Synergistic Effects of Natural Products and Commercial Antibiotics: A Systemic Study 2010-2015

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ABSTRACT

Context: The 'antimicrobial resistant era' requires advances in the approaches and technologies to find new treatments. The enhancement of the antimicrobial activity of Commercially Available Drugs (CADs) by Natural Products (NPs) has successful mixtures (e.g., clavulanic acid and amoxicillin).

Objective: To systematically review reports of synergistic effects of CADs and NPs against opportunistic microbial strains from 2010 to April 2016.

Methods: The databases and search engines PubMed, Medline, Scifinder, Scopus, Science Direct and Google Scholar were systematically searched. Among the keywords utilized were: synergistic effects natural products and antibiotics, botanicals and antibiotics bioassays, plant extracts interaction with antibiotics and antibiotic adjuvant bioassays. Only synergistic results were tabulated and analyzed according to CADs, NPs and strains.

Results: A set of 76 studies that reported *in vitro* synergistic effects of CADs and NPs against gram-positive or gram-negative bacteria or fungi opportunistic strains was found. From the 60 reports on antibacterial adjuvants, the most frequent designs involved beta-lactamics or aminoglycosides against Methicillin Resistant *Staphylococcus aureus*. The assayed NPs encompassed extracts or fractions from 22 different species distributed worldwide (45% extracted with non-polar solvents) and 33 purified compounds (flavonoids, other polyphenols and alkaloids).

Conclusion: NPs as potential drug hits for antimicrobial adjuvants had been found and should continue in the drug discovery pipeline. The field certainly would benefit of advances in purification technologies, especially for polar extracts and bioassay platforms.

Keywords: Natural products, Antimicrobial resistance, Synergistic effects, Natural products, Infectious disease.

INTRODUCTION

The challenge of the antimicrobial resistant era

For almost half a century the treatment scenario had been straightforward for a particular set of infectious diseases in humans, animals, and plants [1-3]. The evolutionary pressure that represented the systematic administration of antimicrobials-together with its involuntary mismanagement due to a variety of reasons-in concert with the evolution intrinsic to microbe's life has been yielding resistant strains [4,5]: microbes evolved diversifying their 'hunt' strategies (Figure 1)[5-21]. The consolidation of the Antimicrobial Resistance Era (ARE) represents one step backwards regarding the previous victory over the infectious diseases; this leads to the risk of the infectious diseases recurrence as the first cause of death worldwide [5,22-24]. Beyond that, they ARE effects extrapolate to immune-compromised patients veterinary and plant management [23,25].
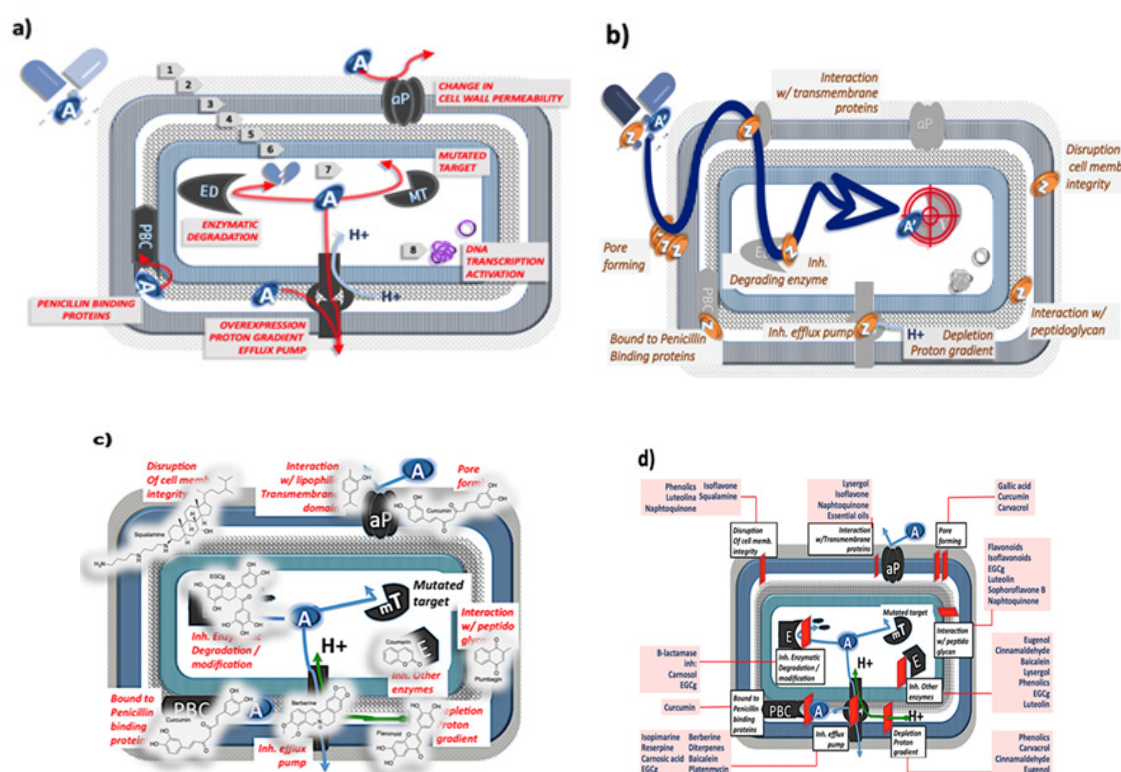
Figure 1: (a) Depiction of examples of molecular mechanisms of antibiotic resistance and antibiotic adjuvant interactions with natural products at a cellular level; examples of molecular mechanisms of antibiotic resistance, which are sites of potential interactions of phytochemicals with a generic resistant gram (-) bacteria.

Note: From extracellular space to inside A=antibiotic; 1: Extracellular space, 2: Alginate; 3: Outer cell membrane; 4: Peri-plasmic space; 5: Peptidoglycan wall; 6: Inner cell membrane; 7: Inner cellular space; 8: Chromosomic and plasmid DNA, (b) Examples of antimicrobial resistance mechanisms that can reduce the intrabacterial drug accumulation and adjuvant molecules with generic gram-negative bacteria, such that facilitates the encounter of antibiotic with its target, which are sites of potential interactions with natural product compounds Note: In blue oval A': A given antibiotic compound (no mutated target), In orange oval Z: A given adjuvant agent(s), highlighted in red: The target of the antibiotics,(c) Potential interactions of NPs with subcellular components of a given Gram negative resistant bacteria,(d) Compilation of NPs and their proposed mechanism of action at subcellular level.

To tackle the ARE, the WHO had established guidelines and priorities, including the development of new drugs and pesticides [25]. So, it can be said that the combat has begun; as it is going on is becoming evident that the challenge is larger than we thought at the beginning, and more bio inspiration is welcomed [26]. It is on creative scaffolds where nature plays on our side, since natural products often defy a medicinal chemist's imagination. The Multi Targets Therapies (MTT), as complex as they can be, is emerging as a viable option to be studied with rationale and random screenings (not necessarily with mechanisms of action based on one drug–one target or polypharmacology, example: 'magic bullet drug') [27]. Combination Therapy (CT), a type of MTT is one feasible option to combat the ARE (example: clavulanic acid–amoxicillin and treatments including immune system stimulation) [27-31]. Phytopharmaceuticals A could be seen an MTT-CT option because a derivative from a given medicinal plant can contain a set of key components with a variety of targets, or one natural product can interact with several targets. But natural products as a CT option has a its own challenges to solve, example: expressly in quality control assurance, to innovate in the research pipelines and information organization to assure the achievement of drug candidates [32,33].

A type of CT-research is the screening for natural products that can potentiate the antimicrobial activity of Commercially Available Drugs (CADs), example: Additive and synergistic effects. Natural products ranging from purified compounds to raw extracts, which can also have anti–inflammatory and immune-stimulant activities. Scientific literature was reviewed to gain insight on the state of synergistic studies of NPs and Commercially Available Drugs (CADs). Only re-

ports that fell in the criteria below described were analyzed [5-21] (Figure 1). (The Indian governmental drug regulation agency defines Phytopharmaceuticals as a fraction of an extract from a given medicinal plant or its parts. Such that the fraction has been purified and standardized in its composition of at least four compounds, which have been detected and quantified. It encompasses the preparation administration for internal or external use in humans or animals, with purpose of diagnosis, treatment, mitigation, or prevention of any disease or disorder. Parenteral administration is excluded).`

METHDOLOGY

The literature search parameters were defined as following;

Databases and search engines

National Center for Biotechnology Information (NCBI)-PubMed Central, Scifinder, Scopus, Sciencedirect [34-37]. In the cases in which the search engine also yielded recommended articles related to the found article, follow up of such studies was performed.

Publication date

In the range from 2010 up to April 2016.

Targeted content

Antimicrobial activity evaluation of Commercially Available Drugs together with Natural Extracts against Opportunistic microbes (CAD and NE–OM), such that yielded synergistic effects results which data analysis included either FICI or a statistical comparison between control and test groups. Also, analog results from testing the main component(s) of any given natural extract were also included.

Keywords and phrases

The keywords applied to start the literature search in the different databases and search engines were: synergistic effects natural products and antibiotics, botanicals and antibiotics bioassays, plant extracts interaction with antibiotics, and antibiotic adjuvant bioassays.

Exclusion criteria

Results classified as either antagonist, additive, or non–interaction effects of CADs and NPs tests; results classified as either enhancement or modulating effects of CADs and NPs tests, such that were reported without statistical analysis, such that it was not possible to conclude if synergistic effects were observed; only the NPs (either as extract or purified components from it) were tested for antimicrobial activity; mixture of two or more NPs, even if those yielded antimicrobial synergistic effects; mixture of two or more CADs, even if those yielded Antimicrobial synergistic effects.

Method

Results obtained by the micro dilution method were included; the design could be either by Fractional Inhibitory Combination Index-FICI determination, or if the differences between control and mixture were established as statistically significant (Another commonly utilized assay is the bioactive compound(s) disk diffusion assay, which is a useful screening tool, but has the drawback of the unknown diffusion factor for NPs, which can become a limiting factor when searching for synergistic effects, example: yielding false negative results. In some cases, a pre-screening panel was performed applying the agar diffusion test. It should be noted that deviations or disagreement between the results of those two methods can be observed, in some cases due to the low solubility or low diffusion of the analyte) [38].

RESULTS AND DISCUSSION

A total of 76 scientific reports of antimicrobial synergistic effects of NPs and CADs (NPs+CADs) were found, 89.5% are in PubMed database, being the years 2014 and 2015 with the highest publication frequency (Figure 2). Reports topics were distributed as follows: 60 related to antibacterial, four to antifungals and 12 to interaction mechanisms studies at cellular level. Studies were performed predominantly by interdepartmental collaborations distributed in four continents, only six reports involved international collaborations. The countries that mostly contributed were China, Korea and Brazil (range 10%-15%). Asian countries were represented in about 56% of the articles. The articles were published in a variety of journals that belonged to different editorials.
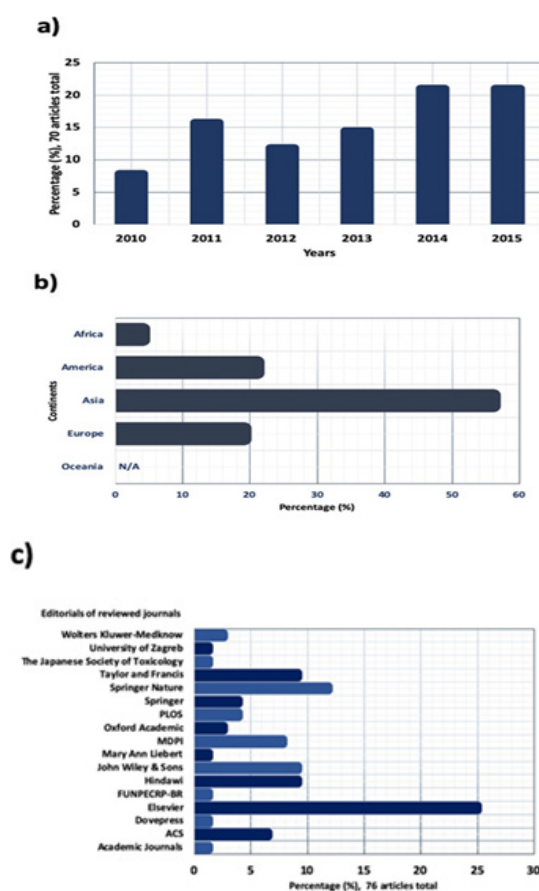
Figure 2: Distribution of the 76 reviewed publications by time, locations and editorials (a) Publication frequency during 2010 to 2016 (April), (b) Approximated continental publication contribution, (c) Article distribution in editorials (usually one editorial hosted more than one journal).

The 60 scientific reports of antibacterial adjuvants, published in 2010 to April 2016 are summarized and are compiled in Tables 1 and 2; in which the convergence of traditional medicinal knowledge and 'Westernized medicine' can be observed. For instance the evaluation of Sami-Hyanglyun-Hwan Korean formulation and ciprofloxacin, or the study of traditional medicinal plants from Cameroon [39,40]. They are proof that the mining Natural Products sense lato (NPs) can still yield useful drug additives to treat resistant nosocomial bacteria(The term natural products Sensulato, as applied in here, includes extracts derived from living organisms as well as purified compounds from such an extracts. These compounds could be obtained from either the direct original source, as biotechnological products, or from organic synthesis, being commercially available, or isolated at laboratory scale). Tables 1 and 2 compile the organism's family and scientific names, potential bioactive compound, organ extracted, polarity of the extracting solvent, the bacterial strains and antibiotics that yielded synergistic effects, comments, and the respective citation. General comments of three parameters are given below: Commercially available drugs tested, bacterial strains assayed, and natural products tested.

Commercially Available Drugs (CADs) from the 47 different drugs tested; 46 were antibiotics from different antibiotic families, and one drug was an antimicrobial with dual activity against bacteria and protozoa (Figure 3); in all the studies, the antimicrobial agents were purchased as at least 95% pure, and not as pharmaceutical formulation. The most frequently assayed antibiotics were (the antibiotic's family is given in parenthesis); ampicillin (beta-lactams), ciprofloxacin (quinolones), and gentamicin (aminoglycosides) (Figure 3). Methicillin Resistant *Staphylococcus aureus*, MRSA, a strain for which new antibiotics are urgently needed [41], was the most common target. The antimicrobial interactions of NPs+CADs were determined by the micro dilution method, usually by the Checkerboard method, applying serial dilutions in the twofold factor mode. The set of CADs encompassed medications that are under control for the emergence resistant strains against those antibiotics; it also included antibiotics that the WHO consider as essential drugs in any health care system [42,43]. The bioassays either tested raw extracts or purified natural products. Among the natural sources investigated were plants across the globe, from which specific plant organs were Lextracted, usually selected based on ethno botanical knowledge (Figures 3, 4 and Table 1) [11,39,40,44-69].
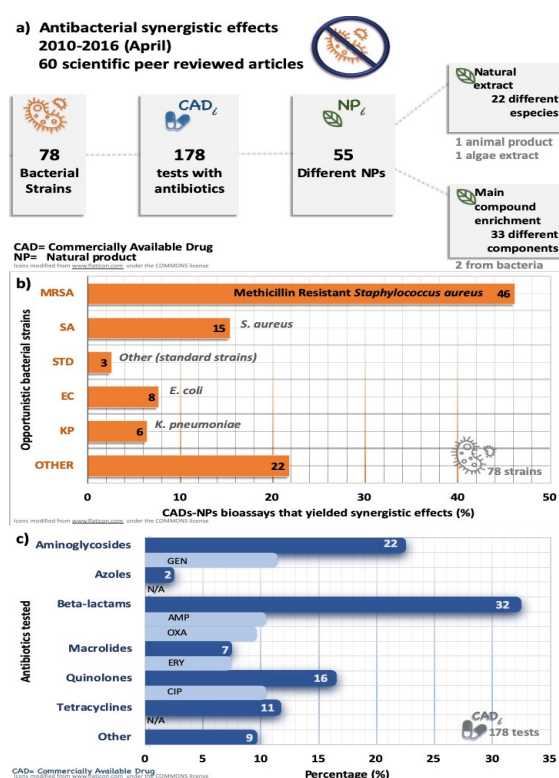
Figure 3: Summary of results of 64 studies of antibiotic synergistic assays of NPs and CADs (a) Overall results were categorized by antibiotics, strains, and NPs, (b) Distribution of bacterial strains that were susceptible to the mixture of NPs and CADs at a synergistic level, (c) Distribution of antibiotics families that yielded synergistic effects with different NPs.
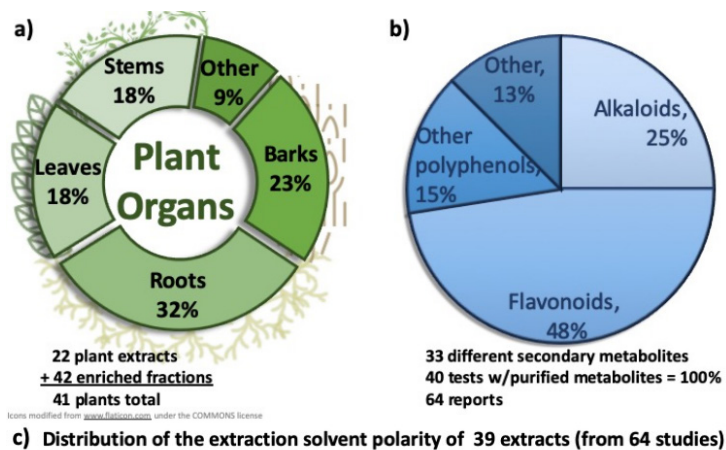
Figure 4 : (a) Plant organs extracted, (b) Distribution of purified natural products assayed in the set of reviewed scientific reports (that yielded synergistic effects with antibiotics), (c) Extraction solvents, ordered according to their dielectric constant (ε).

No plant extracted (plant family)	Potential bioactive compound (Secondary metabolite type)	Plant organ	Extraction solvent	Microbial strain	Antimicrobial agents showing synergistic effects	Comments
Acacia mearnsii (Mimosaceae)	--	B	P	SF (KZN)	ERY, CIP	Antagonism observed.
				PA (ATCC 19582)	ERY, TET,CAM, KAN	
				EC (ATCC 25922)	TET, CIP, KAN	
				BC (ATCC 10702)	TET, CAM	
				SM (ATCC 9986)	TET, CIP	
				EF (KZN)	ERY, AMX,CHL	
				SA (OKI)	ERY, TET,AMX, CIP	
				ST (ATCC 13311)	TET, AMX, CIP,NAL, KAN	
				ML	AMX	
				PV	ERY, TET,AMX, CIP, KAN	
				KP (ATCC 10031)	TET	Antagonism observed.
				PV (ATCC 6830)	TET, ERY	
				SSn (ATCC 29930)	TET, ERY, AMX, CAM	
				EC (ATCC 25922)	TET, CAM,MTZ	
				EF (ATCC 29212)	MTZ, AMX	
				EF (KZN)	AMX	
				BS (KZN)	CAM	
Achyrocline satureioides (Asteraceae)	23-methyl-6-O-desmethyllauricepyrone (Xanthone)	L	P	SA	ERY, GEN	--
Aframonmum sulcatum (Zingiberaceae)	--	S	W	SA	CRO	Antagonism observed other (SS, SE)
				EC	NOR	
Albizia lebbeck (Fabaceae)	--	B	P	SA	AMP	Antagonism strains observed. other (SS, EC,KP)
Alpinia officinarum (Zingiberaceae)	Galangin (Flavonoid)	R	N	SA (DMST 20651)	CEF	--
Baillonella toxisperma (Sapotaceae)	--	B	P	SE	CRO, AMP	Antagonism observed. Other strains (EC, KP,ST)
Bee venom(Apis mellifera, Apidae)	Melittin (Peptide)	--	--	MRSA	OXA, AMK	Study also considered mixture of antibiotic, bee venom, purified melittin and purified phytochemicals

Beilschmiedia cinnamomea (Lauraceae)	--	B	P	MRSA	CAM, FEP,NOR, CIP, ERY	Interaction determined by percentage
Caesalpinia sappan. (Fabaceae)	Brazilin,brazilein, sappanone,(Flavo noids)	Hw	P	MRSA	AMK, GEN	--
Cienfuegosa digitata Cav.(Malvacea)	Alkaloids	L	Alk	MRSA, ARSA	AMP	--
Citrus Hystrix (Rutaceae)	Essential oil	L + P	EO	PG, SM, SSg	CLO	Evaluation for mouth wash
Combretum molle (Combretaceae)	--	B	P	EC, EA	KAN	--
Cordia verbenaceae	--	L	N	MRSA, PA,PA	GEN,NEO	Based on statistical
Croton campestris (Euphorbiaceae)	--	L	P	EC(27)	GEN	Based on statistical
Dichrostachys glomerata (Fabaceae)	0	Fr	P	MRSA	CAM, TET,NOR	--
Ecklonia cava (Lessoniaceae,Brown algae)	--	"L"	NP, P	MRSA	AMP	--
Hibiscus sabdariffa (Malvaceae)	--	Fl	A	HA	CLA, MTZ	--
Hypericum japonicum (Hypericaceae)	Taxifolin-7-O-alpha- L- rhamnopyranosi de (Flavonoid)	S	P	MRSA	CAZ, LVX	--
Hyptis martiusii (Lamiaceae)	--	L	P	MRSA	KAN	--
Manuka honey	--	--	--	MRSA	TET, MUP	Produced by bee pollinization of Manuka tree flowers (*Leptospermum scoparium*)
Myracrodruon urundeuva (Anacardiaceae)	--	B	P	SA, BS, KP, EC	GEN	--
		L	EO	SA 358	GEN	--
Nauclea pobeguinii (Rubiaceae)	--	R	M	SS	CRO, AMP,AMX, NOR	--
				SE	AMP	
				ST	CRO	
				SA	CRO, AMP	
				KP	AMP, AMX	
				EC	NOR	
Ocimum basiliscum L. (Lamiaceae)	Probably linalool	L	EO	PA, SA	CIP	--
Piper arboreum (Piperaceae)	--	L	N	EC 27	AMK, GEN	Based on statistical analysis
Portulaca oleracea L. (Portulacaceae)	Linoleic andoleic acids (Fatty acids)	S	P	MRSA	ERY	--
Psidium guineense (Swartz)(Myrtaceae)	--	L	W	MRSA	FOX	--
Rheum palmatum (Polygonacea)	Emodin flavonoid	R	I	MRSA	AMO,OXA	--

Table 1: Summary of plants screened for antibiotic modulatory effects of commercially available drugs against nosocomial bacteria, which yielded synergistic effects.

They are in the list for essential drugs in a health care system, recommended by the WHO and under the resistant surveillance projects by CDC and the British Society for Antimicrobial Chemotherapy, BSAC as well. The criteria for antibiotic selection varied from study to study, and often were not clearly stated In general, the most common motivations described in the articles' introductions were: a) The antibiotics' toxicity would be reduced by lowering effective concentration (For instance, the associated toxicities to the most frequently studied antibiotics are: i) Ampicillin can induce the development of asthma; ii) Ciprofloxacin can affect the circulatory system in 1% of the cases, and tendinitis. Also, occasional anaphylactic reactions due to beta–lactamics administration have been reported. iii) Aminoglycosides (Example: gentamicin) can cause ototoxicity (5%-10%), which can affect, adults, children and fetus; they can interact with other medications enhancing the toxicity effects), b) The economic burden associated to infectious diseases would be alleviated by shortening the dosages and hospitalization expenses, and c) the reverse the antibacterial resistance can potentially be achieved by exploring natural products bioactivity [70-73] (Table 2 and Figure 3).

No.Antimicrobial active principle (Secondary metabolite type)	Origin	Obtained from	Microbial strain	Antimicrobial agents showing synergistic effects	Comments
Baicalein (Flavonoid)	*Scutellaria baicalnsis Georg*	Sigma-aldrich	MRSA	CIP	--
Berberine (Isoquinoline alkaloid)	*Berberis spp.*	Sigma-aldrich	SE ATCC	LNZ, FOX,PEN, LCM, AMX	not FICI method
		Sigma-aldrich	SE		
Berberine, 8-acetonyl- dihy-droberberine (Isoquinoline alkaloids)	*Berberis sp., Mahonia sp.* (Berberidaceae), Coptis chinensis	Sigma-aldrich, and chemical derivatization	MRSA	AZM, LVX	mecA and SCCmec genes were detected at molecular level
Benzylisothiocyanate	Plants of the order Brassicales. Mustard	Sigma-aldrich	EF ATCC51299	VAN	--
Biochanin (Flavonoid)	Cicer arietinum (Fabaceae) chick	Sigma-aldrich	MRSA	CIP	--
Chalcones (Open chain flavonoids)	Wide spread in Nature, e.g.Leguminosae	synthetized	MRSA	CIP	Three chalcones were inhibitors of NorA efflux pump
Curcumin (Open chain flavonoid)	*Curcuma longa* (Zingiberaceae) the rhizome, polar extract	Isolated from the rhizome, polar extract	MRSA	ATPase inhibitors, detergents, and other compounds	--
			MRSA	OXY, AMP, CIP, NOR	--
Diosmetin (Flavonoid)	Ubiquitous flavonoid, e.g. Citrus limon (Rutaceae) Lemon	Sigma-Aldrich	MRSA	ERY	Inhibits ABC-pump and pyruvate kinase
Embelin (Benzoquinone)	*Embelia schimperi Vatke* (Primulaceae)	Sigma-Aldrich	MRSA, SA	OXA	--
Epigallo-catechin gallate (Flavonoid-Polyphenol)	*Camelia sinensis*	Sigma-Aldrich	MDRSA	OXY	--
		Sigma-Aldrich	MRSA	OXA, TET	--
		Sigma-Aldrich	ESBL-KP	CIP	--
Galangin baicalein quercitin (Flavonoids)	*Alpinia officinarum* (Zingiberaceae)	Purified from plant. Sigma-Aldrich	PRSA	CAZ	Metabolic reversion of resistant phenotype
Gallic-acid-grafted-chitosan	From Crustaceaen exoeskeleton chitin	Synthetized	MRSA	AMP, PEN	--
C-6-Geranylated flavonoids	*Paulownia tomentosa* (Pawloniaceae)fruits	Synthetized	MRSA	OXA	--
Isojacareubin (Xanthone)	*Hypericum japonicum*	Isolated from aerial parts	MRSA	CAZ, LVX	--

Luteolin (Flavonoid)	Ubiquitous phenolic compound	Sigma-Aldrich	MRSA	--	ATPase inhibitor
lysergol (Alkaloid)	*Ipomoea muricata* (Convulvulaceae)	Isolated from the plant's seeds	EC, NAREC, MDREC	TET	--
Magnolol, honokiol. (Diallylbiphenols)	*Magnolia officinalis* (Magnoliaceae)	Isolated from the plant's root	MRSA	AMK, ETM, GEN, FOS	--
5-O-methylglovanon (Isoprenylated flavonoid)	*Glycosmis spp* (Rutaceae)	Isolated from plant and also synthesized	ARSA, ARSE	AMP	No antagonistic effects were observed with other tested strains
Platensimycin, platencin (Terpenoid)	*Streptomyces platensis.* (bacteria)	isolated from culture	MRSA	Antibiotic hit	Pump inhibitor, project under drug development by Merck
Plumbagin (Naphtoquinone)	*Plumbago spp* (Plumbaginaceae)	Sigma-Aldrich	MRSA	OXA	--
Protosappanin A protosappanin B (Polyphenolic lactone)	*Caesalpinia sappannm* (Fabaceae)	Purified from the heartwood	MRSA	AMK, GEN	Metabolic reversion of resistant
Quercetin, luteolin, rhamnetin, octyl gallate (Flavonoids and other polyphenols)	Ubiquitous phenolic compounds	Sigma-Aldrich	CP	Ca^{2+} modulators (isradipine, verapamil, thapsigargin)	--
Quercetin, morin, rutin (Flavonoids)	Ubiquitous phenolic compounds	Sigma-Aldrich	MRSA, SA ATCC 43400	AMX, AMP, CPD, CRO, IPM, MTH	--
Quercitin (Flavonoid)	Ubiquitous flavonoid	Sigma-Aldrich	SA, MRSA	FUS, MNO,RIF	--
Salvianolic acid (Trimer of a polyphenyl propanoic acids)	*Salvia miltiorrhiza Bunge* (Lamiaceae)	Shanghai Greenvalley Pharmaceutical Co., Ltd, Shanghai, Chin	MRSA	AMP, AMK	--
Sanguinarine (Benzophenanthridine alkaloid)	*Sanguinaria canadensis* (Papaveraceae), root	Sigma-Aldrich	MRSA	CIP, NOR,OXA	--
Silibinin(Flavonolignan)	*Silybum marianum* (Asteraceae), seeds	Sigma-Aldrich	MRSA	OXA, AMP	--
Sodium new houttuyfonate (Sulfonic acid, surfactant)	*Houttuynia cordata* (Saururaceae)	Isolated from plant	MRSA	OXA, CF,MEM, NTM	--
Sophoraflavanone B (Flavonoid)	*Desmodium caudatum* (Fabaceae)	Sigma-Aldrich	MRSA	AMP, OXA, GEN, CIP, NOR	--

Table 2: Summary of purified natural products screened for antibiotic modulatory effects of commercially available drugs against nosocomial bacteria that yielded synergistic effects.

Bacterial strains usually were clinical isolates with a specific resistance (no full antibiogram reported). In the reviewed reports, the utilization of standard strains were the exception more than the rule (3% of the tested strains) [44-46, 48,56,63,74-76]. In almost all the cases, resistant opportunistic bacteria were the target; the most frequently strain tested were clinical isolates of MRSA. MRSA is in the WHO list of High Priority Bacteria that requires research and development of new antibiotics [41], and the CDC classifies MRSA as Serious Threat Level [25]. In 2014, MRSA was included among the US government National Targets for Combating Antibiotic-Resistant Bacteria, aiming to reduce by half (to at least 50%) the bloodstream infections caused by MRSA. *Shigella flexneri*, an intestinal pathogen, was the only pathogenic bacteria tested in the set of publications reviewed. Several of the studied strains belong to the so-called ESKAPE pathogens species (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumonia, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter*) [77]. The ESKAPE strains are a set of antibiotic-resistant pathogenic bacteria that represents new paradigms regarding pathogenesis, transmission, and resistance opportunistic bacteria not only represents a challenge in the ARE but can serve as model for other pathogenic strains that affect not only humans but animals and plants [78]. Further tests of the extracts that yielded synergistic effects with CADs against ESKAPE pathogens would allow assessing their potential as drug candidates, as well as cytotoxicity assays. Gram-negative bacteria still represents a medicinal chemistry challenge.

Even though gram–negative strains were included in the assays, synergistic effects were found mostly for gram-positive bacteria. The alkaloids lysergol and squalamina, flavonoids quercetin, lutein, rhamnetin, and other polyphenols octal gallate, benzylisothiocyanate, epigallo-catechin gallate, and the vitamin alpha-tocopherol yielded synergistic effects against gram-negative bacteria. At the same time, the cases of selective synergistic and antagonistic effects can be of interest for bio fermentation purposes [44,45]. The characterization of the tested strains should be pursued to be as complete as possible. Most of the studies were exploratory and further microbiology tests could be performed in collaborations with international collaborations (for instance CO-ADD initiative could be an interesting option).

Natural Products (NPs) were classified in two categories: Raw extracts and main component of a given botanical extract. Which were: i) purified from the raw extract and whose molecular structure was elucidated, ii) prepared by total synthesis, or iii) commercially available NPs (Table 2) [8,12-14,18,19,46,48,50,74-76,79-99].NPs derived from other kingdoms were less frequently found, example: honey, algae, bacterial derived drug leads: platencimicyn and platencin. Raw extracts: The compiled descriptors were: organism's family and scientific names, organ extracted, and polarity of the extracting solvent (Figure 4). The plant organ most frequently analyzed were the stems, the aerial parts extracted summed up to 35% Reports exploring flower extracts were not found; while the phenological state (example: seeding, flowering) or collection date were uncommonly reported. The natural source and extract's polarity were usually chosen based on references from traditional medicinal knowledge or ethno pharmacology. Other criteria were the follow up of given specie, genera, or family; and one study aimed the exploration of side products from industrial processes of an essential oil producing plant [40].

The extraction procedure was described in all the studies (maceration, infusion, reflux, or soxhlet methods). The majority of tested extracts were fractions (or subfractions) extracted with low-polarity solvents. For instance, the alcoholic extracts were further

fractionated, the non–polar subfraction was then bio assayed (example: ethyl acetate sub–fraction). Several techniques were utilized to analyze the extracts composition, example: a thin layer chromatography, high performance liquid chromatography coupled to mass spectrometry. However, such analyses were not consistently included (Non-polar extracts were usually redissolved in Di-Methyl Sulf-Oxide (DMSO), and then tested for their bioactivity. DMSO is not present in the final drug formulations, but it is commonly utilized to dissolve low polarity compounds in aqueous systems, instead of non-polar solvents such as hexane. DMSO by itself has a metabolic effect in cells that can affect the outcome bioactivity results 44. Control experiments quantifying solvents effects were not generally clearly stated in the studies summarized in Tables 1 and 2. This type of experiments could even be considered as a ternary mixture: NPs, CAA, and the solvent. Other approaches for suspension or dissolution of NPs are needed in order to reduce false positives or potency overestimation of a given NPs.)

Polar extracts, the less utilized in the given reports set, can contain bioactive compounds that are already soluble [40,44,45,49]. However, the intricate nature, especially of aqueous extracts, has been limiting their exploitation. It is important to develop technologies that can allow for a more feasible purification process of polar extracts. Chemical analysis and further bioactivity characterization should be pursued for the extracts. For example, the activity of *Acacia mearnsii* bark polar-extract had been explored against a variety of ESKAPE strains, further characterization may lead to find drug hits and understanding of the antagonistic effects observed for some of the strains studied [44,45].

Main components of a given botanical extract or purified NPs

whose molecular structure have been elucidated were described including i) common chemical name; ii) compound classification according to the secondary metabolites family; iii) plant of origin; iv) if the substance was purified from the

raw extract, prepared, or commercially available (Tables 1 and 2). The family most frequently tested was flavonoids (mostly chalcones and flavanones). To date some of them are included in dietary supplements that are not necessarily approved by the Food and Drug Administration (FDA). For instance, quercetin in vitamin C supplements that claim to boost the immune response [30]. Flavonoids show a variety of bioactivities besides antibacterial with a mid-intensity effect, that is anti-inflammatory and antiviral activities. Efforts toward understand their mechanism of action at a molecular level are ongoing [31].

The adjuvant antimicrobial activity space for alkaloidal family was less explored. The 25% of the NPs families explored for that bioactivity. Three alkaloids were clearly defined as druggable structures: Berberine, Platensymicin, Platencin (Tables 1 and 2). These three alkaloids showed stronger adjuvant potency with a more specific effect than the set of flavonoids tested , the latter two are being considered to be antibiotics and are under further development by Merck

pharmaceuticals (Table 2) [74,79,80,87]. The mechanism of action of these three compounds has been proposed to be orthogonal to those of the CADs. Further functionalization of their alkaloidal skeletons may allow improvement of their medicinal chemistry [74,79,80,87].

The dominant supplier of natural products, by either natural extract purification or organic synthesis preparation, was Sigma-Aldrich Company. It is worth to mention that the aqueous extract obtained as a side product of the industrial essential oil extraction, the so called hydrolyte was tested which fits in the bio prospecting framework [40].

Successful examples of CADs+NPs, at *invitro* level, hint that the enhancement of the antibacterial activity of CADs is a worthy approach to keep exploring and expanding. As it is further exploited, some challenges need to be addressed (or taken in account): a) A given extract can yield synergistic effects for some strains while antagonistic for other strains. b) The reproducibility is an issue related to the intrinsic complexity of natural extracts. c) The information included in the scientific reports can be more consistent across literature, e.g: extract's composition, control experiments, strain profile. d) The need of widespread a high throughput analysis platform to speed up the analysis in each stage of the NPDD. e) Funding related to bio prospecting studies, as

well as human resources, are required. f) Innovative approaches, aiming to increase the success rate of plausible medications derived from NPs and CADs mixtures need to keep developing.

Examples of NPs mechanisms of action that are orthogonal to the antibiotics' mechanism of action

Proposed interactions of NPs with bacterial components are depicted in Figure 1. Curcumin, Epi-Gallo-Catechin gallate (EGCg) and Berberine are examples of natural products whose mechanism of action have been frequently studied at sub–cellular level. The

main component in the curcuma spice, curcumin, disrupted the bacterial cell membrane, probably by intercalating in the lipid bilayer [6,81,100]. The main antioxidant component in green tea, EGCg, inhibited beta-lactamase (an antibiotic degrading enzyme); EGCg interacts with cell membrane components, disrupting the cell membrane integrity [81,88,101]. The alkaloid berberin inhibited the efflux pumps with medium specificity a resistance mechanism enabling the antibiotics to remain longer periods inside the bacteria to interact with the desired target [64,79,92,102,103]. Bacterial cell membrane disruption is a non-specific interaction, saponins, other polyphenols and flavonoids [8], and essential oils can exert this effect as well [44,9].

Bacteriostatic effects of polyphenolic compounds such as coumarins, gallic acid, and flavonoids are attributed to: i) To precipitate proteins, a non-specific interaction that reduces the bacterial metabolism; ii) To act as enzymatic reversible inhibitors, reducing the bacterial metabolism, especially if the targets are housekeeping processes; iii) To deplete the bacterial proton motive force and electron flow, thus reducing the amount of the biochemical energy available, which can induce a non–reproductive state in the bacterial metabolism [104-106]. As more information on these mechanisms of actions is gathered, the medicinal chemistry of NPs as drug candidates can be improved. Assays aiming a high throughput approach for the exploration of NPs mechanisms of action against bacteria have been developed, for instance the NPs interactions with peptidoglycan, ATPase's inhibitors, efflux pump inhibitors, membrane disruption [107-109]. Studies on inhibitors of bacterial biofilm formation require more attention-from the NPs perspective [110].

Antifungal synergistic effects: Four reports were found testing a total of 12 opportunistic strains including filamentous fungi (Table 3) [111-114]. Three extracts and two purified natural products yielded synergistic effects with azole type antifungals (Table 3).

Plant extracted effects	Antimicrobial active principle	Parts	Fraction polarity	Microbial strain	Antimicrobial agents showing synergistic	Comments
Extracts assayed against fungi						
Andrographis paniculata	--	W	N,P	AN, CA, CT, MC	--	--
Quercus ilex	Phenolic compounds: Procyanidin B3, Prodelphinidin C	L	--	AFm, AN, PF, PO	BIF	--
Quercus ilex	Phenolic compounds: Procyanidin B3, Prodelphinidin C	L	--	Afl, PF, PO	KET	--

Purified natural products assayed against fungi						
Luehea paniculata	Berberine	Sigma-	--	CA, CG, CKf,	MIC, FLU	--
	-	Aldrich	--	CKr, CP, CT	--	--
	Rosmarinic acid, Vitexin flavonoid	--	--	CT	FLU	abstract
Luehea paniculata	Berberine	Sigma-	--	CA, CG, CKf,	MIC, FLU	--
	-	Aldrich	--	CKr, CP, CT	--	--
	Rosmarinic acid, Vitexin flavonoid	--	--	CT	FLU	abstract

Note: Strains: AFL: Aspergillus Flavus; AFm: Aspergillus Fumigatus; AN: Aspergillus Niger; CA: Candida Albicans; CG: Candida Glabrata; CKr: Candida Krusei; CKf: Candida Kefyr; CP: Candida Parapsilosis; CT: Candida T; MC: Microsporus Canis; PN: Penicillum Funiculosum; PO: Penicillium Ochrochloron; Antimicrobials: BIF: Bifonazole; FLU: Fluconazole; KET: Ketoconazole; MIC: Miconazole.

Table 3: Summary of plants screened for antimicrobial modulatory effects of commercially available drugs that yielded synergistic effects against fungi.

CONCLUSION

The field of antimicrobial synergistic effects is active and yielding drug hits and extracts with promising bioactivities and mechanisms of action. In order to advance in the drug discovery research pipeline is important to pursue the full characterization of the natural products and strains tested; for which inter and multidisciplinary collaborations at local and international level are needed and funding agencies institutions should enhance support, not only by the grants but by facilitating the interactions of scientist from different areas such that more collaborations can emerge. Interactions of this scientific community with industries that utilize plants as raw materials can help to identify potential niches for further plant waste exploitation, contributing to form circular economies in the agroindustry.

Compilation of the reports of synergistic effects of NPs and CADs

The compiled reports of binary mixtures of natural products and commercially available drugs, NPs+CADs, that yielded synergistic effects against opportunistic bacteria were divided in two types of natural products, plant extracts and purified natural products and a section of fungicide.

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