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Phytochemical Screening, Antioxidant, and Antimicrobial Properties of Sequential Extracts of Stems of *Arcangelisia flava*

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In the Philippines, *Arcangelisia flava*, also known as albotra, has been used as an alternative treatment for a variety of diseases, including tumors, myoma, diabetes, and reproductive disorders. Its medicinal properties are attributable to the phytochemicals it contains. This study aimed to characterize the phytochemicals responsible for the antioxidant and antimicrobial activities of *A. flava* stem extracts. Using sequential extraction with solvents of increasing polarity, three crude extracts were obtained: ethyl acetate, methanolic, and water extract. The extraction yield per gram of dried extract were 0.47 ± 0.65, 5.70 ± 0.44, and 5.85 %, respectively. Total phenolic content of ethyl acetate and methanolic extract were 162.30 ± 6.88 and 177.57 ± 23.31 mgGAE/g extract, respectively. Phytochemical analysis detected the presence of alkaloids and saponins in the three extracts. Ethyl acetate extract was also found to contain terpenoids, flavonoids, and cardiac glycosides. The antioxidant assays also showed different trend in the antioxidant capacity of the three crude extracts. In DPPH, water extracts had the highest antioxidant activity of 0.714 ± 0.006 mmol Trolox equivalent per gram of dried extract (TEAC/g) and is significantly different to the values of AEC and AMC. Meanwhile, in the CUPRAC and FRAP assay, methanolic extract had the highest antioxidant activity compared to ethyl acetate and water extract. In addition, the disc and well diffusion method revealed that at concentrations of 10 mg/mL and 50 mg/mL, the methanolic extract exhibited antibacterial activity against *Staphylococcus aureus* and antifungal activity against *Candida tropicalis*. Antimicrobial activities may be possible because of the phytochemicals that have been identified in these extracts of *A. flava*. This study suggests that the methanolic extract of *A. flava* stems is an excellent candidate for the purification and isolation of compounds responsible for antioxidant and antimicrobial activity.

Keywords: Antioxidant; antimicrobial; phytochemicals*; Arcangelisia flava*; sequential extraction

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Various diseases, including diabetes, cardiovascular disease, Alzheimer's disease, retinopathy, cancers, and microbial infections, threaten society's health. Today, scientists are investigating secondary plant metabolites, also called phytochemicals, as a natural source of antioxidants and antimicrobial agents. They are widely distributed in edible and medicinal plants, and discovering their function in pharmaceuticals could increase the value of various plant species. Anti-inflammatory, anti-aging, anti-atherosclerosis, and anti-cancer properties are exhibited by natural antioxidants, in particular polyphenols and flavonoids [1]. In addition, numerous studies have shown that phytochemicals isolated from plants exhibit potent antimicrobial properties and may act against bacterial resistance [2, 3, 4]. An imbalance between oxidants and antioxidants may be caused, among other things, by the overproduction of free radicals in the human body as a result of natural biochemical reactions and external attacks resulting from stress, smoking, and unbalanced diets. It is therefore essential to supplement the diet with antioxidants from phytochemicals [5, 6]. Likewise, phytochemical components such as phenols, poly-phenols, quinones, and flavonoids are responsible for the antimicrobial property of plants. It is known that some wood-containing extractives may inhibit the activities of termites, bacteria, and fungi [7]. For instance, the wood extract of the endemic tree *Hymenaia stigonocarpam* contains synergistic phytochemicals that are responsible for its high antimicrobial and antioxidant activity [8].

In the year 2020, Dapar [9] and his team recorded the indigenous medicinal plant knowledge and practices of Manobo tribe in the Mindanao region, Philippines. One of their reported plants is the woody vine called *Arcangelisia flava*, which, according to their interview, has been used as an alternative treatment for tumors, myoma, diabetes, tonics, respiratory disease complex, diarrhea, dyspepsia, ulcers, appetite enhancers,

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delayed menstruation, and dysmenorrhea with the side effect of abortion in pregnant women. In other countries such as Thailand and Malaysia, the stem of *A. flava* is used as infused teas. The scientific evidences of *A. flava's* bioactivity through in vitro, in vivo, and in silico studies are thoroughly documented throughout Southeast Asian countries. In fact, in our previous study, we evaluated the stems of *A. flava* collected from Quezon, Philippines [10]. It has been reported that the ethanolic extract of *A. flava* stem exhibited promising antioxidant and antibacterial properties and this is possibly due to the different phytochemicals detected such as saponins, tannins, flavonoids, steroids, terpenoids, and cardiac glycosides. However, in order to determine with a high level of accuracy the phytochemicals that are responsible for its bioactivity, [11] suggest using sequential extraction with varying polarities. This would be the step that would come before purification. Furthermore, several distinct antioxidant assays can also be used simultaneously to evaluate the antioxidant activities of plant-derived phytochemicals. In general, a single antioxidant assay is not enough to evaluate the total antioxidant capacity of biological samples, since many factors are not taken into consideration. The DPPH (2,2-diphenyl-1-picryl-hydrazine-hydrate) free radical method is the simplest, rapid and can quantify antioxidants in complex biological systems, however; DPPH represents a poor model for radical quenching in vivo and in food samples. Meanwhile, CUPRAC (cupric reducing antioxidant capacity method is also widely applicable antioxidant capacity assay and could express the 'total antioxidant' as a nutritional index and has been found to effectively measure the antioxidant activity of phenolic acids, hydroxycinnamic acids, flavonoids, carotenoids, and anthocyanins. Lastly, FRAP (ferric ion reducing antioxidant power) method can screen a wide range of biological samples including organic extracts of drugs, foods, and plants however, this method is non-specific [12].

This study aims to build on previous findings [10] by employing sequential extraction to further characterize the bioactive phytochemicals. In addition to the DPPH and FRAP methods used in our previous report, the cupric reducing antioxidant capacity (CUPRAC) assay was used in this study to determine the 'total antioxidant' as a nutritional index for food applications. Moreover, in our previous study, the concentration used for evaluation for antimicrobial activity was not defined. To better assess its bioactivity, two extract concentrations of 10 mg/mL and 50 mg/mL were tested against different microbial strains.

EXPERIMENTAL

Chemicals and Materials

Arcangelisia flava stem as raw material, AR grade ethyl acetate, AR grade methanol, distilled water, Folin-Ciocalteu reagent, 7.5 % sodium carbonate, gallic acid, 2,2-diphenyl-1-picryl-hydrazine-hydrate (DPPH), 300 mM acetate buffer (pH3.6), 2,4,6, tripyridyl-s-triazine, HCl, ferric chloride, copper (II) chloride, ammonium acetate buffer (pH 7.0), neocuproine (2,9-dimethyl-1, 10-phenanthroline), ethanol. For antimicrobial activity, different indicators strains from the Philippine National Collection of Microorganisms (PNCM) were used: *Pseudomonas aeruginosa* (PA1335), *Escherichia coli* (EC1634), *Bacillus subtilis* (BS1679) and *Staphylococcus aureus* (SA1582).

Plant Collection and Preparation

Arcangelisia flava was collected from Pagbilao, Quezon, Philippines on March 2021. Following the procedure described in [13], the wood samples were cut, air-dried for several weeks, and ground using Wiley Mill (N02, Arthur Thomas Co. USA). The ground samples were sieved using a mechanical shaker to produce a coarse powder sample with a particle size between 250 and 400 microns.

Sequential Extraction

The ground sample of *A. flava* was defatted with hexane, followed by a 72-hour maceration with ethyl acetate, resulting in the production of AEC extract. The residue was then macerated with methanol to produce AMC extract, and water was used as a solvent to extract more polar compounds, yielding AWC extract. To remove organic solvents from crude extract samples, a rotary evaporator (Buchi Rotavapor R-100) and vacuum glass desiccator were utilized. The water was removed from the extract using freeze-drier (Hinotek, BK-FD12S(-56/-80).

Phytochemical Screening

The resulting extracts of *A. flava* were subjected to the phytochemical screening procedures employed in the previous study [10].

Determination of Total Phenolic Content

Folin-Ciocalteu reagent were used for the analysis of total phenolic content (TPC). About 0.5 mL of the extract was mixed with 0.5 mL of Foli-Ciocalteu reagent. The solution was kept at 25 °C for 5 to before the addition of 2 mL of 7.5 % sodium carbonate solution. After 30 minutes, the absorbance was measured at 725 nm using UV-Vis Spectrophotometer (UV-1700 Shimadzu). The calibration curve ranged from 0.0-1.0 mg/ mL of gallic acid with coefficient of determination (R^2) of 0.998. The TPC was expressed as mg gallic acid equivalents per gram of extract (mgGAE/g extract).

DPPH Radical Scavenging Assay

The procedure was employed following [14] with some modifications. The working solution used was a

methanolic DPPH• solution (98 mg/L). A portion (950 µL) of the working solution was added to the concentrated extracts or standard solutions. The sample was incubated for 30 minutes and absorbance was read at 515 nm against methanol (blank). Trolox solutions were used for calibration and antioxidant activity were expressed as Trolox equivalent antioxidant capacity (TEAC) or n mmol TE/L. The calibration curve ranged from 0.0-1.00 mMol Trolox with coefficient of determination (R^2) of 0.993.

FRAP Assay

The procedure used was based on [15] with some modifications. The stock solutions consisted of 300mM acetate buffer (pH 3.6), 10mM, 2,4,6, tripyridyl-s-triazine (TPTZ) in 40 mM of HCl and 2- mM ferric chloride solution. The fresh working solution was prepared by mixing 25 mL of acetate buffer, 2.5 mL of TPTZ, and 2.5 mL of $FeCl_3$.6H2O. And the temperature was maintained at 37 ºC before use. The various concentrations of extract compound and Trolox (10-50 ug/mL) were allowed to react with 2 mL of FRAP solution for 30 min in the dark condition. The absorbance was recorded at 593 nm. The calibration curve ranged from 0.0-1.00 mMol Trolox with coefficient of determination (R^2) of 0.997.

CUPRAC Assay

The procedure described by [16] was employed. Three solutions were prepared: solution A was prepared by dissolving copper (II) chloride in distilled water in order to prepare a solution containing 0.010 M Cu(II), solution B contained ammonium acetate buffer pH 7.0 which was prepared by dissolving ammonium acetate in distilled water and solution C contained 0.0075 M neocuproine (2,9-dimethyl-1,10-phenanthroline) in ethanol. Prior to the determination, solution A, solution B and solution C were mixed. The reaction mixture was left for 30 minutes in the dark and then the absorption was measured at 450 nm. Standard solutions of Trolox (0.100–0.800 mmol/L) were used for the calibration and the results were expressed as Trolox equivalent antioxidant capacity (TEAC) or n mmol TE/L. The calibration curve ranged from 0.0-1.00 mMol Trolox with a coefficient of determination (R^2) of 0.996.

Disk-Diffusion Method for Antimicrobial Activity

Discs made from filter paper and with a diameter of 6 mm were soaked overnight in DMSO solution of extracts (10 mg/mL) and (50 mg/mL), and then placed on agar plates inoculated with the test microbes. The resulting plates were incubated at 37ºC to allow the growth of microbes. After 24 hours, the plates were removed, and the inhibition zones (mm) were measured and recorded [17].

Well Diffusion Method for Antimicrobial Activity

The procedure used in the previous study [10] were applied to the resulting *A. flava* extracts. Briefly, a stock culture was incubated at 30 °C until the turbidity of 0.5 MacFarland standard was achieved. Then, the turbidity of the test bacterial suspension was compared with that of 0.5 Mac Farland (vigorously shaken before use) against a white background with a contrasting black line under adequate light. The turbidity was reduced by adding a sterile broth. Forty microliters (40 µL) of the culture were introduced to soft agar overlay. Around 6 mm wells were bored into the agar overlays where the ethanolic extracts were introduced. Fifty microliters (50 µL) of ethanolic extracts were introduced and made to diffuse, and the plates were incubated for 18–24 h at 35 ± 2 °C. The diameter of the zones of inhibition was measured and recorded [17].

Statistical Analysis

All experiments were carried out in triplicate and data were expressed as means ± standard deviation (SD). Analysis of variance (ANOVA) was performed to determine the significance among the treatments. Duncan Multiple Range Test (DMRT) was then run to differentiate each treatment. Significant differences (α=0.05) between the activities of the samples was determined by one-way analysis of variance (ANOVA). P value <0.05 was considered as significant.

RESULTS AND DISCUSSION

Extraction Yield and Total Phenolic Content

The collected *A. flava* stems were cut, air-dried, and pulverized with a grinder and sieve shaker. This was done to break down the tissues and cellular components, exposing the phytochemicals to the solvent. In addition, size reduction enhances the surface area, which improves the mass transfer of phytochemicals from the ground sample to the solvent [18]. According to [19] the optimal extraction particle size is between 400 and 595 microns. The particle size employed in the current study is 425 microns, which is comparable to the optimal size reported in the literature.

The effects of ethyl acetate, methanol, and water on the extraction yield and total phenolic content of *A. flava* were investigated. The results shown in Table 1 indicate that *A. flava* ethyl acetate crude extract (AEC) had the lowest extraction yield and is significantly different from the *A. flava* methanolic crude extract (AMC). However, there were no sample replicates for the *A. flava* water crude extract AWC; its value is comparable to that of AMC. A relatively higher yield of methanolic and water extracts implies that the *A. flava* stem sample contained more polar than nonpolar chemical components.

Table 1. Data on extraction yield and total phenolic content of different crude extracts of *A. flava*.

Extract	Extraction Yield, %	Total Phenolic Content, mgGAE/ g extract
AEC	0.47 ± 0.65^{a}	162.30 ± 6.88^{a}
AMC	5.70 ± 0.44^{b}	177.57 ± 23.31^{a}
AWC	5.85*	no data

*no replication was done

Evaluation of total phenolic content, following the method of Folin–Ciocalteu was initially done to assess the general chemical profile of crude extracts. The results showed that AMC had the higher total phenolic content of 177.57 mgGAE/ g extract but this value is not significantly different to that of AEC. This may be due to the presence of phenolic molecules that readily dissolved in methanol. The sequential extraction employed in the current study was similar to the work of [20] in which they extracted the leaves of *A. flava* using hexane, ethyl acetate, and methanol. In their study, the extraction yield as well as the total phenolics yield of the methanolic leaf extract is higher compared to that of ethyl acetate. Further, they have reported that these extracts, particularly methanolic extract have high α-glucosidase inhibition, implying their anti-diabetic property. The leaves and stem of *A. flava* contain different phytochemical constituents, but their similar extraction and phenolic yield trends are noteworthy.

Phytochemical Screening

Crude extracts of *A. flava* were characterized using qualitative phytochemical tests. As shown in Table 2, AEC extract mostly contained saponins, terpenoids, and glycosides. Meanwhile, AMC extract contained saponins, tannins, flavonoids, and alkaloids. Lastly, AWC extract contained the same phytochemicals as AMC except that flavonoid was detected on it. The findings are consistent with our previous report on the phytochemicals of *A. flava* stem ethanolic extract [10]. The occurrences of these phytochemicals were also observed by [21] in the roots of *A. flava* collected in Indonesia.

In the current study, saponins were detected in all *A. flava* extracts, implying that there are different kinds of saponins with different solubilities. According to [22], while saponins are mostly extracted from water and alcohols, there are also saponins that can be extracted by different organic solvents such as chloroform, benzene, ethyl acetate, and glacial acetic acid. As in the case of this study, saponins were also detected in ethyl acetate extract. Saponins are the natural surface active or foaming agent of plants; however, their use has been limited due to their traditional connotation as "antinutritional factors" [23], and in some cases, their bitter taste has prevented their application. Nonetheless, as research advances, saponins, as naturally occurring sugar-conjugated compounds, have been reported to have a wide range of biological activities. Medicinal properties, anti-microbial and antiviral activity are among these activities [24, 25]. Likewise, tannins that were detected in *A. flava* methanolic and water extracts were once reported as "antinutrients" because they can precipitate proteins, inhibit digestive enzymes, and reduce vitamin and mineral utilization [26]. In recent years, tannins have been shown to promote health benefits depending on the composition of the food diet, as tannins have the ability to form complexes with carbohydrates, proteins, and certain mineral ions in foods [27-28].

Table 2. Phytochemical analysis of crude extracts of *A. flava.*

Phytochemicals	*A. flava* extracts		
	AEC	AMC	AWC
Tannins	-	+	+
Saponins (Frothing test)	+	+	+
Flavonoids	-	+	-
Terpenoids (Salkowski test)	+	-	-
Cardiac Glycosides (Keller-Killiani test)	+	-	-
Carbohydrates (Molisch's test)	-	-	-
Alkaloids (Wagner's test)	+	+	+

The only extract that contained flavonoids was *A. flava* methanolic extract. As reported by [29] flavonoids were measured in the methanolic extract, along with hexane and ethyl acetate extract of leaves of *A. flava.* They have reported that methanolic extract had the highest flavonoids content of around 280 Quercetin Equivalent per gram of sample (QE/g) and exhibited the DPPH free radical scavenging ability compared to the hexane and ethyl acetate extracts.

Terpenoids as well as cardiac glycosides were also detected in ethyl acetate extract but not in methanolic and water extract of *A. flava.* Probably because these molecules are smaller and are most likely non-polar compared to those of other phytochemicals. According to the review of [30], one sesquiterpenoid called aduncin and then other furanoditerpenes were identified in the genus of Arcangelisia. Meanwhile, four megastigane glycosides were also identified and are reported in the literature [31]. Terpenoids and cardiac glycosides are both structurally diverse and are widely distributed in nature [32, 33] and have great potential in many industrial applications. However, there have been reports of the safety and efficacy of cardiac glycolsides as drugs, as poisonings and accidental deaths have been observed. In the presence of multiple diseases, therapeutic dosage, and its actual mechanism must be considered [34, 35]. In all *A. flava* extracts in the current study, the occurrence of alkaloids was detected. This is expected since alkaloids are the most dominant phytochemicals that have been identified in the genus of Arcangelisia [30]. In fact, there are a total of 28 alkaloids have been identified throughout the years [36,37,38,39,40,41]. Alkaloids are promising drug candidates, but their toxicity must be thoroughly investigated. In total, different phytochemical compounds were detected in *A. flava* extracts, and it is possible that these chemicals possess a broad spectrum of bioactivities, as reported in other studies of the genus Arcangelisia.

Antioxidant Activity

The antioxidant capacity of crude extracts of *A. flava* using different assays was expressed as Trolox equivalents (mMol TEAC/g). In all antioxidant assays and among the crude extracts, AMC had the highest antioxidant activity. It can be noted that AMC had higher total phenolic content as discussed earlier.

The DPPH assay measures the free radical scavenging ability of antioxidant species either by donating electrons or by transferring hydrogen to DPPH [42, 43]. Using the DPPH assay, the highest activity was observed on AWE at 0.714 ± 0.006 mmol TEAC/g dried extract), followed by AMC and AEC at 0.365 ± 0.007 and 0.079 ± 0.006 TEAC/g dried extract, respectively. The reported antioxidant capacity is based on the capability of phytochemicals in the extracts to either donate electrons or hydrogen.

The results agree with our recent study in which we reported that ethanolic extract of stem of *A. flava* is moderately active as a source of antioxidants with IC50 value 58.62 ug/mL. The same result was also reported by [44]. In addition, another study from Indonesia reported that the methanolic extract of the leaf of *A. flava* has almost 80 % inhibition against DPPH radical which is higher compared to its ethyl acetate extract.

The CUPRAC method relies on the reducing potential of antioxidants, particularly in the reduction of Cu (II) to Cu resulting in color changes from light blue to orange-yellow measured at 450 nm [45,46]. Among the extracts, the AMC showed the highest activity of value of 0.755 ± 0.013 mmol TEAC/g followed by AEC and AWC with relative values of 0.425 ± 0.014 and 0.343 ± 0.004 mmol TEAC/g dried extract, respectively. The bioactivity that caused the reduction of Cu (II) to Cu (I) is either due to the electron transfer or the chelating power of phytochemicals present in these extracts. This is the first time to report the antioxidant capacity of *A. flava* extracts using the CUPRAC method.

The FRAP assay is a common electron transfer method that measures the reduction of ferric ion ($Fe3^{+}$)-ligand complex to the intensely blue ferrous (Fe^{2+}) complex by antioxidants in an acidic medium [47]. In the present study, the AMC had the highest antioxidant capacity of 0.653 ± 0.086 followed by AWC and AEC with values of 0.144 ± 0.014 and 0.107 ± 0.005 mmol TEAC/g dried extract. The anti-oxidant activity of ethanolic *A. flava* extract was also measured using the FRAP method in our previous study and we reported that it has 0.87 ± 0.07 mMol TEAC/g dried extract. The latter value is relatively greater than that of the current study. Possibly because *A. flava* extract underwent sequential extraction and resulted in phytochemicals separation and in consequence, the antioxidant capacity of ethyl acetate, methanolic, and water extracts was reduced in comparison to a single crude ethanolic extract.

Table 3. Antioxidant capacity of *A. flava* extracts using different antioxidant methods.

A. *flava* extract	DPPH mmol TEAC/g	CUPRAC mmol TEAC/g	FRAP mmol TEAC/g
AEC	0.079 ± 0.006^{c}	0.425 ± 0.014^{b}	0.107 ± 0.005^{b}
AMC	0.365 ± 0.007^{b}	0.755 ± 0.013^{a}	0.653 ± 0.086^{a}
AWC	0.714 ± 0.006^{a}	0.343 ± 0.004^{c}	0.144 ± 0.014^{b}

Table 4. Zone of inhibition of crude extracts against different microbial strains using disk diffusion method

	Inhibition zones against indicator strains (mm)			
Sample	*P. aeruginosa*	*E. coli*	*B. subtilis*	*S. aureus*
AEC (10 mg/mL)	0	0	0	0
AEC (50 mg/mL)	0	0	7.0 ± 0.00	7.0 ± 0.00
AMC (10 mg/mL)	0	0	0	7.0 ± 0.00
AMC (50 mg/mL)	0	0	0	10.2 ± 1.0
AWC (10 mg/mL)	0	0	0	0
AWC (50 mg/mL)	0	0	0	0
DMSO (control)	0	0	0	0

Antimicrobial Activity

A. flava extracts were initially screened for antimicrobial activity using the disk diffusion method. This disk diffusion method is straightforward and the official method utilized by numerous clinical microbiology laboratories for routine antimicrobial susceptibility testing [48]. The initial results as shown in Table 4 reveals that most of the extracts had no zone of inhibition against different microbial strains, with no positive control used during the analysis. In spite of that result, it should be noted that AMC at both concentrations of 10 mg/mL and 50 mg/mL had activity against *S. aureus* as well as AEC (50 mg/mL) against *B. subtilis* and *S. aureus.*

The antimicrobial activities of *A. flava* ethyl acetate and methanolic extracts from the disk diffusion method were verified using the well diffusion method to obtain a precise zone of inhibition. The results as shown in Table 5 revealed that AMC possessed antimicrobial activity in both low (10 mg/mL) and high (50 mg/mL) concentrations. A concentration of 10 mg/mL AMC showed bioactivity against the growth of *S. aureus* and the zone of inhibition was increased when a higher concentration of 50 mg/mL was used. Likewise, at same concentrations, the AMC was also active against the growth of *C. tropicalis.* The antimicrobial activities are possible due to the phytochemicals discussed earlier.

In our previous study, we reported that the growth of *S. aureus* and *C. tropicalis* are immediately susceptible to the *A. flava* ethanolic extract with an undetermined concentration. Antimicrobial activity is strongly influenced by the extract's concentration or dosage [49] and in this present study, we report that *A. flava* methanolic extract had notable antimicrobial activity against *S. aureus* and *C. tropicalis* at both 10 mg mL and 50 mg/mL concentration. Meanwhile, [10] reported that using well diffusion, the water extract of at least 2 % of *A. flava*, had antimicrobial activity against *S. aureus* with a zone of inhibition range of 15.08-6.32 mm. The current study confirms that *A. flava* possessed both antibacterial and antifungal properties. It is recommended that the presented results must be further validated using the minimum inhibition concentration, which is the lowest concentration of extract that prevents visible microbial growth [51,52].

CONCLUSION

In conclusion, phytochemicals, antioxidant, and antimicrobial activities of sequential ethyl acetate, methanolic, and water extracts of *A. flava* were evaluated. The *A. flava* methanolic extract had the highest total phenolic content and antioxidant activities as revealed by DPPH, FRAP, and CUPRAC methods. In addition, at a concentration of 50 mg/mL AMC, its antimicrobial activity against *S. aureus* and *C. tropicalis* was the highest among all *A. flava* extracts. To isolate and identify the bioactive substances, additional purification is required.

Table 5. Zone of inhibition of crude extracts against different microbial strains using well diffusion method.

	Inhibition zones against indicator strains (mm)				
Sample	*P. aeruginosa*	*E. coli*	*B. subtilis*	*S. aureus*	*C. tropicalis*
AMC (10 mg/mL)	7.0 ± 0.0	9.67 ± 2.3	0	14.5 ± 1.4	26.83 ± 2.3
AMC (50 mg/mL)	9.33 ± 2.1	9.5 ± 2.3	14.33 ± 1.0	22.8 ± 2.6	30 ± 1.8
AEC (50 mg/mL)	10.83 ± 1.2	7 ± 0.0	13.5 ± 5.2	7.0 ± 0.0	19.7 ± 0.8

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