Review Article

Efficacy of *Mikania micrantha* Extract against Methicillin-resistant *Staphylococcus aureus*: A Mini Review

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Submission Date: 03-06-2021; Revision Date: 25-07-2021; Accepted Date: 29-07-2021

ABSTRACT

The development of antimicrobial resistance remains to be a major health concern imposing the need for new and effective antimicrobials. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a multidrug-resistant bacteria showing resistance to common antibiotics (i.e. methicillin, penicillin, oxacillin, amoxicillin,). This mini-review sought to evaluate the proven antimicrobial properties of *Mikania micrantha* extract as stated by previous studies that will help us assess for the future studies. This is focused on summarizing the active metabolites and phytochemical compounds that can inhibit MRSA, the minimum inhibitory concentrations of the extract and its active compounds, and the challenges of using the extract as an antibacterial agent. The sources for the literature were ResearchGate, PubMed, ScienceDirect, and Google Scholar. A total of 30 out of 317 international literatures were included and published in English from 2011 and beyond. The results obtained presented the major phytochemical constituents and mechanism action of *M. micrantha* (i.e. sesquiterpene lactones, diterpenes, flavonoids, phenolic compounds). It holds probable antibacterial value against MRSA based on the reported MIC of the extract as a whole and its active compounds. The use of *M. micrantha* as an antibacterial agent is limited by its varying growth stability due to climatic factors, long lag period during its expansion, and the insolubility of its active constituents. In this regard, the state of *M. micrantha* is not at equilibrium which may directly affect the growth condition of the species thereby affecting its progression. Further evidence-based studies are still required to address the challenges encountered by previous studies in developing *M. micrantha* extract as an antimicrobial agent against MRSA.

Key words: Antibiotic resistance, Antimicrobial property, MRSA, Methicilin, Mikania micrantha

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INTRODUCTION

Over the years, infections caused by antibiotic-resistant strains have emerged as a global public health concern prompting the growing interest in developing new antimicrobial agents. The development of antimicrobial resistance restricts the number of potential antibiotics especially if empirical prescription is necessary for the treatment of infections.[1] Plant products occupy major parts of discovered microbial compounds given its abundance, diversity, and distribution in different places. People from remote communities use plant-based products as alternative medicine as their only source of medication in lieu of expensive pharmaceuticals sold in the market. Natural products can provide a wide range of diverse compounds as sources of drugs in addressing this problem.[2] The Philippines is abundant with diverse plant species, some have shown therapeutic properties, which are feasible sources of natural products for possible drug development. Additionally, the therapeutic use of plant-based products is a practice evident in the remote communities of the country, where they are used as sources of medication in lieu of

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DOI: 10.5530/ajbls.2021.10.40

pharmaceuticals sold in the market. An example of an alternative plant-based medicine is the invasive weed *Mikania micrantha*.[3] *Mikania micrantha*, an invasive weed first recorded in the Philippines in 1838, can thrive in various environmental conditions due to its ability to express phenotypic variation of physiological traits.[4] It exhibits antimicrobial and anti-inflammatory properties as seen in past studies. Specifically, it has shown relative antimicrobial properties against most gram positive and gram-negative bacteria including common pathogens such as *Staphylococcus aureus* and *Streptococcus pyogenes* as well as to *Pseudomonas aeruginosa* and *Mycobacterium smegmatis*.[5] Fungicidal properties against *C. albicans* have also been identified.[6] Most of these reference studies were performed within Asia, but there is a limited number of studies on the antimicrobial activity of *M. micrantha* from the Philippines. Considering these, it would be useful to assess the antibacterial activity of *M. micrantha* against the commonly isolated virulent bacteria such as *Staphylococcus aureus*, particularly to its highly virulent antibiotic-resistant counterpart Methicillin-resistant *Staphylococcus aureus* (MRSA) in the Philippine context, where research literature is lacking.

Staphylococcus aureus is an opportunistic gram-positive bacteria which causes skin and wound infections including folliculitis, furuncles, carbuncles, and bullous impetigo.[7] These infections commonly affect immunocompromised patients with prolonged stay in the hospital after invasive medical surgeries. Over the years, *S. aureus* has emerged to be a threatening hospital and community-acquired infection due to its resistance to common antibiotics such as penicillin, amoxicillin, oxacillin, and methicillin.[8] The pathogenicity of *S. aureus* can be attributed to its different virulence factors which include enterotoxins, cytolytic toxins, enzymes, and protein A. One specific enzyme that greatly contributes to its virulence is the beta-lactamase, which hydrolytically destroys beta-lactam bonds of penicillin and cephalosporins destroying these drugs' antimicrobial activities.[9,10] Aside from its traditional resistance mechanisms, a special feature of *S. aureus* pathogenesis is its ability to survive on both biotic and abiotic surfaces in the biofilm state making it a leading cause of human infection.[11]

Methicillin-resistant *Staphylococcus aureus* was first observed in 1960, shortly after methicillin, a semi-synthetic beta-lactam antibiotic was introduced into clinical practice in response to the growing penicillin resistance to *S. aureus*.[12] Since then, MRSA has been shown to be resistant to a host of other commonly used antibiotics such as methicillin, penicillin, oxacillin, amoxicillin, cephalosporins, aminoglycosides, macrolides, sulfonamides, and rifampicin. Thus, MRSA has emerged as a widespread infection in community and hospital settings and is responsible for 10-fold more infections than all multidrug resistant (MDR) Gram-negative pathogens combined.[11] Due to successful clones, community-acquired MRSA has become the principal type of MRSA infection for the past 10-20 years.[13] The World Health Organization[14] recently classified MRSA as one among the twelve priority pathogens that pose the greatest threat to human health for which new antibiotics are urgently needed. The constant threat of antimicrobial resistance such as that of MRSA emphasizes the need for continued research on possible sources of antimicrobial agents for development of bactericidal agents of pharmacologic value. Hence, we aim to determine the phytochemicals present in *Mikania micrantha* extract that may be directly attributed to its antimicrobial properties and the minimum inhibitory concentration of this extract required to be effective against methicillin-resistant *S. aureus* for subsequent microbial infection control. This mini review aimed to evaluate the proven antimicrobial properties of *Mikania micrantha* extract and its efficacy against MRSA by comparing and summarizing results from previous published studies. Specifically, it seeks to identify the constituent phytochemicals present in *Mikania micrantha* that exhibit antibacterial properties against MRSA and the minimum inhibitory concentration the extract and its active compounds have exhibited. This review also seeks to summarize the challenges of using *M. micrantha* extract as an anti-MRSA agent stated from previous studies.

The summarized results involving the pharmacological value of *M. micrantha* may lead to the development of low-cost products such as topical antimicrobials capable of killing methicillin-resistant *S. aureus*. The researchers may acquire useful information and skills that are essential in their future practice as medical technologists. Furthermore, this study can provide interest and a springboard for future researchers for a more comprehensive research.

MATERIALS AND METHODS

Literature Search

The research journals and articles utilized in conducting this mini-review were retrieved from the four different online databases which include ResearchGate, PubMed, ScienceDirect, and Google Scholar. The search keywords used are the following: [MRSA OR methicillin-resistant *S. aureus*], [*Mikania micrantha* OR *M. micanthra* plant OR *M. micanthra* extract], [phytochemicals], [minimum inhibitory concentration OR MIC] and [Philippines].

Upon entering the appropriate keywords the following number of studies were presented by the databases: Google Scholar (248), ScienceDirect (6), PubMed (7), and ResearchGate (56).

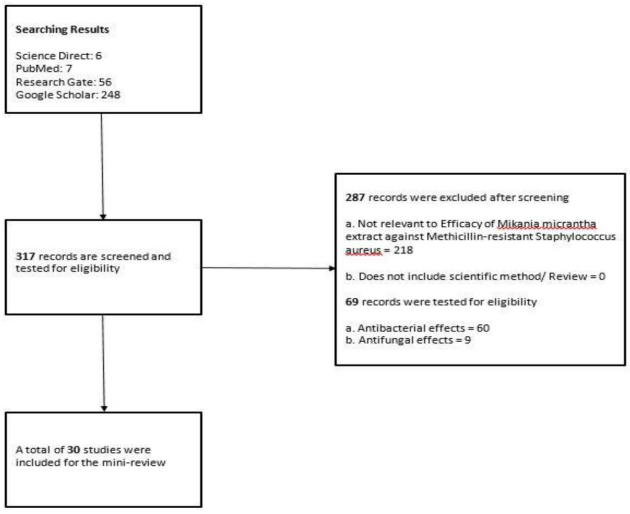

Figure 1: Flow diagram of the study selection process.

Eligibility Criteria

The detailed outline on the flow diagram on the study selection process is presented in Figure 1. The studies included in this mini-review focused on researches addressing the antibacterial properties of *Mikania micrantha* and those that provided quantitative results on the minimum inhibitory concentration required. The authors considered selecting full text studies published from the last 10 years up to the present (2011-2021) and those that are reported in the English language. The articles selected are those with experimental research design and with more than 10 references. There was no restriction in the countries where the studies were conducted but Asian studies were prioritized. Exclusion criteria applied by the authors include any study that has no correlation with Mikania micrantha and its antimicrobial properties against MRSA. The researchers applied limitations on the database by excluding research studies that are not reported in full text and not in the English language.

Table 1: Summary of phytochemicals constituents *M. micrantha* extracts.[15]

Extract	Alkaloid Test	Flavonoid test			Tannin and polyphenol test	Saponin test		Triterpernoids test
	Wagner test (formation of cloudy sedimen)	Wilstatter-Sianidin test	Batesmith test	Metcalf test	Gelatin test	FeCl3 test	Foam test (formation of bee dane)	Salkowski test
				Mikania Micrantha				
CME	(++++)	–	–	–	(+++)	(++++)	–	(+++++)
HE	–	–	–	–	(++)	–	–	–
EAE	–	–	–	–	(+++)	(+++)	(+)	(+++)
CE	–	–	–	–	–	(++++)	(+++++)	(++++)
C:ME	–	–	–	–	–	–	–	(+)
BE	–	–	–	–	–	–	(+++++)	–
AE	(++)	–	–	–	–	–	(+)	–
F1	–	–	–	–	–	–	(++)	(++++)
F2	–	–	–	–	–	–	(++++)	(+++)
F3	–	–	–	–	(+++)	–	(+++)	–
F4	–	–	–	–	(+++)	–	(+++++)	(++)
F5	–	–	–	–	(++)	(++++)	–	(+)

Notes. CME = Crude Methanolic Extract, HE = Hexane Extract, EAE = Etnyl Acetate Extract, CE = Chloroform Extract, CME = (Chloroform:Methanol) Extract, BE = Buthanol Extract, AE = Aqueous Extract. Scores: (+++++) – copiously present; (++++) – present ; (+++) – moderately present; (++) – weekly present; (+) / (-) – No activity
Adapted from "Antibacterial and Phytochemical Investigations of Mikania micrantha H.B.K. (Asteraceae) From Sabah, Malaysia" by Matawali A, Lee P, How S, Azlan G, 2016. Transactions on Science and Technology, 244-250. Copyright 2016 by Scribbr.

Table 2: Phytochemical constituents of different plant parts of *M. micrantha*.[16]

Biological activity	Plant parts	Effective doses	Targets	Nature of actions	Class of compounds	Compounds
Anti-bacterial	Whole Plant	200 mg/ml	*B.subtilis* MTCC441, *B.cereus* MTCC430, *S.aureus* MTCC96, *E.coli* MTCC739, *P.aeruginosa* MTCC1688, and S.epidermidis MTCC435	Inhibited both gram positive and negative stains with similar potency	Alkaloids, phenolics, tannins, steroids, and glycosides	-
		20 µl	*P.aeruginosa, S.typhii, S.aureus,* and *S.pneumoniae*	Inhibited both gram positive and gram negative bacteria	Tannins, polyphenols, alkaloids, saponins, and triterpenoids	-
		300 µg/disc	*S.aureus, B.cereus*, *E.coli* and *S.sonnii*	Mild to moderate inhibitory activity against four bacteria		-
	Leaf and	500 µg/ml (MIC)b	*S.aureus* MTCC1927	Moderate inbibitory		-
	Flower	1000 µg/ml (MBC)c	and *S.pyogenes* MTCC3160	potency against both bacteria	Alkaloids, flavonoids, and tannins	-
			S.epidermis ATCC12228	Significant inhibitory activity against S.epidermis	Sesquiterpene lactones	Deoxymikanolid, scandenolide, dihydroscandenolide, mikanolide, dihydromikanolide, and m-methoxy benzoic method
	Leaf	62.5 – 125 mg/l (MIC) 125 – 250 mg/l (MBC)	*S.aureus*, *B.subtilis, M. luteus, B.cereus, R.solanacearum, X.oryzae* pv. *oryzae, X.campestris* pv. vesicatoria and *X.campestris* pv. citri	Antibacterial effect against all bacterial isolates	-	-
	Stem, Leaf, Inflorescence	0.5 – 4.0 µg/µl (MIC)	*B.subtilis* ATCC6633, and *E.coli* ATCC6051	Both strains were sensitive to all three plant extracts	-	-

Adapted from "Towards the antimicrobial, therapeutic and invasive properties of Mikania micrantha Kunth: a brief overview." by Sheam, M., Haque, Z., and Nain, Z., 2020. Journal of Advanced Biotechnology and Experimental therapeutics. 3(2):92 (doi: 10.5455/jabet.2020.d112). Copyright 2020 by Scribbr.

Notes. b = Minimum inhibitory concentration, c = minimum bactericidal concentration;

RESULTS

Constituent Phytochemicals present in M. micrantha extract that exhibit anti-MRSA activity

Mikania micrantha extract shows antimicrobial activity attributed to its bioactive phytochemical compounds (Table 1), particularly, it shows varying spectrums of anti-bacterial activity depending on the plant part from which the chemicals were extracted. *M. micrantha* has been shown to be effective against *S. aureus* using 200 mg/ml and 20 ml of whole plant parts (with varying phytochemical compounds for each dose concentration: see below), 500 mg/ml (MIC) and 1000 mg/ml (MBC) of leaf and flower extract, and 62.55-125 mg/l (MIC) and 125-250 mg/l (MBC) of leaf extract only.[16] Another study

demonstrates an inhibition zone of *M. micrantha* leaf ethanol extract at a concentration of 30 mg/mL versus *S. aureus*.[17] This may be attributable to the presence of phenol and flavonoid compounds in the leaves of *M. micrantha*. Table 2 shows the phytochemical constituents of different plant parts of *M. micrantha* in detail.

Different pharmacologically active compound groups such as sesquiterpene lactones, diterpenes, flavonoids and phenolic compounds present in *M. micrantha* are responsible for its antibacterial (Table 3), anticancer, and allelopathic response activities. The major antimicrobial activity elements present in *M. micrantha* are sesquiterpene lactones which include mikanolidem dihydromikanolide, n-methoxy benzoic acid, deoxy mikanolide, scandenolide, and dihydro scandenolide. Additionally, various *M. micrantha* plant part extracts contain polyphenols such as tannins and flavonoids and steroid-related molecules such as triterpenoids and saponins that have all shown varying levels of antimicrobial properties.[16] The presence of these phytoconstituents suggests an antimicrobial potential of the sample.

Minimum Inhibitory Concentration of *M. micrantha* extract for Methicillin-resistant *S. aureus*

The minimum inhibitory concentration (MIC) of *M. Micrantha* essential oil extract was determined as resistant against *S. aureus* at MIC is 32 µg/mL.[5] This is in comparison with oxacillin and cefoxitin (methicillin) resistance where *S. aureus* is susceptible at ≤ 2 µg/ml and resistant at ≥ 4 µg/ml for oxacillin and susceptible at ≤ 4 µg/ml and resistant at ≥ 8 µg/ml for cefoxitin.[18] About 86% of the *M. micrantha* flower oil extract consists of sesquiterpenoids. A number of sesquiterpenoids possess antibacterial activity against *S. aureus*, one of which is caryophyllene oxide with an MIC of 0.073 mg/mL against *S. aureus* and similarly trans-caryophyllene

Table 3: Antibacterial activity (%inhibition of growth) of ethanol extracts tested against 17 strains of MDR *Staphylococcus.*

Code no	Family	Tested	Solvent	Nature of the extract	Yield (mg) per gram	Presence of phytochemicals by TLC			
N-343	Compositae	Flower	Ethanol	Sticky		47	Alkaloids, terpenoids		
Strain	NE-345	NE-384	NE-306	NE-341	NE-321	NE-391	NE-386	NE-381	NE-383
KS1	86	69	105	112	102	105	113	29	20
KS3	56	94	90	70	122	95	72	83	92
KS6	56	68	98	108	97	110	115	59	62
KS8	78	110	9	53	114	0	110	121	94
KS9	88	72	108	88	104	98	115	67	95
KS16	66	84	102	59	126	78	113	7	96
S62	41	61	111	54	127	42	72	75	90
S64	93	94	95	112	93	119	105	-3	-29
S69	95	90	100	78	90	58	115	48	45
AV4	44	34	93	64	108	90	90	12	93
AV6	109	25	112	100	106	41	88	35	41
AV18	76	109	91	110	87	115	111	30	-9
KH115	97	99	108	67	85	35	79	86	98
796N	74	110	78	106	82	107	103	84	100
ATCC6538	71	112	59	96	101	97	114	100	45
JCSC1435	109	99	50	67	35	104	110	-2	51
LMG10273	112	107	88	24	87	110	111	73	91

Notes. Percentages are underlined if growth inhibition exceeds 50%; all percentages were rounded to the nearest integer. Antibiotic ciprofloxacin (20 µg/ml) showed 100% inhibition against *S.aureus* Rosenbach. Adapted from "Indian medicinal plant extracts to control multidrug-resistant *S.aureus*, including biofilms." by Panda, S., Das, R., Lavigne, R., & Luyten, W, 2020.
South African Journal of Botany, 128, 283–291. (https://doi.org/10.1016/j.sajb.2019.11.019). Copyright 2020 by Scribbr.

(9.1% of the oil extract) has MIC and MBC of 0.25 mg/mL against *S. aureus*. Additionally, nerolidol and bisabolol are sesquiterpene alcohols that have demonstrated prophylactic effect against *S. aureus*, through enhancement of antibacterial activity of six commonly used antibiotics—ciprofloxacin, clindamycin, erythromycin, gentamicin, tetracycline, and vancomycin—against the pathogen, effectively sensitizing *S. aureus* to these drugs (Saikia *et al.* 2020). In a study by Panda *et al.*[19] *M. micrantha* ethanol extract derived from its flower portion was found to be effective in inhibiting the growth of *S. aureus* strains KS6, S64 and AV18 (all resistant to methicillin, penicillin, macrolide-lincosamide-streptogramin, aminoglycosides and tetracycline). Similarly, *M. micrantha* ethanol extract also exhibited antibacterial activity against *S. aureus* strains KS8, S69, AV6 and 796 N (resistant to methicillin, penicillins, aminoglycosides and tetracycline and sensitive to macrolide-lincosamide-streptogramin).

M. micrantha leaf extract contains deoxy mikanolide, scandenolide, dihydroscandenolide, mikanolide, dihydromikanolide, and m - methoxy benzoic acid. All compounds show antimicrobial activity against *S. aureus* with MIC and MBC values ranging from 62.5 to 1000 µg/mL. Of the six compounds, deoxy mikanolide shows the highest antibiotic activity against *S. aureus* at 62.5 µg/mL.[20,21] A flavonoid compound—quercetin has shown strong antibacterial activity against *S. aureus* with an MIC of 6.25 µg/mL.[20] Additionally, when tested against MRSA strains, quercetin shows synergism with rifampicin and ciprofloxacin. This flavonoid compound was observed in *M. micrantha* as quercetin-3-O-diglucoside and quercetin 30,40,7-trimethyl ether-3-sulfate.[21] Moreover, previous studies have shown that flavonoids, particularly its carbonylic region, produces anti-MRSA activity against MRSA strains AM-51, AM-72 and AM-172.[22]

Tannins exhibited bactericidal and inhibitory effects exhibited all tested MRSA at a concentration of 0.78 and 1.56 mg/mL. In a similar study conducted by Basri *et al.*[23] the interaction between tannin in combination with selected antibiotics was investigated to evaluate its anti-MRSA action. The MIC value of tannin and oxacillin was the same i.e. 31.25 µg/ml. Moreover, the combination of tannin and oxacillin indicated partial synergism against MRSA ATCC 33591 with a Fractional Inhibitory Concentration index value of 0.0625 (Table 4).

Adapted from "Antagonistic Effect of Tannin on Oxacillin Efficacy Against Methicillin-Resistant *Staphylococcus aureus* (MRSA) by Time-kill Assay" by Basri, D., Abdullah N., Khairon, R., Ishak S., and Zin, N.,2015. *Middle East Journal of Scientific Research*. 23(10):2470-2478. (doi: 10.5829/idosi.mejsr.2015.23.10.10167. Copyright 2015 by Scribbr.

Percentage reduction in MIC and FIC values for oleanolic acid, ursolic acid, beta-boswellic acid, and cycloastragenol triterpenoids each in combination with cefradine and vancomycin are shown below.

DISCUSSION

The *Mikania micrantha* kunth (mile-a-minute) is a major invasive creeper. It grows rapidly and inhibits the growth of other plants by smothering them. Research medicinal plants is the most widely studied area around the world for their contribution to healthcare because of the diverse pharmacological activities, antimicrobial, anticancer, anti-inflammatory, anti-nociceptive, anti-oxidant, anti-diabetic, analgesic and anti-parasitic.[24] In the context of phytotherapy, the genus *Micrantha* under *Asteraceae* family is one among the best-selling natural products in the world used in popular medicine.[25] This genus has been extensively studied in recent decades because of its diverse chemical compositions making it a good candidate for development of drugs and therapeutics.[26] One pathogenic bacterium that is quite dangerous and causes infection both sporadically and endemically is *Staphylococcus aureus*. This gram-positive *Staphylococcus aureus* bacteria colonize human skin and mucous membranes and are the most common cause of skin infections in the world with varying degrees of infection. *S. aureus* infections were resistant to various oral or intravenous antibiotics, such as penicillin, methicillin, cephalosporins, tetracyclines, chloramphenicol, methicillin, sulfonamides and vancomycin.[27] A wide portfolio of mechanisms including chromosomal mutation and horizontal gene transfer have contributed to its rapidly evolved antimicrobial resistance. The genetic mutations that alter the target DNA gyrase thereby reducing drug accumulation can cause *S. aureus* to become drug resistant.[10] Aside from its traditional resistance mechanisms, a special feature of *S. aureus* pathogenesis is its ability to survive on both biotic and abiotic surfaces in the biofilm state making it a leading cause of human infection.[11] Particularly, methicillin-resistant *S. aureus* (MRSA) has emerged as a widespread pathogen and is responsible for 10-fold more infections than all multi-drug resistant (MDR) Gram-negative pathogens combined.[11] With MRSA being one of the leading causative agents of nosocomial infections, Galar and colleagues[28] cited reports correlating increased hospital mortality due to prosthetic valve endocarditis (PVE)

Table 4: Determination of MIC and MBC values of tannin and oxacillin against MRSA ATCC 33591.

	MIC		MBC		Control	
Concentration (µg/ml)	Tannin	Oxacillin	Tannin	Oxacillin	Positive	Negative
2000	–	–	–	–	+	–
1000	–	–	–	–	+	–
500	–	–	–	–	+	–
250	–	–	–	–	+	–
125	–	–	–	–	+	–
62.50	–	–	+	–	+	–
31.25	–	–	+	–	+	–
15.63	+	+	+	+	+	–
7.81	+	+	+	+	+	–
3.91	+	+	+	+	+	–

Notes. + represents presence of growth (turbid well), – represents absence of growth (clear well); positive control – bacterial suspension and Mueller-Hinton broth, negative control – tannin/oxacillin and Mueller-Hinton broth

among patients who had undergone valve surgery. However, MRSA-related infections may occur even without invasive procedures. Hence, the need for effective disinfection processes involving environmental surface materials in the healthcare setting must be emphasized to reduce the risk of transmission of various healthcare-associated pathogens including MRSA which may occur via direct contact or through fomites.[29]

The independent acquisition of *Staphylococcal cassette* chromosome mec (SCCmec) which contains the genes that encode for proteins rendering the bacterium resistance to most β-lactam antibiotics have resulted in different MRSA clones.[30] It is hypothesized that the spread of MRSA occurs by at least two mechanisms. The first mechanism is the spread of existing resistant clones and second is the acquisition of SCC*mec* by a methicillin-sensitive *S. aureus* (MSSA) strain. One particular strain, MRSA ATCC BAA-44, is known to be resistant to 18 clinically relevant antibiotics.[31] Of the antibiotics effective against MRSA, vancomycin is the best drug of treatment against severe MRSA infections.[32] The bactericidal action of vancomycin involves binding to the D-alanyl-D-alanine of cell wall peptidoglycan precursor molecules to inhibit elongation and cross-linking of peptides, leading to cell death.[33] Over time, the increasing instances of *S. aureus* resistance to vancomycin has produced vancomycin-intermediate *S. aureus* (VISA) and vancomycin-resistant *S. aureus* (VRSA). VISA is a result of continual mutations of WalKR, GraSR, and VraSR genes in *S. aureus* while VRSA is facilitated by *van* gene clusters which code for D-alanyl-D-lactate and D-alanyl-D-serine ligases that replace D-alanyl-D-alanine on the cell wall thus rendering vancomycin's bactericidal activity as ineffective.[34] Another antibiotic that works against Gram-positive bacteria such as resistant *Staphylococcus, Streptococcus and Enterococcus spp.* is linezolid, which is an oxazolidinone antibiotic.[35] Linezolid binds to the 23S site of rRNA on the 50S subunit thus inhibiting the formation of the 70S complex preventing protein synthesis leading to cell death. This unique bactericidal mechanism excludes the possibility of cross-resistance with other antibiotics.[36] Daptomycin is a cyclized lipopeptide produced by *Streptomyces roseosporus* as a secondary metabolite. It is a novel and strong broad-spectrum antibiotic used for treating infections by multidrug resistant bacteria such as MRSA and vancomycin-resistant enterococci (VRE). Daptomycin bactericidal activity is dependent on its action on Ca^{2+} ions on bacterial cell membranes which involves substituting Ca^{2+} for divalent cations thus causing the destruction of the membrane electric potential leading to cell death. This mechanism of action is unique to other antibiotics such as vancomycin and linezolid, thus there is no cross-resistance.[37] Whole plant extract contains tannins and triterpenoids with antibacterial activity against *S. aureus* and probable activity against its multidrug resistant counterpart MRSA.[16] Leaf and flower parts contain flavonoids, which are polyphenols that are able to disrupt bacterial cell walls which results in multiple component inhibition.[16] Tannins are water-soluble polyphenols that easily penetrate microbial cell walls due to its polar nature rendering its antimicrobial effect.[38] In the study of Adnan *et al.*[39] tannins exhibited cell wall disruption,

Table 5: Combination testing of antibiotics and compounds against methicillin-sensitive *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus*.

Compound			CI								RS							
			MSSA				MRSA				MSSA ATCC 29213				MSSS ATCC 43300			
T	A	MIC[T]	MIC[A]	FIC[T]	FIC[A]	FIC[S]	MIC[T]	MIC[A]	FIC[T]	FIC[A]	FIC[S]	MIC[T]	MIC[A]	FIC[T]	FIC[A]	FIC[S]	MIC[T]	MIC[A]	FIC[T]	FIC[A]	FIC[S]
OA	VA	7 ± 1.4	0.31 ± 0	0.35	0.17	0.52	22 ± 6	1 ± 0.2	0.27	0.17	0.44	2 ± 0	0.5 ± 0	0.125	0.25	0.375	16 ± 0	0.5 ± 0	0.25	0.125	0.375
CY	VA	3 ± 0.3	0.75 ± 0.1	0.18	0.4	0.58	4 ± 0	1.5 ± 0.1	0.13	0.25	0.38	4 ± 0	0.5 ± 0	0.25	0.25	0.5	4 ± 0	1 ± 0	0.12	0.25	0.375
UA	VA	0.9 ± 0.1	0.12 ± 0	0.11	0.06	0.17	2 ± 0	2 ± 0	0.12	0.34	0.46	0.5 ± 0	0.5 ± 0	0.125	0.25	0.325	2 ± 0	1 ± 0	0.12	0.25	0.375
BBA	VA	21±7	1 ± 0.3	0.45	0.54	0.99	64 ± 11	2 ± 0	0.5	0.34	0.84	16 ± 0	0.5 ± 0	0.5	0.25	0.75	64 ± 0	2 ± 0	0.5	0.5	1
OA	CE	7.5 ± 1	1 ± 0.6	0.37	0.1	0.47	16 ± 7	8 ± 1	0.2	0.17	0.37	4 ± 0	0.5 ± 0	0.25	0.25	0.5	8 ± 0	4 ± 0	0.12	0.125	0.24
CY	CE	6.5 ± 0.7	3.2 ± 0.3	0.4	0.34	0.74	-	-	-	-	-	8 ± 0	0.5 ± 0	0.5	0.25	0.75	-	-	-	-	-
UA	CE	1.1 0.1	0.5 ± 0	0.14	0.05	0.19	8 ± 0	8 ± 0	0.17	0.17	0.67	0.5 ± 0	0.5 ± 0	0.25	0.25	0.5	4 ± 0	2 ± 0	0.25	0.12	0.37
BBA	CE	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Note. MIC represented as mean±SEM. Mean for the clinical isolates is calculated from eight different strains. Mean for reference strains is calculated from three values obtained by three independent experiments. T=Triterpenoid, A=Antibiotic, OA=Oleanolic acid, VA=Vancomycin, CY=Cycloastragenol, UA=Ursolic acid, BBA=Beta-boswellic Acid, CE=Cefradine, MICT=MIC of triterpenoid in combination expressed in µg/ml, MICA=MIC of antibiotic in combination expressed in µg/ml, FICT=Fractional inhibitory concentration of triterpenoid, FICA=Fractional inhibitory concentration of antibiotic, FICS=Sum of fractional inhibitory concentration, MSSA=Methicillin-sensitive *Staphylococcus aureus*, MRSA=Methicillin-resistant *Staphylococcus aureus*, -=Combination was ineffective at the concentrations which were tested. CI=Clinical isolates, RS=Reference strains, MICs=Minimum inhibitory concentrations, ATCC=American Type Culture Collection, SEM=Standard error of mean.Adapted from "*In vitro* effectiveness of triterpenoids and their synergistic effect with antibiotics against *Staphylococcus aureus* strains." By Hamza M, Nadir M, Mehmood N, and Farooq A., 2016.Indian Journal of Pharmacology. 48(6):710-714. (doi: 10.4103/0253-7613.194851). Copyright 2016 by Scribbr.

release of cytoplasmic contents and a decrease in cellular volume in the morphology and ultrastructure of MRSA treated with this phytochemical. It was found out that tannin exhibited equal anti-MRSA potency as oxacillin.[23] Moreover, the combination of tannin and oxacillin indicated partial synergism against MRSA ATCC 33591 with a Fractional Inhibitory Concentration index value of 0.0625 (Table 5).[23] Triterpenoids are compounds derived from six isoprenoid units of 30 carbons. In a study by Hamza *et al.*[40] oleanolic acid and cycloastragenol triterpenoids showed potency against *S. aureus*. Results show that oleanolic or ursolic acid triterpenoids may be used in combination with cefradine (a drug not suitable to treat MRSA infections as it shows MIC resistance at > 32 µg/ml) to reduce its MIC concentration by 83-87% ($P < 0.01$). Similarly, triterpenoids in combination with vancomycin (a drug suitable for MSSA and MRSA) reduces its MIC by 46-94% against MSSA and MRSA ($P < 0.05$). Triterpenoids when used independently exert a weaker effect on MSSA and MRSA compared to the antibiotics, but when used in combination with cefradine and vancomycin show synergism by reducing the MIC of both the triterpenoids and the antibiotics.[40] The continuing threat of Methicillin-resistant *Staphylococcus aureus*, owing to its steady capacity to adapt and give rise to drug-resistant strains necessitates a comprehensive approach. This includes epidemiologic surveillance along with the development of novel antimicrobial agents with potent bioactivity to yield improved clinical outcomes.[41] The lack of in-depth studies regarding Mikania micrantha's molecular mechanisms that direct its biological actions hinders successful drug development.[16] Thorough analysis of Mikania micrantha's toxicity is still needed to pave the way for the development of oral medication from the plant.[42]With regards to consistency in the antimicrobial activity demonstrated, issues may arise that may be attributed to the lack of solubility of the active constituents of *M. Micrantha*, depending on the extraction method employed.[43] The state of *M. micrantha* is not at equilibrium due to varying environmental conditions which may directly affect the growth condition of the species. The bioclimatic variables of the dry and cold temperature brought about by climate change can significantly affect the progression of the species. Hence, this poses a risk of yielding inconsistent data if a long-term study is conducted.[4] Banerjee and colleagues also discussed the long lag period of *M. micrantha* during its expansion due to its isolation to small populations and lack of

reliable quantitative data regarding its diversity; hence making it hard to obtain these species in the local setting. Although the Philippines possess a wide array of medicinal plants that may provide alternative remedies, extensive research on and application of traditional medicine is yet to be explored, particularly the species being studied.[2]

CONCLUSION

Mikania micrantha is shown to have diverse pharmacological, antimicrobial, anticancer, anti-inflammatory, anti-nociceptive, antioxidant, anti-diabetic, analgesic and anti-parasitic properties. It hosts a number of phytochemicals of therapeutic value which contribute to such activities. Its major phytochemical constituents include sesquiterpene lactones, diterpenes, flavonoids and phenolic compounds. Sesquiterpenoids make up a majority of *M. micrantha* extract: caryophyllene oxide in particular showed an MIC of 0.073 mg/mL *S. aureus* while trans-caryophyllene showed an MIC and MBC of 0.25 mg/mL. The mikanolides, notably deoxy mikanolide, scandenolide, dihydroscandenolide, mikanolide, dihydromikanolide, and m - methoxy benzoic acid showed substantial antimicrobial activity against *S. aureus* with MIC and MBC values ranging from 62.5 to 1000 µg/mL. Flavonoids like quercetin have shown strong antibacterial activity against *S. aureus* with an MIC of 6.25 µg/mL, while tannin polyphenols exhibited bactericidal and inhibitory effects at a concentration of 0.78 and 1.56 mg/mL. Thus the data indicates probable antibacterial activity of *M. micrantha* against *S. aureus* in a range of varying susceptibility. Moreover, *M. micrantha* ethanol extract was found to be effective against MRSA strains resistant to about five commonly used antibiotics. Additionally, tannins, triterpenoids and sesquiterpene alcohols nerolidol and bisabolol have all exhibited synergism with commonly used antibiotics against *S. aureus* and MRSA thus reducing resistance to these drugs.

Collectively, most bioactive substances sourced from *M. micrantha* extract exhibited antibacterial activity against *S. aureus* with some exhibiting activity against MRSA. The corresponding MICs for each compound are variable and those with MICs greater than commonly used antibiotics may not be used independently as a therapeutic source. Since some aforementioned compounds have shown synergism with antibiotics, administration of combined therapy may improve the pharmacologic properties of the extract and may have potential in reducing drug dosage in clinical use. In effect, *M. micrantha* extract may be a promising source of antibacterial compounds for therapeutic use against *S. aureus* and MRSA. While some bioactive substances show activity against *S. aureus* and MRSA, it is yet to be discovered if the extract as a whole is substantially effective against MRSA compared to commonly used antibiotics. Furthermore, the number of clinical and pharmacologic studies concerning *M. micrantha* and *S. aureus* as well as MRSA is low. While this review compiles and assesses key studies concerning the phytochemical constituents of *M. micrantha* and its activity against *S. aureus*, further studies may provide information about the antibacterial mechanisms of action and therapeutic activity of *M. micrantha* constituents against *S. aureus* and MRSA in the future. In line with this, isolation of pure compounds must be considered to determine the metabolites responsible for their bioactivities and to detect the presence of synergistic interactions among them. Consequently, the compounds mentioned may be employed in the formulation of novel drugs and disinfectants in the future.

ACKNOWLEDGEMENT

The authors would like to express their gratitude to their parents and families for the moral and unending support. Without their untiring love this would not be possible. The authors also would like to thank the faculty of the Department of Medical Technology, Far Eastern University for their academic guidance.

Authors' Contributions

All authors contributed to the data extraction and analysis, draft, and revision of the review. All of them gave final approval of the version to be passed and agreed to be accountable for all aspects of the work.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Cite this article: Baguingan SAB, Macadangdang RRJ, Bermejo JP, Bondoc PE, Cartina MLL, Liwa ANS, *et al*. Efficacy of *Mikania micrantha* Extract against Methicillin-resistant *Staphylococcus aureus*: A Mini Review. Asian J Biol Life Sci. 2021;10(2):280-90.