic bags in desiccators at room temperature until further use [7].

2.3. Ultrasound-assisted extraction (UAE)

A method modified by [8] was used during the extraction process. Five grams of the pulverized plant materials were placed in 100 mL of 80% ethanol and sonicated at the sonication power of 20 kHz. The sonication probe immersed into the extraction vessel during the process to ensure maximum power transfer. The sonication was set for 15 minutes at pulse-on pulse-off times of 20 and 5 seconds, respectively. After the sonication time, the mixture was filtered, and the filtrate was concentrated in a rotary evaporator to remove the solvent. The concentrated filtrate was later placed in a fume hood to ensure the evaporation of the remaining solvent in the extract.

2.4. Screening for phytochemicals content

2.4.1. Alkaline reagent test for flavonoid. About 50 mg of each extract was mixed with 5 mL of dilute ammonia solution and filtered. Then, concentrated H_2SO_4 was added to the filtrate and monitored for the formation of a yellow colour which will indicate the presence of flavonoids in the extracts [9].

2.4.2. Wagner's test for alkaloids. The plant extract (50 mg) was dissolved in a small volume of dilute HCl and filtered. Then was followed by a gentle addition of few drops of Wagner's reagent to the filtrate

along the side of the test tube. The solution was monitored for the development of reddish-brown precipitates which indicates the presence of alkaloids in the extract [10].

2.4.3. Salkowski's test for glycoside. The plant extract (2 mL) was diluted in chloroform (2 mL), followed by the addition of a few drops of concentrated H_2SO_4. The mixture was observed for the formation of a reddish-brown color to indicate the presence of glycosides in the extract [11].

2.4.4. Tannin test. The plant extract (50 mg) was weighed and dissolved in distilled water (d.H_2O) before adding a few drops of $FeCl_3$ solution. The development of green precipitates after mixing indicates the presence of tannins in the extract [12].

2.4.5. Steroid test. The plant extract (50 mg) was dissolved in 2 mL each of chloroform and concentrated H_2SO_4 and allowed for some seconds. The presence of steroids is manifested by the formation of red colour in the lower layer of the mixture [11].

2.4.6. Froth test for Saponin. The plant extract (50 mg) was dissolved in d.H_2O and later made up the volume to 20 mL with d.H_2O. Then, the mixture was vigorously shaken for 15 min and observed for the formation of stable foam of at least 2 cm length to indicate the presence of saponins [10].

2.4.7. Ferric chloride test for phenolics. The plant extract (50 mg) was dissolved in d.H_2O (5 mL), followed by the addition of a few drops of ferric chloride (5%). The mixture was monitored for the formation of a bluish-black colour to indicate the presence of phenolics in the extract [10].

2.5. Microbial culture preparation

The five plant extracts were evaluated for antimicrobial activities against pure cultures of *S. aureus*, *B. subtilis*, *E. coli*, *P. vulgari*s, and *Candida albicans.* The microbes were sourced from the Microbiology Unit of FIST laboratory, UMP, Malaysia. The bacteria were cultured in Nutrient broth while the fungi were cultured in potato dextrose broth media (PDB). Both cultures were incubated for 24 h at 37 and 30 °C, respectively. After the incubation period, the optical density (OD) of microbial suspension was determined at 600 nm. An OD reading of 0.5 McFarland standard was considered to represent a microbial concentration of 10^8 CFU/mL [10].

2.6. Determination of antimicrobial activity

The extracts were evaluated for antimicrobial activity at different concentrations of 200, 400 and 600 mg/mL. The desired concentrations of the extracts were prepared in 80% ethanol and stored in the chiller to avoid evaporation. The disc diffusion method was used to screen the extracts for antimicrobial activity. A drop of each microbial suspension containing about 10^8 CFU/mL (determined by hemocytometer) was gently spread on the Mueller-Hinton agar (MHA) and PDA using a sterile cotton bud. Then, 6 mm diameter discs were sterilized at 121℃ for 15 min and loaded with the extracts at different concentrations. Chloramphenicol (30 mg) and fluconazole (25 mg) were used as positive controls. The discs were dried and placed in contact with the surface of the plates containing the organisms, labeled, and incubated for 24 h (bacteria) and 48 h (fungi) at 37℃. The experiments were done in replicates and the results were reported as the diameter (mm) of the inhibition zones [10].

2.7. Determination of antioxidant activity

DPPH (2,2-diphenyl-1picrylhydazyl) scavenging assay: The antioxidant capability of the extracts was determined based on their DPPH free radical scavenging power [13]. The stock DPPH solution was prepared by dissolving DPPH (24 mg) in 100 mL of absolute methanol. The solution was covered with foil and stored at 20°C. For the working solution, the stock solution was further diluted with absolute methanol to the absorbance reading of 0.950 ± 0.02 at 517 nm. The extract solution was prepared by dissolved the crude extracts in absolute methanol at the concentration of 1mg/mL and further diluted for the analysis.

The test was performed by adding 1 mL of DPPH working solution into 100 µL of the extract solution prepared in different concentrations (10, 40, 100, 200, 500, 600 and 700 µg/mL). The mixtures were shaken and placed in the dark for 10 min at room temperature. The absorbance (Ab) of the mixture was read at 517 nm against a methanol blank. The decrease in the absorbance reading was correlated with the radical scavenging potential of extracts. The IC_{50} values of the extracts were calculated as the concentration of the extracts required to stabilize 50% of the radical population. Ascorbic acid was used as the positive control while the DPPH radical scavenging activity of the extracts was calculated using the relation:

$$\text{DPPH (\%)} = \frac{\text{Ab (control)} - \text{Ab (sample)}}{\text{Ab (control)}} \text{X } 100$$

Where Ab (control) is the absorbance of the control, and Ab (sample) is the absorbance of the test sample. The antioxidant activity of the combined extracts was also determined. The mixture prepared by mixing the extract of *P. granatum* and *O. stamineus* in 3 different ratios of 1:1, 2:1, and 1:2. The Absorbance of the different combination ratios was also read at 517 nm and the IC_{50} value of each combination was calculated as earlier described.

2.8. Data analysis

The data obtained from the experiments were analyzed carefully by calculating their mean and standard deviations. The IC_{50} values were calculated via linear regression analysis.

3. Results and discussion

3.1. Phytochemical screening

From the results of the phytochemicals screening, the presence of medically important phytochemicals was detected in the ethanolic extract of the studied plants. Glycosides were present in all the extracts while alkaloids were absent from all the extracts except in *E. longifolia.* Phenolics and tannins were present in all the samples except *E. longifolia*. Steroids were not detected in the ethanolic extract of *O. stamineus* and *A. bilimbi* while saponin was absent from both *E. longifolia* and *M. nigra* extracts (Table 2). Belkacem *et al.* [14] reported the extraction of phytochemicals from *P. granatum* fruit using ethanol as the extraction solvent. The absence of alkaloids and flavonoids in some of the extracts might be due to the non-suitability of ethanol as the extraction solvent for these group of compounds since Sharma *et al.* [15] reported the successful extraction of alkaloids and flavonoids using methanol and water. The ethanol extract of *P. granatum* contained most of the screened phytochemicals; however, *O. stamineus* extract contained all the phytochemical groups except alkaloids, steroid, and flavonoid [16].

Table 2: The preliminary phytochemical screening on the five plant extracts under test.

Phytochemical test	**The plant extract**				
	P. granatum	*O. stamineus*	*E. longifolia*	*M. nigra*	*A. bilimbi*
Phenolic	+	+	-	+	+
Saponin	+	+	-	-	+
Alkaloids	-	-	+	-	-
Glycoside	+	+	+	+	+
Tannin	+	+	-	+	+
Steroid	+	-	+	+	-
Flavonoids	-	-	+	+	+

(+): indicates presence; (-): indicates absence.

The extracts of *E. longifolia* revealed the presence of alkaloids, glycosides, steroids and flavanoids compounds while phenolic, saponins, and tannins were absent. Different types of chemicals were used to

extract the plant, yet, all the extracts showed negative saponin tests, indicating that *E. longifolia* might contain little or no saponin content. Some studies have explained that tannins and phenolic compounds were present in *E. longifolia* but certain factors such as the plant source can cause some differences in their phytochemicals content [10]. *M. nigra* has been reported to contain alkaloids [17]. According to Hussain *et al.* [18], *M. nigra* does not contain saponin but the outcome of this study showed the presence of phenolics, saponin, glycoside, tannins, and flavonoids in *M. nigra* while alkaloids and steroids were not detected. Makky *et al.* [19] reported the presence of alkaloids and steroids in ethanolic extract of *A. bilimbi*.

3.2. Antimicrobial activity

To evaluate the antimicrobial efficacy of the extracts, discs containing ethanolic extract of the five plants at three different concentrations were placed onto the media inoculated with five different microbial pathogens. The varying degrees of the antimicrobial potentiality of the five plant extracts were presented in Table 3.

Chloramphenicol (30 mg) was used as the standard positive control and it showed inhibition zones of diameter ranging from 20-24.67mm against all the tested organisms. The result showed the most potent antibacterial activity against *S. aureus, B. subtilis* and *P. vulgaris* to be attributed to *P. granatum* extract at 400 mg/mL (diameter of inhibition zone = 12 ± 2.5 mm, 7.7± 0.5 mm, and 9.3± 1.3 mm at 400, 600, and 800 mg/mL, respectively). *M. nigra* extract was effective against only *S. aureus* (inhibitory zone of 8.0 ± 0.8 mm at 400 mg/mL), followed by *P. vulgaris* (inhibitory zone of 3.3 ± 4.7 mm at 400 mg/mL). *E. longifolia*, *A. bilimbi,* and *O. stamineus* extracts showed the lowest inhibitory activities to most of the tested microorganisms. *E. coli* was resistant to all the plant extracts except *E. longifolia* which was effective at the concentration of 200 mg/mL (inhibition zone = 3.0 ± 4.2 mm). The resistance of the Gram-negative organisms to the extract could be attributed to the outer membrane in the cell wall of the bacteria which prevented the penetration of hydrophilic compounds into the cells [20]. Tannins are one of the phytochemicals in *P. granatum* which have been revealed to prevent microbial growth [21]. The phenolic compounds for antimicrobial mechanisms in *P. granatum* are involved in reactions with microbial cell membrane proteins which will stimulate protein precipitation-mediated cell death and enzymes inhibition [22]. As per Souza *et al.* [23], the compounds present in *M. nigra* that inhibits bacterial growth are phenolics and flavonoids. As *M. nigra* has high steroids, flavonoids and alkaloids content, it is commonly used in herbal medicine [24] [18-19]. *O. stamineus* exhibits good antimicrobial properties [20]. Flavonoid and phenolic compounds present in *O. stamineus* can successfully inhibit bacterial growth. *S. aureus* has been reported to be sensitive to plant phytochemicals [16]. *E. logifolia* is also believed to be an effective antimicrobial agent even when extracted with different solvents [25]. According to Das *et al.* [26], *A. bilimbi* has effective antimicrobial activity especially against *E. coli* and *C. albicans*. The results of this study showed less effectiveness of *E. longifolia* possibly due to the extraction solvent and technique used. Some of the plant extracts gave the best yields when polar solvents are used for the extraction [10]. The antibiotic used as a control was effective against all the bacteria except *B. subtilis* and *C. albicans* which showed resistance to all the plant extracts and fluconazole. Similarly, chloramphenicol produced no zones of inhibition against *E. coli* and *B. subtilis* (Figure 1).

Table 3: Antimicrobial activity of five plant extracts at different concentrations against different microorganisms. The data were express in term of mean ± standard deviation.

Microbial isolates	Extract Conc. (mg/mL)	Inhibition zone diameter (mm±SD)					
		E. longifolia	*P. granatum*	*A. bilimbi*	*M. nigra*	*O. stamineus*	Chloramphenicol (30 mg)
S. aureus	600	0.0	12± 0.82	0.0	7.33± 0.47	7.67± 0.47	24.67± 1.25
	400	0.0	12± 2.45	0.0	8±0.82	2.67± 3.77	24± 1.41
	200	0.0	11± 0.82	3±4.24	5.33±4.71	0.0	21± 1.41
B. subtilis	600	0.0	7.67± 0.47	0.0	0.0	0.0	0.0
	400	0.0	5.67± 4.20	0.0	0.0	0.0	0.0
	200	0.0	5.67± 4.20	0.0	0.0	0.0	0.0
P. vulgaris	600	0.0	9.33± 1.25	0.0	0.0	3± 4.24	21± 1.41
	400	0.0	6.67± 4.99	0.0	3.33± 4.71	0.0	21.67± 0.47
	200	0.0	9± 2.16	0.0	3.33± 4.71	0.0	20± 1.63
E. coli	600	0.0	0.0	0.0	0.0	0.0	21.67± 0.47
	400	0.0	0.0	0.0	0.0	0.0	21.33± 0.47
	200	3 ± 4.24	0.0	0.0	0.0	0.0	20.67±0.94
							Fluconazole (25 mg)
C. albicans	600	0.0	0.0	0.0	0.0	0.0	0.0
	400	0.0	0.0	0.0	0.0	0.0	0.0
	200	0.0	0.0	0.0	0.0	0.0	0.0

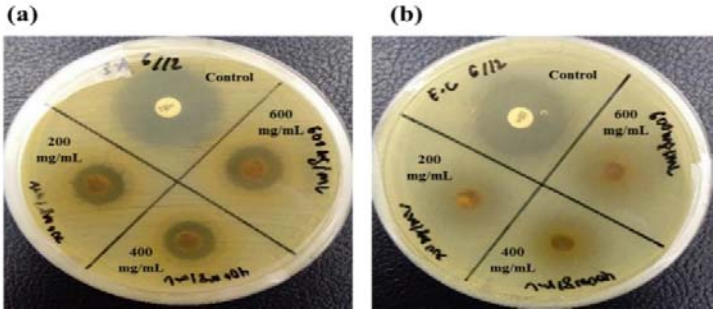

Figure 1: Growth inhibition zone of *P. granatum* against (a) *S. aureus* and (b) *E. coli*.

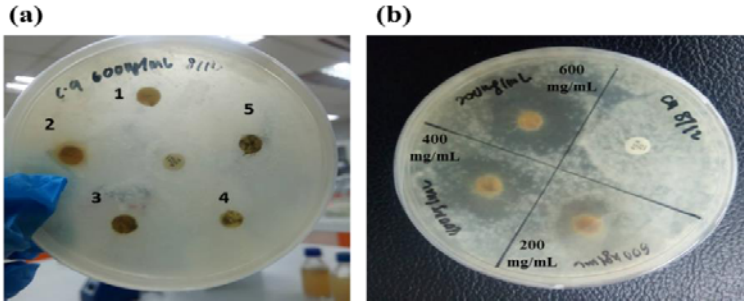

Figure 2: Growth inhibition zone of plant sample against *C. albicans* of all five plant extracts (a); and the mixture of *E. longifolia* and *M. nigra* (b) at different concentrations. 1: *E. longifolia*; 2: *P. granatum*; 3: *A. bilimbi*; 4: *M. nigra*; 5: *O. stamineus.*

The antimicrobial efficacy of the combined extracts of *P. granatum* and *M. nigra* at different concentrations was shown in Table 4. All the combination ratios of the plant extracts showed activity against all the microbial isolates except *E. coli* possibly due to nature of the bacterial cell wall. *S. aureus* showed the highest sensitivity at 600 mg/mL (inhibition zone = 12.0 ± 1.4mm) while the lowest inhibition zone of 5.7 ± 4.0 mm was recorded against *P. vulgaris* at 200 mg/mL. However, *C. albicans* was successfully inhibited at all the concentrations despite its resistance to the individual extracts. Fluconazole did not show any activity as it is a fungistatic agent rather than a fungicide. So, treatment with this agent can result in the development of acquired resistance. The impact of different Upc2p mutations on *ERG11* expression in *C. albicans* and its fluconazole-resistant phenotype and reported that the sustained production of the azole target annuls the activity of fluconazole, resulting in resistance [27] (Figure 2).

Table 4: Antimicrobial activity of plant extract mixture of *P. granatum* and *M. nigra* at different concentrations.

Microbial isolate	**Extract conc. (mg/mL)**	**Inhibition zone diameter (mm±SD)**	
		Extract mixture of *P. granatum/M. nigra*	Chloramphenicol (30 mg)
S. aureus	600	12± 1.4	22.7± 1.2
	400	11.7± 0.5	NA*
	200	7.7± 5.4	NA
B. subtilis	600	9.7± 0.5	0.0
	400	9.0± 0.8	NA
	200	7.7± 0.5	NA
P. vulgaris	600	11.3± 0.5	21.7± 2.6
	400	9.7± 0.5	NA
	200	5.7± 4.0	NA
E. coli	600	0.0	21.3±1.7
	400	0.0	NA
	200	0.0	NA
			Fluconazole (25 mg)
C. albicans	600	10.0± 0.8	NA
	400	8.7± 0.5	0.0
	200	8.3±0.5	NA

NA*: Not applicable.

3.3. Antioxidant assay

The free radical scavenging activity of the five plant extracts was assessed on DPPH radicals. The disappearance of DPPH radicals was observed due to the effect of the antioxidants in the extracts. Different concentrations of the extract caused the fading of the purple color of the DPPH solution to yellow. DPPH radicals are usually used to study the antioxidant activity of extracts as it can accept electrons and get stabilized [28]. DPPH is a purple stable free radical with an adsorption band at 517 nm and can be reduced to yellow by accepting electrons from antioxidants [29]. Figure 3 depicts the ability of different concentrations (10-1000 µg/mL) of the plant extracts to scavenge DPPH free radicals compared to the ability of ascorbic acid. From the table. Ascorbic acid presented the lowest IC_{50} value (37.5 µg/mL). Ascorbic acid is one of the potent naturally-occurring antioxidants in the biological system [30]. As per Arrigoni & Tullio, [31], efficient biological antioxidants are supposed to: (a) exist in good quantities in the cell, (b) react with several free radicals, (c) be ideal for regeneration other than just reacting with free radicals. These properties are typical for ascorbic acid, making it a good antioxidant for the cells of almost all aerobic species. Among the five plant extracts, the radical scavenging activity of *P. granatum* was almost equivalent to that of ascorbic acid at the concentration of 500 µg/mL and above. This proved that *P. granatum* extract possesses antioxidant properties like ascorbic acid at high concentration. On the other hand, *O. stamineus* extract only showed good scavenging activity at 700 µg/mL. Statistically, *P. granatum*

and *O. stamineus* extracts were able to inhibit the formation of DPPH free radicals at the inhibition percentages of 95.40 and 90.20 %, respectively at 700 µg/mL. These results showed *P. granatum* and *O. stamineus* extracts to contain higher inhibitory compounds which may act as primary antioxidants and react with free radicals. Some studies reported that tannins possessed antioxidants and antiradical activities. Tannins are antinutrient of plant origin due to their ability to cause protein precipitation, lower the availability of vitamins & minerals, and inhibit digestive enzymes. It can be considered as the "health-promoting" components in foods derived from plants and beverages. Tannins do not function only as primary antioxidants, it also functions as a secondary antioxidant [32].

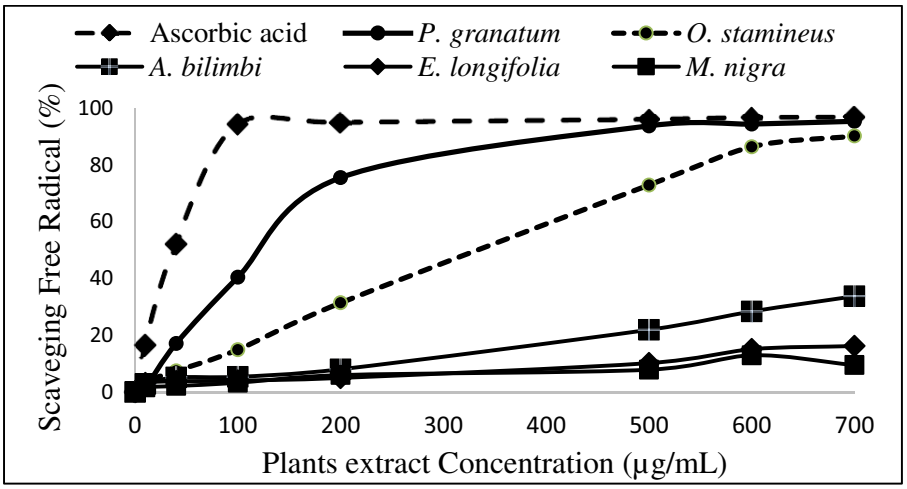

Figure 3: The DPPH free radical scavenging activity of the five plant extracts at different concentrations.

As the plants extract with the highest scavenging activity, *P. granatum* must contain phytochemicals that exhibit antioxidant properties, such as flavonoid and tannin. *P. granatum* extract does not contain flavonoid but rich in the other phytochemicals; hence, it presented the highest scavenging activity. This result can be explained by certain circumstances, such as the dependence of antioxidant activities on the type of extraction solvent used, the presence of other antioxidants of different chemical properties, as well as the compound polarity in the extraction solvent. To enhance the extraction of phytochemicals, the mixture of methanol and ethanol has been reported in several studies [33]. Contrarily, the scavenging activity of *A. bilimbi, E. longifolia,* and *M. nigra* was observed to be 33.78 %, 16.24 %, and 9.51 %, respectively at 700 µg/mL. These activities are weak to be considered antioxidant activities. Comparing with the positive control, the scavenging activity of the three extracts showed significant differences to that of ascorbic acid (more than 62.98%), indicating that these extracts have weak antioxidant activities. The scavenging activity of plant extracts has been reported to increase with the extract concentrations. The IC_{50} values of all the samples were calculated and reported as the concentration of the extracts required to scavenge 50% of the DPPH population [34]. Table 5 showed that *P. granatum* extract exhibited the highest antioxidant activity (IC_{50}= 120.2), followed by *O. stamineus* (IC_{50}= 330.0). Studies have shown that the IC_{50} values can decrease as the free radical scavenging activity of the extract increases. No IC_{50} values were recorded for *E. longifolia*, *A. bilimbi*, and *M. nigra* extracts as they exhibited poor antioxidant activity. This study also showed all the studied extracts to exhibit some levels of activity against DPPH radical even though *P. granatum* and *O. stamineus* extracts exhibited the best radical scavenging activity compared to *E. longifolia*, *A. bilimbi*, and *M. nigra*. From the observed antioxidant activities, it can be concluded that *P. granatum* and *O. stamineus* showed the best antioxidant activities and as such, were combined and investigated for improved antioxidant activity.

Table 5: IC_{50} values of individual plant extracts samples.

Samples	IC_{50} values (µg/mL)
Ascorbic acid	37.5
P. granatum	120.2
O. stamineus	330.0
E. longifolia	ND
A. bilimbi	ND
M. nigra	ND

ND: Not detected.

3.4. Antioxidant activities of the extract mixtures

The extracts of *P. granatum* and *O. stamineus* were combined at three different combination ratios (1:1, 2:1, 1:2) and studied for antioxidant activity. The results showed the extract combinations at the respective ratios to exhibit DPPH radical scavenging activities of 94.31, 91.80 and 87.79%, respectively at the concentration of 700 µg/mL. The combination ratio of 1:1 showed the highest radical scavenging activity while the other two combination ratios showed comparable radical scavenging activities to that of ratio 1:1. These results indicate that all the combination ratios of the extracts exhibited good antioxidant activities (Figure 4). The IC_{50} values of the extract combinations showed ratio 1:1 to exhibit the lowest IC_{50} value (82 µg/mL), followed by ratio 1:2 (196.0 µg/mL) and ratio 2:1 (255.0 µg/mL). The low IC_{50} value of combination ratio 1:1 showed that it has good antioxidant activity (Table 6). The good antioxidant activity of the extract combination at different ratios could be due to the interaction of different phytochemicals contained in both extracts.

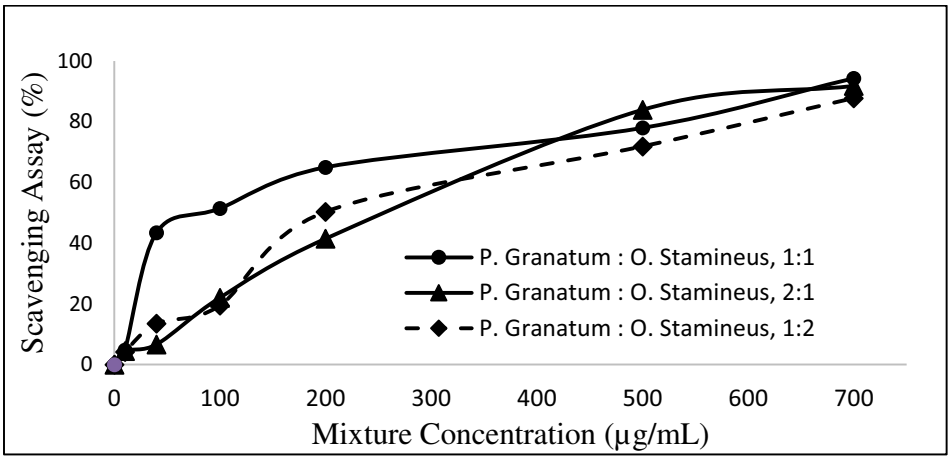

Figure 4: Scavenging activities of the different ratios of mixture *P. granatum* and *O. stamineus* extracts at different concentrations.

Table 6: IC_{50} values of *P. granatum* and *O. stamineus* mixture extracts at different ratios.

The ratio mixture of *P. granatum and O. stamineus* extracts	IC_{50} value (µg/mL)
1:1	82.0
1:2	196.0
2:1	255.0

4. Conclusion

In conclusion, the outcome of this study suggests that the five plant extracts have the potential to be used as antioxidant and antimicrobial compounds. The current report will provide preliminary findings for the selection of these plants for further antioxidant and antimicrobial studied which might be necessary to characterize, isolate, and purify the bioactive compounds they contain for onward use as complementary and alternative medicine in treating various diseases. The five extracts contained glycoside 100 %, phenolic and tannin compounds 80%, steroid 60%, flavonoids and alkaloids 20%. *P. granatum* extract showed better antimicrobial activity (60%) against all the microbial isolates, while, *M. nigra* and *O. stamineus* were effective at only 40%. *E. longifolia* and *A. bilimbi* showed only 20% antimicrobial activity while none of the extracts had antifungal activity. Regarding the combined extracts, the mixture exhibited 80% antimicrobial activity and some levels of antifungal activity.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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