MedCrave
Step into the World of Research
International Journal of Complementary & Alternative Medicine

Research Article

Open Access

Systematic review and meta-analysis of plant-derived antimicrobials in WHO priority pathogens

Abstract

Background: Antimicrobial resistance (AMR) threatens the effective prevention and treatment of a growing range of infections and requires immediate intersectoral action. A report into the global burden of AMR estimated that AMR contributed to 700,000 deaths in 2016 and is expected to reach 10 million deaths annually by 2050. Plant-derived antimicrobials (PDAms) have demonstrated the ability to combat multi-drug-resistant (MDR) pathogens and hope exists that they may help control AMR. This review investigated and recorded the antimicrobial activity (AMA) of whole plant ethanolic and methanolic extracts in WHO priority pathogens (WHO PPs) reported between 2000 until present. The review collated studies on PDAms tested on WHO PP laboratory isolates, determined efficacious PDAms, and grouped PDAms by WHO PP and antimicrobial susceptibility test (AST).

Methods: A comprehensive search of *PubMed* and *Google* databases using a combination of MeSH and free text terms was conducted from April 28th - May 8th 2020 of *in-vitro* studies determining the AMA of angiosperms in WHO PPs. Endpoints included minimum inhibitory concentration (MIC), zone of inhibition (ZOI) and half-maximal inhibitory concentration (IC50). This systematic review and meta-analysis was reported according to PRISMA guidelines. A modified STROBE-AMS checklist was applied to included studies. Limitations are that a double screening selection process is generally Scholar recommended.

Results: Data were pooled and computed in a meta-analysis. Thirty-four studies on PDAm-WHO priority specific data were identified**.** Effective PDAms were grouped by WHO PP and AST. PDAm activity was demonstrated in 50% of WHO PPs. *Piper betle L*. demonstrated the greatest AMA in six WHO PPs (50%). Methicillin-resistant *Staphylococcus aureus* (MRSA) was the most commonly studied WHO PP (69.6%), returning forty-three PDAms. No studies were returned on clarithromycin-resistant *Helicobacter pylori,* fluoroquinolone-resistant *Campylobacter* spp. fluoroquinolone-resistant *Salmonellae,* penicillin-non-susceptible *Streptococcus pneumoniae*, ampicillin-resistant *Haemophilus influenzae* and fluoroquinolone-resistant *Shigella* spp.

Conclusion: This synthesis of the available studies and identification of the quality data may help to inform and prioritise future research on PDAms in WHO PPs.

Volume 15 Issue 2 - 2022

Carina Harkin
University College Cork, Ireland

Correspondence: Carina Harkin, University College Cork, Cork City, 24 Waterlane, Galway city, Co. Galway, Ireland, Email carahealth@gmail.com

Received: March 30, 2022 | **Published:** April 12, 2022

Abbreviations

AMA, antimicrobial activity; AMR, antimicrobial resistance; AMRIC, the antimicrobial resistance and infection control division; AMU, antimicrobial use; AB, antibiotics; AR, antibiotic resistance; AST, antimicrobial susceptibility testing; CPE, carbapenemase-producing enterobacterales; CLSI, clinical and laboratory standards institute; CAM, complementary and alternative medicine; EUCAST, european committee on antimicrobial susceptibility testing; *E. coli*, ESBL-*E.Escherichia coli*; ESBL-E, extended-spectrum β-lactamase-producing enterobacterales; GPB, gram positive bacteria; GNB, gram negative bacteria; IC50, half maximal inhibitory concentration; HCAIs, healthcare associated infections; HPSC, health protection surveillance centre; MRSA, methicillin-resistant *staphylococcus aureus*; MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration; MDR, multidrug resistant; MDRO, multidrug resistant organisms; PDAms, plant-derived antimicrobials; QS, quorum sensing; R&D, research and development; RMAs, resistance-modifying agents; STROBE-AMS, STROBE for antimicrobial stewardship; SSIs, surgical site infections; VRE, vancomycin-resistant Enterococci; WHO, world health organisation; WHO PPs, WHO priority pathogens; ZOI, zone of inhibition

Background

Antimicrobial Resistance (AMR) occurs when microorganisms, including bacteria, fungi, viruses and parasites, adapt to become resistant to their counterpart antimicrobial drugs. Antimicrobial-resistant microbes are found in people, animals, food, water, soil and air, and can spread from person to person or person to animal. The spread is magnified by poor infection control, sanitation and food handling.[1] Overprescribing antimicrobials is the main driver of AMR.[2] A report by Jim O'Neill report entitled 'The Review on Antimicrobial Resistance' found that AMR contributed to 700 000 deaths in 2016 and is predicted to reach 10 million deaths annually by 2050. The foreword of this report admitted that this figure did not account for the secondary effects of AMR such as the risks imposed by surgery.[3] Additionally, former UK Chief Medical Officer (CMO) Sally Davies highlighted the magnitude of underreporting of AMR deaths and called for AMR to be put on relevant death certificates.[4] A Royal Institute of International Affairs review on AMR progress, estimated a cost to the global economy of US$100 trillion by 2050.[5]

Antibiotic resistance

Antibiotic Resistance (AR) is a subset of AMR and applies to bacteria becoming resistant to antibiotics.[6] In 2017 the WHO published a WHO priority pathogens (WHO PPs) list of AR bacteria requiring urgent research and development (R&D) for new antibiotics.[7] The WHO PPs are a catalogue of twelve families of bacteria that are categorised into three priority levels. The WHO PP list was developed in collaboration with the Division of Infectious Diseases Tübingen

University whereby a multicriteria decision analysis was used to prioritise these AR bacteria. These criteria included infections with the highest mortality, length of hospital stay required, how frequently resistance is encountered once community spread occurs, the ease of zoonotic or human-to-human transmission, preventability through hygiene practices and or vaccines, therapeutics, and whether new AB were already in the R&D channel.[8] Table 1 describes the WHO Priority Pathogens (Appendix 1 & Appendix 2).

Table 1 WHO Priority Pathogens

Priority 1 Critical	*Acinetobacter baumannii* (carbapenem-resistant *A. baumannii*)
	Pseudomonas aeruginosa (carbapenem-resistant *P. aeruginosa*)
	Enterobacterales (carbapenem-resistant) [carbapenemase-producing *Enterobacterales* (CPE) and extended-spectrum β-lactamase-*producing Enterobacterales* (ESBL-E) *[Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae* (*K. pneumoniae)]*
	Enterococcus faecium (vancomycin-resistant *E. faecium)* [vancomycin-resistant Enterococci (VRE)]
	Staphylococcus aureus (methicillin-resistant [MRSA], vancomycin-intermediate *S. aureus*)
Priority 2 High	*Helicobacter pylori* (clarithromycin-resistant *H. pylori*)
	Campylobacter spp. (fluoroquinolone-resistant)
	Salmonellae (fluoroquinolone-resistant)
	Neisseria gonorrhoeae (cephalosporin-resistant, fluoroquinolone-resistant *N. gonorrhoeae*)
	Streptococcus pneumoniae (penicillin-non-susceptible *S. pneumoniae*)
Priority 3 Medium	*Haemophilus influenza* (ampicillin-resistant *H. influenza*)
	Shigella spp. (fluoroquinolone-resistant)

Healthcare-associated infections

The Society of Hospital Medicine (SHM) describes the WHO PPs as the principle nosocomial pathogens.[9] Healthcare-associated infections (HCAIs) lead to an increase in morbidity, mortality and healthcare costs and are a public health emergency. The WHO estimates hundreds of millions of patients are affected by HACAIs annually, yet admits the difficulty in gathering reliable data means the true burden is unknown.[10] The WHO rank HCAIs as the fifth greatest threat to global health and one of the top ten causes of hospital deaths worldwide.[11] AMR is a key driver of HCAIs and increases associated morbidity and mortality.[12] The leading cause of HCAIs is a group of common multidrug-resistant (MDR) bacteria known as ESKAPE pathogens (*E. faecium, S. aureus, K. pneumoniae, A. baumannii, P. aeruginosa* and Enterobacter spp.).[13] The ESKAPE pathogens are extended-spectrum beta-lactamase (ESBL)-producing, the latter four are Gram-negative bacteria (GNB) and all are WHO PPs.[14] As the principal ESBL-Es, *E. coli and K. pneumoniae* have been chosen for the purpose of this review.[15]

A 2017 Point Prevalence Survey of HCAIs and antimicrobial use (AMU) in European Acute Care Hospitals identified the following WHO PPs, methicillin-resistant Staphylococcus aureus (MRSA), vancomycin-resistant Enterococci (VRE), extended-spectrum β lactamase (ESBLs) and carbapenem-resistant or carbapenemase-producing Enterobacterales (CPE), as the pathogens associated with higher healthcare costs, increased length-of-stay and higher mortality.[16] The Antimicrobial Resistance and Infection Control (AMRIC) division section of Ireland's Health Protection Surveillance Centre (HPSC) state the pathogens associated with AMR in Ireland are CPE, VRE, *E. coli, K. pneumoniae, P. aeruginosa* and *N. gonorrhoeae*.[17]

Considering again that the O'Neill report estimate of 10 million deaths annually did not account for the secondary effects of AMR, it is important to contemplate the potential additional global burden. Surgical site infections (SSIs) for example, are one of the most common HCAIs and are associated with increased morbidity and mortality.[18] 77% of surgical patient deaths are related to a SSI.[19] Globally, 300 million people undergo operations annually with 31% resulting in a SSI.[20] Given that between 39% and 51% of SSI pathogens are AR, the figure of 10 million deaths annually is potentially substantially underestimated.[21]

Sepsis is another secondary effect of AMR, with AMR posing a major challenge in sepsis treatment. Paradoxically, antimicrobial stewardship programmes and sepsis are at odds, with delayed, incomplete and ineffective treatment driving AMR and increasing sepsis risk.[22] Current estimates are that 20% of all global deaths are sepsis-related. A review into the global burden of sepsis states that AMR may be a major driver of community and hospital-acquired sepsis.[23] A recent study estimated that in 2017 there were 48.9 million cases of sepsis worldwide and 11 million deaths. Previous global sepsis burden estimates were significantly lower at 30 million cases leading to 6 million deaths.[24] The WHO global priority pathogens list is one of the WHO projects aimed to prevent, diagnose and treat sepsis.[25] A study looking at the contribution of sepsis to mortality in patients with sepsis from two independent cohorts, the Kaiser Permanente Northern California (KPNC) and the Healthcare Cost and Utilization Project (HCUP) Nationwide Inpatient Sample (NIS), found that sepsis contributed to 1 in every 2 to 3 hospital cohort deaths.[26] The reviewers of a 2015 report, 'Just Say Sepsis', by the National Confidential Enquiry into Patient Outcome and Death (NCEPOD) found that in cases where sepsis was not mentioned as a cause of death on the death certificate that it should have been in 48/59 (81.4%) of cases.[27]

Plant-derived antimicrobials

The O'Neill report describes the potential for both vaccines and alternatives to lower antimicrobial use (AMU). These alternatives include phage therapy, lysins, antibodies, probiotics, and peptides and immune stimulation; described specifically as boosting the patient's natural immune system.[3] Herbal medicine is a complementary and alternative medicine (CAM). Herbal Medicine boosts the patient's natural immune system. Herbal immunomodulators have been shown to stimulate the innate and adaptive defence mechanisms of the host to attune the activity of the immune system, thus decrease the inflammatory response.[28]

Pharmacognosy describes the study of plants as drug sources. It is estimated that 25% of modern drugs are directly or indirectly plant-derived. Additionally, more than one third of all FDA-approved new molecular entities (NMEs) are of natural origin.[29,30] 120 plant secondary metabolite phytochemicals have been isolated and identified, with 80% showing a positive correlation between modern therapeutic and traditional use.[31,32] In herbal pharmacology, the sixteen main phytochemical groups are alkaloids, anthocyanins, anthraquinones, cardiac glycosides, coumarins, cyanogenic glycosides, flavonoids, glucosinolates, phenols, saponins and tannins.[33] Phytochemical bioactivity is of particular clinical value as plant strategies to deal with microorganisms adapt over time hence are generally considered not to confer resistance.[34,35] Herbal resistance in clinical bacterial isolates has been demonstrated however and needs to be considered. *E. coli* herbal resistant plasmid for example, has been shown to replicate and be expressed in human urinary tract *S. aureus*.[36,37]

Ethnopharmacology offers a wide array of plant-derived antimicrobials (PDAms) and increasing numbers of pharmaceutical companies are focusing on botanical drug development.[38,39] Evidence suggests PDAms display broad-spectrum activity against both Gram-positive bacteria (GPB) and GNB, and can attenuate bacterial virulence.[40,41] PDAms have exhibited activity against virulence factors including quorum sensing (QS), biofilms, motility, toxins, pigments, enzymes and bacterial surfactants.[42,43] Antibiotic discovery is not keeping up with the evolution of resistance and urgency exists to find novel antimicrobial agents. Plant secondary metabolite phytochemicals and whole plant extracts have demonstrated their potential as either stand-alone antibacterials or as potentiators of conventional antibacterial agents. As yet however, the therapeutic application of PDAms remains to be clinically proven.

The relevance of *in-vitro* antimicrobial susceptibility testing (AST) in ascertaining the antimicrobial activity (AMA) of plants cannot be overemphasised. Due to use of various non-standardised approaches including inoculum preparation method, inoculum size, growth medium, atmosphere, temperature, incubation duration and end-point determination however, comparisons between results are difficult. ASTs themselves have certain advantages and disadvantages. Aiming to ensure an accurate experimental approach is used that can allow a comparison of results, accepted and approved standards are published by the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST), two global organisations that establish interpretive criteria for *in-vitro* susceptibility data.[44]. Table 2 provides a summary of the pros and cons of *in-vitro* ASTs used in PDAms.

A group of international experts came together through a joint initiative by the European Centre for Disease Prevention and Control (ECDC) and the Centers for Disease Control and Prevention (CDC) to create a standardized international terminology with which to describe acquired resistance profiles in *Staphylococcus aureus*, Enterococcus spp., Enterobacteriaceae (other than *Salmonella* and *Shigella*), *Pseudomonas aeruginosa and Acinetobacter spp*., all bacteria often responsible for healthcare-associated infections and prone to multidrug resistance. Clinical Laboratory Standards Institute (CLSI), the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the United States Food and Drug Administration (FDA).[45]

A variety of herbal preparation methods exists also. The European Medicine Agency and the European Directorate for the Quality of Medicines and HealthCare state the most commonly used solvents are ethanol, methanol and water. Ethanol and methanol are frequently used in herbal extraction techniques such as maceration, percolation and soxhlet extraction as their polarity maximises phytochemical extraction and complies with their European Pharmacopoeia monographs.[46, 47]

Previous systematic reviews on the cidal activity of PDAms in MDR-bacteria exist, however these review the phytochemical classes of plant-derived resistance-modifying agents (RMAs) to potentiate other antibacterial agents. None of these reviews are on WHO PP specific susceptible strains of bacteria.[48-50] As no reviews on PDAMs and WHO specific PPs exist, this systematic review and meta-analysis is a worthwhile study and may contribute to the body of existing research.

Aims

The aim of this review was to collate information on PDAms tested on WHO PP bacterial isolates and to determine the most efficacious PDAms.

Objectives

Study objectives were to conduct a systematic review and meta-analysis of the data in order to answer the research question.

Hypothesis

PDAms have demonstrated efficacious AMA in WHO PPs.

Review question

In applying the feasible, interesting, novel, ethical, and relevant (FINER) Cochrane standards and the PICO technique requiring consideration of patient population, intervention (exposure), comparison (control) and outcome, the research question is which PDAms have demonstrated AMA in WHO PPs and which of these has demonstrated the greatest AMA in each WHO PP.[51,52]

Methods

Study design

This systematic review and meta-analysis was reported according to the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) framework.[53].

Study Selection

Table 3 displays the inclusion/exclusion criteria that set the boundaries for the review.

Table 2 Summary of *in-vitro* ASTs used in PDAms: Pros and Cons

AST	Pros	Cons
Diffusion Methods		
Agar disk diffusion *also known as* Kirby-Bauer test (J. Hudzicki, 2009; p.1)	Widely used to determine the AMA of plants. Provides zones of inhibition (ZOI) (Balouiri M. et al. 2016; p. 74). Standards provided by the CLSI and EUCAST are qualitative; can be quantitative according to CLSI categories of susceptible, intermediate and resistant (Jorgensen and Ferraro, 2009; p.1751). ZOI can be compared with that of different antibiotic standards in an antibiogram and described as susceptible, intermediate or resistant (Balouiri M. et al. 2016; p. 72).	Not all fastidious bacteria can be tested accurately however standardisation allows testing of certain fastidious bacterial pathogens (Balouiri M. et al., 2016; p. 72). Impossibility to measure amount of test compound diffused into agar medium. Not appropriate to determine MIC; MIC values are approximated using stored algorithms (Balouiri M. et al. 2016; p. 72).
Agar well diffusion	Widely used to determine the AMA of plants. Provides ZOI (Balouiri M. et al. 2016; p. 74).	
Thin-layer chromatography (TLC)–bioautography	Used to separate complex mixtures into bioactive compounds (Balouiri M. et al. 2016; p. 74).	Bioautography is not a quantitative measure of antimicrobial activity *(Suleiman, M.M., et al.*, 2009, p. 67).
Dilution methods		
Broth microdilution method	Quantitative measurement. The gold standard for minimum inhibitory concentration (MIC) determination (Lallemand E.A. et al., 2016; p 1). Provides the most consistent results, allowing determination of relative species sensitivities and relative AMA of each medium (King T. et al., 2008; p. 1423). *Can be used to calculate the* half maximal inhibitory concentration (IC50), the concentration of a drug required to inhibit 50% of a microorganism *in vitro*. IC50 is a more accurately definable measure than the *MIC* and standard error is easily calculable (Soothill J.S. et al.,1992; p. 137).	
Broth macrodilution method	MIC determination.	Tedious, manual and high risk of error (Balouiri M. et al. 2016; p. 75).
Agar dilution method	Agar dilution is preferred over broth dilution in determining MIC when the compound/ extract being tested is coloured and masks microbial growth detection in the liquid medium (Balouiri M. et al. 2016; p. 76). Possible to estimate concentration of test compound in agar medium therefore the most commonly used (Balouiri M. et al. 2016; p. 76). Standards provided by CLSI and EUCAST (Balouiri M. et al. 2016; p. 75).	

Table 3 Inclusion/exclusion criteria

Criteria	Inclusion	Exclusion
Population	WHO PP species and isolate identified. *E. coli* and *K. pneumoniae* as most prominent carbapenem-resistant and ESBL-producing bacteria. *Enterobacterales*.	Isolate not identified. Non human pathogens. Non-WHO PPs.

Table Continued...

Criteria	Inclusion	Exclusion
Intervention	Crude plant methanol (CH_3OH) or ethanol (C2H6O) extracts only.	Acetone, dichloromethane, chloroform, hexane, ethyl acetate solvent. Essential oils. Counterpart phytochemical or bioactive compound. Honey, gums, resins and juices. Endophytes. Nanoparticles.
Comparator	No intervention. Conventional antimicrobial.	NA
Outcomes	Qualitative ZOI in mm, or *susceptible*, *intermediate*, and *resistant*. MIC. IC50.	Minimum *Bactericidal* Concentration (MBC).
Study Design	**Diffusion Methods** Agar disk-diffusion. Agar well-diffusion. **Dilution Methods** Broth microdilution/ microtitre broth microdilution.	Bioassay-guided fractionation ASTs on fungi, lichen (archaea and protists). Synergism with antibiotics. In-vivo tests. TLC-bioautography studies. Antiviral studies. Synergistic combination studies.
Other	Free PMC studies available or PDFs received through Research Gate requests.	Full text links unavailable Reviews/Theses Pre 2000

Search strategy

Table 4 displays the search strategies for bibliographic databases used in conducting the systematic review.

Information sources

The MeSH database was consulted to identify concepts and choose appropriate terms and combinations of free-text and controlled-vocabulary terms. Prior to establishing these search criteria, a scoping exercise was used to ensure that the search terms 'drug resistance, microbial' returned individual WHO PPs and the search term 'angiosperms' returned the most diverse group of PDAms being flowering plants (mosses and ferns were excluded). An online literature search of *PubMed /Google Scholar* bibliographic databases was conducted from April 28th –May 8th 2020 using a combination of MeSH and free text terms. The exact search terms used in both *PubMed* and *Google Scholar* were; Drug Resistance, Microbial, Angiosperms, Antimicrobial Tests; returning 1115 and 17100 results respectively. It was intended that 1000 results only be downloaded from *Google Scholar.* In the event where "Drug Resistance, Microbial" alone did not return a particular WHO PP, the specific pathogen was searched in addition although this tactic did not produce any additional returns.

Study records

Data management

Google scholar was set at 10 results per page. When activity was flagged as suspicious a virtual private network (VPN) service provider was used to change the IP address and the CAPTCHA I am not a robot checked. The Zotero plug-in was used to export citations. Only 98 page results were accessible, totalling 980 returns in total rather than the planned 1000. All *PubMed* results were imported into Endnote, 200 at a time, using "send to citation manager" providing 1115 results in total. Endnote was used to store, manage, merge and remove duplicates.

Selection process

First level single screening

A first level screening of titles and abstracts was conducted and inclusion/exclusion criteria applied.[54]

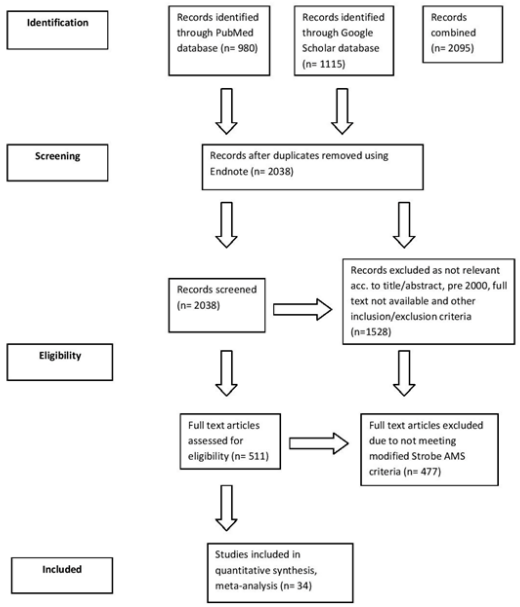

Figure 1 Study flow diagram based on Moher D et al.,[53] PRISMA 2009 Flow Diagram.

Study	Item #	Recommendation	Reported Yes	Page #	Relevant text from manuscript
Title and Abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract (b) Provide in the abstract an informative and balanced summary of what was done and what was found			
Introduction					
Background Rationale	2	Explain the scientific background and rationale for the investigation being reported			
Objectives	3	State specific objectives, including any pre-specified hypotheses			
Methods					
Study Design	4	Present key elements of study design early in the paper			
Settings	5	Describe the setting			
Subjects	6	Identification of specific laboratory biobanks Identification of specimen as multidrug resistant (MDR) bacteria			
Intervention	7	Reporting of Plant derived antimicrobial (PDAm) species name, part used. Identification of extraction solvent used ethanol / methanol.			
Antimicrobial Susceptibility Test (AST)	8	(a) Reports Clinical & Laboratory Standards Institute (CLSI) or European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines are met. (b) Describes AST method used (diffusion or dilution) including inoculum preparation method, inoculum size, growth medium, atmosphere, temperature, duration of incubation and end-point determination.			
Data sources/ measurement	9	Describes how antimicrobial activity was measured. MIC/MBC/ZOI			
Statistical methods	10	Describe all statistical methods used.			
Results					
Interventions	12	Provides results of AST (MIC/MBC/ZOI)			
Descriptive Data	13	Provides description of these results in terms of qualitative/quantitative.			
Main results	14	Results provide qualitative data ZOI in mm or alternatively quantitative data MICS and ZOI described as *susceptible, intermediate* and *resistant*			
Discussion					
Key Results	15	Summarise key results with reference to study objectives			
Limitations	16	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias			
Interpretations	17	Give a cautious overall interpretation of results and other relevant evidence. Conclusions match results			
Other					
Funding	18	Give the source of funding and the role of the funders for the present study			

Figure 2 Modified STROBE-AMS checklist.

Second level screening

Full text articles were critically appraised using a specifically devised modified STROBE for antimicrobial stewardship (STROBE-AMS) checklist for PDAm ASTs. The modified STROBE was used to assess the internal and external validity of the study, reduce the risk of bias and assure methodological quality and quality of evidence.[55,56] See Figure 2: modified STROBE-AMS checklist.

Table 4 Search strategies for bibliographic databases [98]

	Concept 1	Concept 2	Concept 3	Concept 4
Key concepts	**Antimicrobial Resistance**	**Botanical Medicine**	**Anti-Bacterial**	**Antimicrobial susceptibility tests**
Free text terms / natural language terms	Antibiotic resistance, Multidrug-resistance, Multidrug-resistant organisms.	Herbal Medicine, Chinese Herbal Medicine. Angiosperms. Phytotherapy. Plant extracts.	Antibacterial, Bacteriostatic, Bactericidal.	Minimum Inhibitory Concentration (MIC), Antibiotic sensitivity, Antibiotic susceptibility. Microbial Sensitivity Test. Minimum Inhibitory Concentration
Controlled vocabulary terms / Subject terms (MeSH terms, Emtree terms)	Drug Resistance, Microbial (NCBI [Internet] Drug Resistance, Microbial, 1963)	Drugs, Chinese Herbal. Plant Preparations Plants, Medicinal (NCBI [Internet]. Drugs, Chinese Herbal, 1988)	Anti-Bacterial Agents (NCBI [Internet] Drug Resistance, Microbial, 1963)	(NCBI [Internet] Microbial Sensitivity Tests. 2020) Clinical Laboratory Techniques. Disk Diffusion Antimicrobial Tests. (Library Cochrane, Advanced Search MeSH terms., Microbial Sensitivity Tests 2020)

Data collection processes

Data was extracted. Included studies were grouped by WHO PP and AST result. Eight texts were received through Research Gate PDF requests with one only meeting the modified Strobe-AMS criteria.

Data items

The PICO items for which data were sought included WHO PP, whole PDAm methanol or ethanol extracts only and PDAm activity in WHO PP demonstrated.

Outcomes and prioritisation

The primary outcome was to retrieve a sufficient number of studies determining the AMA of PDAms in WHO PPs. The secondary outcome was to determine the most efficacious PDAm in each WHO PP. Full data is included in the appendices.

Data synthesis and analysis

Data were pooled by pathogen, plant, AST and computed in a meta-analysis.

Figure 1 shows the study flow diagram based on Moher D et al., PRISMA 2009 Flow Diagram.

Results

34 studies on PDAm-WHO priority specific data were identified. PDAm activity was demonstrated in 50% of WHO PPs. *Piper betle* L. demonstrated the greatest AMA in 7 out of the 13 WHO PPs (53.9%). *S. aureus* was the most commonly studied pathogen (69.6%), returning 43 PDAm species. Results data are tabulated below.

Priority 1

PDAm activity was demonstrated in all three priority 1 pathogens; carbapenem-resistant *A. baumannii* and *P. aeruginosa,* and in ESBL-producing and carbapenem-resistant *E. coli* and *K. pneumoniae* Enterobacter spp. 4 out of 34 (11.8%) studies returned confirmed PDAm activity in carbapenem-resistant *A. baumannii*. 7 PDAms demonstrated AMA. *Piper betel* L. demonstrated both the lowest MIC and greatest ZOI.[57-60] Table 5 indicates that *Piper betle* L. demonstrated the greatest AMA against carbapenem resistant *A. baumannii* with the lowest MIC of 406 µg/ml.[57] *Terminalia bellirica* demonstrated a similar MIC of 500 µg/ml.[58] Table 6 indicates that *Martynia annua* L. IC50 was 256 µg/ml. Table 7 indicates *Piper betle* L. demonstrated the highest ZOI of 24mm.[57]

4 out of 34 (11.8%) studies returned confirmed PDAm activity in carbapenem-resistant *P. aeruginosa*. 7 PDAms demonstrated AMA. *Piper betel* L. demonstrated both the lowest MIC and greatest ZOI.[57-59, 61] Table 8 indicates that *Piper betle* L. demonstrated the greatest AMA in the average of 3 strains, exhibiting the lowest MIC of 260 µg/ml.[57] *Plectranthus barbatus* and *Terminalia bellirica* demonstrated equal MICs of 500 µg/ml.[58,59] Table 9 indicates that *Piper betle* L. demonstrated the largest ZOI of 21.33mm followed by *Terminalia bellirica* of 17.55 mm and then *Nigella sativa* L. of 13mm.[57, 58, 61]

Priority 1: Critical

Table 5 MIC Results: *Acinetobacter baumannii* (carbapenem-resistant)

Plant part and solvent used	MIC µg/ml
Piper betle L. (leaf ethanol)	406 (mean of 5 MDR *A.baumannii* strains) (Valle DL Jr. et al., 2016; p.9)
Terminalia bellirica (fruit methanol)	500 (Dharmaratne MPJ. et al., 2018; p. 8)
Piper betle L. (leaf methanol)	526.4 (mean of 5 MDR *A.baumannii* strains) (Valle DL Jr. et al., 2016; p.9)
Rosmarinus officinalis (leaf ethanol):	1000 (Assis FV. et al., 2018; p. 1667)
Plectranthus barbatus (leaf ethanol):	2000 (mean of 2 *A.baumannii* strains) (Assis FV. et al., 2018; p. 1667)
Ocimun basilicum (leaf ethanol):	2000 (Assis FV. et al., 2018; p. 1667)
Mentha spp. (leaf ethanol):	2000 (Assis FV. et al., 2018; p. 1667)

Table 6 IC50 Results: *Acinetobacter baumannii* (carbapenem-resistant)

Plant part and solvent used	IC50 µg/ml
Martynia annua L. (leaf ethanol):	256 (Khan MF. et al., 2018; p. 6)

Table 7 ZOI Results: *Acinetobacter baumannii* (carbapenem-resistant)

Plant part and solvent used	ZOI mm
Piper betle L. (leaf ethanol)	24 (mean of 5 MDR *A.baumannii* strains) (Valle DL Jr. et al., 2016; p.7)
Terminalia bellirica (fruit methanol)	17 (mean of 2 *A.baumannii* strains) (Dharmaratne MPJ. et al., 2018; p. 7)

Table 8 MIC Results: *Pseudomonas aeruginosa* (carbapenem-resistant)

Plant part and solvent used	MIC µg/ml
Piper betle L. (leaf ethanol)	260 (Valle DL Jr. et al., 2016; p.9)
Terminalia bellirica (fruit methanol)	500 (Dharmaratne MPJ. et al., 2018; p. 8)
Plectranthus barbatus (leaf ethanol)	500 (Assis FV. et al., 2018; p. 1667)
Mentha spp. (leaf ethanol)	2000 (Assis FV. et al., 2018; p. 1667)
Ocimun basilicum (leaf ethanol)	>2000 (Assis FV. et al., 2018; p. 1667)
Rosmarinus officinalis (leaf ethanol)	>2000 (Assis FV. et al., 2018; p. 1667)

Table 9 ZOI Results: *Pseudomonas aeruginosa* (carbapenem-resistant)

Plant part and solvent used	ZOI mm
Piper betle L. (leaf ethanol)	21.33mm (Valle DL Jr. et al., 2016; p.7)
Terminalia bellirica (fruit methanol)	17.55 (Dharmaratne MPJ. et al., 2018; p. 7)
Nigella sativa L. (leaf methanol)	13mm P < 0.001 (Salman MT. et al., 2009; p.99)

13 out of 34 (38%) studies confirmed PDAm activity in Enterobacterales species. Of these Enterobacterales species, 6 out of 34 studies were returned for *E. coli* and 7 out of 34 studies were returned for *K. pneumoniae*. 4 out of 34 (11.8%) studies returned confirmed PDAm activity in ESBL-producing *E. coli*. 7 PDAms demonstrating AMA. *Nauclea latifolia* demonstrated the lowest MIC and *Piper betel* L. demonstrated the greatest ZOI.[57,58, 62, 63] Table 10 indicates that *Nauclea latifolia* demonstrated the lowest MIC of 32 µg/ml. Table 11 indicates that *Piper betle* L. demonstrated the greatest ZOI of 20mm. 2 out of 34 (5.9%) studies confirmed PDAm activity in carbapenem-resistant *E. coli*. 2 PDAms demonstrated AMA. *Croton campestris* demonstrated the lowest MIC.[64, 65] Table 12 indicates that *Croton campestris* demonstrated the lowest MIC of 512 µg/ml.

4 out of 34 (11.8%) studies returned confirmed PDAm activity in ESBL-producing *K. pneumoniae*. 4 PDAms demonstrated AMA. *Piper betel* L. demonstrated both the lowest MIC and greatest ZOI.[57,59,66] Table 13 indicates that *Piper betle* L. demonstrated the lowest MIC of 625µg/ml. Table 14 indicates that *Martynia annua* L. demonstrated an IC50 of 256 µg/ml. Table 15 indicates that *Piper*

betle L. demonstrated the highest ZOI of 20mm. 3 out of 34 (8.8%) studies returned confirmed PDAm activity in carbapenem-resistant *K. pneumoniae*. 8 PDAms demonstrated AMA. *Piper betel* L. demonstrated both the lowest MIC and greatest ZOI.[57, 59, 66] Table 16 indicates that *Piper betle* L. demonstrated the lowest MIC of 390µg/ml. Table 17 indicates that *Alstonia scholaris* demonstrated the lowest mean IC50 of 4060 µg/ml. Table 18 indicates that *Piper betle* L. demonstrated the largest ZOI of 23mm.

Table 10 MIC Results: *Escherichia coli* (ESBL-producing)

Plant part and solvent used	MIC µg/ml
Nauclea latifolia (stem bark methanol)	32 (Tekwu EM.,et al, 2012; p. 269)
Albizia gummifera (stem bark and leaves methanol)	128 (Tekwu EM. et al, 2012; p. 269)
Ficus exasperate (leaf methanol)	128 (Tekwu EM. et al., 2012; p. 269)
Ricinodendron heudelotii (stem bark methanol)	312 (Tekwu EM. et al., 2012; p. 269)
Piper betle L. (leaf ethanol)	312 (Valle DL Jr. et al., 2016; p.9)
Terminalia bellirica (fruit methanol)	>500 (Dharmaratne MPJ. et al., 2018; p. 8)
Vitex doniana (bark methanol)	7142 (Ouattara A. et al., 2013; p 99)

Table 11 ZOI Results: *Escherichia coli* (ESBL-producing)

Plant part and solvent used	ZOI mm
Piper betle L. (leaf ethanol)	20 (Valle DL Jr. et al., 2016; p.7)
Albizia gummifera (stem bark and leaves methanol)	16–20 (Tekwu EM., et al, 2012; p. 268)
Ficus exasperate (leaf methanol)	16–20 (Tekwu EM., et al, 2012; p. 268)
Nauclea latifolia (stem bark methanol)	16–20 (Tekwu EM., et al, 2012; p. 268)
Ricinodendron heudelotii (stem bark methanol)	11–15 (Tekwu EM., et al, 2012; p. 268)
Vitex doniana (bark methanol)	13 (Ouattara A., et al., 2013; p 97)
Terminalia bellirica (fruit methanol)	12 (Dharmaratne MPJ. et al., 2018; p. 7)

Table 12 MIC Results: *Escherichia coli* (carbapenem-resistant)

Plant part and solvent used	MIC µg/ml
Croton campestris (leaf methanol)	512 (Matias EF. et al., 2011; p.307)
Murraya paniculata (leaf ethanol)	1024 (Menezes IR. et al., 2015; p. 5)

Table 13 MIC Results: *Enterobacterales: Klebsiella pneumoniae* (*ESBL-producing*)

Plant part and solvent used	MIC µg/ml
Piper betle L. (leaf ethanol)	625 (Valle DL Jr. et al., 2016; p.9)
Terminalia bellirica (fruit methanol)	>5000 (Dharmaratne MPJ. et al., 2018; p. 8)
Vitex doniana (bark methanol)	71420 (Ouattara A. et al., 2013; p 99)

Table 14 IC50 Results: *Klebsiella pneumonie* (*ESBL-producing*)

Plant part and solvent used	IC50 µg/ml
Martynia annua L. (leaf ethanol):	256 (Khan MF. et al., 2018; p. 6)

Table 15 ZOI Results: *Klebsiella pneumoniae* (*ESBL-producing*)

Plant part and solvent used	ZOI mm
Piper betle L. (leaf ethanol)	20 (Valle DL Jr. et al., 2016; p.7)
Terminalia bellirica (fruit methanol)	12 (Dharmaratne MPJ. et al., 2018; p. 7)
Vitex doniana (bark methanol)	11 (Ouattara A. et al., 2013; p 97)

Table 16 MIC Results: *Klebsiella pneumoniae* (carbapenem-resistant)

Plant part and solvent used	MIC µg/ml
Piper betle L. (leaf ethanol)	390 (Valle DL Jr. et al., 2016; p.9)
Rosmarinus officinalis (leaf ethanol)	500 (Assis FV. et al., 2018; p. 1667)
Plectranthus barbatus (leaf ethanol)	>2000 (Assis FV. et al., 2018; p. 1667)
Ocimun basilicum (leaf ethanol)	>2000 (Assis FV. et al., 2018; p. 1667)
Mentha sp. (leaf ethanol)	>2000 (Assis FV. et al., 2018; p. 1667)

Table 17 IC50 Results: *Klebsiella pneumoniae* (carbapenem-resistant)

Plant part and solvent used	Mean IC50 values 8 hr (µg/ml)
Alstonia scholaris (stem ethanol)	4060 µg/ml (Bonvicini F. et al., 2014; p. 3)
Tinospora cordifolia (stem ethanol)	7070 µg/ml (Bonvicini F. et al., 2014; p. 3)
Crataeva nurvala (stem ethanol)	>12500 µg/ml (Bonvicini F. et al., 2014; p. 3)

Table 18 ZOI Results: *Klebsiella pneumoniae* (carbapenem-resistant)

Plant part and solvent used	ZOI mm
Piper betle L. (leaf ethanol)	23 (Valle DL Jr. et al., 2016; p.7)

Priority 2

PDAm activity was demonstrated in 3 out of 6 priority 2 pathogens; vancomycin-resistant *E. faecium*, methicillin-resistant and vancomycin-intermediate *S. aureus* and cephalosporin-resistant and fluoroquinolone-resistant *N. gonorrhoeae*. 3 out of 34 (8.8%) studies confirmed PDAm activity in vancomycin-resistant *E. faecium*. 5 PDAms demonstrated AMA. *Piper betel* L. demonstrated both the lowest MIC and greatest ZOI.[57, 60,67] Table 19 indicates that Piper betle L. demonstrated the lowest MIC of 64.67µg/ml. Table 20 indicates that *Adiantum capillus, Artemisia absinthium and Zanthoxylum armatum* demonstrated the same IC50 of 256 µg/ml. Table 21 indicates that *Piper betle* L. demonstrated the largest ZOI of 28.3 mm.

23 out of 34 (66.7%) studies confirmed PDAm activity in MRSA. 43 plant species demonstrated AMA.[57, 58, 60, 62, 65-83] 3 of these studies reported results in mean IC50 values.[60,66,82] *Tecoma stans* demonstrated the lowest MIC, *Castanea sativa* demonstrated the lowest IC50 and *Piper betel* L. demonstrated the greatest ZOI. Table 22 indicates that *Tecoma stans* demonstrated the lowest MIC of 7.81 µg/ml. Table 23 indicates that *Castanea sativa* demonstrated the lowest IC50 of 16 µg/ml. Table 24 indicates that *Piper betle* L. demonstrated the largest ZOI of 32.7mm. Table 24 also indicates that *Thuja orientalis* and *Psidium guajava* had similar ZOIs of >29mm. 1 out of 34 (2.9%) of studies confirmed PDAm activity in vancomycin-intermediate *S. aureus*. 9 PDAms demonstrated AMA. *Punica granatum* and *Anogeissus acuminate* equally demonstrated the lowest MIC and the greatest ZOI.[84] Table 25 indicates that Anogeissus acuminate and *Punica granatum* demonstrated the lowest MIC of 290µg/ml. Table 26 indicates that Anogeissus acuminate also demonstrated the largest ZOI of 27 mm followed closely by *Punica granatum* at 26mm.

No studies were returned on priority 2 clarithromycin-resistant *H. pylori*, fluoroquinolone-resistant *Campylobacter* spp. and fluoroquinolone-resistant *Salmonellae*. 1 out of 34 (2.9%) studies confirmed PDAm activity in fluoroquinol one-resistant *N. gonorrhoea*. 2 PDAms demonstrated AMA. *Tagetes erecta* L and *Myrteola nummularia* demonstrated equal ZOI of 27 mm.[85] Table 27 indicates that *Tagetes erecta* L and *Myrteola nummularia* demonstrated equal ZOI of 27 mm.

1 out of 34 (2.9%) studies confirmed PDAm activity in both fluoroquinolone-resistant and cephalosporin-resistant *N. gonorrhoeae*. The fluoroquinolone-resistant and cephalosporin less sensitive WHO K and L strains were equally highly sensitive to all 15 plant extracts tested. The AMA of the plant extracts against *N. gonorrhoeae* was detected by measuring the ZOI. The percentage inhibition was then determined by considering inhibition by penicillin (0.5 IU) and ciprofloxacin (1 g) to be 100% for antibacterial activity and comparing the extracts ZOIs with these standards.[86] Table 28 indicates that all *N. gonorrhoeae* (cephalosporin-resistant, fluoroquinolone-resistant) were highly sensitive to all 15 PDAms with the percentage inhibition of the ZOI being ≥100%.

Priority 2: High

Table 19 MIC Results: *Enterococcus faecium* (vancomycin-resistant)

Plant part and solvent used	MIC µg/ml
Piper betle L. (leaf ethanol)	64.67 (Valle DL Jr. et al., 2016; p.9)

Table 20 IC50 Results: *Enterococcus faecium* (vancomycin-resistant)

Plant part and solvent used	IC50 µg/ml
Adiantum capillus (whole plant ethanol)	256 (Khan MF. et al., 2018; p. 6)
Artemisia absinthium (aerial parts ethanol)	256 (Khan MF. et al., 2018; p. 6)
Zanthoxylum armatum (fruit ethanol)	256 (Khan MF. et al., 2018; p. 6)

Table 21 ZOI Results: *Enterococcus faecium* (vancomycin-resistant)

Plant part and solvent used	ZOI mm
Piper betle L. (leaf ethanol)	28.3 (Valle DL Jr. et al., 2016; p.7)
Cinnamomum porrectum (stem bark methanol)	7.5 ± 0.7071(Buru AS. Et al., 2014; p. 3)

Table 22 MIC Results: *Staphylococcus aureus* (methicillin-resistant)

Plant part and solvent used	MIC µg/ml
Tecoma stans (leaf ethanol)	7.81 (Bakr RO. et al., 2019; p. 5)
Nauclea latifolia (stem bark methanol)	64 (Tekwu EM. et al, 2012; p. 269)
Zingiber officinale (rhizome ethanol)	68.45 (Karuppiah and Rajaram, 2012; p. 600)
Allium sativum (cloves ethanol)	78.90 (Karuppiah and Rajaram, 2012; p. 600)
Quercus infectoria (gall ethanol)	80 (Wan Nor Amilah WA. et al., 2014; p. 5)
Terminalia fagifolia (stem bark methanol)	100 (de Araujo AR. et al., 2015; p. 6)
Piper betle L. (leaf ethanol)	167.14 (Valle DL Jr. et al., 2016; p.9)
Punica granatum (seeds ethanol)	250 (all 16 MRSA strains) (Machado TB. et al. p. 282)
Tabebuia avellanedae (wood ethanol)	250 (all 16 MRSA strains) (Machado TB. et al. p. 282)
Terminalia bellirica (fruit ethanol)	250 (Dharmaratne MPJ. et al., 2018; p. 8)
Albizia gummifera (stem bark and leaves methanol)	256 (Tekwu EM. et al, 2012; p. 269)
Hygrophila auriculata (root methanol)	500 (Savitha and Yoganant, 2016; p. 198)
Jasminum sambac (leaf ethanol)	500 (Al-Hussaini and Mahasneh, 2009; p. 3430-31)
Jasminum sambac (flower ethanol)	500 (Al-Hussaini and Mahasneh, 2009; p. 3430-31)
Lavandula angustifolia (flower)	500 (Al-Hussaini and Mahasneh, 2009; p. 3430-31)
Laurus nobilis (fruit ethanol)	500 (Al-Hussaini and Mahasneh, 2009; p. 3430-31)

Table Continued...

Plant part and solvent used	MIC µg/ml
Laurus nobilis (leaf ethanol)	500 (Al-Hussaini and Mahasneh, 2009; p. 3430-31)
Laurus nobilis (bark ethanol)	500 (Al-Hussaini and Mahasneh, 2009; p. 3430-31)
Populus nigra (leaf)	500 (Al-Hussaini and Mahasneh, 2009; p. 3430-31)
Rosmarinus officinalis (leaf ethanol)	500 (Al-Hussaini and Mahasneh, 2009; p. 3430-31)
Tecoma capensis (flower ethanol)	500 (Al-Hussaini and Mahasneh, 2009; p. 3430-31)
Ficus exasperate (leaf and stem bark methanol)	512 (Tekwu EM. et al, 2012; p. 269)
Ricinodendron heudelotii (stem methanol)	512 (Tekwu EM. et al, 2012; p. 269)
Chuquiraga straminea (flowers methanol)	533.3 µg/ml [average 3 strains] (Mendiondo ME. et al., 2011; p. 966)
Cinnamomum porrectum (stem bark methanol)	625.00 ±0 (Buru AS. Et al., 2014; p. 7)
Cinnamomum iners (stem bark methanol)	625.00 ±0 (Buru AS. Et al., 2014; p. 7)
Sonchus oleraceus (ariel ethanol)	750 (Al-Hussaini and Mahasneh. 2009; p. 3430-31)
Laurus nobilis (flower ethanol)	1000 (Al-Hussaini and Mahasneh. 2009; p. 3430-31)
Rosmarinus officinalis (flower ethanol)	1200 (Al-Hussaini and Mahasneh, 2009; p. 3430-31)
Populus alba (leaf)	1500 (Al-Hussaini and Mahasneh, 2009; p. 3430-31)
Tecoma capensis (leaf ethanol)	1500 (Al-Hussaini and Mahasneh, 2009; p. 3430-31)
Aloe barbadensis (leaf ethanol)	1560 (Dahiya and Purkayastha 2010;p. 447)
Azadirachta indica (leaf ethanol)	1560 (Dahiya and Purkayastha 2010;p.447)
Ocimum sanctum (leaf ethanol)	1560 (and Purkayastha 2010;p.447)
Scrophularia striata (whole plant methanol)	3100 (Zangeneh M. et al., 2017; p. 42)
Origanum vulgare (leaf ethanol)	3120 (Dahiya and Purkayastha 2010;p. 447)
Rosmarinus officinalis (flower ethanol)	3120 (Dahiya and Purkayastha 2010;p. 447)
Thymus vulgaris (leaf ethanol)	3120 (Dahiya and Purkayasth 2010;p. 447)
Rafflesia kerrii Meijer (perigone lobe ethanol)	6000 [average 5 MRSA isolates] (Hirunpetcharat C. et al., 2018; p. 43)
Piper sarmentosum (leaf methanol)	50000 (Fernandez L. et al., 2012; p. 109)

Table 23 IC50 Results: *Staphylococcus aureus* (methicillin-resistant)

Plant part and solvent used	IC50 µg/ml
Castanea sativa (leaf methanol)	16 (Quave CL et al., 2015; p.13)
Adiantum capillus (aerial parts ethanol)	256 (Khan MF. et al., 2018; p. 6)
Martynia annua L. (fruit ethanol)	256 (Khan MF. et al., 2018; p. 6)
Swertia chirata (whole plant ethanol)	256 (Khan MF. et al., 2018; p. 6)
Zanthoxylum armatum (fruit ethanol)	256 (Khan MF. et al., 2018; p. 6)
Tinospora cordifolia (fruit ethanol)	12280 [10.81 – 13.95] (Bonvicini F. et al., 2014; p. 3)
Alstonia scholaris (fruit ethanol)	8080 (7.25 – 9.00) (Bonvicini F. et al., 2014; p. 3)
Crataeva nurvala (fruit ethanol)	6150 (4.75– 7.85) (Bonvicini F. et al., 2014; p. 3)

Table 24 ZOI Results: *Staphylococcus aureus* (methicillin-resistant)

Plant part and solvent used	ZOI mm
Piper betle L. (leaf ethanol)	32.7 (Valle DL, Jr. et al., 2016; p.7)
Thuja orientalis (leaf ethanol)	29.80±0.71 (Chakraborty S. et al., 2018; p.354)
Psidium guajava (leaf ethanol)	29.69±0.78(Chakraborty S. et al., 2018; p.354)
Hygrophila auriculata (root methanol)	22 (Savitha and Yoganant, 2016; p. 198)
Terminalia bellirica (fruit ethanol)	20.5 (Dharmaratne MPJ. et al., 2018; p. 7)
Scrophularia striata (whole plant methanol)	19 (Zangeneh M. et al., 2017; p. 42)
Tecoma stans (leaf ethanol)	18.3±0.25 (Bakr RO. et al., 2019; p. 5)
Ocimum sanctum (leaf ethanol)	18.10±0.10 (Dahiya and Purkayasth 2010;p. 446)
Laurus nobilis (bark ethanol)	18±0.7 (Al-Hussaini R and Mahasneh AM. 2009; p. 3429)
Aloe barbadensis (leaf ethanol)	17.90±0.35 (Dahiya and Purkayasth 2010;p. 446)
Azadirachta indica (leaf ethanol)	16.96±0.10 (Dahiya and Purkayasth 2010;p. 446)
Jasminum sambac (flower ethanol)	16±0.7 (Al-Hussaini and Mahasneh, 2009; p. 3429)
Laurus nobilis (leaf ethanol)	16±0.7 (Al-Hussaini R and Mahasneh AM. 2009; p. 3429)
Lavandula angustifolia (flower)	15±0.7 (Al-Hussaini and Mahasneh, 2009; p. 3429)
Origanum vulgare (leaf ethanol)	14.90±0.05 (Dahiya and Purkayasth 2010;p. 446)
Allium sativum (cloves ethanol)	14.55±0.20 (Karuppiah and Rajaram 2012; p. 599)
Rosmarinus officinalis (flowers ethanol)	14.00±0.90mm (Dahiya and Purkayastha 2010;p. 447)
Thymus vulgaris (leaf ethanol)	13.76±0.20 (Dahiya and Purkayasth 2010;p. 446)
Zingiber officinale (rhizome ethanol)	13.55±0.20 (Karuppiah and Rajaram 2012; p. 599)
Quercus infectoria (gall ethanol)	13.33 ± 0.33 P value 0.52 (Wan Nor Amilah WA. et al., 2014; p. 4)
Rosmarinus officinalis (flower ethanol)	13±0.7 (Al-Hussaini R and Mahasneh AM. 2009; p. 3429)
Tecoma capensis (flower ethanol)	12.50.7 (Al-Hussaini R and Mahasneh AM. 2009; p. 3429)

Table Continued...

Plant part and solvent used	ZOI mm
Rosmarinus officinalis (leaf ethanol)	12±2.1 (Al-Hussaini and Mahasneh,2009; p. 3429)
Laurus nobilis (fruit ethanol)	12±2.1 (Al-Hussaini R and Mahasneh AM. 2009; p. 3429)
Albizia gummifera (stem bark and leaves methanol):	11–15 mm (Tekwu EM., et al, 2012; p. 268)
Ficus exasperate (leaf and stem bark methanol)	11–15 (Tekwu EM., et al, 2012; p. 268)
Nauclea latifolia (stem bark methanol)	11–15 (Tekwu EM., et al, 2012; p. 268)
Ricinodendron heudelotii (stem methanol)	11–15 (Tekwu EM., et al, 2012; p. 268)
Laurus nobilis (flower ethanol)	11±0.0 (Al-Hussaini R and Mahasneh AM. 2009; p. 3429)
Rafflesia kerrii Meijer (perigone lobe ethanol)	11.1 (Hirunpetcharat C. et al., 2018; p. 43) [average 5 MRSA isolates]
Cinnamomum porrectum (stem bark methanol)	10.5 ± 0.7071 (Buru AS. Et al., 2014; p. 7)
Populus nigra (leaf)	10±1.4 Al-Hussaini and Mahasneh, 2009; p. 3429)
Sonchus oleraceus (ariel ethanol)	101.4 (Al-Hussaini R and Mahasneh AM. 2009; p. 3429)
Piper sarmentosum (leaf methanol)	10.0±0.0 (p=0.0018) (Fernandez L. et al., 2012;p. 109)
Cinnamomum iners (stem bark methanol)	9.5 ± 0.7071 (Buru AS. Et al., 2014; p. 7)
Tecoma capensis (leaf ethanol)	8.01.4 (Al-Hussaini and Mahasneh, 2009; p. 3429)
Populus alba (leaf)	8.0±1.4 (Al-Hussaini and Mahasneh, 2009; p. 3429)

Table 25 MIC Results: *Staphylococcus aureus* (vancomycin-intermediate)

Plant part and solvent used	MIC µg/ml
Anogeissus acuminate (leaf bark methanol)	290 (Mishra MP. et al., 2017; p.90)
Punica granatum (leaf, bark, fruits methanol)	290 (Mishra MP. et al., 2017; p.90)
Soymida febrifuga (leaf, bark methanol)	670 (Mishra MP. et al., 2017; p.90)
Terminalia chebula (leaf methanol): MIC µg/ml, ZOI 23 mm	1510 (Mishra MP. et al., 2017; p.90)
Azadirachta indica (leaf methanol)	3410 (Mishra MP. et al., 2017; p.90)
Bauhinia variegate (leaf methanol)	3410 (Mishra MP. et al., 2017; p.90)
Tribulus terrestris (leaf, bark methanol): MIC µg/ml, ZOI 21 mm	3410 (Mishra MP. et al., 2017; p.90)
Boerhaavia diffusa (leaf, root methanol)	4270 (Mishra MP. et al., 2017; p.90)
Tinospora cordifolia (leaf, fruits methanol):	4270 (Mishra MP. et al., 2017; p.90)

Table 26 ZOI Results: *Staphylococcus aureus* (vancomycin-intermediate)

Plant part and solvent used	ZOI mm
Anogeissus acuminate (leaf bark methanol)	27 (Mishra MP. et al., 2017; p.89)
Punica granatum (leaf, bark, fruits methanol)	26 (Mishra MP. et al., 2017; p.89)
Soymida febrifuga (leaf, bark methanol)	25 (Mishra MP. et al., 2017; p.89)
Terminalia chebula (leaf methanol)	23 (Mishra MP. et al., 2017; p.89)
Bauhinia variegate (leaf methanol)	21 (Mishra MP. et al., 2017; p.89)
Tribulus terrestris (leaf, bark methanol)	21 (Mishra MP. et al., 2017; p.89)
Azadirachta indica (leaf methanol)	20 (Mishra MP. et al., 2017; p.89)
Tinospora cordifolia (leaf, fruits methanol)	19 (Mishra MP. et al., 2017; p.89)
Boerhaavia diffusa (leaf, root methanol)	17 (Mishra MP. et al., 2017; p.89)

Table 27 ZOI Results: *Neisseria gonorrhoeae* (fluoroquinolone-resistant)

Plant part and solvent used	ZOI mm
Tagetes erecta L. (flower methanol)	> 20 mm (Ruddock PS. et al. 2011; p.84)
Myrteola nummularia (aerial parts methanol)	> 20 mm (Ruddock PS. et al. 2011; p.84)

Table 28 ZOI Results: *Neisseria gonorrhoeae* (cephalosporin-resistant, fluoroquinolone-resistant

Plant part and solvent used	ZOI a No inhibition. b 1–50% inhibition. c 51–99% inhibition. d ≥100% inhibition
Adhatoda vasica (roots methanol)	highly sensitive ≥100% inhibition (Shokeen, Bala and Tandon, 2009; p. 88-9)
Albizzia lebbeck (stem bark methanol)	highly sensitive ≥100% inhibition (Shokeen, Bala and Tandon, 2009; p. 88-9)
Alangium salviifolium (seeds methanol)	highly sensitive ≥100% inhibition (Shokeen, Bala and Tandon, 2009; p. 88-9)
Anethum sowa (fruits methanol)	highly sensitive ≥100% inhibition (Shokeen, Bala and Tandon, 2009; p. 88-9)
Azadirachta indica (leaf methanol)	highly sensitive ≥100% inhibition (Shokeen, Bala and Tandon, 2009; p. 88-9)
Cedrela toona (leaf methanol)	highly sensitive ≥100% inhibition (Shokeen, Bala and Tandon, 2009; p. 88-9)
Drynaria peregrine (stem bark methanol)	highly sensitive ≥100% inhibition (Shokeen, Bala and Tandon, 2009; p. 88-9)
Eugenia camaldulensis (leaf methanol)	highly sensitive ≥100% inhibition (Shokeen, Bala and Tandon, 2009; p. 88-9)
Euphorbia hirta (entire plant methanol)	highly sensitive ≥100% inhibition (Shokeen, Bala and Tandon, 2009; p. 88-9)
Michelia champaca (stem bark methanol)	highly sensitive ≥100% inhibition (Shokeen, Bala and Tandon, 2009; p. 88-9)
Phyllanthus fraternus (entire plant methanol)	highly sensitive ≥100% inhibition (Shokeen, Bala and Tandon, 2009; p. 88-9)
Plumeria rubra (leaves, stem and stem bark [3 separate preparations] methanol)	highly sensitive ≥100% inhibition (Shokeen, Bala and Tandon, 2009; p. 88-9)

Table Continued...

Plant part and solvent used	ZOI a No inhibition. b 1–50% inhibition. c 51–99% inhibition. d ≥100% inhibition
Ricinus communis (stem and roots [2 separate preparations] methanol)	highly sensitive ≥100% inhibition (Shokeen, Bala and Tandon, 2009; p. 88-9)
Salvadora persica (leaves and stem [2 separate preparations] methanol)	highly sensitive ≥100% inhibition (Shokeen, Bala and Tandon, 2009; p. 88-9)
Urginea indica (bulbs methanol)	highly sensitive ≥100% inhibition (Shokeen, Bala and Tandon, 2009; p. 88-9)

Priority 3

No studies were returned on penicillin-non-susceptible *S. pneumoniae*, ampicillin-resistant *H. influenza* and fluoroquinolone-resistant *Shigella* spp.

Priority 3: Medium

No studies on penicillin-non-susceptible *S. pneumoniae*, ampicillin-resistant *H. influenza* and fluoroquinolone-resistant *Shigella* spp. were returned.

Table 29 Main Summary Findings

Priority 1	% of total studies	Nu. PDAms	PDAm with Lowest MIC	MIC µg/ml	PDAm with Largest ZOI	ZOI mm
***A. baumannii* (carbapenem-resistant)**	11.8	7	*Piper betle L.* (Valle DL, Jr. et al., 2016; p.9)	406	*Piper betle L.* (Valle DL Jr. et al., 2016; p.7)	24
***Pseudomonas aeruginosa* (carbapenem-resistant)**	11.8	5	*Piper betle L.* (Valle DL Jr. et al., 2016;p.9)	260	*Piper betle L.* (Valle DL Jr. et al., 2016;p.7)	21.3
Enterobacterales						
E. coli ***TOTAL***	17.7					
E. coli (ESBL-producing**)**	11.8	7	*Nauclea latifolia* (Tekwu EM. et al., 2012; p. 269)	32	*Piper betle L.* (Valle DL Jr. et al., 2016;p.7)	20
E. coli **(carbapenem-resistant)**	5.9	2	*Croton campestris* 512 (Matias EF. et al., 2011; p.307)	512	Not results returned	N/A
K. pneumoniae TOTAL	20.6					
K. pneumoniae (ESBL-producing**)**	11.8	4	*Piper betle L.* (Valle DL, Jr. et al., 2016; p.9)	625	*Piper betle L.* (Valle DL Jr. et al., 2016; p.7)	20
K. pneumoniae **(carbapenem-resistant)**	8.8	8	*Piper betle L.* (Valle DL, Jr. et al., 2016; p.9)	390	*Piper betle L.* (Valle DL Jr. et al., 2016; p.7)	23
Priority 2						
E. faecium	8.8	5	*Piper betle L.* (Valle DL, Jr. et al., 2016; p.9)	64.67	*Piper betle L.* (Valle DL Jr. et al., 2016; p.7)	28.3
S. aureus TOTAL	69.6					
***S. aureus* (methicillin-resistant)**	66.7	43	*Tecoma stans* (Bakr RO. et al., 2019; p. 5)	80	*Piper betle L.* (Valle DL Jr. et al., 2016; p.7)	32.7
***S. aureus* (vancomycin-intermediate)**	2.9	9	*Anogeissus acuminate and Punica granatum* (Mishra MP. et al., 2017; p.90)	290	*Anogeissus acuminate* (Mishra MP. et al., 2017; p.89)	27
***H. pylori* (clarithromycin-resistant)**	0					
***Campylobacter* spp. (fluoroquinolone-resistant)**	0					
***Salmonellae* (fluoroquinolone-resistant)**	0					
N. gonorrhoeae	5.8					
***N. gonorrhoeae* (fluoroquinolone-resistant)**	2.9	2	No results returned	N/A	*Tagetes erecta* and *Myrteola nummularia* (Ruddock PS. et al. 2011; p.84)	>20
***N. gonorrhoeae* (cephalosporin-resistant, fluoroquinolone-resistant)**	2.9	15	No results returned		15 Indian plant Species (Shokeen, Bala and Tandon, 2009; p.88-9)	≥100% Inhibition

Table Continued...

Priority 1	% of total studies	Nu. PDAms	PDAm with Lowest MIC	MIC µg/ml	PDAm with Largest ZOI	ZOI mm
Priority 3						
***S. pneumoniae* (non penicillin-non-susceptible)**	0					
***H. influenza* (ampicillin-resistant)**	0					
***Shigella* spp. (fluoroquinolone-resistant)**	0					

Discussion

Promising results were found for Priority 1 Critical Pathogens, including carbapenem-resistant *A. baumannii* and *P. aeruginosa*, Carbapenemase-producing Enterobacterales (CPE) and extended-spectrum beta-lactamase producing Enterobacterales (ESBL-E). Furthermore, results were found for vancomycin-resistant *E. feacium*, a vancomycin-resistant Enterococcus (VRE), another principal nosocomial pathogen. As zero studies were returned on clarithromycin-resistant *H. pylori*, fluoroquinolone-resistant *Campylobacter* spp., fluoroquinolone-resistant *Salmonellae*, penicillin-non-susceptible *S. pneumoniae*, ampicillin-resistant *H. influenza* and fluoroquinolone-resistant *Shigella* spp. as this review analysed over 2000 studies, it can be safely postulated that further research on these pathogens is urgently required.

Moreover and frustratingly, many studies identified PDAm activity in MDR bacterial isolates yet could not be included in the analysis as they were not WHO PP specific. Such studies either confirmed sensitivity to the antibiotics mentioned in the WHO PP list or did not test these specific antibiotics. For example, three studies referred to MDR *A. baumannii* and identified multiple antibiotic-resistant isolates however did not specify carbapenem resistance.[69, 78, 84] Four studies referred to MDR *P. aeruginosa*, identifying many antibiotic-resistant isolates using the multiple antibiotic resistance (MAR) index that tests ciprofloxacin, vancomycin and methicillin, however did not specify carbapenem-resistance.[62,72,78,84] Eight studies referred to MDR *E. coli*., identifying multiple antibiotic-resistant isolates however did not specify carbapenem-resistance or ESBL-producing.[59,72,75, 80, 84, 87-89] Five studies referred to MDR *K. pneumoniae* as per MAR index, again identifying multiple antibiotic-resistant isolates however failed to specify carbapenem-resistance or ESBL-production.[62,72,75, 84, 87] Three studies confirmed PDAm activity in drug resistant *Salmonellae*, yet strains were not identified as fluoroquinolone resistant. One study identified the *Salmonellae* strain as chloramphenicol-resistant however fluoroquinolones were not tested.[62] Another study identified *Salmonellae* strains as chloramphenicol-resistant however these were susceptible to the fluoroquinolone ciprofloxacin.[90] All *Salmonellae* strains in one study were identified as MDR however were also identified as susceptible to ciprofloxacin.[88] Whilst no studies on penicillin-non-susceptible *S. pneumoniae* were returned, one study confirmed PDAm activity in drug resistant *S. pneumoniae* however the strain identified was the macrolide erythromycin and penicillin was not tested.[90]

Piper betel L. was the most frequently mentioned plant antimicrobial. *Piper betel* L belongs to the Piperaceae family. The leaves are used in traditional medicine for the treatment of a variety of ailments.[91] Notably it is one ingredient of 'betel quid' or 'paan' and is commonly chewed in Asia as a mild stimulant.[92] In addition to its antibacterial properties, studies have shown that *Piper betel* L. is anticaries, antifungal, antioxidant, anti-inflammatory, antiprotozal, antiulcer, chemoprotective, gastroprotective, hepatoprotective, immunomodulatory and larvicidal.[93] Whilst the phytochemical and pharmacological profiling of *Piper betel* L is well established, and biotechnological interventions have aimed to improve the cash value of this botanical, as yet, no biotechnological interventions have progressed toward the development of a medical product.[94, 95]

It is vitally important to note that this review is on laboratory studies (*in-vitro*) only not by choice, but as this is the only evidence available. Botanical drug development is only emerging. Recently, an antibiotic-producing strain of Streptomyces sp. TM32 was isolated from of the medicinal plant *Curcuma longa* L.[96] Whilst the US Food and Drug Administration (FDA) has established a clear regulatory pathway for botanical drugs, to date, only two drugs have been approved.[97] It is the hope that the information obtained in these pre-clinical phase trials may one day work towards clinical testing in humans, yet until these laboratory studies progress through to clinical trials, the scientific community is a long way off ameliorating microbial resistance to drugs in humans using PDAms.

Further limitations are that a double screening selection process is generally recommended to enable the uncertainty to be quantified with more confidence and to eliminate bias. Whilst CLSI/ EUCAST have standardised AST procedures there can be no absolute surety that comparisons are meaningful. This research would benefit by expanding search criteria to include plant bioactive compounds and alternative solvents.

Strengths and weaknesses

Risk of bias, quality of evidence assignment

Some PDAms demonstrated a MIC >2000 µg/ml hence were resistant but as results were so limited, these were included. Whilst a checklist was used for unbiased decision making, a risk of bias still exists.

Conclusions

AMR is one of the greatest threats to global health. It is promising that PDAms have demonstrated AMA in all WHO Priority 1 Critical and the majority of Priority 2 High pathogens. It is particularly promising that the principal nosocomial infections, the ESKAPE pathogens, MRSA, CPE, ESBL-E and VRE are represented, as addressing these presents the greatest opportunity to reduce HCAIs. Research on the Priority 2 High pathogens clarithromycin-resistant *H. Pylori*, fluoroquinolone-resistant *Campylobacter* spp., fluoroquinolone-resistant *Salmonellae* and in all Priority 3 Medium pathogens is urgently required. Given the public health emergency posed by the WHO PPs, future PDAm studies should prioritise these specific resistant bacterial isolates. This review has highlighted that PDAms have demonstrated *in-vitro* anti-bacterial activity in WHO PPs and contributed to the body of

data. As it stands, the therapeutic application of PDAms remains to be clinically proven. Given the global burden of AR and the urgent need for novel antibacterial agents, and considering that preclinical plant antimicrobial studies have demonstrated promise perhaps it is pertinent that some allocation of the funding mentioned in the O'Neill report be directed toward finding novel antimicrobial agents from plants, with an aim to progress botanical drug development to tackle drug-resistant infections globally. Most certainly, more research on PDAms in WHO specific PPs is urgently required.

Declarations

Ethics approval and consent to participate.

The SPH Ethics Department of UCC approved the research project on the 28th April 2020.

Consent for Publication

Not applicable.

Availability of data and material

The authors confirm that the data supporting the findings of this study are available within the article appendices.

Competing Interests

The author declares that there is no conflict of interest.

Funding

No funding was allocated to this project.

Author's Contributions

Carina Harkin contributed to the design and implementation of the research protocol and to the writing of the manuscript.

Acknowledgements

The author would like to thank Dr. Brendan Palmer and Dr Éilis O'Reilly from the Dept. of Epidemiology and Public Health University College Cork (UCC) for their direction and feedback.

References

1. Antimicrobial resistance. Geneva: World Health Organization; 2020.
2. Shallcross LJ, Davies DSC. Antibiotic overuse: a key driver of antimicrobial resistance. *Br J Gen Pract*. 2014;64(629):604–605.
3. O'Neill J. Tackling drug resistant infections globally: final report and recommendations. London, UK: Jointly commisioned by the Wellcome Trust and HM Government; 2016.
4. O'Dowd A. Death certificates should record antimicrobial resistance as cause of deaths, says CMO. *BMJ.* 2018;362:k3832.
5. Security DCCCoGH. Review of progress on antimicrobial resistance: background and analysis. London: the royal institute of international affairs chatham house; 2019.
6. Cambridge Uo. antimicrobial resistance university of Cambridge 2020.
7. WHO. WHO publishes list of bacteria for which new antibiotics are urgently needed Geneva, Switzerland 2017 Feb 27.
8. Tacconelli E, Carrara E, Savoldi A, et al. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic–resistant bacteria and tuberculosis. *Lancet Infect Dis*. 2018;18(3):318–327.
9. Dotinga R. Hospital infections top WHO's list of priority pathogens. The Hospitalist 2017 Apr 25.
10. WHO. The burden of health care–associated infection worldwide 2020.
11. WHO. Ten threats to global health in 2019.
12. Peleg AY, Hooper DC. Hospital–acquired infections due to gram–negative bacteria. *N Engl J Med*. 2010;362(19):1804–13.
13. Santajit S, Indrawattana N. mechanisms of antimicrobial resistance in ESKAPE Pathogens. *BioMed Res Int*. 2016;2016:2475067.
14. Mulani MS, Kamble EE, Kumkar SN, et al. Emerging strategies to combat ESKAPE pathogens in the era of antimicrobial resistance: a review. Front Microbiol. 2019;10:539.
15. Brolund A. Overview of ESBL–producing enterobacteriaceae from a nordic perspective. *Infect Ecol Epidemiol.* 2014;4(1):24555.
16. 2011–2016 HPSC. point prevalence survey of hospital–acquired infections & antimicrobial use in european acute care hospitals 2017.
17. (HPSE) HPSC. Antimicrobial resistance and infection control (AMRIC) Division 2020.
18. ECDC. Healthcare–associated infections: surgical site infections – annual epidemiological report for 2017: European Centre for Disease Prevention and Control; 2019.
19. Mangram AJ, Horan TC, Pearson ML, et al. guideline for prevention of surgical site infection, 1999. Centers for disease control and prevention (CDC) hospital infection control practices advisory committee. Am J Infect Control. 1999;27(2):97–132.
20. Abbas M, Pittet D. Surgical site infection prevention: a global priority. J Hosp Infect. 2016;93(4):319–322.
21. Teillant A, Gandra S, Barter D, et al. Potential burden of antibiotic resistance on surgery and cancer chemotherapy antibiotic prophylaxis in the USA: a literature review and modelling study. *Lancet Infect Dis*. 2015;15(12):1429–1437.
22. Fitzpatrick F, Tarrant C, Hamilton V, et al. Sepsis and antimicrobial stewardship: two sides of the same coin. *BMJ Qual saf*. 2019;28(9):758–761.
23. Rudd KE, Kissoon N, Limmathurotsakul D. The global burden of sepsis: barriers and potential solutions. Crit Care. 2018;22(1):232.
24. James SLea. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. The Lancet. 2018;392(10159):1789–1858.
25. Kelley E. WHO work on sepsis In: Group SC, editor. Geneva, Switzerland: The WHO service delivery and safety department (SDS); 2017.
26. Liu V, Escobar GJ, Greene JD, et al. Hospital deaths in patients with sepsis from 2 independent cohorts. JAMA. 2014;312(1):90–92.
27. NCEPOD. Just Say Sepsis! London: UK Sepsis Trust; 2015.
28. Shukla S, Bajpai VK, Kim M. Plants as potential sources of natural immunomodulators. Reviews in Environmental Science and Bio/Technology. 2014;13(1):17–33.
29. Calixto JB. The role of natural products in modern drug discovery. *An Acad Bras Cienc.* 2019;91 Suppl 3:e20190105.
30. Patridge E, Gareiss P, Kinch MS, et al. An analysis of FDA–approved drugs: natural products and their derivatives. Drug Discov Today. 2016;21(2):204–207.
31. Zhao Y, Wu Y, Wang M. Bioactive substances of plant origin. In: Cheung PCK, Mehta BM, editors. Handbook of Food Chemistry. Berlin, Heidelberg: Springer Berlin Heidelberg; 2015. p. 967–1008.

32. Fabricant DS, Farnsworth NR. The value of plants used in traditional medicine for drug discovery. Environ Health Perspect. 2001;109 (Suppl 1):69–75.
33. Patel V, Patel R. The active constituents of herbs and their plant chemistry, extraction and identification methods. Journal of Chemical and Pharmaceutical Research. 2016;2016:1423–1443.
34. Palaniappan K, Holley RA. Use of natural antimicrobials to increase antibiotic susceptibility of drug resistant bacteria. *Int J Food Microbiol.* 2010;140(2–3):164–168.
35. Abreu AC, Coqueiro A, Sultan AR, et al. Looking to nature for a new concept in antimicrobial treatments: isoflavonoids from Cytisus striatus as antibiotic adjuvants against MRSA. *Scientific Reports*. 2017;7(1):3777.
36. Vadhana P SB, Bharadwaj M, Singh SV. Emergence of Herbal Antimicrobial Drug Resistance in Clinical Bacterial Isolates. Pharm Anal Acta. 2015;6:1–7.
37. Tong YQ, Xin B, Zhu L. Transfer of herb–resistance plasmid from escherichia coli to staphylococcus aureus residing in the human urinary tract. *Jundishapur J Microbiol.* 2014;7(3):e15056.
38. Asaduzzaman M. Antimicrobial resistance: an urgent need for a planetary and ecosystem approach. Lancet Planet Health. 2018;2(3):e99–e100.
39. Ahn K. The worldwide trend of using botanical drugs and strategies for developing global drugs. BMB Rep. 2017;50(3):111–116.
40. Upadhyay A, Upadhyaya I, Kollanoor–Johny A, et al. Combating pathogenic microorganisms using plant–derived antimicrobials: a minireview of the mechanistic basis. *Biomed Res Int.* 2014;2014:761741.
41. Al–Mariri A, Safi M. *In vitro* antibacterial activity of several plant extracts and oils against some gram–negative bacteria. Iran *J Med Sci*. 2014;39(1):36–43.
42. Silva LN, Zimmer KR, Macedo AJ, et al. Plant natural products targeting bacterial virulence factors. *Chemical reviews*. 2016;116(16):9162–236.
43. Asfour HZ. Anti–Quorum Sensing Natural Compounds. *J Microsc Ultrastruct*. 2018;6(1):1–10.
44. Satlin MJ, Lewis JS, II, Weinstein MP, et al. Clinical and Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) position statements on polymyxin B and colistin clinical breakpoints. *Clin Infect Dis*. 2020;71(9):e523–e529.
45. Magiorakos AP, Srinivasan A, Carey RB, et al. Multidrug–resistant, extensively drug–resistant and pandrug–resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect*. 2012;18(3):268–281.
46. Agency EM. Questions and answers on quality of herbal medicinal products/traditional herbal medicinal products. Committee on Herbal Medicinal Products (HMPC); 2018.
47. EDftQoMH (EDQM). Guide for the elaboration of monographs on herbal drugs and herbal drug preparations. European Pharmacopoeia; 2007.
48. Subramani R, Narayanasamy M, Feussner K–D. Plant–derived antimicrobials to fight against multi–drug–resistant human pathogens. *3 Biotech*. 2017;7(3):172.
49. Abreu A, McBain A, Simões M. Plants as sources of new antimicrobials and resistance–modifying agents. *Nat Prod Rep*. 2012;29:1007–1021.
50. Khameneh B, Iranshahy M, Soheili V, et al. Review on plant antimicrobials: a mechanistic viewpoint. *Antimicrobial Resistance & Infection Control*. 2019;8(1):118.
51. Thomas J KD, McKenzie JE, Brennan SE, et al. Determining the scope of the review and the questions it will address. In: Higgins JPT TJ, Chandler J, Cumpston M, editors. Cochrane Handbook for Systematic Reviews of Interventions: Determining the scope of the review and the questions it will address. Cochrane Handbook for Systematic Reviews of Interventions version 6.0 (updated July 2019); 2019.
52. Richardson W, Wilson, MC, Nishikawa J, et al. The well–built clinical question: A key to evidence–based decisions. *ACP J Club*. 1995;123:A12–A13.
53. Moher D, Liberati A, Tetzlaff J, et al. Preferred reporting items for systematic reviews and meta–analyses: The PRISMA statement. *PLOS Medicine*. 2009;6(7):e1000097.
54. Carol L, Julie G, Simon B. Searching for and selecting studies. Cochrane Handbook for Systematic Reviews of Interventions: Cochrane; 2019.
55. Bern Uo. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE). Statement Bern University of Bern; 2009.
56. Tacconelli E, Cataldo MA, Paul M, et al. STROBE–AMS: recommendations to optimise reporting of epidemiological studies on antimicrobial resistance and informing improvement in antimicrobial stewardship. *BMJ Open*. 2016;6(2):e010134.
57. Valle DL, Cabrera EC, Puzon JJ, et al. Antimicrobial activities of methanol, ethanol and supercritical CO2 extracts of Philippine *Piper betle* L. on clinical isolates of gram positive and gram negative bacteria with transferable multiple drug resistance. *PLoS One*. 2016;11(1):e0146349.
58. Dharmaratne MPJ, Manoraj A, Thevanesam V, et al. Terminalia bellirica fruit extracts: in–vitro antibacterial activity against selected multidrug–resistant bacteria, radical scavenging activity and cytotoxicity study on BHK–21 cells. *BMC Complement Altern Med*. 2018;18(1):325.
59. Assis FV, Siqueira FL, Goncalves IE, et al. Antibacterial activity of Lamiaceae plant extracts in clinical isolates of multidrug–resistant bacteria. *An Acad Bras Cienc*. 2018;90(2):1665–1670.
60. Khan MF, Tang H, Lyles JT, et al. Antibacterial properties of medicinal plants from Pakistan against multidrug–resistant ESKAPE pathogens. *Frontiers in pharmacology*. 2018;9:815.
61. Salman MT, Khan RA, Shukla I. A Study of Nigella sativa Linn. Seeds for antimicrobial activity against multidrug resistant clinical strains of Pseudomonas aeruginosa. J Ethnopharmacol. 2009;142(1):265–273.
62. Tekwu EM, Pieme AC, Beng VP. Investigations of antimicrobial activity of some Cameroonian medicinal plant extracts against bacteria and yeast with gastrointestinal relevance. *Journal of ethnopharmacology*. 2012;142(1):265–273.
63. Ouattara A, Coulibaly A, Adima AA, et al. Exploration of the antistaphylococcic activity of Vitex doniana (Verbenaceae) stem bark extracts. *Scholars Academic Journal of Pharmacy (SAJP)*. 2013.
64. Matias EF, Santos KK, Almeida TS, et al. Phytochemical prospection and modulation of aminoglycoside antibiotic activity by Croton campestris A. *Chemotherapy*. 2011;57(4):305–309.
65. Menezes IR, Santana TI, Varela VJ, et al. Chemical composition and evaluation of acute toxicological, antimicrobial and modulatory resistance of the extract of Murraya paniculata. *Pharm Biol*. 2015;53(2):185–191.
66. Bonvicini F, Mandrone M, Antognoni F, et al. Ethanolic extracts of Tinospora cordifolia and Alstonia scholaris show antimicrobial activity towards clinical isolates of methicillin–resistant and carbapenemase–producing bacteria. *Nat Prod Res*. 2014;28(18):1438–1445.
67. Buru AS, Pichika MR, Neela V, et al. *In vitro* antibacterial effects of Cinnamomum extracts on common bacteria found in wound infections with emphasis on methicillin–resistant Staphylococcus aureus. J Ethnopharmacol. 2014;153(3):587–595.
68. Zangeneh M, Najafi F, Tahvilian R, et al. Assessment of *In Vitro* Antibacterial Properties of the Hydroalcoholic Extract of Scrophularia striata Against Staphylococcus aureus (ATCC No. 25923). *International Journal of Pharmacognosy and Phytochemical Research*. 2017.

69. Wan Nor Amilah WA, Masrah M, Hasmah A, et al. *In vitro* antibacterial activity of Quercus infectoria gall extracts against multidrug resistant bacteria. *Trop Biomed*. 2014;31(4):680–688.

70. Fernandez L, Daruliza K, Sudhakaran S, et al. Antimicrobial activity of the crude extract of Piper sarmentosum against methicilin–resistant Staphylococcus aureus (MRSA), Escherichia coli, ect. *Eur Rev Med Pharmacol Sci*. 2012;16(3):105–111.

71. Al–Hussaini R, Mahasneh AM. Microbial growth and quorum sensing antagonist activities of herbal plants extracts. *Molecules (Basel, Switzerland)*. 2009;14(9):3425–435.

72. Karuppiah P, Rajaram S. Antibacterial effect of Allium sativum cloves and Zingiber officinale rhizomes against multiple–drug resistant clinical pathogens. *Asian Pac J Trop Biomed.* 2012;2(8):597–601.

73. Hirunpetcharat C, Wichantuk P, Diraphat P, et al. Antibacterial activity of Rafflesia kerrii Meijer extracts against hospital isolates of methicillin–resistant Staphylococcus aureus (MRSA). *Journal of Thai Interdisciplinary Research*. 2018;13(1):40–45.

74. Savitha S, Yogananth N. Antibacterial screening on phytochemical extracts of *Hygrophila auriculata* (Neer mulli) on MRSA (methicillin resistant *Staphylococcus aureus*)–an *in vitro* study. *Int J Adv Res Biol Sci*. 2016;3(12):196–199.

75. DPaP S. Phytochemical screening and antimicrobial activity from five Indian medicinal plants against human pathogens. *Middle East Journal of Scientific Research.* 2010;5(6):477–482.

76. Bakr RO, Fayed MAA, Salem MA, et al. Tecoma stans: Alkaloid Profile and Antimicrobial Activity. *J Pharm Bioallied Sci.* 2019;11(4):341–347.

77. Chakraborty S, Afaq N, Singh N, et al. Antimicrobial activity of *Cannabis sativa*, *Thuja orientalis* and *Psidium guajava* leaf extracts against methicillin–resistant *Staphylococcus aureus. Journal of integrative medicine.* 2018;16(5):350–357.

78. Su PW, Yang CH, Yang JF, et al. Antibacterial Activities and Antibacterial Mechanism of *Polygonum cuspidatum* Extracts against Nosocomial Drug–Resistant Pathogens. *Molecules (Basel, Switzerland).* 2015;20(6):11119–111130.

79. Mendiondo ME, Juareza BE, Zampini C, et al. Bioactivities of Chuquiraga straminea sand with. *Natural product communications*. 2011;6(7):965–968.

80. Sousa EO, Almeida TS, Rodrigues FF, et al. Lantana montevidensis Briq improves the aminoglycoside activity against multiresistant Escherichia coli and Staphylococcus aureus. *Indian journal of pharmacology*. 2011;43(2):180–182.

81. de Araujo AR, Quelemes PV, Perfeito ML, et al. Antibacterial, antibiofilm and cytotoxic activities of Terminalia fagifolia Mart. extract and fractions. *Ann Clin Microbiol Antimicrob*. 2015;14:25.

82. Quave CL, Lyles JT, Kavanaugh JS, et al. *Castanea sativa* (European Chestnut) Leaf Extracts Rich in Ursene and Oleanene Derivatives Block *Staphylococcus aureus* Virulence and Pathogenesis without Detectable Resistance. *PLoS One*. 2015;10(8):e0136486.

83. Machado TB, Pinto AV, Pinto MC, et al. *In vitro* activity of Brazilian medicinal plants, naturally occurring naphthoquinones and their analogues, against methicillin–resistant Staphylococcus aureus. *International journal of antimicrobial agents.* 2003;21(3):279–284.

84. Mishra MP, Rath S, Swain SS, et al. *In vitro* antibacterial activity of crude extracts of 9 selected medicinal plants against UTI causing MDR bacteria. *Journal of King Saud University–Science*. 2017;29(1):84–95.

85. Ruddock PS, Charland M, Ramirez S, et al. Antimicrobial activity of flavonoids from Piper lanceaefolium and other Colombian medicinal plants against antibiotic susceptible and resistant strains of Neisseria gonorrhoeae. *Sex Transm Dis.* 2011;38(2):82–88.

86. Shokeen P, Bala M, Tandon V. Evaluation of the activity of 16 medicinal plants against Neisseria gonorrhoeae. *International journal of antimicrobial agents.* 2009;33(1):86–91.

87. Tabassum H, Ali MN, Al–Jameil N, et al. Evaluation of antibacterial potential of selected plant extracts on bacterial pathogens isolated from urinary tract infections. *Int J Curr Microbiol App Sci*. 2013;2(10):353–368.

88. Sadiq MB, Tarning J, Aye Cho TZ, et al. Antibacterial Activities and Possible Modes of Action of Acacia nilotica (L.) Del. against Multidrug–Resistant Escherichia coli and Salmonella. *Molecules (Basel, Switzerland).* 2017;22(1).

89. Barreto FS, Sousa EO, Rodrigues FFG, et al. Antibacterial activity of Lantana camara Linn Lantana montevidensis Brig extracts from Cariri–Ceara, Brazil. *Journal of Young Pharmacists*. 2010;2(1):42–44.

90. A Ewansiha JU, A Garba, Samuel Alimi, et al. Therapeutic Potency of *Citrus limon* (L) Burm. F. (Lemon) PeelExtract Against Some Disease Causing Microorganisms. *J International Journal of Research*. 2016;4:30–39.

91. Madhumita M, Guha P, Nag A. Bio–actives of betel leaf (*Piper betle* L.): A comprehensive review on extraction, isolation, characterization, and biological activity. Phytotherapy Research.

92. Gupta PC, Ray CS. Epidemiology of betel quid usage. *Ann Acad Med Singap*. 2004;33(4 Suppl):31–36.

93. Fazal F, Mane P, Rai M, Thilakchand K, et al. The phytochemistry, traditional uses and pharmacology of Piper Betel. linn (Betel Leaf): A pan–asiatic medicinal plant*. Chinese journal of integrative medicine.* 2014.

94. Nagori K, Singh M, Alexander A, et al. *Piper betle* L.: A review on its ethnobotany, phytochemistry, pharmacological profile and profiling by new hyphenated technique DART–MS (Direct Analysis in Real Time Mass Spectrometry). *Journal of Pharmacy Research.* 2011;4:2991–2997.

95. Das S, Parida R, Sriram et al. Biotechnological intervention in betelvine (*Piper betle* L.): A review on recent advances and future prospects. *Asian Pacific Journal of Tropical Medicine.* 2016;9(10):938–946.

96. Quinn GG, Banat A, Abdelhameed A, et al. Streptomyces from traditional medicine: sources of new innovations in antibiotic discovery. *Journal of medical microbiology*. 2020;69.

97. Ilag L. Botanicals and its extracts: A new drug discovery and development paradigm for chronic and age–related illnesses. *Medical Research and Innovations*. 2018;2.

98. Julian Higgins SG. Controlled vocabulary and text words. cochrane handbook for systematic reviews of interventions: John Wiley & Sons 2019.