Vol. 15(1), pp. 56-63, January, 2021
DOI: 10.5897/JMPR2020.7031
Article Number: 3935B0665881
ISSN 1996-0875

http://www.academicjournals.org/JMPR

ACADEMIC JOURNALS
expand your knowledge

Journal of Medicinal Plants Research

Full Length Research Paper

Chemical composition and antibacterial activity against *Escherichia coli* of extracts of a common household plant

Maria K. Kozar[1], Joanna G. Kondylis[2], Katerina A. Drouvalakis[3] and Leland G. Kozar[4*]

[1]Wilcox High School, Santa Clara, California, USA.
[2]Nueva High School, San Mateo, California, USA.
[3]U.C. Berkeley, Berkeley, California, USA.
[4]Beckman Center, Stanford University, Stanford, California, USA.

Received 16 August, 2020; Accepted 29 December, 2020

Plants have long been a source for a wide variety of compounds with medicinal properties; of significant interest are plant antimicrobial compounds. Given the continual increase in bacterial resistance, it is imperative that new antimicrobial compounds need to be discovered. The purpose of this study was to screen some common household plants for antibacterial activity, specifically against *Escherichia coli*. Leaves of 16 different household plants were subjected to methanol-water extraction and the dried extract was analyzed for antibacterial activity using the standard Kirby-Bauer disk diffusion technique. Of all the plants tested, only the Zebra plant (*Aphelandra squarrosa*) showed a strong antibacterial activity against *E. coli*. This Zebra plant extract was further analyzed by gas chromatography/mass spectrometry (GC/MS). A variety of known antibacterial compounds including glycerine 46%, phytol 10%, palmitic acid 9%, hydroquinone 6%, linoleic acid 6%, catechol 4% and decanoic acid 3% were shown to be present in the Zebra plant extract along with other compounds whose structure could not be identified at this time. This provides additional avenues for future research especially in the identification of these unknown compounds present in this plant extract which may contribute to its antibacterial activity.

Key words: Chemical composition, antibacterial activity, *Aphelandra squarrosa* extracts, *Escherichia coli.*

INTRODUCTION

Ongoing scientific research of the importance of plants in human health has led to the identification of more than 9,000 native plants and about 1,500 new species with antimicrobial properties (Swamy et al., 2016). While these numbers appear large, they constitute only a fraction of the estimated 391,000 plant species (Willis, 2016) known to date.

Plants contain a wide variety of common phytochemicals, such as flavonoids, alkaloids, tannins, and terpenoids which possess antimicrobial and antioxidant properties (Talib and Mahasneh, 2010; Savoia, 2012).

*Corresponding author. E-mail: kozar@stanford.edu. Tel: 1-(650) 725-4483.

These phytochemicals can disrupt microbial membranes, impairing cellular metabolism, control biofilm formation, inhibit bacterial capsule production, weaken bacterial virulence and even reduce microbial toxin production (Upadhyay et al., 2014; Abreu et al., 2012). The continued study of plant-based derivatives and therapies is particularly important given that a large percentage of the world-wide population depends on them for medicine and healthcare (World Health Organization, 2019). Of the 141 antibacterial drugs to receive approval between 1981 and 2014, over 58% acquired the label of “natural product”, and in the past 33 years, 47% of approved drugs have been directly or indirectly linked to natural products (Newman and Cragg, 2016), with 11% of these medicines derived from native flowering plant species (Veeresham, 2012). With the increase in antibiotic resistance by bacteria, plants are being seen as a reservoir of potential pharmaceuticals (Gupta and Birdi, 2017). These developments explain the renewed interest in natural product-based drug discovery (Atanasov et al., 2015). Even when a product does not result in a direct medical application, it can aid the process of drug discovery by serving as a chemical template for the synthesis of other novel substances (Newman, 2008; Veersham, 2012).

In search of new plant candidates, it is preferred to find medicinal compounds in the regenerating portion of a plant, such as the leaves, thereby preserving the plants longevity and productivity over a period of time. The aim of the current study was to screen for potential antibacterial activity against *E. coli* in the leaves of common household plants. *E. coli* was selected as the bacterial target since it is a prevalent bacterium responsible for common ailments such as urinary tract infection, food-poisoning, gastroenteritis, and even some forms of meningitis and pneumonia (Croxen et al., 2013).

MATERIALS AND METHODS

Plant selection

The entire PubMed database was searched using a list of 150 commonly available plants compiled from the local SummerWinds Nursery (Palo Alto, California) to find species that had not been analyzed for antibacterial phytochemicals. From this list, 16 plants (listed in Table 1) were chosen for testing, along with positive plant controls, fennel and poinsettia known to possess antibacterial activity (Cowan, 1999; Sharif et al., 2015). Plants were obtained between the months of January and April of 2018; plant identification was based on the documentation attached to each plant by the nursery along with independent verification in consultations with Prof. Arthur Grossman, Carnegie Institute, Department of Plant Biology, and databases plants.jstor.org, plants.sc.egov.usda.gov and garden.org. A filter disk devoid of plant extract was used as a negative control.

Drying and preparation of plant leaves

Freshly collected leaves were washed with tap water to remove any dust, and then shade dried at room temperature for 7 days. The shade dried plant materials were ground to a coarse powder and subsequently used for phytochemical extraction and GC/MS quantification.

Preparation of plant extract for antibacterial testing

One gram of ground plant leaves was added to 10 ml of a 4:1 methanol-water mixture and allowed to sit at room temperature for 24 h (Harborne, 1998; Eloff, 1998). The extract was passed through a 0.45 µm syringe filter into 150 mm × 15 mm sterile petri dishes. The filtrate was allowed to evaporate to dryness at room temperature. This dried plant extract was used to test for antibacterial activity.

Bacterial strain

The antibacterial potency of each plant extract was evaluated using the K-12 strain of *Escherichia coli* (Code 15-5068) obtained from Carolina Biological. Cells were inoculated and incubated at 37°C in Luria-Bertani (LB) (Thermo Fisher Scientific) broth for 12 h prior to plating. This culture was then diluted 1:100 with distilled water prior to plating.

Antibacterial activity test

The Kirby-Bauer disk diffusion method was used to test for antibacterial activity (Bauer et al., 1966; Hudzicki, 2009). Twenty-five ml of LB agar medium was poured into 100 mm × 15 mm sterile petri dishes. After the agar set, plates were marked into quadrants for the testing of four plant samples per plate. Using sterile techniques, 100 µl of the 1:100 diluted bacterial culture was then added to the agar plates and spread evenly across the entire plate surface using a sterile cell spreader. An individual 6 mm filter disk was moistened with sterile water and then swabbed across the dried plant extract. The filter disk containing the plant extract was then placed on the surface of the agar plate, ensuring complete contact with the agar. This procedure was repeated for each plant extract. The plates were subsequently incubated at 35°C for 24 h. The presence of an inhibition zone was measured and recorded (Table 1); the absence of bacterial growth around the filter disk was an indication of antibacterial activity. Each plant was tested at least twice, and each run included both the positive (fennel and poinsettia) and negative (plain filter paper) controls.

Preparation of plant sample for GC/MS

To prepare samples for GC/MS analysis, the same extraction procedure was used as for the antibacterial assay. 1.0 g of dried *Aphelandra squarrosa* leaves was added to 10 ml of a 4:1 methanol-water mixture and allowed to sit for 24 h. The extract was then passed through a 0.45 µm filter, and evaporated to dryness to remove any water that could damage the chromatography column. This evaporate was reconstituted in 0.1 ml of methanol, and 1.0 µl of this solution was subsequently injected into an Agilent 7890A GC/MS. A different extraction technique was used to extract more hydrophobic molecules than could be obtained with methanol-water. One gram of dried *A. squarrosa* leaves was added to 10 ml of methanol and allowed to sit at room temperature for 24 h. The extract was passed through a 0.45 µm syringe filter, and then evaporated to dryness. This evaporate was then reconstituted in 0.1 ml of methanol, and 1.0 µl was injected into an Agilent 7890A GC/MS.

Table 1. Antibacterial activity against *E. coli* of common household plant leaf extracts.

	Plant	Measurement (mm)	Average (mm)	Strength (NI/strong)
1	*Xerochrysum bracteatum* (Mohawk Yellow)	6, 6	6	NI
2	*Felicia Armelbides* (Agthea San Luis)	6, 6	6	NI
3	*Heuchera* (Purple Petticoats)	6, 6	6	NI
4	*Verbena Tapien* (Blue Violet)	6, 7	6.5	NI
5	*Osteospermum ecklonis* (African Daisy)	6, 6	6	NI
6	*Matthiola incana* (Harmony Purple)	6, 6	6	NI
7	Leucanthemum (Shasta Daisy 'Becky')	6, 7	6.5	NI
8	*Gaillardia* (Arizona Sun)	7, 6	6.5	NI
9	*Brachyscome* (Swan River Daisy)	6, 7	6.5	NI
10	*Lithodora diffusa* (Purple gromwell)	6, 6	6	NI
11	*Blechnum gibbum* (Silver Lady Fern)	6, 6	6	NI
12	*Pellaea rotundifolia* (Button Fern)	8, 6	7	NI
13	*Didymochlaena truncatula* (Red Fern)	6, 6	6	NI
14	*Caladium bicolor*	6, 6	6	NI
15	*Aphelandra squarrosa* (Zebra Plant)	15, 13	14	Strong
16	*Soleirolia soleirolii* (Baby's Tears)	6, 6	6	NI
Positive	*Euphorbia pulcherrima* (Poinsettia)	15, 18; 20, 23	19	Strong
Positive	*Foeniculum vulgare* (Fennel)	22, 25	23.5	Strong
Negative	No plant extract	6, 6, 6, 6	6	NI

Each plant species was tested in duplicate. The average zone of inhibition (in mm) is shown. (<8 mm is considered to have no antibacterial activity (no inhibition (NI)); 8-12 is weak antibacterial activity; >12 mm is strong antibacterial activity). The zone of inhibition measurement includes the disk diameter of 6 mm.

GC/MS procedure

The GC/MS analysis of the methanol and methanol-water extracts of *A. squarrosa* leaves were performed using an Agilent 7890A GC/MS instrument employing the following conditions: a standard DB5–MS Capillary non-polar column (30 m × 0.25 mm × 0.25 µm) with a stationary phase of (5%-phenyl)-methylpolysiloxane. Helium was used as a carrier gas at a constant flow rate of 11.3 psi (1 ml/min). The initial oven temperature was 37°C for 4 min, then increasing at a rate of 20°C/min to a final temperature of 320°C for 7 min. Total run time was 25 min. The fractions obtained from gas chromatography were then analyzed by mass spectrometry to identify the fragmentation patterns (Rukshana et al., 2017; Thangavel et al., 2015). Mass range was m/z 50–550. Identification of phytochemical compounds was achieved through retention time and mass spectrometry by comparing the mass spectra of unknown peaks with those stored in the Agilent ChemStation™ GC/MS library (version 2.00.493).

RESULTS

Antibacterial screening of household plants

Sixteen different household plant species were tested for antibacterial activity against the K-12 strain of *E. coli* by the disk diffusion method. An evaluation of the antibacterial activity of the plant extracts—the mean diameter of the zones of inhibition—is recorded in Table 1. The two positive controls, *Foeniculum vulgare* (fennel) and *Euphorbia pulcherrima* (poinsettia), exhibited the largest inhibition zones of antibacterial activity against the *E. coli*. Of these sixteen plants tested, only the Zebra plant (*A. squarrosa)* exhibited significant antibacterial activity against *E. coli*. *A. squarrosa* displayed an average distinct inhibition zone of 14 mm. The inhibition zone of *A. squarrosa* was comparable to those of the positive controls, fennel and poinsettia, being on average of 23.5 and 19 mm respectively.

GC/MS analysis

The phytochemical components of *A. squarrosa* were further analyzed by GC/MS. Since water could not be injected into the GC/MS column, two different extract preparations of the plant were analyzed. The first was a 4:1 methanol-water extract that was evaporated to dryness and then reconstituted in pure methanol prior to analysis by GC/MS. The results are shown in Figure 1 and Table 2. This extract showed several different compounds (Table 2) including glycerine (46%), cyclohexanetetrol (12%), hydroquinone (6%), catechol (4%), 3,7-dimethyl-6-octen-1-ol (3%), capric acid (3%), butyl isopropyl ether (2%), 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-Pyran-4-one (1%), and methyl vinyl ketone (1%), all of which are water soluble. There were three other significant peaks that could not be identified with high probability by the ChemStation™ software.

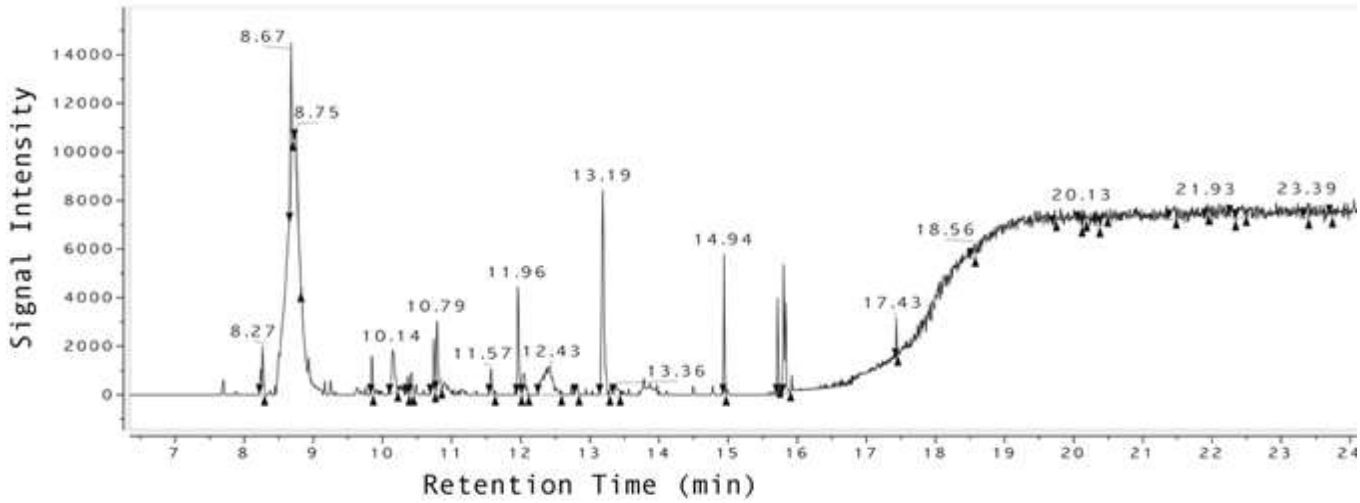


Figure 1. Gas chromatography of methanol-water extract of Zebra plant leaves.

The second extraction method utilized pure methanol to extract additional hydrophobic compounds. This methanolic extract produced a different set of compounds as shown in Figure 2 and Table 3. 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-Pyran-4-one was present in both the methanol-water and the pure methanol extracts. Otherwise, the pure methanol extraction yielded phytol (10%), palmitic acid (9%) and linoleic acid (6%); all long chain hydrocarbon compounds not particularly soluble in water. There were also seven other significant peaks that could not be identified with high probability from their retention time and fragmentation pattern using the ChemStation™ software. The percent abundance of the various peaks was calculated by the ChemStation™ software. The total abundance from Tables 2 and 3 does not add up to 100% because only peaks with more than 1% abundance were listed. There were many peaks identified by the software that appeared to be little more than background noise and were not further analyzed.

DISCUSSION

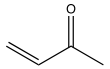
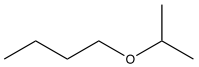
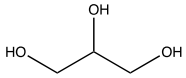
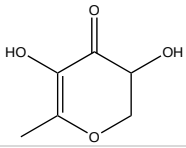
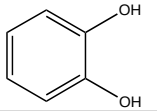
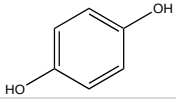
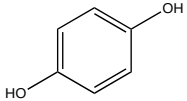
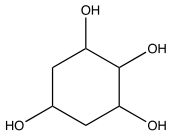
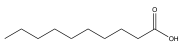
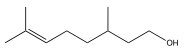
Dried leaves from a variety of common household plants were processed in a 4:1 methanol-water solution, and these extracts were tested for antibacterial activity using the disk diffusion method. This extraction solution was chosen because it has been shown to extract a wider variety of phytochemicals than other solvents (Harborne, 1998; Eloff, 1998; Banu and Catherine, 2015). Therefore, majority of the plant phytochemicals potentially responsible for any antibacterial activity were extracted using this method.

Of the 16 plants tested, only one, *A. squarrosa,* exhibited significant antibacterial activity to *E. coli*. Due to its striped leaves, this plant is commonly known as the Zebra plant. *A. squarrosa* is a species in the Acanthaceae family, a native to the tropical forests of Brazil. Traditionally, the leaves from this family have been used in the treatment of a variety of conditions ranging from poisonous bites (Awan et al., 2014; Muthu et al., 2006), fever, headache, vertigo, wounds (Mia et al*.,* 2009), ulcers (Ignacimuthu et al., 2008), to bronchial disease (Krishnaraju et al., 2005). However, specifically for *A. squarrosa,* there has been no medicinal use reported to date.

A literature search beyond PubMed found a study by Roia and Smith (1977) who used methanolic extracts of the whole Zebra plant, finding antibacterial activity against *Proteus vulgaris* but not *E. coli*. Pierre et al. (2015), also report no activity against *E. coli,* although a different extraction solvent (mixture of methanol and dichloromethane) was used. In contrast, a study by Khalili et al. (2012) reports low antibacterial activity of this plant against *E. coli* using methanol extractions. However, Khalili et al. do not indicate which part of the plant is used; a positive plant control is not incorporated in the study; and in addition, there is no characterization of the compounds in the plant extract. When harvesting a plant for medicinal purposes, it is important to use only the leaves, as they are able to regenerate, thereby preserving the plant for further harvesting. Our study, however, used the leaves of *A. squarrosa* and has shown significant antibacterial activity against *E. coli*.

Further analysis of the *A. squarrosa* methanol-water leaf extracts by GC/MS identified compounds with known antibacterial activity: methyl vinyl ketone (Kai et al., 2012), glycerine (Nalawade et al*.,* 2015), the phenolic compounds catechol and hydroquinone (Kocacaliskan et al., 2006), capric acid (Royce et al., 2013), and citronellol (Guimaraes et al., 2019). The analysis of the straight methanolic leaf extract identified phytol (Lee et al., 2001),

Table 2. Phytochemicals found in methanol-water extract of Zebra plant leaves.

Peak	RT (min)	% Area	Name of compound	CAS	Molecular formula	Molecular weight	Probability (%)	Chemical structure	Biological activity
1	8.23	1	Methyl Vinyl Ketone	78-94-4	C_4H_6O	70	42		antibacterial Kai et al. (2012)
2	8.27	2	Butyl isopropyl ether	1860-27-1	$C_7H_{16}O$	116	79		
3	8.67	46	Glycerine	56-81-5	$C_3H_8O_3$	92	54		antibacterial Nalawade et al. (2015)
4	9.84	1	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-Pyran-4-one	28564-83-2	$C_6H_8O_4$	144	83		antioxidant Yu et al. (2013)
5	10.1	4	Catechol	120-80-9	$C_6H_6O_2$	110	72		antibacterial Kocacaliskan et al. (2006)
6.1	10.74	2	Hydroquinone	123-31-9	$C_6H_6O_2$	110	38		antibacterial Cowan (1999)
6.2	10.79	4	Hydroquinone	123-31-9	$C_6H_6O_2$	110	67		antibacterial Cowan (1999)
7	11.96	4	Unknown						
8	13.19	12	1,2,3,5-Cyclohexanetetrol	136936-97-5	$C_6H_{12}O_4$	148	15		potential osmotic regulator Garza-Sanchez et al. (2008)
9	14.94	3	n-Decanoic acid, capric acid	334-48-5	$C_{10}H_{20}O_2$	172	31		antibacterial Royce et al. (2013)
10	15.72	3	(±)-3,7-dimethyl-6-Octen-1-ol	106-22-9	$C_{10}H_{20}O$	156	9		antibacterial Santos et al. (2019) Guimaraes et al. 2019
11	15.80	4	Unknown						
12	15.82	2	Unknown						

RT denotes retention time.

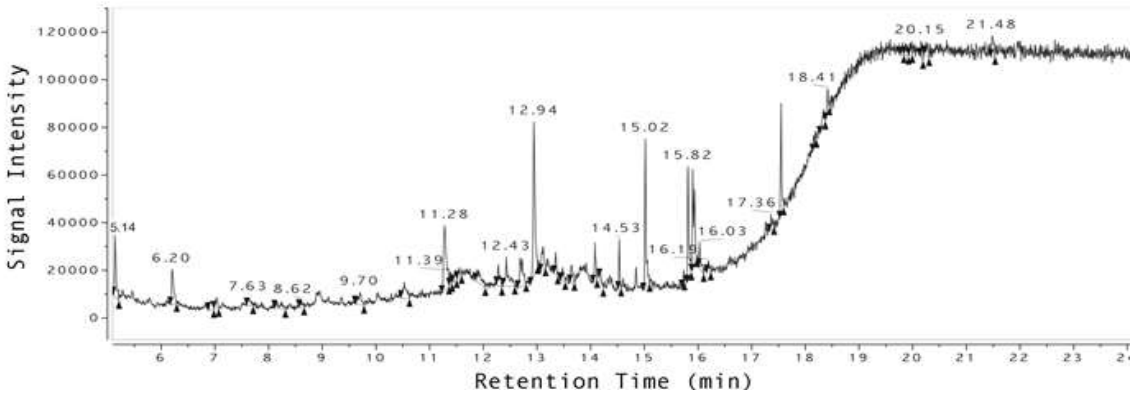
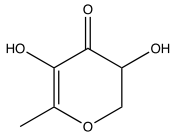
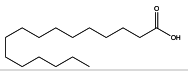
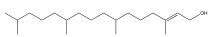
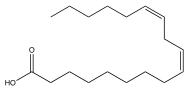


Figure 2. Gas chromatography of methanol extract of Zebra plant leaves.

Table 3. Phytochemicals found in methanolic extract of Zebra plant leaves RT denotes retention time.

Peak	RT (min)	% Area	Name of compound	CAS	Molecular formula	Molecular weight	Probability (%)	Chemical structure	Biological activity
1	6.20	5	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	28564-83-2	$C_6H_8O_4$	144	78		Antioxidant Yu et al. (2013)
2	11.28	13	Unknown						
3	12.26	1	Unknown						
4	12.41	1	Unknown						
5	12.68	2	Unknown						
6	12.94	18	Unknown						
7	14.08	3	Unknown						
8	14.53	3	Unknown						
9	15.02	9	Palmitic Acid	57-10-3	$C_{16}H_{32}O_2$	256	60		Antibacterial Knothe et al. (2019)
10	15.82	10	Phytol	150-86-7	$C_{20}H_{40}O$	296	31		Antibacterial Lee et al. (2001)
11	15.90	6	Linoleic acid	60-33-3	$C_{18}H_{32}O_2$	280	21		Antibacterial Knothe et al, (2019)

and the long chain fatty acids; palmitic and linoleic acid (Huang et al., 2011; Desbois and Smith, 2010; Dilika et al., 2000). It is of significance to note that the more polar methanol-water solvent was able to extract more polar compounds (such as the quinones, catechol and hydroquinone), while the less polar methanol solvent was able to extract a long chain alcohol (phytol), and fatty acids (palmitic acid and linoleic acid). This demonstrates the importance of using both polar and non-polar solvents for the identification of available phytochemicals in a sample.

These aforementioned compounds are found in other plants (Knothe et al., 2019, Santos et al., 2019) and are not unique to the *A. squarrosa* plant. The cyclohexanetetrol identified in the methanol-water preparation potentially functions as an osmotic regulator, as similar chemical structures serve this function in other plants (Garza-Sanchez et al., 2008). Comparing the two solvent leaf extracts, only one identifiable compound was present in both, namely the pyran. This pyran compound, has been shown to be an antioxidant, and most likely formed during the drying process (Yu et al*.,* 2013).There were, however, additional peaks in the gas chromatogram for both the methanol and methanol-water extracts that were not found in the known chemical database. The characterization of these entities requires further analytical studies, something that is beyond the scope of the current study.

Conclusion

The current study screened a cohort of common household plants for antibacterial activity against *E. coli*, identifying *A. squarrosa* as having significant antibacterial properties in its leaf material. GC/MS analysis of the *A. squarrosa* leaf extracts identified several known compounds that could account for the observed antibacterial activity. The chromatography also showed peaks whose identity could not be determined by the current ChemStation™ chemical database. Further studies are needed for the characterization of these unknown phytochemicals.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors appreciate the biology lab of the Nueva High School for the use of their facility for bacterial cultures, and the Stanford Mass Spectrometry facility for the generous use of their equipment. They are also grateful to Prof. Arthur Grossman of the Carnegie Institute, Department of Plant Biology, for helpful suggestions.

REFERENCES

Abreu AC, McBain AJ, Simões M (2012). Plants as sources of new antimicrobials and resistance-modifying agents. Natural Product Reports 29:1007-1021. https://doi.org/10.1039/c2np20035j

Atanasov AG, Waltenberger B, Pferschy-Wenzig EM, Linder T, Wawrosch C, Uhrin P, Temml V, Wang L, Schwaiger S, Heiss EH, Rollinger JM, Schuster D, Breuss JM, Bochkov V, Mihovilovic MD, Kopp B, Bauer R, Dirsch VM., Stuppner H (2015). Discovery and resupply of pharmacologically active plant-derived natural products: A review. Biotechnology Advances 33(8):1582-1614. https://doi.org/10.1016/j.biotechadv.2015.08.001

Awan AJ, Ahmed CB, Uzair M, Aslam MS, Farooq U, Ishfaq K (2014). Family Acanthaceae and genus Aphelandra: Ethnopharmacological and phytochemical review. International Journal of Pharmacy and Pharmaceutical Sciences 10:44-55.

Banu KS, Cathrine L (2015). General Techniques Involved in Phytochemical Analysis. International Journal of Advanced Research in Chemical Science 2(4):25-32.

Bauer AW, Kirby WMM, Sherris JC, Turck M (1966). Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology 45(4):493-496. https://www.ncbi.nlm.nih.gov/pubmed/5325707

Cowan MM (1999). Plant Products as Antimicrobial Agents. Clinical Microbiology Reviews 12(4):564-582. https://dx.doi.org/10.1128/CMR.12.4.564

Croxen MA, Law RJ, Scholz R, Keeney K, Wlodarska M, Finlay BB (2013). Recent Advances in Understanding Enteric Pathogenic *Escherichia coli*. Clinical Microbiology Reviews 26(4):822-880. https://dx.doi.org/10.1128/CMR.00022-13

Desbois AP, Smith VJ (2010). Antibacterial free fatty acids: activities, mechanisms of action and biotechnological potential. Applied Microbiology and Biotechnology 85(6):1629-1642. https://doi.org/10.1007/s00253-009-2355-3

Dilika F, Bremner PD, Meyer JJ (2000). Antibacterial activity of linoleic and oleic acids isolated from *Helichrysum pedunculatum*: a plant used during circumcision rites. Fitoterapia 71(4):450-452. https://doi.org/10.1016/S0367-326X(00)00150-7

Eloff JN (1998). Which extractant should be used for the screening and isolation of antimicrobial components from plants? Journal of Ethnopharmacology 60(1):1-8. https://doi.org/10.1016/s0378-8741(97)00123-2

Garza-Sanchez F, Chapman DJ, Cooper JB (2008). *Nitzschia Ovalis* (Bacillariophyceae) Mono Lake strain accumulates 1,4/2,5 cycolhexanetetrol in response to increased salinity. Journal of Phycology 45(2):495-403. https://doi.org/10.1111/j/1529-8817-2009-00667.x

Guimaraes AC, Meireles, LM, Lemos MF, Guimaraes MCC, Endringer DC, Fronza M, Scherer R (2019). Antibacterial Activity of Terpenes and Terpenoids Present in Essential Oil. Molecules 24:2471-2483. https://doi.org/10.3390/molecules24132471

Gupta PD, Birdi TJ (2017). Development of botanicals to combat antibiotic resistance. Journal of Ayurveda and Integrative Medicine 8:266-275. https://dx.doi.org/10.1016/j.jaim.2017.05.004

Harborne JB (1998). Phytochemical Methods – 3rd Edition. Chapman and Hall, London pp. 4-7.

Huang CB, Alimova Y, Myers TM, Ebersole JL (2011). Short- and medium-chain fatty acids exhibit antimicrobial activity for oral microorganisms. Archives of Oral Biology 56(7):650-654. https://doi.org/10.1016/j.archoralbio.2011.01.011

Hudzicki J (2009). Kirby-Bauer disk diffusion susceptibility test protocol. American Society for Microbiology. Retrieved December 11, 2020, from https://asm.org/getattachment/2594ce26-bd44-47f6-8287-0657aa9185ad/Kirby-Bauer-Disk-Diffusion-Susceptibility-Test-Protocol-pdf.pdf

Ignacimuthu S, Ayyanar M, Sankarasivaraman K (2008). Ethnobotanical study of medicinal plants used by Paliyar tribals in Theni district of Tamil Nadu, India. Fitoterapia 79:562-568. https://doi.org/10.1016/j.fitote.2008.06.003

Kai H, Hirashima K, Matsuda O, Ikegami H, Winkelmann T, NakaharaT, Iba K (2012). Thermotolerant cyclamen with reduced acrolein and

methyl vinyl ketone. Journal of Experimental Botany 63(11):4143-4150. https://doi.org/10.1093/jxb/ers110
Khalili RM, Norhayati A, Fazari GM, Azlina M, Zahili MT, Zubaidi LA (2012). Screening of seven types Terengganu herbs for their potential antibacterial activity against selected food microorganisms. Borneo Science Journal 31:9-23.
Knothe G, Razon L, de Castro ME (2019). Fatty acids, triterpenes and cycloalkanes in ficus seed oils. Plant Physiology and Biochemistry 135:127-131. https://doi.org/10.1016/j.plaphy.2018.11.030
Kocacaliskan I, Talan I, Terzi I (2006). Antimicrobial activity of catechol and pyrogallol as allelochemicals. Zeitschrift für Naturforschung C, 61(9-10):639-642. https://doi.org/10.1515/znc-2006-9-1004
Krishnaraju AV, Rao TVN, Sundararaju D, Vanisree M, Tsay HS, Subbaraju GV (2005). Assessment of bioactivity of Indian medicinal plants using brine shrimp (*Artemia salina*) lethality assay. International Journal of Applied Science and Engineering 3:125-34.
Lee W, Woo ER, Lee DG (2001). Phytol has antibacterial property by inducing oxidative stress response in Pseudomonas aeruginosa. Free Radical Research 50(12):1309-1318. https://doi.org/10.1080/10715762.2016.1241395
Mia MMK, Kadir MF, Hossan MS, Rahmatullah M (2009). Medicinal plants of Garo tribe inhabiting the Madhupur forest region of Bangladesh. American-European Journal of Sustainable Agriculture 3:165-71.
Muthu C, Ayyanar M, Raja N, Ignacimuthu S (2006). Medicinal plants used by traditional healers in Kancheepuram District of Tamil Nadu, India. Journal of Ethnobiology Ethnomedicine 2:1-10. https://doi.org/10.1186/1746-4269-2-43
Nalawade TM, Bhat K, Sogi SH (2015). Bactericidal activity of propylene glycol, glycerine, polyethylene glycol 400, and polyethylene glycol 1000 against selected microorganisms. Journal of International Society of Preventative and Community Dentistry 5(2):114-119. https://doi.org/10.4103/2231-0762.155736
Newman DJ (2008). Natural products as leads to potential drugs: an old process or the new hope for drug discovery? Journal of Medicinal Chemistry 51:2589-2599. https://doi.org/10.1021/jm0704090
Newman DJ, Cragg GM (2016). Natural products as sources of new drugs over the 30 years from 1981 to 2014. Journal of Natural Products 79:629-661. https://doi.org/10.1021/acs.jnatprod.5b01055
Pierre LL, Moses MN, Mubanga CP, Harrison S (2015). Phytochemical screening and in vitro antibacterial activity of *Odontonema strictum* (Acanthaceae) against selected bacteria. International Journal of Development Research 5(6):4655-4659. https://www.journalijdr.com/sites/default/files/issue-pdf/3182.pdf
Roia FC, Smith RA (1977). The antibacterial screening of some common ornamental plants. Economic Botany 31:28-37. https://doi.org/10.1007/BF02860649
Royce LA, Liu P, Stebbins MJ, Hanson BC, Jarboe LR (2013). The damaging effects of short chain fatty acids on *Escherichia coli* membranes. Applied Microbiology and Biotechnology 97:8317-8327. https://doi.org/10.1007/s00253-013-5113-5
Rukshana MS, Doss A, and Kumari Pushpa Rani, TP (2017). Phytochemical screening and GC-MS analysis of leaf extract of *Pergularia daemia* (Forssk) Chiov. Asian Journal of Plant Science and Research 7(1):9-15.
Santos PL, Matos JPSCF, Picot L, Almeida JRGS, Quintans JSS, Lucindo J, Quintans-Júnio LJ (2019). Citronellol, a monoterpene alcohol with promising pharmacological activities - A systematic review. Food and Chemical Toxicology 123:459-469. https://doi.org/10.1016/j.fct.2018.11.030
Savoia D (2012). Plant-derived antimicrobial compounds: alternatives to antibiotics. Future Microbiology 7(8):979-90. https://doi.org/10.2217/fmb.12.68
Sharif HB, Mukhtar MD, Mustapha Y, Lawal AO (2015). Preliminary Investigation of Bioactive Compounds and Bioautographic Studies of Whole Plant Extract of *Euphorbia pulcherrima* on *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, and *Pseudomonas aeruginosa.* Advances in Pharmaceutics, Volume 2015. Article ID 485469. https://dx.doi.org/10.1155/2015/485469
Swamy KM, Akhar, MS, Sinniah, UR (2016). Antimicrobial properties of plant essential oils against human pathogens and their mode of action: An updated review. Evidence-Based Complementary and Alternative Medicine, volume 2016: Article ID 3012462, 21 pages. https://doi.org/10.1155/2016/3012462
Talib WH, Mahasneh AM (2010). Antimicrobial, cytotoxicity and phytochemical screening of Jordanian plants used in traditional medicine. Molecules 15:1811-1824. https://doi.org/10.3390/molecules15031811
Thangavel A, Balakrishnan S, Alakshmi V, and Duraisamy S (2015). Phytochemical screening, gas chromatography-mass spectrometry (GC-MS) analysis of phytochemical constituents and antibacterial activity of *Coriandrum sativum* seeds. International Journal of Pharmacy and Pharmaceutical Sciences 7(9):153-159.
Upadhyay A, Upadhyaya I, Kollanoor-Johny A, Venkitanarayanan K (2014). Combating pathogenic microorganisms using plant-derived antimicrobials: a minireview of the mechanistic basis. BioMed Research International. Volume 2014, Article ID 761741. https://doi:10.1155/2014/761741
Yu X, Zhao M, Liu F, Zeng S, Hu J (2013). 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(1):397-403. https://doi.org/10.1016/j.foodres.2012.12.044
Veeresham C (2012). Natural products derived from plants as a source of drugs. Journal of Advanced Pharmaceutical Technology & Research, 3(4): 200–201. https://doi.org/10.4103/2231-4040.104709
Willis KJ (2016). State of the World's Plants, 2016. Retrieved December 10, 2020, from. https://stateoftheworldsplants.org/2017/report/SOTWP_2017.pdf
World Health Organization (2019). WHO global report on traditional and complementary medicine 2019. World Health Organization. Retrieved December 10, 2020, from https://apps.who.int/iris/handle/10665/312342.