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Antibacterial Activity of *Acacia* spp. Leaves Extracts against *Xanthomonas oryzae* pv. *oryzae* and Screening for Active Phytochemical Contents

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Abstract: *The in vitro study revealed the ethyl acetate and methanol leaf extracts from both Acacia species had shown inhibition against Xanthomonas oryzae* pv. *oryzae (Xoo). Acacia auriculiformis ethyl acetate (AAEA) and A. mangium methanol (AMMH) leaf extract at concentration 200 mg/mL showed the largest diameters of inhibition zone (DIZ) produced that were 33.33 and 25.78 mm compared to other concentrations used. Bacteriostatic study showed that the minimum concentration required by AAEA to inhibit Xoo was 3.13 mg/mL while 1.56 mg/mL was required by AMMH. Bactericidal activity showed that the minimum concentrations required to kill Xoo were 6.25 and 12.5 mg/mL, respectively for AAEA and AMMH. The minimum inhibitory concentration (MIC) index suggests that AAEA leaf extract possesses bactericidal effect while AMMH leaf extract possesses bacteriostatic effect on Xoo. Ultrastructural studies on the effect of AAEA and AMMH leaf extract on Xoo cells revealed that both Acacia leaf extracts altered the normal cell of Xoo by causing lysis, loss of rigidity, malformation, and death. The study on active chemical contents for both Acacia leaf extracts by GC-MS revealed the presence of mostly terpenes esters, alcohols and other volatile organic compounds.*

Keywords: *Acacia auriculiformis, Acacia mangium, antibacterial, GC-MS, Xanthomonas oryzae pv. Oryzae*

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I. Introduction

Acacia species are robust pioneer plants and consisted of 1250 species which are mainly shrubs and trees. Approximately, 700 of *Acacia* species are identified as an indigenous species in Australia, 170 species in Africa and 18 other species are widespread while the remaining are more localized [1]. In Malaysia, two species of *Acacia* namely *Acacia auriculiformis* and *A. mangium* are recorded and introduced by Sabah Softwoods Berhad for its pulps and timbers to substitute the *Pinus caribaea* for pulp industry. However, both species are now found to grow wildly by the roadside and idle lands. *Acacia* has been reported rich with valuable antimicrobial properties against a wide range of pathogens, especially towards clinical pathogens. Various parts of *Acacia* were extracted using various solvents with different polarities and tested against various pathogens like fungi, bacteria, nematodes, and viruses.

Acacia is rich with phytochemicals contents like cardiac glycosides, cylitols, amines, alkaloids, fatty acids, terpenes, flavonoids, saponins, hydrolysable and condensed tannins, non-protein amino acids, fluoroacetate, and phytosterols [2]. On the other hand, certain phytochemicals are found less in mimosoid legumes and the compounds are glucosinolates, naphtoquinones, coumarins, anthraquinones, liginans, acetylenes, stilbenes, and atypical fatty acids. *Acacia auriculiformis* was demonstrated to have galactose, methylglucuronic acid, arabinose, rhamnose, and glucuronic acids. These compounds have been reported to possess CNS activities such as spermicidal and filaricidal activities due to the presence of triterpenoid, saponins, and tannins [3]. The pharmacological properties of *A. auriculiformis* have been investigated and proven to possess antifilarial, antihelmintic, and microbicidal activities [4]. Meanwhile, *A. mangium* has been reported to have flavonoid compounds such as 3,4',7,8-tetrahydroxyflavanone and 4',7,8-trihydroxyfavanone, and that these compounds exhibited antifungal activities against wood rotting fungi [5]. However, the antimicrobial properties of both species against rice pathogens have never been discovered. The present study was conducted to further explore the new potential of *Acacia* to be employed in plant disease control program.

Bacterial leaf blight caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is the most important and destructive bacterial disease of rice worldwide. It causes severe damage to rice production up to 50-70% during

serious infection. Although various disease controls have been introduced, no effective disease control could be claimed as a sole solution for controlling this pathogen until now. Nevertheless, synthetic chemicals and antibiotics are the most popular controlling measures applied on the field to overcome the disease infection. Many reports have shown that environment and human health fare badly and these are partly due to the use of synthetic chemicals. In addition, their use has been restricted due to their carcinogenicity, teratogenicity, high and acute residual toxicity, long degradation period, environmental pollution, and possible side effects on human health through food chain [6][7][8]. Therefore, the aims of this study were to conduct a phytochemical profiling and determine the antibacterial potency of *Acacia* species using leaf extracts against *Xoo*.

II. Materials And Methods

2.1 Pure Culture

Pure culture *Xoo* used in this study was obtained from the bacteriology laboratory, Department of Plant Protection, Universiti Putra Malaysia (UPM), Malaysia. This culture was previously isolated from the infected rice with typical symptoms of bacterial leaf blight disease at Kuala Selangor, Malaysia.

2.2 Extraction of *Acacia* leaves

The leaves of *A. auriculiformis* and *A. mangium* were collected from the UPM Equine Centre. The fresh leaves were cut into small pieces using a pair of scissors. The leaves were then air dried in a drying oven at 35°C for 14 days. The dried leaves were grounded to a fine powder using the grinding mill equipped with a sieve size of 0.20 mm. The leaf powder was kept in air tight container for storage until further use.

The leaf powder was extracted with n-hexane, chloroform, ethyl acetate, and methanol. The extraction was performed in the increasing order of solvent polarity. The sequence of extraction is shown in Figure 1 [9]. Each extract was filtered through Whatman No. 1 filter paper. The filtrates were concentrated at 40°C and 180 rpm using rotary evaporator.

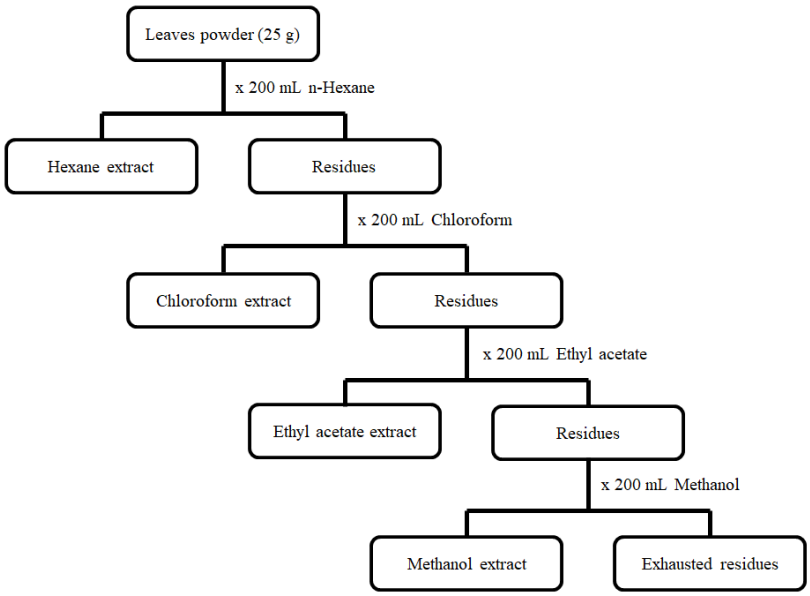

Figure 1. Flowchart of the Extraction Procedures for *Acacia* Leaves

The yield percentage (%) of the crude extracts after rotary evaporation was determined based on the formula: [(weight of final extract/weight of powdered sample) x 100] [10]. The organoleptic properties of each crude extract were observed and recorded for comparison. An appropriate amount of solvents were poured into the flask to dissolve the extracts. The stock concentration was prepared at 400 mg/mL for each extract and kept in separate clean screw cap bottles, labelled, and stored in the refrigerator for further use.

2.3 Antibacterial Activity of *Acacia* Leaf Extracts against *Xanthomonas oryzae* pv. *oryzae*

Xanthomonas oryzae pv. *oryzae* (*Xoo*) isolate was maintained on peptone sucrose agar (PSA) and incubated at 30°C for 48 hours [11]. A stock solution of the extracts prepared earlier was diluted with respective solvents using the two-fold dilution techniques. Concentrations of 200, 100, 50, and 25 mg/mL were prepared and kept in separate clean label screw cap bottles.

The two-day old *Xoo* culture was harvested from the PSA using a glass rod in 10 mL sterile distilled water (SDW) and vortexed. The optical density (O.D) value was adjusted using microplate spectrophotometer to

0.1 $O.D_{600}$ as standard *Xoo* suspensions. About 35 µL of *Xoo* suspensions were pipetted into 0.7% soft agar (SA) at 40°C [12], overlaid onto solidified Mueller-Hinton agar (MHA) prepared earlier, and left to solidify for 15 minutes. Six wells with a diameter of 6 mm were punched through a cork borer to remove the agar. A 50 µL of different extract concentration was filled in each well. The positive control (streptomycin sulfate at 0.2 mg/mL concentration) and negative control (respective solvents) were filled into the respective wells. The plates were left inside the laminar flow for 30 minutes before incubation at 30°C for two days.

Results were expressed as a diameter of inhibition zone (DIZ) produced around the treatment and control wells. Diameter of inhibition zones were measured in millimeter (mm) using a ruler. Each experiment was run three times with three replications. The mean of DIZ was statistically evaluated using one-way ANOVA as well as Tukey's Honestly Significant Difference post hoc test using SAS software. The mean of DIZ was compared between controls and the same concentration for both *Acacia* leaves extract.

2.4 Minimum Inhibitory and Bactericidal Concentration Determination

The minimum inhibitory concentration (MIC) was conducted based on the lowest concentration from the previous antibacterial activity study that was able to show inhibition against *Xoo*. The MIC was performed in 96-wells flat-bottom microtiter plate using 2-fold microdilutions technique. A 35 µL of the standardized *Xoo* suspension was pipetted into all the wells containing test solutions. The negative control (MHB + diluted *Acacia* leaf extracts + *Xoo* suspension) and positive control (MHB + *Xoo* suspension) well was prepared at the end of the microtiter plate. The plate was incubated at 30°C for two days inside the incubator. After the incubation period, a 50 µL of sterile 1.5% 2,3,5-tetrazolium chloride (TTC) solution was pipetted into all the wells and was incubated for another four hours. The wells with no color change were taken and recorded as the MIC value. The minimum bactericidal concentration (MBC) was performed by taking out 10 µL of the well contents with no color changes and was sub-cultured onto a fresh MHA plate using the drop plate method for MBC assay [13]. The plate was incubated for two days at 30°C. The content of the wells no bacterial growth on an MHA plate was taken as the MBC value. Based on MIC and MBC results, the MIC index was calculated using the formula; MBC/MIC [14].

2.5 Determination on Active Chemical Contents of *Acacia* Leaf Extracts by GC-MS

Both *Acacia* leaf extracts showing potent antibacterial were subjected for GC-MS to determine the active chemical constituents. *Acacia* leaf extracts was prepared at a concentration of 200 mg/mL and filtered through syringe filter 0.45 µm. The filtrates were analyzed using Shimadzu GCMS-QP2010 Ultra.

III. Results

3.1 Yield and organoleptic properties of *Acacia* leaf extracts

In this study, *A. auriculiformis* and *A. mangium* leaf extracts were yielded with different concentrations. The highest yield (%) of the crude extracts of *A. auriculiformis* and *A. mangium* were methanol with 7.76% and 9.12%, respectively. This was then followed by chloroform, n-hexane and ethyl acetate (Table 1). These results indicated that *Acacia* leaves of both species yielded higher polar compounds compared to the non-polar and semi-polar compounds.

Table 1. Yield (%) of the crude extracts of *Acacia auriculiformis* and *Acacia mangium* leaves using different types of solvents

Species	n-Hexane	Chloroform	Ethyl acetate	Methanol
Acacia auriculiformis	1.44%	1.92%	1.04%	7.76%
Acacia mangium	1.24%	1.92%	1.08%	9.12%

Many reports revealed that polar compounds are much easier to be extracted from plant parts compared to the non-polar and semi-polar compounds. The results obtained from the present study showed the similar result previously reported before [15][16]. Plants comprised of large amounts of polar compounds [17], hence explained the high yield (%) obtained from *A. auriculiformis* and *A. mangium* using methanol as a solvent. Polar solvent acted by opening the plant cell wall then allowed the solvents to penetrate and extract the cell components thoroughly [18].

Findings from this study showed that both of the non-polar and semi-polar solvents yielded at low concentration (1.04-1.92%). This indicated that *A. auriculiformis* and *A. mangium* leaves contain less amount of non-polar and semi-polar compounds. This might also be due to the degree of polarity of the solvent itself. For example, the polarity of chloroform is higher than n-hexane. Therefore, chloroform could be more effective in extracting the compounds than n-hexane. A study confirmed that chloroform is the best solvent for plant lipids due to its high polarity properties [19]. Previous reports showed that ethyl acetate is an intermediate solvent that brought out the miscibility of the non-polar and polar compounds [20][21]. The type of solvents used for

extraction does not only affect the yield (%) of the extracts but also modifies the organoleptic properties (Table 2). The color of the crude extracts with chloroform, n-hexane, and ethyl acetate is dark green. Meanwhile, methanol leaf extract is dark brown in color. According to the previous studies, the dark green colors produced by the leaf extracts were associated with the color of chlorophyll. Meanwhile, dark brown color is associated with the presence of betacyanins which are reddish to violet betalain pigments [22][23][24].

Table 2. Organoleptic properties of *Acacia* spp. leaves extracts using various types of solvents

Species	Properties	n-Hexane	Chloroform	Ethyl acetate	Methanol
Acacia auriculiformis	Color	Dark green	Dark green	Dark green	Dark brown
	Odor	Pleasant	Pleasant	Unpleasant	Pleasant
	Consistency	Dry	Dry	Sticky	Sticky
Acacia mangium	Color	Dark green	Dark green	Dark green	Dark brown
	Odor	Pleasant	Pleasant	Unpleasant	Pleasant
	Consistency	Dry	Dry	Sticky	Sticky

All crude extracts possessed pleasant odor except for ethyl acetate extract. Ethyl acetate extracts produced vinegar smell, while other extracts such as n-hexane, chloroform, and methanol gave leafy like odor. The consistency of the extracts produced using non-polar, semi-polar, and polar solvents, was different from each other. Hexane and chloroform extracts were in a solid state while ethyl acetate while methanol was in a semi-solid state. It is important to note that solvent polarities play important roles in determining the types of plant compounds with different properties; hence may influent the quality and properties of the extracts obtained [25].

3.2 Antibacterial Activity of *Acacia* Leaf Extracts

DIZ zone produced by both *Acacia* extracts with ethyl acetate and methanol increased in ascending order and was parallel with the extract concentration as shown in Table 3. The *A. auriculiformis* ethyl acetate (AAEA) leaf extracts demonstrated the highest DIZ of 9.89, 18.898, 28.89, and 33.33 mm at concentrations of 25, 50, 100, and 200 mg/mL, respectively compared to *A. auriculiformis* methanol leaf extracts. The *A. auriculiformis* methanol (AAMH) leaf extracts only started to demonstrate inhibition against *Xoo* at 50 mg/mL concentration of. There was no DIZ recorded at 25 mg/mL concentration by AAMH. Hence, this result indicated that AAEA was more effective compared to AAMH since AAEA was able to demonstrate inhibition towards *Xoo* at the lowest 25 mg/mL concentration.

Table 3. Diameter of inhibition zone of *Acacia* spp. leaves extracts against *X. oryzae* pv. *oryzae*

Concentrations (mg/mL)	Controls		*Acacia auriculiformis*		*Acacia mangium*	
	Positive	Negative	Ethyl acetate	Methanol	Ethyl acetate	Methanol
25	18.33±1.15^{a}	0.00±0.00^{d}	9.89±0.19^{c}	0.00±0.00^{d}	15.22±0.69^{b}	17.89±0.96^{a}
50	18.33±1.15^{ab}	0.00±0.00^{d}	16.78±0.84^{b}	5.56±2.70^{c}	18.22±0.19^{ab}	20.22±0.69^{a}
100	18.33±1.15^{c}	0.00±0.00^{e}	28.89±0.19^{a}	15.55±0.69^{d}	19.89±0.19^{c}	23.44±1.02^{b}
200	18.33±1.15^{c}	0.00±0.00^{d}	33.33±0.88^{a}	18.56±0.51^{c}	20.56±0.39^{c}	25.78±1.65^{b}

Note: Positive control (streptomycin sulfate, 0.2 mg/mL) and negative control (ethyl acetate and methanol). Means were compared among controls and *Acacia* spp. ethyl acetate and methanol leaf extracts for each concentrations with N = 3 and Pr>F = <0.0001. Means with the same letter represented no significant differences among treatments.

Different effects were recorded for *A. mangium* leaf extracts. The results showed that the methanol extract (AMMH) demonstrated the better size of DIZ (17.89, 20.22, 23.44, and 25.78 mm) compared to ethyl acetate extracts (AMEA) at various concentrations. AMEA recorded DIZ values of 15.22, 18.22, 19.89, and 20.56 mm at 25, 50, 100, and 200 mg/mL concentrations. The size of DIZ produced by AMEA was slightly lower.

The antibacterial activities of different solvents revealed that the non-polar solvents such as n-hexane and chloroform failed to inhibit *Xoo* at tested concentrations even at the highest 200 mg/mL concentration. The increase in extract concentrations produced larger inhibition zone represented by clear halo zone that formed around the wells as shown in Figure 2. A similar effect was observed for both *Acacia* leaves extracted with the same types of solvents.

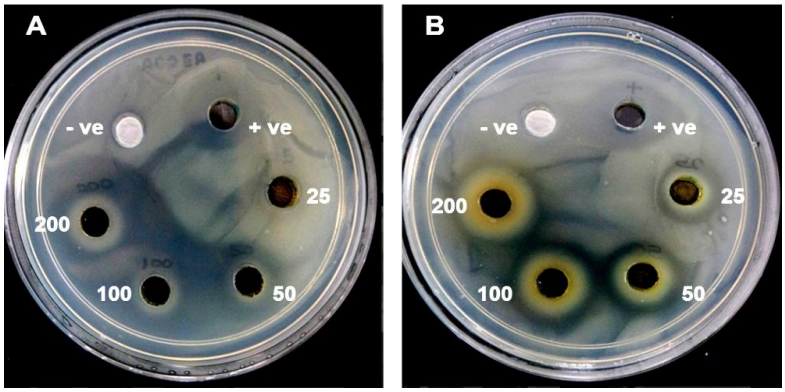

Figure 2. Antibacterial activity of *A. auriculiformis* ethyl acetate (A) and *A. mangium* methanol leaf extract (B) against *X. oryzae* pv. *oryzae*

3.3 Determination of MIC and MBC of *Acacia* Leaf Etxracts

MIC and MBC assessments demonstrated that the lowest concentration of AAEA was required to control the growth and completely kill the *Xoo* cells of 3.13 and 6.25 mg/mL, respectively. Meanwhile, the AMMH leaf extracts demonstrated MIC and MBC values of 1.56 and 12.5 mg/mL, respectively. The MIC index 2 and 8 were recorded for AAEA and AMMH, respectively (Table 4).

Table 4. MIC, MBC and MIC index of *Acacia* spp. leaves extracts against *X. oryzae* pv. *oryzae*

Extracts	Concentrations (mg/mL)		Index
	MIC	MBC	MIC
AAEA	3.13	6.25	2
AMMH	1.56	12.5	8

Note: AAEA = *A. auriculiformis* ethyl acetate leaf extract and AMMH = *A. mangium* methanol leaf extract.

The MIC index obtained suggested that AAEA and AMMH possessed bactericidal and bacteriostatic effects against *Xoo*. In the micro dilution technique, the inhibition of *Xoo* was determined by the colour appearance of the well contents. It was due to the colour changes produced by TTC solution. The well with none viable bacterial cells demonstrated no colour change while the wells containing viable cells produced red colour formazan (Figure 3).

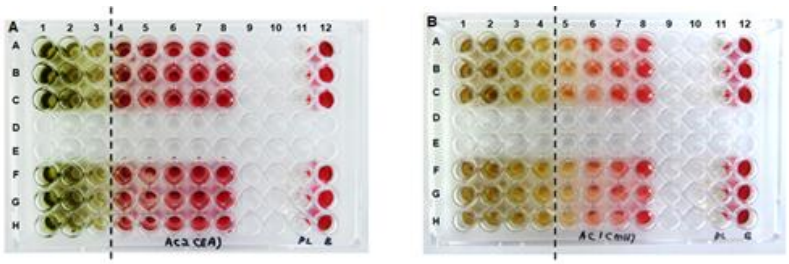

Figure 3. Minimum inhibitory concentration of *Acacia* spp. leaves extracts against *X. oryzae* pv. *oryzae*

The MIC index was assessed in order to determine whether the extracts at particular concentrations were bacteriostatic or bactericidal. Based on the data presented in Table 4, both Acacia extracts showed MIC index of 2 and 8. This value indicated that the MBC value was two and eight times higher than the MIC value. Hence, these results revealed that AAEA and AMMH leaf extracts possess bactericidal and bacteriostatic effects toward *Xoo*. The evaluation of MIC index was based on the findings of [26] and [27] who showed that when the MBC/MIC value was ≤4, the extract possessed bactericidal effect while when the MBC/MIC value was >4, the extract possessed bacteriostatic effect against the tested pathogen.

Determination of Active Organic Compounds in *Acacia* Leaf Extracts by GC-MS

GC-MS analysis of active chemical contents of AAEA and AMMH revealed the presence of different amount of plant compounds in both Acacia extracts (Table 5). The AAEA leaf extracts possessed more compounds than the AMMH leaf extracts. This might be due to the polarity characteristics of each *Acacia* leaf extracts that take place during the extraction process. Methanol was reported to extract only polar based compounds thus yielding limited plant compared to ethyl acetate. Both AAEA and AMMH leaf extracts

indicated that the presence of some numerous active compounds was identified and reported to be present in other plant extracts. The combinations of these chemical compounds might contribute to the effective antibacterial properties of both leaf extracts against *Xoo*.

Table 5. Active chemical contents of *Acacia* spp. leaves extracts by GC-MS

Extracts	Compounds	Biological Activities	References
AAEA	Propanoic acid, ethyl ester	Antimicrobial; antifungal	[28] [29]
	2,3-Butanediol	Induce plant defenses; growth promotion, salt tolerance, drought tolerance, enhance disease resistance, induced systemic resistance	[30] [31]
	1,2-Ethanediol, monoacetate	Stimulant, antiseptic, carminative, dysentery, digestive	[32]
	1-Acetoxy-2-propanol	Antimicrobial	[33]
	1,2-Ethanediol, diacetate	Antimicrobial; antibacterial	[28] [34]
	Benzyl alcohol	Antimicrobial and free radical scavenging	[35]
	1,2,3-Propanetriol, 1-acetate	Antibacterial; antimicrobial	[36] [37]
	1-Tridecene	Antibacterial, free radical scavenging; antimicrobial	[38] [39]
	Ketone, methyl 2-methyl-1,3-oxothiolan-2-yl	Antifungal	[40]
	Resorcinol	Antimicrobial; antioxidant, antibacterial, anticancer	[41] [42]
	n-Pentadecanol	Antibacterial; antibiofilm	[43] [44]
	Phenol, 2,4-bis(1,1-dimethylethyl)-	Inhibit quorum sensing; antifungal	[45] [46]
	3-Buten-2-one, 4-(4-hydroxy-2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)-	Antimicrobial, anticancer, antioxidant; antibacterial	[47] [48]
	Pentadecanal-	Antimicrobial; antibacterial, antioxidant	[49] [50]
	Octacosanol	Antimicrobial; anti-inflammation	[51] [52]
	2-Cyclohexen-1-one, 4-hydroxy-3,5,5-trimethyl-4-(3-oxo-1-butenyl)-	Antifungal; antibiofilm	[53] [54]
	Neophytadiene	Antimicrobial; antibacterial	[55] [56]
	Palmitic acid	Antibacterial; antioxidant, anticholinesterase, antimicrobial	[57] [58]
	Phytol isomer	Antibacterial; trematocidal	[59] [60]
	E,E,Z-1,3,12-Nonadecatriene-5,14-diol	Antifungal; antibacterial	[61] [62]
	Phthalic acid, di(6-methylhept-2-yl) ester	Antioxidant	[63]
	Supraene	Antioxidant, antibacterial, antitumor, pesticide, immunostimulent	[64]
	Hexacontane	Not found	-
	Solanesol	Anti-inflammation, anti-ulcer, antioxidant, antibacterial	[65]
	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-	Antibacterial; antioxidant, anti-inflammatory	[66] [67]
	Triacontyl acetate		
	.gamma.-Tocopherol	Antioxidant, antibacterial; anticancer, anti-inflammatory, cardioprotective	[68] [69]
	Farnesyl bromide	Anti-malarial; antimicrobial	[70] [71]
	Vitamin E	Antioxidant, antimicrobial; anti-inflammatory	[72] [73]
	Stigmasta-7,16-dien-3-ol, (3.beta.,5.alpha.)-	Insecticidal	[74]
	.beta.-Amyrin	Antioxidant, antibacterial; antimicrobial, antimutagenicity	[75] [76]
	Lup-20(29)-en-3-one	Therapeutic agent for hypopigmentation; antifungal	[77] [78]
	Methyl commate b	Antimicrobial, anti-inflammatory; antifungal	[79] [80]
	dl-.alpha.-Tocopherol	Antioxidant; radical scavenger, anti-tumor	[81] [82]

AMMH	1,2,3-Propanetriol, 1-acetate	Antibacterial; antimicrobial	[36] [37]
	1-Butanol, 3-methyl-, acetate	Antibacterial, antioxidant; antifungal	[83] [84]
	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	Antimicrobial; antioxidant	[85] [86]
	Undeca-1,3,5-triene	Antimicrobial, cytotoxic; antioxidant	[87] [88]
	Resorcinol	Antimicrobial; antioxidant, antibacterial, anticancer	[41] [42]
	Ethanol, 2-(pentyloxy)-, acetate	Not found	-
	Guaiacol <4-vinyl->	Biocidal activity, macro-biofoulants; antimicrobial, antioxidant	[89] [90]
	Megastigmatrienone	Aroma; cytotoxic	[91] [92]
	.Beta.-asarone	Anti MRSA, antibacterial; genotoxicity, mutagenicity	[93] [94]
	Hexadecanoic acid, methyl ester	Antibacterial, antifungal, antioxidant	[95]
	Palmitic acid	Antibacterial; antioxidant, anticholinesterase, antimicrobial	[57] [58]
	Linolenate <methyl->	Antimicrobial; antioxidant	[96] [97]
	Benzyl .beta.-d-glucoside	Not found	[98]
	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl	Not found	[99]
	Phthalic acid, di(2-propylpentyl) ester	Antimicrobial	[100]
	dl-.alpha.-Tocopherol	Antioxidant; radical scavenger, anti-tumor	[81] [82]
	Methyl commate b	Antimicrobial, anti-inflammatory; antifungal	[79] [80

Note: AAEA = *A. auriculiformis* ethyl acetate leaf extract; AMMH = *A. mangium* methanol leaf extract

IV. DISCUSSION

The study of antibacterial activities confirmed the potential of both AAEA and AMMH against *Xoo*. These findings are in accordance with previous studies conducted on antibacterial activities extracted from different plant parts and species of *Acacia* against various species of *Xanthomonas*. The aqueous extracts of bark and seed of *A. arabicae*, *A. catechu*, and *A. fernesiana* inhibited *X. campestris* pv. *campestris* [101], while water, ethyl alcohol, and ethyl acetate extracts of leaf, bark, and root of *A. nilotica* inhibited *X. malvacearum* [102], while the aqueous, methanol and ethanol extracts of *A. nilotica* inhibited *X. axonopodis* pv. *punicae* [103]. Other *Acacia* species like *A. mellifera*, *A. arabica*, *A. senegal*, *A. nilotica*, *A. aroma*, *A. ataxacantha*, and *A. mearnsii* were reported to be effective against clinical and multidrug resistant pathogenic bacteria. The antibacterial properties of *Acacia* plants have been related to antimicrobial compounds such as glycosides, tannins, phenolic compounds, terpenoids, alkaloids, and flavonoids [104][105][106][107][108][109].

Interestingly, the MIC value of AAEA recorded two-fold higher than AMMH. However, the MIC value of AMMH obtained in this study was in agreement with MIC values obtained from other plant extracts and essential oils against *Xanthomonas* spp. For instance, the MIC value of *Rosa damascene* to be effective against *X. axonopodis* pv. *vesicatoria* (XV88-5, XV56, and XV97-2) was 1.5 mg/mL [110]. Meanwhile, the fruit rinds and leaves of *Thevetia peruviana* acquired concentration ranging from 0.25 to 1.00 mg/mL and 0.5 to 1.25 mg/mL against bacterial pathogens as their MIC and MBC values [111]. The Gram-negative bacteria like *Xanthomonas* usually require higher MIC and MBC values. This was proven when there was a report showed that the MIC and MBC values of 87.5 mg/mL of plant extracts of *Achyranthes aspera*, *Datura stramonium*, and *Vernonia amygdalina* were required against *X. campestris* pv. *musacearum* [112]. In the same study, they demonstrated that the *Agarista salicifolia* and *Pycnostachys abyssinica* were the most potent plant extracts, which require the lowest MIC and MBC values (10 mg/mL). Some microorganisms need a high concentration of plant extracts to be killed or inhibited due to the cell wall components of the particular microbes [113]. Specific concentrations of plant extracts were needed in order to ensure that the mechanisms of action would take place such as disturbing DNA replication or impairing cellular interaction and membrane integrity through cellular leakage of nucleotides and proteinaceous materials [27].

The MIC and MBC assays were proven to be a very important analysis in providing information regarding the bacteriostatic and bactericidal effects of leaf extracts. Both assays were based on the general visualization of antibacterial agent reaction against the tested pathogen. More importantly, this technique does not require high expenditure yet provides reproducible results and needs a small quantity of plant extracts [27]. These conditions have been correlated to the ability of the viable cells in reducing the 2,3,5-triphenyltetrazolium chloride (TTC) to form red formazan. The concentration of red formazan is proportioned to the viable active cells of the microbes [114].The combination of various plant compounds detected by GC-MS suggested that

these compounds possessed synergistic effects against *Xoo* pathogen. The plant crude extracts offered better *in-vitro* and *in-vivo* biological activities compared to pure compounds isolated from the crude extracts at an equivalent dose. The mixture of various compounds in plant extracts possessed multi-factorial effects and synergistic interaction that assured dynamic biological activities of crude extracts [115].

V. Conclusion

This study confirmed that the ethyl acetate and methanol leaf extracts of *A. auriculiformis* and *A. mangium* possess antibacterial potency against *X. oryzae* pv. *oryzae*. The size of inhibition zones produced by plant extracts is proportioned with the concentration of the extracts used. The MIC and MBC assays showed that these extracts possessed bacteriostatic and bactericidal effects against *Xoo*. Phytochemical determination by GC-MS led to the identification of various compounds which possess multiple biological activities against *Xoo*. The GC-MS analysis revealed the presence of terpenes, esters, alcohols and other volatile organic compounds.

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References

[1]. B. Hayward, Section 1 Background, in B. Hayward (Ed.), *The acacia tree: a sustainable resource for Africa*, (Penzance: Rowes the Printers, 2004) 1.
[2]. D.S. Seigler, Phytochemistry of *Acacia* – sensu lato, *Biochemical Systematics and Ecology, 31(8),* 2003, 845-873.
[3]. S. Garai, and S.B. Mahato, Isolation and structure elucidation of three triterpenoid saponins from *Acacia auriculiformis*, *Phytochemistry, 44(1),* 1997, 137-140.
[4]. S. Chaki, B. Ghosh, S. Bandyopadyhay, M. Mookerjee, S. Das, and S.G. Dastidar, Detection of various phytochemical compounds from seeds of *A. auriculiformis* for possibilities of obtaining potent antimicrobial agents, *International Journal of Biology and Pharmaceutical Research, 6(2),* 2015, 120-128.
[5]. R. Mihara, K.M. Barry, C.L. Mohammed, and T. Mitsunaga, Comparison of antifungal and antioxidant activities of *Acacia mangium* and *A. auriculiformis* heartwood extracts, *Journal of Chemical Ecology, 31(4),* 2005, 789-804.
[6]. A.Y. Guan, C.L. Liu, M. Li, H. Zhang, Z.N. Li, and Z.M. Li, Design, synthesis and structure-activity relationship of novel coumarin derivatives, *Pest Management Science, 67(6),* 2011, 647-655.
[7]. D.N. Enyiukwu, A.N. Awurum, and J.A. Nwaneri, Efficacy of plant-derived pesticides in the control of myco-induced postharvest rots of tubers and agricultural products: A review, *Net Journal of Agricultural Science, 2(1),* 2014, 30-46.
[8]. P. Tripathi, and N.K. Dubey, Exploitation of natural products as an alternative strategy to control postharvest fungal rotting of fruit and vegetables, *Postharvest Biology and Technology, 32(3),* 2004, 235-245.
[9]. G. Brusotti, I. Cesari, A. Dentamaro, G. Caccialanza, and G. Massolini, Isolation and characterization of bioactive compounds from plant resources: The role of analysis in the ethnopharmacological approach, *Journal of Pharmaceutical and Biomedical Analysis, 87(18),* 2014, 218-228.
[10]. S. Janjua, M. Shahid, and F. Abbas, Phytochemical analysis and *in vivo* antibacterial activity of root peel extract of *Raphanus sativa* L. var niger, *Advancement in Medicinal Plant Research, 1(1),* 2013, 1-7.
[11]. T. Berić, M. Kojić, S. Stanković, L. Topisirović, G. Degrassi, M. Myers, V. Venturi, and D. Fira, Antimicrobial activity of *Bacillus* sp. natural isolates and their potential use in the biocontrol of phytopathogenic bacteria. *Food Technology and Biotechnology, 50(1),* 2012, 25-31.
[12]. W. Wangkangwan, S. Boonkerd, W. Chavasiri, K. Sukapirom, K. Pattanapanyasat, N. Kongkathip, T. Miyakawa, and C. Yompakdee, Pinostrobin from *Boesenbergia pandurata* is an inhibitor of Ca^{2+}- signal-mediated cell-cycle regulation in the yeast *Saccharomyces cerevisiae*, *Bioscience, Biotechnology, and Biochemistry, 73(7),* 2009, 1679-1682.
[13]. J.M. Andrews, Determination of minimum inhibitory concentrations, *The Journal of Antimicrobial Chemotheraphy, Suppl 1,* 2001, 5-16.
[14]. P. Akbary, *In vitro* inhibitory activity of the leaf methanol extract of green tea (*Camellia sinensis*) against *Lactococcus garvieae* and *Aeromonas hydrophila* isolated of rainbow trout (*Oncorhynchus mykiss*), *Advances in Microbiology, 4,* 2014, 829-834.
[15]. M.F.A. Razak, P.K. Yong, Z.M. Shah, L.C. Abdullah, S.S. Yee, and I.T.C.S. Yaw, The effects of varying solvent polarity on extraction yield of *Orthosiphon stamineus* leaves. *Journal of Applied Sciences, 12(11),* 2012, 1207-1210.
[16]. L. Tomsone, Z. Kruma, and R. Galoburda, Comparison of different solvents and extraction methods for isolation of phenolic compounds from horseradish roots (*Armoracia rusticana*), *International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnological Engineering, 6(4),* 2012, 236-241.
[17]. P.S. Widyawati, T.D.W. Budianta, F.A. Kusuma, and E.L. Wijaya, Difference of solvent polarity to phytochemical content and antioxidant activity of *Pluchea indica* Less leaves extracts, *International Journal of Pharmacognosy and Phytochemical Research, 6(4),* 2014, 850-855.
[18]. R. Tir, P.C. Dutta, and A.Y. Badjah-Hadj-Ahmed, Effect of the extraction solvent polarity on the sesame seeds oil composition, *European Journal of Lipid Science and Technology, 114(12),* 2012,1427-1438.
[19]. E.M. Grima, M.J.I. González, and A.G. Giménez, Solvent extractions for microalgae lipids, in M. Borowitzka, and N. Moheimani (Eds.), *Algae for Biofuels and Energy*, 5 (Dordrecht: Springer, 2012) 187-205.
[20]. B.I. Okeleye, P.O. Bessong, and R.N. Ndip, Preliminary phytochemical screening and *in vitro* anti-*Helicobacter pylori* activity of extracts of the stem bark of *Bridelia micrantha* (Hochst., Baill., Euphorbiaceae), *Molecules, 16(12),* 2011, 6193-6205.
[21]. S. Visht, and S. Chaturvedi, Isolation of natural products, *Current Pharma Research, 2(3),* 2012, 584-599.
[22]. T. Nakashima, T. Araki, and O. Ueno, Photoprotective function of betacyanin in leaves of *Amaranthus cruentus* L. under water stress, *Photosynthetica, 49(4),* 2011, 497-506.

[23]. P. Sasongko, N. Laohankunjit, and O. Kerdhoechuen, Evaluation of physicochemical properties of plant extracts from *Persicaria odorata*, *Agricultural Science Journal, 42(2),* 2011, 333-336.

[24]. R. Abarca-Vargas, C.F..P. Malacara, and V.L. Petricevich, Characterization of chemical compounds with antioxidant and cytotoxic activities in *Bougainvillea x buttiana* Holttum and Standl, (var. Rose) extracts, *Antioxidants, 5(4),* 2016, 45.

[25]. S.A. Emmanuel, O.O. Olajide, S. Abubakar, I.D. Idowu, A.T. Orishadipe, and S.A. Thomas, Phytochemical and antimicrobial studies of methanol, ethyl acetate, and aqueous extracts of *Moringa oleifera* seeds, *American Journal of Ethnomedicine, 1(5),* 2014, 346-354.

[26]. G.A. Pankey, and L.D. Sabath, Clinical relevance of bacteriostatic versus bactericidal mechanisms of action in the treatment of Gram-positive bacterial infections, *Clinical Infectious Diseases, 38(6),* 2004, 864-870.

[27]. L. Fernandez, K. Daruliza, S. Sudhakaran, and R. Jegathambigai, Antimicrobial activity of the crude extract of *Piper sarmentosum* against methicilin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli*, *Vibrio cholera* and *Streptococcus pneumoniae*, *European Review for Medical and Pharmacological Sciences, 16,* 2012, 105-111.

[28]. D. Bukvicki, D. Gottardi, A.K. Tyagi, M. Veljic, P.D. Marin, L. Vujisic, M.E. Guerzoni, and L. Vannini, *Scapania nemorea* liverwort extracts: Investigation on volatile compounds, *in vitro* antimicrobial activity and control of *Saccharomyces cerevisiae* in fruit juice, *LWT-Food Science and Technology, 55(2),* 2014, 452-458.

[29]. C. Rujjanawate, Treatment of tinea corporis with *Tacca chantrieri*'s extract, *Sakon Nakhon Rajabhat University Journal of Science and Technology, 8, 2016,* 115-121.

[30]. H.S. Yi, Y.R. Ahn, G.C. Song, S.Y. Ghim, S. Lee, G. Lee, and C.M. Ryu. Impact of a bacterial volatile 2,3-butanediol on *Bacillus subtilis* rhizosphere robustness, *Frontiers in Microbiology, 7(993),* 2016.

[31]. C.N. Kanchiswamy, M. Malnoy, and M.E. Maffei, Bioprospecting bacterial and fungal volatiles for sustainable agriculture, *Trends in Plant Science, 20(4),* 2015, 206-211.

[32]. S.M. Sharma, and D.G. Bhadange, Review on study of medicinal uses of some aromatic plants, *World Journal of Microbiology and Biotechnology,* 2013, 1-14.

[33]. M. Aldunate, D. Srbinovski, A.C. Hearps, C.F. Latham, P.A. Ramsland, R. Gugasyan, R.A. Cone, and G. Tachedjian, Antimicrobial and immune modulatory effects of lactic acid and short chain fatty acids produced by vaginal microbiota associated with eubiosis and bacterial vaginosis, *Frontiers in Physiology, 6(164),* 2015.

[34]. S.B. Ge, T. Jiang, L. Wang, W.X. Peng, D.L. Li, and Y. Furuta, TD-GC-MS analysis on antibacterial and safety characteristics of volatiles from bamboo during cavitation, carbonization and activation for public health, *Journal of Pure and Applied Microbiology, 9(3),* 2015, 1907.

[35]. C. Shanmugam, G. Sivasubramaniam, P. Bera, K. Baskaran, R. Balachander, and V.R. Parameswaran, Erratum to: Antimicrobial, free radical scavenging activities and catalytic oxidation of benzyl alcohol by nano-silver synthesized from the leaf extract of *Aristolochia indica* L.: a promenade towards sustainability, *Applied Nanoscience, 6(5),* 2016, 725.

[36]. B.M. Haroun, A.A. Elghamry, E.H. Abdel-Shakour, S.M. Elmorsy, and Y.H. Zahem, Determination of bioactive compounds of *Punica granatum punicaceae* and studying its antimicrobial activities against bacteria isolated from El-Manzala water treatment planet, *World Rural Observations, 6,* 2014, 79-86.

[37]. L. Song, M. Cao, C. Chen, P. Qi, N. Li, D. Wu, J. Peng, X. Wang, M. Zhang, G. Hu, and J. Zhao, Antibacterial activity of *Pyrrosia petiolosa* ethyl acetate extract against *Staphylococcus aureus* by decreasing hla and sea virulence genes. *Natural Product Research, 31,* 2017,1347-1350.

[38]. A. Jerbi, S. Zehri, R. Abdnnabi, N. Gharsallah, and M. Kammoun, Essential oil composition, free-radical-scavenging and antibacterial effect from stems of *Ephedra alata alenda* in Tunisia, *Journal of Essential Oil Bearing Plants, 19(6),* 2016, 1503-1509.

[39]. F. Granados-Chinchilla, E. Villegas, A. Molina, and C. Arias, Composition, chemical fingerprinting and antimicrobial assessment of Costa Rican cultivated guavas (*Psidium friedrichsthalianum* (O. Berg) Nied. and *Psidium guajava* L.) essential oils from leaves and fruits, *Natural Products Chemistry and Research, 4(5),* 2016.

[40]. M.Z.M. Salem, Y.E. Zidan, N.M.N.E. Hadidi, M.M.A. Mansour, and W.A.A.A. Elgat, Evaluation of usage three natural extracts applied to three commercial wood species against five common molds, *International Biodeterioration and Biodegradation, 110,* 2016, 206-226.

[41]. I. Tamura, K. Kagota, Y. Yasuda, S. Yoneda, J. Morita, N. Nakada, Y. Kameda, K. Kimura, N. Tatarazako, and H. Yamamoto, Ecotoxicity and screening level ecotoxicological risk assessment of five antimicrobial agents: triclosan, triclocarban, resorcinol, phenoxyethanol and p-thymol, *Journal of Applied Toxicology, 33(11),* 2013, 1222-1229.

[42]. C. Kantar, H. Akal, B. Kaya, F. Islamoğlu, M. Tűrk, and S. Sasmaz, Novel phthalocyanines containing resorcinol azo dyes; synthesis, determination of pKa values, antioxidant, antibacterial and anticancer activity, *Journal of Organometallic Chemistry, 783,* 2015, 28-39.

[43]. K. Muthuraj, A. Shalimol, K.T. Sivapriya, and N. Nagarajan, Screening of active phytocompounds by GC-MS analysis and *in vitro* antibacterial activity of endemic plant *Pogostemon mollis* Benth, *International Journal of Recent Advances in Multidisciplinary Research, 2(7),* 2015, 534-539.

[44]. I.M. Everlyne, T.P. Darsini, and S.A. Yadav, Unraveling antibiofilm potency of *Strobilanthes kunthiana* Nees T Anderson ex Benth against throat-infectious methicillin-resistant *Staphylococcus aureus*, *Indo American Journal of Pharmaceutical Research, 6(6),* 2016, 5707-5716.

[45]. A.R. Padmavathi, B. Abinaya, and S.K. Pandian, Phenol, 2,4-bis(1,1-dimethylethyl) of marine bacterial origin inhibits quorum sensing mediated biofilm formation in the uropathogen *Serratia marcescens*, *Biofouling – The Journal of Bioadhesion and Biofilm Research, 30(9),* 2014, 1111-1112.

[46]. G. Rangel-Sánchez, E. Castro-Mercado, and E. Garcia-Pineda, Avocado roots treated with salicylic acid produce phenol-2,4-bis (1,1-dimethylethyl), a compound with antifungal activity, *Journal of Plant Physiology, 171(3-4),* 2014, 189-198.

[47]. J.M. Ahamath, and J. Sirajudeen, Phytochemical studies and anti-bacterial activities of *Amaranthus spinosus* L., *World Journal of Pharmacy and Pharmaceutical Sciences, 3(12),* 2014, 1692-1697.

[48]. H.M.F. Madkour, M.A. Ghareeb, M.S. Abdel-Aziz, O.M. Khalaf, A.M. Saad, A.K. El-Ziaty, and M. Abdel-Mogib, Gas chromatography-mass spectrometry analysis, antimicrobial, anticancer and antioxidant activities of n-hexane and methylene chloride extracts of *Senna italica*, *Journal of Applied Pharmaceutical Science, 7(6),* 2017, 23-32.

[49]. M.S. Owolabi, O.A. Lawal, N.S. Dosoky, P. Satyal, and W.N. Setzer, Chemical composition, antimicrobial, and cytotoxic assessment of *Mitracarpus scaber* Zucc. (Rubiaceae) essential oil from southwestern Nigeria, *American Journal of Essential Oil and Natural Product, 1(1),* 2013, 4-6.

[50]. L. Iauk, R. Acquaviva, S. Mastrojeni, A. Amodeo, M. Pugliese, M. Ragusa, M.R. Loizzo, F. Menichini, and R. Tundis, Antibacterial, antioxidant and hypoglycaemic effects of *Thymus capitatus* (L.) Hoffmanns. et Link leaves' fractions, *Journal of Enzyme Inhibition and Medicinal Chemistry, 30(3),* 2015, 360-365.

[51]. Q.Y. Zeng, J. Wu, and P.C. Lin, Chemical composition and antimicrobial activity of the essential oil from *Epilobium angustifolium*, *Chemistry of Natural Compounds, 52(6),* 2016, 1113-1115.

[52]. T. Guo, Q. Lin, X. Li, Y. Nie, L. Wang, L. Shi, W. Xu, T. Hu, T. Guo, F. Luo, Octacosanol attenuates inflammation in both RAW264.7 macrophages and a mouse model of colitis, *Journal of Agricultural and Food Chemistry, 65(18),* 2017, 3647-3658.

[53]. M. Kanagarajan, T.P.D. Deivamarudachalam, S. Ponnuraj, and D. Jaganathan, Synergistic effect of ethno medicinal plants against biofilm forming *Streptococcus pyogenes* isolated from upper respiratory tract infection, *International Journal of Phytomedicine, 8(2),* 2016, 208-216.

[54]. P. Jain, H.P. Sharma, F. Basri, K. Priya, and P. Singh, Phytochemical analysis of *Bacopa monnieri* (L.) Wettst. and their anti-fungal activities, *Indian Journal of Traditional Knowledge, 16(2),* 2017, 310-318.

[55]. N. Ceyhan-Gűvensen, and D. Keskin, Chemical content and antimicrobial properties of three different extracts of *Mentha pulegium* leaves from Mugla region, Turkey, *Journal of Environmental Biology, 37(6),* 2016, 1341-1346.

[56]. [Q. Wei, L. Kong, J. Zhang, C. Ma, X. Wu, X. Li, and H. Jiang, Essential oil composition and antibacterial activity of *Lindera nacusua* (D. Don) Merr, *Natural Product Research, 30(23),* 2016, 2704-2706.

[57]. A. Ertas, M. Boga, I. Gazioglu, Y. Yesil, N. Hasimi, C. Ozaslan, H. Yilmaz, and M. Kaplan, Fatty acid, essential oil and phenolic compositions of *Alcea pallida* and *Alcea apterocarpa* with antioxidant, anticholinesterase and antimicrobial activities, *Chiang Mai Journal of Science, 43(1),* 2016, 89-99.

[58]. E.P. Ivanova, S.H. Nguyen, Y. Guo, V.A. Baulin, H.K. Webb, V.K. Truong, J.V. Wandiyanto, C.J. Garvey, P.J. Mahon, D.E. Mainwaring, and R.J. Crawford, Bactericidal activity of self-assembled palmitic and stearic fatty acid crystals on highly ordered pyrolytic graphite, *Acta Biomaterialia, 59(1),* 2017, 148-157.

[59]. R.K. Devi, S. Vasantha, A. Panneerselvam, N.V. Rajesh, and N. Jeyathilakan, Phytochemical constituents and *in vitro* trematocidal activity of *Blechnum orientale* Linn. against *Gastrothylax crumenifer*, *Annals of Phytomedicine, 5(1),* 2016, 127-134.

[60]. A. Agarwal, R. Prajapati, S.K. Raza, and L.K. Thakur, GC-MS analysis and antibacterial activity of aerial parts of *Quisqualis indica* plant extracts, *Indian Journal of Pharmaceutical Education and Research, 51(2),* 2017, 329-336.

[61]. Y. Zhang, X. Liu, Y. Wang, P. Jiang, and S.W. Quek, Antibacterial activity and mechanism of cinnamon essential oil against *Escherichia coli* and *Staphylococcus aureus*, *Food Control, 59,* 2016, 282-289.

[62]. Z. Parveen, S. Mazhar, S. Siddique, A. Manzoor, and Z. Ali, Chemical composition and antifungal activity of essential oil from Xanthium strumarium L. leaves, Indian Journal of Pharmaceutical Sciences, 79(2), 2017, 316-321.

[63]. A. Sharma, and D.S. Cannoo, Comparative evaluation of extraction solvents/techniques for antioxidant potential and phytochemical composition from roots of *Nepeta leucophylla* and quantification of polyphenolic constituents by RP-HPLC-DAD, *Journal of Food Measurement and Characterization, 10(3),* 2016, 658-669.

[64]. K. Nivetha, and G. Prasanna, GC-MS and FT-IR analysis of *Nigella sativa* L. seeds, *International Journal of Advanced Research in Biological Sciences, 3(6),* 2016, 45-54.

[65]. R.S. Hu, J. Wang, H. Li, H. Ni, Y.F. Chen, Y.W. Zhang, S.P. Xiang, H.H. Li, Simultaneous extraction of nicotine and solanesol from waste tobacco materials by the column chromatographic extraction method and their separation and purification, *Separation and Purification Technology, 146,* 2016, 1-7.

[66]. Channabasava, M. Govindappa, and N. Chandrashekar, Determination of phytochemicals by GC-MS in two fractions (17 and 21) of methanol extract of *Loranthus micranthus* and their antioxidant and anti-inflammatory activity, *The Natural Products Journal, 5(3),* 2015, 158-175.

[67]. W. Peng, D. Li, M. Zhang, S. Ge, B. Mo, S. Li, and M. Ohkoshi, Characteristics of antibacterial molecular activities in poplar wood extractives, *Saudi Journal of Biological Sciences, 24(2),* 2017, 399-404.

[68]. S. Pandey, and R.K. Gupta, Screening of nutritional, phytochemical, antioxidant and antibacterial activity of *Chenopodium album* (Bathua), *Journal of Pharmacognosy and Phytochemistry, 3(3),* 2014, 1-9.

[69]. S. Pandey, G. Satpathy, and R.K. Gupta, Evaluation of nutritional, phytochemical, antioxidant and antibacterial activity of exotic fruit "*Limonia acidissima*", *Journal of Pharmacognosy and Phytochemistry, 3(2),* 2014, 81-88.

[70]. G.A. Kraus, D. Chaudhary, S. Riley, F. Liu, A. Schlapkohl, M. Weems, and G.J. Phillips, Synthesis of 3-farnesyl salicylic acid, a novel antimicrobial from *Piper multiplinervium*, *Natural Product Communication, 8(7),* 2013, 911-913.

[71]. A.L. Eaton, S. Dalal, M.B. Cassera, S. Zhao, and D.G.I. Kingston, Synthesis and antimalarial activity of Mallatojaponin C and related compounds, *Journal of Natural Products, 79(6),* 2016, 1679-1683.

[72]. D. Compoccia, L. Visai, F. Renò, I. Cangini, M. Rizzi, A. Poggi, L. Montanaro, L. Rimondini, and C.R. Arciola, Bacterial adhesion to poly-(D,L)lactic acid blended with vitamin E: Toward gentle anti-infective biomaterials, *Journal of Biomedical Materials Research, 103(4),* 2014, 1447-1458.

[73]. J. Ortuño, R. Serrano, and S. Bañón, Antioxidant and antimicrobial effects of dietary supplementation with rosemary diterpenes (carnosic acid and carnosol) vs vitamin E on lamb meat packed under protective atmosphere, *Meat Science, 110,* 2015, 62-69.

[74]. J.S. Jung, B.H. Lee, K.H. Mun, and J.Y. Kwon, Analysis of alkaloid components and essential oils in the jasmine tea and insecticidal effects of ethanolic extracts of jasmine tea on house dust mite, *International Journal of Bio-Science and Bio-Technology, 8,* 2016, 387-398.

[75]. I. Verma, and R.K. Gupta, Estimation of phytochemical, nutritional, antioxidant and antibacterial activity of dried fruit of sacred figs (*Ficus religiosa*) and formulation of value added product (Hard Candy), *Journal of Pharmacognosy and Phytochemistry, 4(3),* 2015, 257-267.

[76]. E.M. Lima, D.S. Cazelli, F.E. Pinto, R.A. Mazuco, I.C. Kalil, D. Lenz, R. Scherer, T.U. Andrade, and D.C. Endringer, Essential oil from the resin of *Protium heptaphyllum*: Chemical composition, cytotoxicity, antimicrobial activity, and antimutagenicity, *Pharmacognosy Magazine, 12,* 2016, S42-S46.

[77]. M.O. Villareal, J. Han, K. Matsuyama, Y. Sekii, A. Smaoui, H. Shigemori, and H. Isoda, Lupenone from *Erica multiflora* leaf extract stimulates melanogenesis in B16 murine melanoma cells through the inhibition of ERK1/2 activation, *Planta Medica, 79(3-4),* 2013, 236-243.

[78]. D.J. Rodríguez, F.A. Trejo-González, R. Rodríguez-García, M.L.V. Díaz-Jimenez, A. Sáenz-Galindo, F.D. Hernández-Castillo, J.A. Villarreal-Quintanilla, and F.M. Peña-Ramos, Antifungal activity *in vitro* of *Rhus muelleri* against *Fusarium oxysporum* f. sp. *lycopersici*, *Industrial Crops and Products, 75(Part B),* 2015, 150-158.

[79]. I.A. Freires, V.C. Queiroz, V.F. Furletti, M. Ikegaki, S.M. Alencar, M.C. Duarte, and P.L. Rosalen, Chemical composition and antifungal potential of Brazilian propolis against *Candida* spp., *Journal of Medical Mycology, 26(2),* 2016, 122-132.

[80]. N. Fatima, M. Rizwan, Y.H. Hobani, A.E.S. Marwan, B.V. Kumar, R.A. Sunosi, S.I. Abdulwahab, M.Y. Areeshi, and A. Alvi, Gas chromatography/Mass spectroscopy analysis of *Catha edulis* Forsk, a psycho stimulant revealed potent solvent dependent antimicrobial activity, *Journal of Pharmacognosy and Phytochemistry, 6(2),* 2017, 197-204.

[81]. N.K. Narayanan, K. Kunimasa, Y. Yamori, M. Mori, H. Mori, K. Nakamura, G. Miller, U. Manne, A.K. Tiwari, and B. Narayanan, Antitumor activity of melinjo (*Gnetum gnemon* L.) seed extract in human and murine tumor models *in vitro* and in a colon-26 tumor-bearing mouse model *in vivo*, *Cancer Medicine, 4(11),* 2016, 1767-1780.

[82]. V. Sivakumar, and G. Gayathri, GC-MS analysis of bioactive components from ethanol extract of *Andrographis paniculata*, *World Journal of Pharmacy and Pharmaceutical Sciences, 4(11),* 2015, 2031-2039.

[83]. C. Chaves-López, A. Serio, A. Gianotti, G. Sacchetti, N. Ndagijimana, C. Ciccarone, A. Stellarini, A. Corsetti, and A. Paparella, Diversity of food-borne *Bacillus* volatile compounds and influence on fungal growth, *Journal of Applied Microbiology, 119(2),* 2015, 487-499.

[84]. X. Shen, W. Chen, Y. Zheng, X. Lei, M. Tang, H. Wang, and F. Song, Chemical composition, antibacterial and antioxidant activities of hydrosols from different parts of Areca catechu L. and Cocos nucifera L., Industrial Crops and Products, 96, 2017, 110-119.

[85]. X. Yu, M. Zhao, F. Liu, S. Zeng, and J. Hu, Identification of 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one as a strong antioxidant in glucose–histidine Maillard reaction products, Food Research International, 51, 2013, 397-403.

[86]. L.W. Foo, E. Salleh, and S.N.H. Mamat, Extraction and qualitative analysis of Piper betle leaves for antimicrobial activities, International Journal of Engineering Technology Science and Research, 2, 2015, 1-8.

[87]. Y. Tűmer, N. Asmafiliz, Z. Kiliç, T. Hökelek, L.Y. Koç, L. Açik, M.Y. Yola, A.O. Solak, Y. Öner, D. Dündar, and M. Yavuz, Phosphorus–nitrogen compounds: Part 28. Syntheses, structural characterizations, antimicrobial and cytotoxic activities, and DNA interactions of new phosphazenes bearing vanillinato and pendant ferrocenyl groups, Journal of Molecular Structure, 1049(8), 2013, 112-124.

[88]. G.M. Petrović, J.G. Stamenković, I.R. Kostevski, G.S. Stojanović, V.D. Mitić, and B.K. Zlatković, Chemical composition of volatiles; antimicrobial, antioxidant and cholinesterase inhibitory activity of *Chaerophyllum aromaticum* L. (Apiaceae) essential oils and extracts, *Chemistry and Biodiversity, 14(5),* 2017.

[89]. E.E. Hames-Kocabas, B. Demirci, A. Uzel, and F. Demirci, Volatile composition of Anatolian propolis by headspace-solid-phase microextraction (HS-SPME), antimicrobial activity against food contaminants and antioxidant activity, *Journal of Medicinal Plants Research, 7(28),* 2013, 2140-2149.

[90]. B.A. Omran, N.A. Fatthalah, N.S. El-Gendy, E.H. El-Shatoury, and M.A. Abouzeid, Green biocides against sulphate reducing bacteria and macrofouling organisms, *Journal of Pure and Applied Microbiology, 7(3),* 2013, 2219-2232.

[91]. S. Vlaisavljevic, B. Kaurinovic, M. Popovic, M. Djurendic-Brenesel, B. Vasiljevic, D. Cvetkovic, and S. Vasiljevic, *Trifolium pratense* L. as a potential natural antioxidant, *Molecules, 19,* 2014, 713-725.

[92]. R. Dandekar, B. Fegade, and V.H. Bhaskar, GC-MS analysis of phytoconstituents in alcohol extract of *Epiphyllum oxypetalum* leaves, *Journal of Pharmacognosy and Phytochemistry, 4,* 2015, 149-154.

[93]. S.B. Rajput, M.B. Tonge, and S.M. Karuppayil, An overview on traditional uses and pharmacological profile of *Acorus calamus* Linn. (Sweet flag) and other *Acorus* species, *Phytomedicine, 21(3),* 2014, 268-276.

[94]. A. Kali, Antibiotics and bioactive natural products in treatment of methicillin resistant *Staphylococcus aureus*: A brief review, *Pharmacognosy Review, 9(17),* 2015, 29-34.

[95]. M.Z.M. Salem, H.M. Ali, and M.M. Mansour, Fatty acid methyl esters from air-dried wood, bark, and leaves of *Brachychiton diversifolius* R. Br: antibacterial, antifungal, and antioxidant activities, *BioResources, 9(3),* 2014, 3835-3845.

[96]. A.N. Singab, N.M. Mostafa, O.A. Eldahshan, M.L. Ashour, and M.Wink, Profile of volatile components of hydrodistilled and extracted leaves of Jacaranda acutifolia and their antimicrobial activity against foodborne pathogens, *Natural Product Communications, 9(7),* 2014, 1007-1010.

[97]. J.M. Soares, P.F. Silva, B.M.S. Puton, A.P. Brustolin, R.L. Cansian, R.M. Dallago, and E. Valduga, Antimicrobial and antioxidant activity of liquid smoke and its potential application to bacon, *Innovative Food Science and Emerging Technologies, 38(Part A),* 2016, 189-197.

[98]. T.Y. Suman, D. Elumalai, P.K. Kaleena, and S.R.R. Rajasree, GC–MS analysis of bioactive components and synthesis of silver nanoparticle using *Ammannia baccifera* aerial extract and its larvicidal activity against malaria and filariasis vectors, *Industrial Crops and Products, 47,* 2013, 239-245.

[99]. R. Prabakaran, B. Joseph, and P.N. Pradeep, Phyto medicinal compounds from *Urginea indica* Kunth: A synthetic drugs potential alternative, *British Journal of Pharmaceutical Research, 11(5),* 2016, 1-9.

[100]. H. Jasim, A.O. Hussein, I.H. Hameed, and M.A. Kareem, Characterization of alkaloid constitution and evaluation of antimicrobial activity of *Solanum nigrum* using gas chromatography mass spectrometry (GC-MS), *Journal of Pharmacognosy and Phytotheraphy, 7(4),* 2015, 56-72.

[101]. S.K. Bhardwaj, and J.S. Laura, Antibacterial activity of some plant-extracts against plant pathogenic bacteria *Xanthomonas campestris* pv. *campestris*, *Indian Journal of Agricultural Research, 43(1),* 2009, 26-31.

[102]. S. Fatima, M.R. Baig, M. Baig, and V.B. Kadam, Antimicrobial activity of *Acacia nilotica* (L.) Del. plant extracts against *Xanthomonas malvacearum* bacteria, *International Multidisciplinary Research Journal, 5(6),* 2012, 48-49.

[103]. V.A. Gargade, and D.G. Kadam, Antibacterial activity of some herbal extracts and traditional biocontrol agents against Xanthomonas axonopodis punicae, Journal of International Academic Research for Multidisciplinary, 2(3), 2014, 16-26.

[104]. S. Lalitha, K. Rajeshwara, P.S. Kumar, K. Deepa, and K. Gowthami, In vivo screening of antibacterial activity of Acacia mellifera (BENTH) (Leguminosae) on human pathogenic bacteria, Global Journal of Pharmacology, 4(3), 2010, 148-150.

[105]. C.M. Mattana, S.E. Satorres, A. Sosa, M. Fusco, and L.E. Alcaráz, Antibacterial activity of extracts of *Acacia aroma* against methicillin-resistant and methicillin-sensitive *Staphylococcus*, *Brazilian Journal of Microbiology, 41(3),* 2010, 581-587.

[106]. S.O. Okoro, A.H. Kawo, and A.H. Arzai, Phytochemical screening, antibacterial and toxicological activities of *Acacia senegal* extracts, *Bayero Journal of Pure and Applied Sciences, 5,* 2012, 163-170.

[107]. S.O. Okoro, A.H. Kawo, and A.H. Arzai, Phytochemical screening, antibacterial and toxicological activities of *Acacia nilotica* extracts, *Bayero Journal of Pure and Applied Sciences, 7,* 2014, 105-115.

[108]. O.Y. Aba, I.T. Ezuruike, R.G. Ayo, J.D. Habila, and G.I. Ndukwe, Isolation, antibacterial and antifungal evaluation of α–amyrenol from the root extract of *Acacia ataxacantha* DC, *Scholars Academic Journal of Pharmacy, 4(2),* 2015, 124-131.

[109]. R. Lawrence, E. Jeyakumar, and A. Gupta, Antibacterial activity of *Acacia arabica* (bark) extract against selected multi drug resistant pathogenic bacteria, *International Journal of Current Microbiology and Applied Sciences, 1,* 2015, 213-222.

[110]. E. Basim, and H. Basim, Antibacterial activity of *Rosa damascena* essential oil, *Fitoterapia, 74(4),* 2003 394-396.

[111]. N. Rahman, R. Mahmood, H. Rahman, and M. Haris, Spectrophotometric screening of potent bactericidal property of *Thevetia peruviana* Schum. leaf and fruit rind extracts on clinical and plant pathogens, *International Journal of Applied Science and Biotechnology, 2(4),* 2014, 451-459.
[112]. G. Yemata, and M. Fetene, *In vitro* evaluation of the antibacterial activity of some medicinal plant extracts against *Xanthomonas campestris* pv. *musacearum*, *Ethiopian Journal of Science and Technology, 10(1),* 2017, 17-32.
[113]. A. Banso, Phytochemical and antibacterial investigation of bark extracts of *Acacia nilotica*, *Journal of Medicinal Plants Research, 3(2),* 2009, 82-85.
[114]. S.H. Moussa, A.A. Tayel, A.A. Al-Hassan, and A. Farouk, Tetrazolium/formazan test as an efficient method to determine fungal chitosan antimicrobial activity, *Journal of Mycology, ID 753692,* 2013, 7 pages.
[115]. P. Rasoanaivo, C.W. Wright, M.L. Wilcox, and B. Gilbert, Whole plant extracts versus single compounds for the treatment of malaria: synergy and positive interactions, *Malaria Journal 10(Suppl 1),* 2011, S4.

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