UKaaZ

Annals of Phytomedicine 2(2): 28-46, 2013

ANNALS OF PHYTOMEDICINE
An International Journal

ISSN 2278-9839

Antimicrobial activity of phytopharmaceuticals for prevention and cure of diseases

Mary Grace Jinukuti and Archana Giri

Centre for Biotechnology, Institute of Science and Technology, Jawaharlal Nehru Technological University Hyderabad, Kukatpally, Hyderabad-50085, A.P., India

Received August 27, 2013: Revised September 30, 2013: Accepted October 10, 2013: Published online December 30, 2013

Abstract

Plants have been used for thousands of years to treat various health disorders and to prevent diseases including epidemics. Over the centuries within and among human communities, the knowledge of their healing properties has been transmitted. Attention has been drawn towards the studies on the potential antimicrobial activity of plant-derived substances by pharmaceutical and scientific communities, due to the challenge of growing incidence of drug-resistant pathogens. Recently, focus has been established on the effect of the plant extracts in terms of their microstatic and microcidal action and the spectrum of organism which has enabled exploitation of plants for the treatment of microbial infections in the development of new antimicrobial agents. Antimicrobial action of the plant extract is largely dependent on the techniques employed in the investigations and conclusive results can only be obtained, if methods are standardized and universal. This review focuses on medicinal plants and their active compounds as a useful source of antimicrobial agents and current methods used in the investigation of the efficacy of plants as antimicrobial agents.

Key words: Medicinal plants, phytochemistry, antimicrobial activity, plant extracts, antimicrobial techniques

Introduction

Medicinal plants are used for treating various diseases since prehistory. Around, 250 to 500 thousand plant species are estimated to be present on the planet, out of which 1 to 10% of them are used as food by human beings and other animals (Cowan , 1999). According to World Health Organization (WHO), 80% of the world's population depends mainly on traditional medicines and use plant extracts in traditional treatments (WHO, 1993). This is commonly found in rural areas where synthetic drugs are not available or too expensive to purchase. Some of the crude drugs used in past are still in use in phytotherapeutics. *Cinchona* plant is often used in its natural and unrefined form to treat malaria (Trease and Evans, 1985), although some other herbal drugs are refined by isolating their pure active compounds and their active principles, helped in the advancement of scientific medicine. 20th century, the antibiotic era substantially reduced the threat of infectious diseases, but over the years there is a decrease in microbial susceptibility to existing microbial agents, responsible for drug resistance which is exerting global problem today. Infact, the theme of World Health Day 2011 was: **no action today, no cure tomorrow**. There is an urgent need of new antimicrobial agents to overcome this global problem (Gould, 2008). It was the discovery of penicillin that led to later discoveries of antibiotics such as streptomycin, aureomycin and chloromycetin. Each year, two million people are admitted with bacterial infection in US hospitals, 70% of the cases involve strains that are resistant to atleast one drug (Infectious Diseases Society of America, 2004). Higher plants are a rich source of antibiotics (Trease and Evans, 1972), the antibiotic action of allinine from *Allium sativum* (garlic), or the antimicrobial action of berberine from

Author for correspondence: Professor Archana Giri
Centre for Biotechnology, Institute of Science and Technology, Jawaharlal Nehru Technological University Hyderabad, Kukatpally, Hyderabad-50085, A.P., India
E-mail: archanagiriin@yahoo.co.in
Tel.: +91-09849028367, 040-23156129

goldenseal (*Hydrastis canadensis*). During the period (1981-2006), 109 drugs were approved in which 69% were from natural products and 21% of the antifungal drugs were refined natural derivatives (Newman, 2008).The promising potential of antimicrobial plant derived substances have attracted the attention of pharmaceutical and scientific communities during the last few years (Osbourn, 1996). The primary benefit of plant derived medicines is that they are relatively safer than their synthetic counterparts and offer profound therapeutic benefits and more affordable treatment.

Brief history

Since the dawn of humanity, war against disease has been a part of everyday life and the use of plant materials to treat the sickness is as old as man (Longer and Robinson, 1985). The Ancient Egyptians in 1500 BC, wrote the "Ebers Papyrus" which contain information on around 850 plant medicines, including garlic, juniper, cannabis, castor bean, aloe, and mandrake (Sumner and Judith, 2000). In India, earliest Sanskrit writings such as the Rig Veda, and Atharva Veda are some of the earliest available documents, detailing the medical knowledge that formed the basis of the Ayurveda system (Sumner and Judith, 2000). Ayurveda medicine has used herbs such as turmeric possibly as early as 1900 BC (Aggarwal *et al*., 2007) and many other herbs and minerals in Ayurveda are later described by ancient Indian herbalists such as Charaka and Sushruta during the 1st millennium BC. Shennong, the mythological Chinese Emperor is said to have written the first Chinese pharmacopoeia, the "Shennong Ben Cao Jing" which contain a list of 365 medicinal plants and their uses - including *Ephedra* (the shrub that introduced the drug ephedrine to modern medicine), hemp, and chaulmoogra (one of the first effective treatments for leprosy) (Sumner and Judith, 2000). In the late fifth century B.C., Hippocrates mentioned 300 to 400 medicinal plants (Schultes, 1978). Dioscorides wrote "*De Materia Medica"* in the first century A.D, a medicinal plant catalog which became the prototype for modern pharmacopoeias. During the Early Middle Ages, Benedictine monasteries were the primary source of medical knowledge in Europe and England (Arsdall and Anne, 2002). A 12th-century Benedictine nun wrote a medical text called *Causae et Curae* (Ramos-e-Silva, 1999). The 15th, 16th, and 17th centuries were the great age of herbals, many of them available for the first time in English and other languages rather than Latin or Greek and the first herbal to be published in English was the anonymous *Grete Herball* of 1526. In the modern era, pharmaceuticals currently available to physicians have a long history of use as herbal remedies, including opium, aspirin, digitalis, and quinine. Pharmacologists, microbiologists, botanists, and natural-products chemists are combing the Earth for phytochemicals that can be used for the treatment of various diseases. According to the World Health Organisation, approximately 25% of modern drugs used in the United States have been derived from plants. Around 7,000 medical compounds in the modern pharmacopoeia are derived from plants (Interactive European Network for Industrial Crops and their Applications, 2000-2005).

Antimicrobial properties of medicinal plants

Plant-derived substances have recently gained great interest due to their versatile applications (Baris *et al*., 2006). Medicinal plants are the richest bioresource of drugs of modern medicines , food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Hammer *et al*., 1999). *Baccharis trimera* Less *"carqueja"* showed antimicrobial action on both Gram -positive (*Staphylococcus aureus and Streptococcus uberis*) and Gram-negative (*Salmonella gallinarum and Escherichia coli*) bacterial strains and it was found that the former microorganisms are more sensitive to this herb than the latter, which corroborates previous studies(Avancini *et al*., 2000). Essential oil obtained from stem and leaves of *Achillea millefolium* showed higher antimicrobial activity than its respective extracts .These oils prevented the growth of *Streptococcus pneumoniae, Clostridium perfringes* and *Candida albicans* and inhibited *Mycobacterium smegmatis*, *Acinetobacter lwoffii* and *Candida krussei* to a lesser extent (Candan *et al*., 2003). The phenolic compounds present in ethanol extract of chamomile showed antibacterial properties against *Staphylococcus aureus* (Asolini *et al*., 2006). Essential oils from *Pelargonium graveolens* (geranium) showed low values of minimum inhibitory concentration against *B. cereus* (0.36 mg/ml), *B. subitilis* (0.72 mg/ml) and *S. aureus* (0.72 mg/ml), whereas oils from *Origanum vulgare* (oregano) also showed antimicrobial activity against the same bacteria, in addition to *Escherichia coli*. However, a concentration of 0.35 mg/ml is required to inhibit *B. subitilis* whereas 0.70 mg/ml is necessary to inhibit the other bacteria (Rosato *et al*., 2007) and *Centella asiatica* L. (Suryakala, 2013). The hydroalcoholic extract of *Rosmarinus officinalis* Linn. (rosemary) showed antimicrobial activity against *Streptococcus mitis, Streptococcus sanguinis, Streptococcus mutans, Streptococcus sobrinus* and *Lactobacillus casei* except *S. mitis* (Silva *et al*., 2008). Phytochemicals of plant origin with excellent antimicrobial activity are summarized in Table 1.

Table 1: Plants with antimicrobial activity

S.No	Scientific name	Compound	Class	Activity	Reference
1.	*Abrus precatorius* L.	Hypaphorine Isohemiphloin	Alkaloids Flavonoids	*K. pneumonia,* *S. aureus,* *Mycobacteriun aurum*	Balungile *et al*., 2013
2.	*Adesmia aegiceras*	Quercetin Chlorogenic acid Isovitexin	Polyphenols Flavonoid Cyclic polyol	*E. coli,* *S. aureus,* *S. saprophyticus,* *P. aeruginosa,* *P. mirabilis.*	Agnese *et al.*,2001
3.	*Allium cepa* L.	Allicin	Sulfoxide	*Bacteria*	Vohora *et al*., 1973
4.	*Allium sativum* L.	Allicin, Ajoene	Sulfoxide	*B. subtilis,* *S. aureus,* *Mycobacterium phlei* *S. grisseus,* *E. coli,* *P. aeruginosa,* *K. pneumonia.*	Naganawa *et al*., 1996; San-Blas *et al*., 1993; San-Blas *et al*., 1989
5.	*Aframomum alboviolaceum* (Ridl.) k schum.	Essential oil	Terpenoid	*E. coli,* *E. aerogenes,* *E. cloacae,* *K. pneumonia,* *P. stuartii,* *P. aeruginosa.*	Doriane *et al*., 2013
6.	*Anthemis xylopoda* O. schworz	Essential oils	Terpenoid	*S. aureus,* *E. fecalis,* *B. cereus,* *B. subtilis,* *E. coli,* *K. pneumonia,* *P. vulgaris,* *P. aeruginosa,* *P. fluorescens,* *S. typhimurium.*	Uzel *et al*., 2004
7.	*Ardrographis paniculata* (Burm.f.) Wall ex.Nees	Tannic acid, Gallic acid, Caffeic acid, Vanilli C acid, Ferulic acid, Chlorogenic acid, Cinnamic acid, Salicylic acid	Phenolic acid	*E. coli,* *P. aeruginosa,* *K. pneumonia,* *S. typhimurium,* *E. cloacae,* *S. aureus,* *B.subtilis,* *E.fecalis,* *S.epidermis.*	Somaroy *et al*., 2010

8.	*Artemisia douglasiana* Besser.	Camphor, Artemisia ketone, Artemisia alcohol Hexanal	Terpenoid alkyl aldehyde	*B. cereus, S. aureus, P. aeruginosa, E. coli.*	Setzer *et al*., 2004
9.	*Artemisia species viz., A.scoparia, A.turanica and A.oliveriana*	Monoterpenes, Sesquiterpenes	Terpenes	*B. subtilis, S. aureus, P. aeruginosa,*	Ramezania *et al.*, 2004
10.	*Alpinia galanga* (L.) Wild.	Galangin	Flavonoid	*B. subtilis, E. aerogene, E. fecalis, E. coli, K. pneumonia, P. aeruginosa, S. typhimurium, S. aureus.*	Kiranmayee Rao *et al*., 2010
11.	*Berberis vulgaris* L.	Berberine	Alkaloid	*S. aureus*	McDevitt *et al*., 1996; Omulokoli *et al*., 1997
12.	*Capsicum annuum* L.	Capsaicin	Terpenoid	B. cereus, B. subtilis, C. sporogenes, S. pyogenes.	Cichewicz and Thorpe 1996; Jones and Luchsinger, 1986
13.	*Carum carvi* L.	Thymol	Terpenoid	*S. aureus, P. aeruginosa, E.coli.*	Berkada, 1978; Bose, 1958; Hamburger and Hostettmann, 1991; Scheel, 1972
14.	*Cassia alata* (L.) Roxb	Cassiaindoline	Alkaloid	*E. coli, S. aureus.*	Somchit *et al*., 2003
15.	*Carica papaya* L.	Latex	Mix of terpenoids, organic acids, alkaloids	*E.coli, S. aureus, P. aeruginosa, K. pneumonia.*	Burdich, 1971; Osato *et al*., 1993; Satrija *et al*., 1995
16.	*Citrus sinensis* (L.) Osbeck	_	Terpenoid	*S. aureus, P. aeruginosa, K. pneumonia.*	Stange *et al*., 1993
17.	*Curcuma longa* L.	Curcumin Turmeric oil	Terpenoids	*B. subtilis, M. grisea*	Apisariyakul *et al*., 1995
18.	*Datura innoxia* Mill.	Atropine, Hyoscine, Hyoscyamine	Alkaloids	*S. aureus, E. fecalis B. subtilis.*	Eftekhar *et al*., 2005
19.	*Heliotropium subulatum*	Heliotrine 7-angeloyl heliotrine, Retronecine subulacine-N-oxid	Alkaloids	*E.coli, S.aureus, S. pneumoniae, B. subtilis, B. anthracis.*	Singh *et al*., 2002b

20.	*Hibiscus sabdariffa* L.	Anthocyanins Hibiscus protocatechuic acid	Flavonoids Phenolic	*E. coli, E. aerogenes, E.cloacae, K. pneumonia, P. stuartii, P. aeruginosa.*	Doriane *et al*., 2013
21.	*Ixora coccinea* L.	Lupeol Quercitin Gentisic	Ester Flavonoid Phenolic acid	*E.coli, P. aeruginosa, S. typhimurium, S. aureus and B. subtilis.*	Annapurna *et al*., 2003
22.	*Indigofera* spp.	Anthocyanin	Flavonoid	*K. pneumonia, S. aureus, Mycobacteriun aurum.*	Balungile *et al*., 2013
23.	*Juniperus oxycedrus* L.	Essential oils	Terpenoid	*Bacillus, Escherichia, Staphylococcus, Enterobacter, Acinetobacter, Xanthomonas and pseudomonas.*	Karamana *et al.*, 2003
24.	*Lepista nude*	Linoleic acid	Essential fatty acids	*S. typhimurium, K.pneumonia.*	Dulgera *et al*., 2002
25.	*Malus sylvestris*	Phloretin	Flavonoid derivative	*E.fecalis, S.aureus.*	Hunter and Hull, 1993
26.	*Matricaria chamomilla* L.	Anthemic acid	Phenolic acid	*M. tuberculosis, S. typhi murium, S. aureus* Helminthes.	Bose, 1958; Hamburger and Hostettmann, 1991; Scheel, 1972
27.	*Millettia thonningii* (Schumach) Baker	Alpinumisoflavone	Flavone	*Schistosoma.*	Perrett *et al*., 1995
28.	*Melissa officinalis*	Tannins	Polyphenols	*B. subtilis, S. typhi murium,* Viruses.	Wild, 1994
29.	*Ocimum basilicum*	Essential oils	Terpenoids	*Salmonella*	Wan *et al*., 1998
30.	*Olea europaea*	Hexanal	Aldehyde	*E. coli, K. pneumonia, P. aeruginosa.*	Kubo *et al*., 1995
31.	*Onobrychis viciifolia*	Tannins	Polyphenols	*Ruminal* bacteria	Jones *et al*., 1994
32.	*Piper nigrum*	Piperine	Alkaloid	Fungi, *Lactobacillus.*	Goshal *et al*., 1996
33.	*Petalostemum*	Petalostemumol	Flavonol	*M. intracellulare, B. subtilis , S.aureus.*	Hufford *et al*., 1993

34.	*Quercus rubra*	Quercetin	Flavonoid	*E. faecalis,* *B. cereus,* *S. epidermidis.*	Keating and Kennedy, 1997
35.	*Rabdosia trichocarpa*	Trichorabdal A	Terpene	*Helicobacter pylori*	Kadota *et al*., 1997
36.	*Rhynchosia beddomei*	Essential oils	Terpenoids	*B. subtilis* , *S. aureus,* *E. coli,* *P. aeruginosa.*	Bakshu and Venkat Raju, 2001
37.	*Rubia peregrina* L.	Pentacyclic triterpenes Cyclopeptides	Terpenes peptide	*B. subtilis,* *S. aureus,* *E.coli.*	Ozgena *et al.*,2003
38.	*Salacia beddomei*	Salacinol, Kotalanol	α-glucosidase	*B. subtilis,* *S. aureus,* *E. coli,* *K. pneumoniae,* *P. phosphorescence,* *Aeromonas hydrophylla.*	Deepa and Narmada, 2004
39.	*Santolina chamaecyparissus* L.	Essential oils	Terpenoids	*S.aureus,* *S. epidermidis.*	Suresh *et al*., 1997
40.	*Satureja montana*	Carvacrol	Terpenoid	*S. typhi murium.*	Ali-Shtyeh *et al*., 1997
41.	*Satureja khuzestanica* L.	Essential oils	Terpenoids	*S. aureus.*	Amanloua *et al*., 2004
42.	*Scutellaria barbala*	Essential oils	Terpenoids	*S. aureus,* *E. coli,* *P. aeruginosa,* *S. epidermidis,* *S. heamolyticus,* *S. simulans,* *E. fecalis,* *C. freundii,* *K. pneumonia,* *S. flexneri,* *S. typhi,* *S. paratyphi-A,* *S. liquefaviens,* *S. marcescens,* *S. maltophila.*	Yu *et al*., 2004
43.	*Salacia oblonga* Wall.	Salicic acid di-ethyl bis (tri-methylsilyl) ester	Aliphatic ester	*B. subtilis,* *E. aerogene,* *E. fecalis,* *E. coli,* *K. pneumoniae,*	Anjaneyulu *et al*., 2013

				P. aeruginosa, *S. typhimurium,* *S. aureus,* *E. cloacae,* *S. epidermis,* *L. monocytogenes.*	
44.	*Solanum aculeastrum* Dunal	Essential oils	Terpenoids	*S. epidermis,* *S. aureus,* *B. subtilis,* *K. pneumoniae,* *P. aeruginosa,* *E. coli,* *E. fecalis.*	Wanyonyi *et al*., 2003
45.	*Solidago virgaurea* L.	Quercetin, Kaempferol	Polyphenol Flavonoid	*S. epidermis,* *S. aureus,* *B. subtilis,* *K. pneumoniae,* *P. aeruginosa,* *E. coli, E. fecalis.*	Thiem and olga, 2002
46.	*Strobilanthes callosus* (Nees)	Triterpenoids	Terpenoid	*E.coli,* *S.aureus,* *K.pneumoniae,* *E. cloacae,* *B. thuringiensis.*	Singh *et al*., 2002a
47.	*Vaccinium* spp.	Fructose	Monosaccharide	*E. coli.*	Ofek *et al*., 1996
48.	*Viola tricolor* L.	Vitri peptide A	Cyclic peptide	*S. epidermis,* *S. aureus,* *B. cereus,* *K. pneumoniae,* *P. aeruginosa,* *E. coli,* *E .fecalis.*	Witkowska *et al*., 2005
49.	*Warburgia ugandensis* Sprague	Kaempferol	Flavonoid	*S.aureus, Methicillin resistance S.aureus (MRSA).*	Peter *et al*., 2013
50.	*Withania somnifera* (L.) Dunal	Withaferin A	Withanolide	*S. aureus,* *E. coli,* *P. aeruginosa.*	Singh and Kumar, 2011

Antimicrobial compounds and their mode of action on microbes

Plant-derived compounds with therapeutic value are mostly plant secondary metabolites which are traditionally used for medicinal purposes. They exhibit a wide range of activity according to the species, the topography and climatic condition . Plants contain a wide array of active principles (Assob *et al*., 2011; Arruda *et al.*, 2011), different group of secondary metabolites display varying pharmacological activities.

Phenolics

Phenolic compounds are one of the most diverse groups of secondary metabolites, found in plants and also found in fruits, vegetables, nuts, seeds, stems and flowers as well as

tea, wine, propolis and honey and form an important constituent of human diet. They play an important role in plant growth and reproduction, provide resistance from pathogens and predators and protect crops from disease and preharvest seed germination (Ross and Kasum, 2002).

Mechanism of action

Antimicrobial action of phenolic compounds was related to inactivation of cellular enzymes, which depends on the rate of penetration of the substance into the cell or by membrane permeability changes (Moreno *et al*., 2006). The major factor in the mechanism of antimicrobial action is increase in membrane permeability, where compounds may disrupt membranes and cause a loss of cellular integrity and eventual cell death. *Escherichia coli* is a food borne pathogen that causes enterohemorrhagic infection and, occasionally kidney failure. It is widely used in antimicrobial screening studies, in conjunction with other beneficial and pathogenic bacteria. Multivariate statistical analyses have been applied to confirm similarities and differences among phenolic acids based on their antimicrobial potency. The variations in antimicrobial activities among bacteria may reflect differences in cell surface structures between Gram-negative and Gram-positive species. *Lactobacillus spp.* and *Staphylococcus aureus* (Gram-positive) appeared more susceptible to the action of phenolic acids than Gram-negative bacteria such as *E. coli* and *Pseudomonas aeruginosa*. In *E. coli*, mutations of the lpxC and tolC genes seemed to amplify the phenolic acid antimicrobial mechanisms of action against Gram-negative species. The antimicrobial potential of the phenolic acids against the different microorganisms are influenced by the number and position of substitutions in the benzene ring and the saturated side-chain length. Phenolic acids exhibited greater antimicrobial potency than their corresponding precursors such as the monomers (þ)-catechin, (-) epicatechin and dimers B1 and B2. Active compounds such as thymol, eugenol, and carvacrol cause disruption of the cellular membrane, inhibition of ATPase activity, and release of intracellular ATP and other constituents of several microorganisms such as *E. coli*, *L. monocytogenes*, *Lactobacillus sakei*, *Pseudomonas aeruginosa*, *Salmonella enteritidis*, and *S. aureus* (Lambert *et al*., 2001; Raybaudi-Massilia *et al*., 2009). Oussalah *et al*. (2006) and Gill and Holley (2004) reported that cinnamon oil and cinnamaldehyde stimulated a decrease in the intracellular ATP by ATPase activity without apparent changes on the cell membrane of *E. coli* and *L. monocytogenes*. This might be due to the interaction of cinnamaldehyde with the cell membrane, which may cause enough disruption to disperse the proton motive force by leakage of small ions but without leakage of larger cell molecules such as ATP (Raybaudi-Massilia *et al*., 2009). Organic acids are usually employed in food preservation because of their effects on bacteria. The mode of action of organic acids on bacteria is non-dissociated (non-ionized) and can penetrate the bacterial cell wall and disrupt the normal physiology of certain types of bacteria that is called pH-*sensitive*, which cannot tolerate a wide internal and external pH gradient (Figure 1).

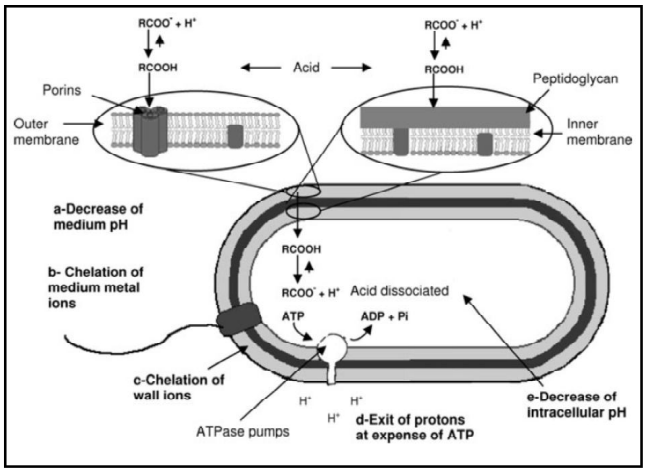


Figure 1: Mechanisms of action of organic acids in a bacterial cell (Raybaudi-Massilia *et al*., 2009).

The passive diffusion of organic acids into the bacteria cause dissociation of acids and lower the bacterial internal pH which leads to the situations which impairs or stops the growth of bacteria. On the other hand, the anionic part of the organic acids that cannot escape the bacteria in its dissociated form, gets accumulated within the bacteria and disrupt many metabolic functions, leading to osmotic pressure increase,that is incompatible for bacterial survival. Essential oils, which are concentrated hydrophobic liquid containing volatile aroma compounds from plants, provide a wide research area. Burt (2004) reported that the antibacterial activity of the essential oils is not carried out by one specific mechanism but acts over several specific targets in the cell. Skandamis *et al*. (1999) have reported the location and mechanism of action of essential oils on bacterial cell such as : degradation of the cell wall, damage to cytoplasmic membrane and membrane proteins (Lambert *et al*., 2001), leakage of contents out of the cell, coagulation of cytoplasm (Burt, 2004), and depletion of the proton motive force (Figure 2). Raybaudi-Massilia *et al*. (2009) reported that the mode of action of essential oils depends on the concentration of phytochemicals, indicating that the low concentrations inhibit enzymes associated with energy production, while higher amounts may precipitate proteins.

Flavonoids are phenolic structures, ubiquitous in photosynthesizing cells (Havsteen, 1983) and are commonly found in fruits, vegetables, nuts, seeds, stems, flowers, tea, wine (Middleton *et al*., 1986), propolis and honey (Grange and Davey, 1990), and represent a common constituent of the human diet (Harborne and Baxter, 1999). The function of flavonoids in flowers is to provide colours, attractive to plant pollinators and in leaves, this compound is believed to promote physiological survival of the plant and protects them from fungal pathogens and UV-B radiation (Harborne and Williams, 2000). Flavonoids are involved in photosensitisation, energy transfer, control of respiration and photosynthesis, morphogenesis and sex determination. (Harborne and Williams, 2000). Flavonoids have been reported to possess many useful properties, *viz.*, anti-inflammatory activity, oestrogenic activity, enzyme inhibition, antimicrobial activity (Havsteen, 1983), antiallergic activity, antioxidant activity (Middleton *et al*., 1986) and cytotoxic antitumour activity (Harborne and Williams, 2000) for which it has become the subject of medical research. For centuries, preparations containing flavonoids are used by physicians in an attempt to treat human diseases (Havsteen, 1983). For example, the plant *Tagetes minuta* (containing quercetagetin-7-arabinosyl-galactoside) has been used extensively in Argentine folk medicine to treat infectious disease (Tereschuk *et al*., 1997). The basic structural feature of flavonoid compounds is the 2-phenyl-benzo pyrane or flavane nucleus, which consists of two benzene rings (A and B), linked through a heterocyclic pyrane ring (C) (Figure 3) (Brown, 1980). Structures of some of the major classes of flavonoids are given in Figure 4.

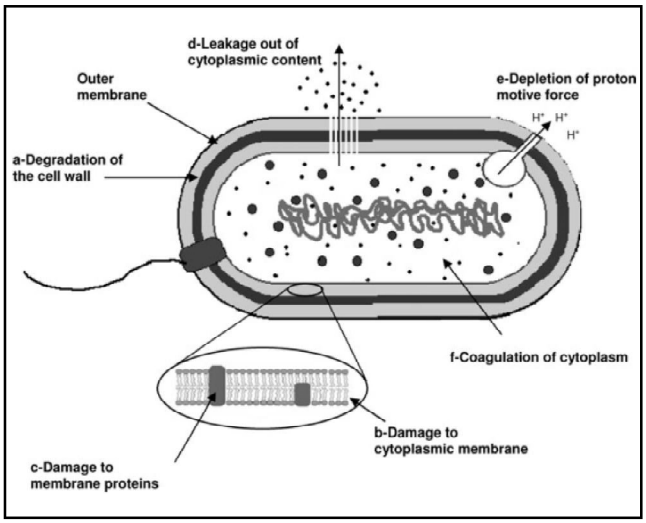


Figure 2: Mechanisms of action of essential oils and their components in a bacterial cell (Burt, 2004).

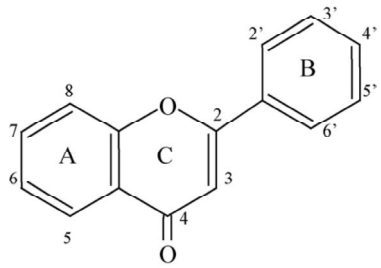


Figure 3: The structure of the flavones (a class of flavonoids) (Harborne and Baxter, 1999).

Flavonoids as antimicrobial agents

Flavonoids are being increasingly documented due to their antibacterial activity. *Hypericum* (Dall'Agnol *et al*., 2003), *Capsella* and *Chromolaena* (El-Abyad *et al*., 1990) are reported to possess antibacterial activity that are rich in flavonoids. Flavonoids having antibacterial activity have been isolated and identified by many research groups, *viz*., apigenin , galangin, genkwanin , sophora flavanone G and its derivatives and other flavones such as flavone glycosides, isoflavones, flavanones, isoflavanones, isofla-vans, flavonols, flavonol glycosides and chalcones.

Mechanisms of action of various flavonoids

In a study, using radioactive precursors showed that DNA synthesis was strongly inhibited by flavonoids such as robinetin, myricetin and (“)-epigallocatechin in *Proteus vulgaris*, whilst RNA synthesis was most affected in *S. aureus*. Protein and lipid synthesis were also affected but to a lesser extent. The inhibitory action on DNA and RNA synthesis depends on the B ring of the flavonoids that play a major role in intercalation or hydrogen bonding with the stacking of nucleic acid bases. Ohemeng *et al*. (1993) screened 14 flavonoids of varying struc ture to determine the inhibitory activity against *Escherichia coli* DNA gyrase, and for antibacterial activity against *Staphylococcus epidermidis*, *E. coli*, *S. aureus*, *Stenotrophomonas maltophilia* and *S. typhimurium*. It was found that *E. coli* DNA gyrase was inhibited to different extents by seven compounds, including quercetin, apigenin and 3,6,7,3$^{\times}$,4$^{\times}$- pentahydroxyflavone. Interestingly, enzyme inhibition was limited to those compounds with B-ring hydroxylation, with the exception (Ohemeng *et al*., 1993) of 7, 8-dihydroxyflavone. The authors proposed that the antibacterial activity of flavonoids was due to their inhibition of DNA gyrase. However, since the level of antibacterial activity and enzyme inhibition did not correlate always, it was suggested that other mechanisms may also be involved. A research team previously had found sophorafla- vanone G to have intensive antibacterial activity against MRSA and *Streptococci* which made them to elucidate the mechanism of action of this flavanone recently. Using liposomal model membranes, the effect of sophoraflavanone

G on membrane fluidity was studied and compared with the less active flavanone naringenin, which lacks 8-lavandulyl and $2^{\times}$-hydroxyl groups. Sophoraflavanone G was shown to increase fluorescence polarisation of the liposomes significantly at concentrations corresponding to the MIC values which indicated an alteration of membrane fluidity in hydrophilic and hydrophobic regions, suggesting that sophoraflavanone G reduced the fluidity of outer and inner layers of membranes. Naringenin also exhibited a membrane effect but at much higher concentrations. This correlation between antibacterial activity and membrane interference suggest that sophoraflavanone G exhibit antibacterial activity by reducing membrane fluidity of bacterial cells. In addition, galangin which effect the cytoplasmic integrity in *S. aureus*, has been investigated by measuring loss of internal potassium. When high cell densities of *S. aureus* were incubated for 12h in media containing 50 g/ml of the flavonol, a 60-fold decrease in the number of CFUs was noted and cells lost ca. 20% more potassium than untreated control bacteria which strongly suggest that galangin induces cytoplasmic membrane damage and potassium leakage. Flavonoids that interfere with energy metabolism was demonstrated by Merck Research Laboratories which recently reported that the flavanone lonchocarpol A inhibits macromolecular synthesis in *Bacillus megaterium* by using radioactive precursors. It was demonstrated that RNA, DNA, cell wall and protein synthesis were all inhibited at concentrations similar to the MIC value.

Alkaloids

Alkaloids are heterocyclic nitrogen compounds which show different antimicrobial activities. The analysis of the leaf extracts of *Gymnema montanum* (Ramkumar *et al*., 2007) and of ethanol extract of *Tabernaemontana catharinensis* root bark revealed an antimicrobial activity in the first case due to an activity, depending upon the chemical composition of the extracts and membrane permeability of the microbes, and in the second case linked to indole alkaloids responsible for the observed antibacterial and antidermatophytic activity. Diterpene alkaloids show antimicrobial properties which are commonly found in plants of the Ranunculaceae group. Berberine which is a hydrophobic cation and an iso-quinoline alkaloid, present in roots and stem-bark of *Berberis* species are widely used in traditional medicine, owing to its activity against bacteria, fungi, protozoa and viruses (Kim *et al*., 2002). It is an excellent DNA intercalator and accumulates in cells driven by the membrane potential (Iwasa *et al*., 2001) .These compounds are active on several microorganisms and target on RNA polymerase, gyrase and topoisomerase IV and on nucleic acid.

Terpenes

Terpenes are compounds also referred to as isoprenoids, and their derivatives, containing additional elements, usually oxygen, are called terpenoids. The antibacterial activity of some monoterpenes (C10), diterpenoids, sesquiterpenes (C15), triterpenoids and their derivatives was recently reviewed (Suryakala and Giri, 2013). The results obtained, illustrate the strong structure-function influence of the antibacterial potential of terpenes. Diterpenoids, and sesquiterpenes, isolated from different plants inhibited the growth of *M. tuberculosis* (Garcia *et al*., 2012; Kurek *et al*., 2011) and exhibited bactericidal activity against Gram-positive bacteria. The mechanism of action of terpenoids is not fully understood, but is speculated to involve membrane disruption by the lipophilic compounds.

Coumarins

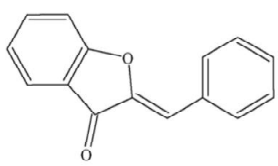
One known coumarin, scopoletin, and two chalcones were isolated as antitubercular constituents of the whole plant, *Fatoua pilosa*. The antimicrobial effectiveness of spices and aromatic plants depend on the kind of plant, its composition and concentration of essential oils. The importance of aroma precursors (cysteine sulfoxides) as a potent biologically active agents was revealed in accordance with the popular use of plants belonging to the *Allium* genus in traditional medicine by analyzing the antimicrobial activity of essential oil of *Allium sphaerocephalon* inflorescenses (Lazarevic *et al*., 2011).

Experimental approaches

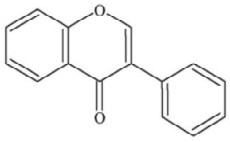
Outcome of susceptibility tests and standardized methods are affected by multiple factors and are more likely to be reproducible than unstandardized methods. When dealing with plant extracts, sometimes it is difficult to compare the results obtained with published results in the literature because several variables influence the results, such as the environmental and climatic conditions under which the plant grew, choice of plant extracts, choice of extraction method, antimicrobial test method and test microorganisms (Nostro *et al*., 2000; Hammer *et al*.,1999).

Plant extract preparation

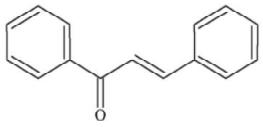
Pharmaceutically, extraction methods involve separation of medicinally active components by using selective solvents with appropriate extraction technology. During extraction, solvents diffuse into the solid plant material and solubilize compounds with similar polarity (Green, 2004). The quality of an extract is influenced by the basic parameters such as: a) the plant part used as starting material, b) the solvent used for extraction and c) the extraction technology. Effect of plant material depends on the nature of the plant material; its origin; degree of processing; moisture content and particle size, while variations in extraction method include type of extraction; time of extraction and temperature. The concentration, polarity and the nature of solvent affects the quantity and secondary metabolite composition of an extract.



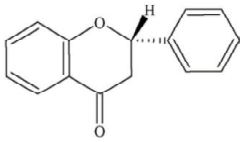
Aurone



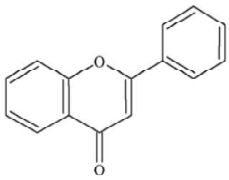
Isoflavone



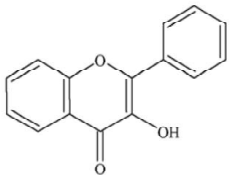
Chalcone



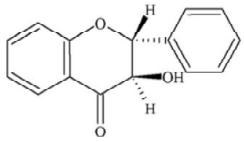
Flavanone



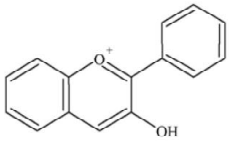
Flavone



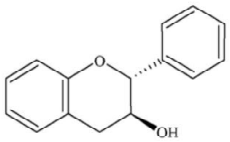
Flavonol



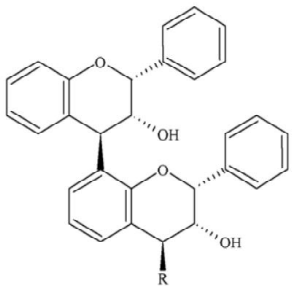
Flavanon-3-ol (also known as 3-hydroxyflavanone or dihydroflavonol)



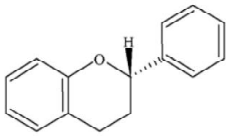
Anthocyanidin



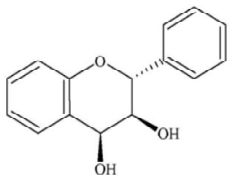
Flavan-3-ol (also known as catechin)



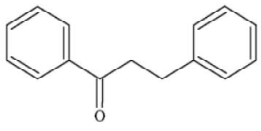
Proanthocyanidin (also known as flavolan or condensed tannin)



Flavan



Flavan-3,4-diol (also known as leucoanthocyanidin)



Dihydrochalcone

Figure 4: (Tim Cushnie *et al.*, 2005): The skeleton structures of the main classes of flavonoids: aurones (Muziol *et al.*,2001; Villemin *et al.*, 1998), isoflavones (Havsteen, 1983), chalcones (Harborne and Baxter, 1999; Xu and Lee, 2001), flavanones (Havsteen, 1983; Harborne and Baxter, 1999), flavones (Havsteen, 1983), flavonols (Havsteen, 1983), flavanon-3-ols (Harborne and Baxter, 1999), anthocyanidins (Harborne and Baxter, 1999, Middleton *et al., 2000*), flavan-3-ols (Havsteen, 1983; Harborne and Baxter 1999), proanthocyanidins (occur as dimers, trimers, tetramers and pentamers; R = 0, 1, 2 or 3 flavan-3-ol structures) (Harborne and Baxter, 1999), flavans (Harborne and Baxter, 1999), flavan-3,4-diols (Harborne and Baxter, 1999) and dihydrochalcones (Harborne and Baxter, 1999).

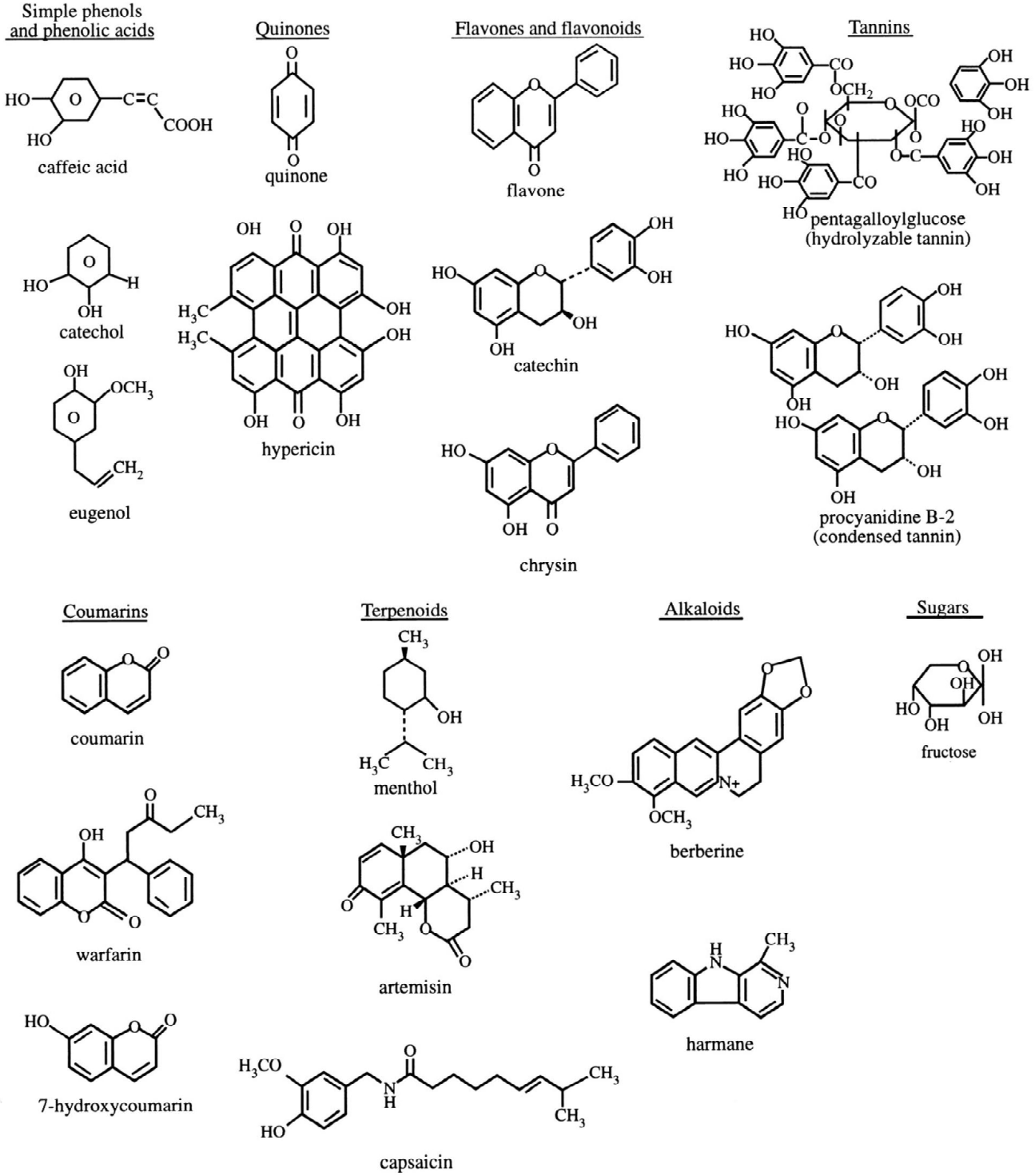


Figure 5: Chemical structures of some antimicrobial compounds (Cowan, 1999)

Plant material

As a source for secondary plant components, fresh or dried plant material can be used. However, dried plant material is used by most of the scientists, working on the chemistry of secondary plant components. Differences in water content may affect solubility of subsequent separation by liquid-liquid extraction and the secondary metabolic components should be relatively stable, especially if it is to be used as an antimicrobial agent. Furthermore, many plants are used in

the dry form or as an aqueous extract by traditional healers. Plants are usually air dried (Dilika *et al*, 1996; Baris *et al*., 2006) but some researchers, dry the plants in the oven at about 40°C for 72h (Salie *et al*., 1996). Plants differ in their constituents, depending on the climatic conditions in which they are growing. The choice of plant material used in the extract preparation is usually guided by the traditional use of the plant and the ease of handling of the different plant parts like the leaves, stems *etc*.

Choice of solvent

The choice of solvent is influenced by what is intended with the extract. Since the end product contain traces of residual solvent, the solvent should be non-toxic and should not interfere with the bioassay. The choice will also depend on targeted compounds. In a study, aqueous acetone was better at extracting total phenolics than aqueous methanol. In another study, out of twenty different solvents, chloroform was found to be the best solvent for the extraction of non-polar, biologically active compounds from the roots of *Angelica archangelica* (Harmala *et al*., 1992). For general phytochemical analysis, the larger the variety of compounds, the extractant will extract the better, because there is a better chance that biologically active compounds will be present in the extract (Eloff, 1998a). Compared to water extracts, plant extracts from organic solvents have been found to give more consistent antimicrobial activity (Parekh *et al*., 2005). Methanol, ethanol, and water are the most commonly used solvents for investigations of antimicrobial activity in plants (Parekh *et al*., 2005; Bisignino *et al*., 1999; Lourens *et al*., 2004; Salie *et al*., 1996; Rojas *et al*., 2006). Dichloromethane has also been used by a number of researchers (Dilika *et al*., 1996; Freixa *et al*., 1996). Some authors use different solvents in a combination to obtain the best solvent systems for extraction (Nostro *et al*., 2000). Acetone, although not a very commonly used solvent, has been used by a number of authors (Dilika et al., 1996; Lourens et al., 2004; Mathkega , 2001). In a study, Masokoa et al. (2007) investigated the antifungal activity of *Combretum* species in which, the extractants used include hexane, dichloromethane, acetone and methanol. They discovered that more chemical compounds from the leaves are extracted by acetone and methanol than other solvents. Saponins are extracted by both acetone and methanol which have antimicrobial activity. Eloff (1998a) studied a variety of extractants for their ability to solubilise antimicrobials from plants. Rate of extraction, ease of removal, toxicity in bioassay, acetone received the highest among all other solvents and gave the lowest minimum inhibitory concentration for Gram -positive organisms tested and the largest number of different components and inhibitors from two plants tested. However, different results may be obtained with other plants and generalization can not be made on the usefulness of acetone as an extractant (Eloff, 1998a).

The extraction methods

Variations in extraction methods are due to the extraction period, solvent used, pH, temperature, particle size and the solvent-to-sample ratio. Homogenization in solvent is the widely used method by researchers investigating antimicrobial activity (Meyer and Dilika, 1996; Parekh *et al*., 2005). Dried plant material is ground in a blender, put in solvent and shaken vigorously for 5 min or left for 24 h after which, the extract is filtered and fresh solvent added to the residue for another 24 h. To obtain an epicuticular extract, some authors suggested the shaking of unhomogenized dry leaves in solvent for about 5 min, followed by filtering and concentrating under reduced pressure (Mathkega, 2001) which actually gave a higher yield and bioactivity than using homogenized (macerated) extract (Dilika *et al*., 1996; Mathkega, 2001). Of interests are the results, obtained in a study by Meyer and Dilika *et al*. (1996), using these methods on the same plant found that the homogenized dichloromethane extract generally had higher activity than the shaken extract of the same solvent.

Antibacterial susceptibility tests

Antibacterial susceptibility testing are performed by either dilution or diffusion methods. The choice of methodology are based on many factors such as relative ease of performance, flexibility, use of automated or semi-automated devices for both identification and susceptibility testing. AST standard tests are conveniently divided into diffusion and dilution methods. Diffusion tests usually include agar well diffusion, agar disk diffusion and bioautography, while dilution methods include agar dilution and broth micro/ macrodilution. Conventional reference methods for AST are broth and agar based methods (Tenover *et al*., 1995).

Agar disk diffusion assay

Agar diffusion techniques have been widely used to evaluate antimicrobial activity of plant extracts (Freixa *et al*., 1996; Salie *et al*., 1996), although there are limitations with the technique. For identification of leads, disk diffusion techniques are suitable but not effective for quantification of bioactivity (Hammer *et al*., 1999; Nostro *et al*., 2000; Langfield *et al*., 2004), which generally do not distinguish bactericidal and bacteriostatic effects. Since MIC can not be determined by these techniques, it is usually used for preliminary screening (Parekh *et al*., 2005; Tepe *et al*., 2004). Some researchers have reported that by the agar diffusion method, MIC values can be obtained (Leite *et al*., 2006), although high activity in the disk diffusion assay does not necessarily correlate to low MIC values in the microtitreplate method (Lourens *et al*., 2004). The agar disk diffusion technique can only be applied to determine the AST of pure substances because the results may be unreliable when it is applied to mixtures, containing different constituents, which exhibit different diffusion rates (Silva *et al*., 2005). In this method,

6 mm paper disks saturated with filter sterilized (Salie *et al*., 1996) plant extract at the desired concentration, are placed onto the surface of a suitable solid agar medium. Usually Muller Hinton is the choice of medium by most of the researchers, although, tryptone soy agar (Lourens *et al*., 2004) or nutrient agar (Doughari, 2006) have sometimes been used by other researchers. Some authors have reported the inoculum sizes of 1×10^8 cfu/ml of bacteria to pre-inoculate the media for inoculating diffusion plates (Baris *et al*., 2006). Some impregnate the disk before placing on the agar (Lourens *et al*., 2004; Salie *et al*., 1996), while others place the disk on the plate first before impregnating (Nostro *et al*., 2000; Baris *et al*., 2006). In a report by Mbata *et al* (2006), the paper disks were soaked in the leaf extract for about 2 h. Basri and Fan (2005) left the disks to dry under a laminar flow cabinet overnight while other authors refrigerate the plates for an hour or two at 4°C to allow pre-diffusion of the extracts from the disk into the seeded agar layer before incubation (Lourens *et al*., 2004; Tepe *et al*., 2004; Schmourlo *et al*., 2004). The plates are then incubated at 37°C for 24 h for bacteria and 48 h for fungi (Salie *et al*., 1996; Baris *et al*., 2006). Zones of inhibition are then measured from the circumference of the disks to the circumference of the inhibition zone (Salie *et al*., 1996).

Agar well diffusion

The agar well diffusion is similar to that of the agar disk diffusion method. A standardized inoculum culture is spread evenly on the surface of gelled agar plates. Using a sterile cork borer wells of between 6 and 8 mm are aseptically punched on the agar, allowing at least 30 mm between adjacent wells and the petridish. Fixed volumes of the plant extract are then introduced into the wells and incubated at 37°C for 24 h for bacteria (Mbata *et al*., 2006).

Bioautography

In this method, the analyte is adsorbed onto a Thin Layer Chromatography (TLC) plate which is different from that of agar diffusion method. Bioautography is a preliminary phytochemical screening technique used to detect active components (Nostro *et al*., 2000; Schmourlo *et al*., 2004). It also overcomes the challenge of isolating antimicrobial compounds from crude extracts with complex chemical components. This method relatively uses very little amount of sample and also determine the polarity of the active compounds (Runyoro *et al*., 2006). In a study by Silva *et al*. (2005), it was concluded that bioautography is a practical, reproducible test and easy to perform when they compared different methods of AST. In the bioautography agar overlay method, the extract of determined amount is applied to silica 60 gel plates and developed with an appropriate solvent system and a suspension of the test bacteria is sprayed onto the TLC plate and incubated at 25°C for 48 h in humid conditions. Microbial indicators (usually tetrazolium salts) are used as a growth detector (Silva *et al*., 2005) and are sprayed onto the plates after which the plates are reincubated at 25°C for 24 h (Dilika *et al*., 1996) and the clear (white) zones on the TLC plate indicate antimicrobial activity of the extracts. Some authors state that this method is not an ideal method for the quantification of bioactivity of plant extracts because TLC causes disruption of synergism between active constituents in an extract, thereby reducing its activity (Schmourlo *et al*., 2004).

Broth microdilution

For determining MICs of large numbers of test samples, the microtitre plate or broth microdilution method have been proved to be a potentially useful technique. This technique has advantages over diffusion techniques because of its increased sensitivity for small quantities of extract, ability to distinguish between bacteriostatic and bactericidal effects and quantitative determination of the MIC (Langfield *et al*., 2004). This method is inexpensive and presents reproducible results and can also be used for a wide variety of microorganisms. In the microtitre plate method, a stock solution of the extract is obtained in solvent usually used for extraction (Grierson and Afolayan, 1999) or in **DMSO** (Salie *et al*., 1996; Nostro *et al*., 2000; Baris *et al*., 2006). Most authors prefer filter sterilizing with a 0.22 or 0.45 μm membrane filter before the procedure (Meyer and Afolayan, 1995; Kianbakht and Jahaniani, 2003). As **diluents** in the wells of the microtitre plate, Mueller Hinton Broth or water are often used before transferring an equal volume of stock solution to the plate. Cation-supplemented Muller-Hinton Broth is recommended by Eucast (2003) for non-fastidious microorganisms. In a study, Kianbakht and Jahaniani (2003) discovered that the MIC values for *Tribulus terrestris* L. did not depend on the type of media used, when comparing the performance of Brain Heart Infusion Broth and Muller-Hinton Broth. Twofold serial dilutions are then made from the first well to obtain a concentration range. For full range MIC 5 to 8 concentrations representing achievable concentrations for the antimicrobial are usually tested (Mendoza, 1998), although some authors have reported from even 3 concentrations. An equal volume of a fixed bacterial culture is added to the wells and incubated at 37°C for 24 h (Lourens *et al*., 2004). Eucast (2003) recommended a temperature of 35-37°C for 16-20 h for nonfastidious organisms (Eucast, 2003). Usually 1×10^6 cfu/ml of inoculum size for the microtitre plate procedure is recommended (Lourens *et al*., 2004). Eucast (2003) also recommended that the plates should be inoculated within 30 min of standardization of inoculum to avoid changes in inoculum density. As an indicator of growth, plates are examined for changes in turbidity. The well that appears clear, is taken as the MIC of the extract. To determine the presence of growth in microtitre plates some researchers use indicators

(Umeh *et al*., 2005) or spectrophotometry (Devienne and Raddi, 2002, Matsumoto *et al*., 2001). To detect the MIC breakpoint indicators such as tetrazolium salts or resazurin dye are added after the incubation period and left for about 6 h and changes in color or absence of color is determined. The use of calorimetric indicators eliminates the need for a spectrophotometric plate reader and avoids the ambiguity associated with visual comparison or measurement of growth inhibition rings on agar plates. To detect the breakpoint, the spectrophotometric method is usually used at absorbance of 620 nm with the negative control as a blank (Salie *et al*., 1996). The concentration at which there is a sharp decline in the absorbance value (Devienne and Raddi, 2002), or the lowest concentration which gives a zero absorbance reading (Salie *et al*., 1996) is deemed to be the MIC. Subculturing of the preparations that have shown no evidence of growth in the MIC determination assay is determined as minimum bactericidal concentration (MBC). These subcultures are made either in broth or in agar plates. In broth, the MBC is regarded as the lowest concentration of extract which does not produce an absorbance reading at 620 nm relative to the negative control (Salie *et al*., 1996). On agar, the lowest concentration showing lack of growth represents the MBC.

Agar dilution assay

The agar dilution test does not show any problems when encountered with the latter such as, sample solution, contamination and determination of MIC breakpoints, which states that the agar dilution test is more versatile than the broth dilution assay (Silva *et al*., 2005). In this method, a stock solution of the extract is prepared which are filter-sterilized (0.22 µm) and then incorporated in molten agar and cooled to 50°C in a water bath, to obtain different concentrations of the extract in the agar. Usually Muller-Hinton is used, although some authors have used nutrient agar (Grierson and Afolayan, 1999; Meyer and Afolayan, 1995). Inoculum preparation also differs between authors, some use overnight culture dilutions of 1:100 (Meyer and Afolayan, 1995) while others use 1:10 (Meyer and Dilika, 1996) in broth. Eucast (2003) recommended an inoculum density of about 10^7 cfu/ml. Some reports suggest leaving the plates overnight, before streaking, to allow the solvent to evaporate (Grierson and Afolayan, 1999). Agar plates are incubated at 37ºC for 24 to 48 h after streaking the organisms in radial patterns. The lowest concentration of the extract inhibiting the visible growth of each microorganism on the agar plate is defined as MIC (Nostro *et al*., 2000; Hammer *et al*., 1999).

Conclusion

Drug- resistant microorganism is a growing global problem today and there is an urgent need of new compounds as antimicrobial agent which are characterized by diverse chemical structures and mechanism of action. Plants are rich sources of secondary metabolites such as, phenols, flavonoids, tannins, alkaloids and terpenoids which have been found to have antimicrobial properties. The study of plants for their medicinal properties is important for morden day medicine, but its usefulness cannot be over emphasized if methods are not standardized to obtain comparable and reproducible results. Phytopharmaceuticals as antimicrobial agents has led to the discovery of bioactive compounds for which ethnopharmacologists, botanists, microbiologists and natural-product chemists are standardizing universal methods to produce effective and safe natural products to treat various diseases.

Conflict of interest

We declare that we have no conflict of interest.

References

Aggarwal, B. B.; Sundaram, C.; Malani, N. and Ichikawa, H. (2007). Curcumin: the Indian solid gold. Adv. Exp. Med. Biol. Advances in Experimental Medicine and Biology, **595**:1-75.

Agnese, A. M.;Perez,C. and Cabrera, J.L.(2001).*Adesmia aegiceras*: antimicrobial activity and chemical study. Phytomedicine, **8**(5):389-394.

Ali-Shtayeh, M. S.; Al-Nuri, M. A.; Yaghmour, R. M. R and Faidi, Y. R. (1997). Antimicrobial activity of *Micromeria nervosa* from the Palestinian area. J. Ethnopharmacol., **58**:143-147.

Amanloua, M.; Frazelib, M. R.; Arvinb, A.; Aminc, H. G. and Farsama, H. (2004). Antimicrobial activity of crude methanolic extract of *Satureja khuzestanica*. Fitoterapia, **75**:768-770.

Annapurna, J.; Amarnath, P. V.; Amar, D.; Ramakrishna, S. V. and Raghavan, K. V. (2003). Antimicrobial activity of *Ixora coccinea* leaves. Fitoterapia, **74**(3):291-3.

Anjaneyulu, M.; Jayaram Prakash, M. and Archana, G. (2013). Phytochemical investigation and antibacterial activity of *Salacia oblonga* Wall ethanolic extract. Ann. Phytomed., **2**(1):102-107.

Apisariyakul, A.; Vanittanakom, N. and Buddhasukh, D. (1995). Antifungal activity of turmeric oil extracted from *Curcuma longa*. J. Ethnopharmacol., **49**:163-169.

Arruda, A. L; Vieira, C. J.; Sousa, D. G.; Oliveira, R. F. and Castilho, R. O. (2011). Bignoniaceae as antibacterial agent. J. Med. Food, **14**: 1604-1608.

Assob, J. C.; Kamga, H. L. and Nsagha, D. S. (2011). Antimicrobial and toxicological activities of five medicinal plant species from Cameroon Traditional Medicine. BMC Complement. Altern. Med., **11**:70-81.

Arsdall and Anne, V. (2002). The Old English Herbarium and Anglo-Saxon Medicine. Medieval Herbal Remedies. Psychology Press, pp:70-71.

Asolini, F. C.; Tedesco, A. M.; Ferraz, C.; Alencar, S. M. and Carpes, S.T. (2006). Antioxidant and antibacterial activities of phenolic compounds in extracts of plants used as tea. Braz. J. Food Technol., **9**(6):209-15.

Avancini, C.A.M.; Wiest, J.M. and Mundstock, E.A. (2000). Bacteriostatic and bactericidal activity of the *Baccharis trimera* (Less.) D.C. Arq. Bras. Med. Vet. Zootec., **52**(3):2304.

Bakshu, L. M. and Venkata Raju, R. (2001). Antimicrobial activity of *Rhynchosia beddomei*. Fitoterapia, **72**(5):579-82.

Balungile, M.; Ashwell, R.; Jeffrey, F. and Johannes, V. (2013). *In vitro* Antimicrobial activity of extracts from plants used traditionally in south Africa to treat tuberculosis and related symptoms. Evidence-Based Complementary and Alternative Medicine, pp:1-8.

Baris, O.; Gulluce, M.; Sahin, F.; Ozer, H.; Kilic, H.; Ozkan, H.; Sokmen, M. and Ozbek, T .(2006). Biological activities of the essential oil and methanol extract of *Achillea Biebersteinii* Afan. Turk. J. Biol., **30**: 65-73.

Basri, D. F. and Fan, S. H. (2005). The potential of aqueous and acetone extracts of galls of *Quercus infectoria* as antibacterial agents. Indian J. Pharmacol., **37**(1):26-29.

Berkada, B (1978). Preliminary report on warfarin for the treatment of herpes simplex. J. Irish Coll. Phys. Surg., **22**(Suppl.):56.

Bisignino, G.; Sanogo, R.; Marino, A.; Aquino, R.; D'angelo, V.; Germano, M. P.; De Pasquale, R. and Pizza, C. (1999). Antimicrobial activities of *Mitracarpus scaber* extract and isolated constituents. Lett. Appl. Microbiol., **30**:105-108.

Bose, P. K. (1958). On some biochemical properties of natural coumarins. J. Indian Chem. Soc., **58**:367-375.

Brown, J. P. (1980). A review of the genetic effects of naturally occurring flavonoids, anthraquinones and related compounds. Mutat. Res., **75**:243-77.

Burdich, E. M. (1971). Carpaine, an alkaloid of *Carica papaya*. Econ. Bot., **25**:363-365.

Burt, S. (2004). Essential oils: their antibacterial properties and potential applications in foods - a review. Int. J. Food Microbiol., **94** (3):223-253.

Candan, F.; Unlu, M.; Tepe, B.; Daferera, D.; Polissiou, M. and Sokmen, A. (2003). Antioxidant and antimicrobial activity of the essential oil and methanol extracts of *Achillea millefolium* subsp. *millefolium Afan*. (Asteraceae). J Ethnopharmacol., **87**(2-3):215-20.

Cichewicz, R. H.; and Thorpe, P. A.(1996). The antimicrobial properties of chile peppers (*Capsicum* species) and their uses in Mayan medicine. J. Ethnopharmacol., **52**:61-70.

Cowan, M.M. (1999). Plant products as antimicrobial agents. Clin Microbiol. Rev., **12**(4):564-82.

Dall'Agnol, R.; Ferraz, A. and Bernardi, A.P. (2003). Antimicrobial activity of some *Hypericum* species. Phytomedicine, **10**:511-516.

Deepa, M. A. and Narmada, B. (2004). Antimicrobial activity of *Salacia beddomei*. Fitoterapia, **75**:589-591.

Devienne, K. F. and Raddi, M. S. G. (2002). Screening for antimicrobial activity of natural products using a microplate photometer. Braz. J. Microbiol., **33**(2):97-105.

Dilika, F.; Afolayan, A. J. and Meyer, J. J. M. (1996). Comparative antibacterial activity of two *Helichrysum* species used in male circumcision in South Africa. S. Afr. J. Bot., **63**(3):158-159.

Dorianne, E. D.;Jaures, A. K.; Jackson, A. S.; Aime, G. F.; Igor, K. V.; Simplice, B. T.; Antoine, H.L. and Nkuete (2013). Antibacterial activity of selected edible plant extracts against multidrug-resistant Gram-negative bacteria. BMC Complementary and Alternative Medicine, **13**:164.

Doughari, J. H .(2006). Antimicrobial activity of *Tamarindus indica* Linn. Trop. J. Pharm. Res., **5**(2):597-603.

Dulgera, B.; Cem, C. and Ergul Gucin, F. (2002). Antimicrobial activity of the macrofungus *Lepista nuda*. Fitoterapia, **73**:695-697.

Eftekhar, F.; Yousefzadi, M. and Tafakori, V. (2005). Antimicrobial activity of *Datura innoxia* and *Datura stramonium*. Fitoterapia, **76**(1):118-20.

El-Abyad, M. S.; Morsi, N. M.; Zaki, D. A. and Shaaban, M. T. (1990). Preliminary screening of some Egyptian weeds for antimicrobial activity. Microbios., **62**:47-57.

Eloff, J. N. (1998a). A sensitive and quick microplate method to determine the minimum inhibitory concentration of plant extracts for bacteria. Plant Med., **64**:711-713.

Eucast (2003). Determination of minimum inhibitory concentrations (MICs) of antibacterial agents by broth dilution. Clin. Microbiol. Infect., **9**(8):1-7.

Freixa, B.; Vila, R.; Vargas, L.; Lozano, N.; Adzet, T. and Caniguera, S. (1996). Screening for antifungal activity of nineteen Latin American plants. Phytother. Res., **12**(6):427-430.

Garcia, A.; Bocanegra-Garcia, V.; Palma-Nicolas, J. P. and Rivera, G. (2012). Recent advances in antitubercular natural products. Eur. J. Med. Chem., **49**:1-23.

Ghoshal, S., Krishna Prasad, B. N and Lakshmi, V. (1996). Antiamoebic activity of *Piper longum* fruits against *Entamoeba histolytica in vitro* and *in vivo*. J. Ethnopharmacol., **50**:167-170.

Gill, A.O., and Holley, R.A. (2004). Mechanisms of bactericidal action of cinnamaldehyde against *Listeria monocytogenes* and of eugenol against *L. monocytogenes* and *Lactobacillus sakei*. Appl. Environ. Microbiol., **70**:5750-5755.

Gould, I. M. (2008). The epidemiology of antibiotic resistance. Int. J. Antimicrob. Agents, **32**(1):2-9.

Grange, J. M. and Davey, R. W. (1990). Antibacterial properties of propolis (bee glue). J. R. Soc. Med., **83**:159-60.

Green, R. J. (2004). Antioxidant activity of peanut plant tissues, Masters Thesis. North Carolina State University. USA.

Grierson, D. S. and Afolayan, A. J. (1999). Antibacterial activity of some indigenous plants used for the treatment of wounds in the Eastern Cape. South Africa. J. Ethnopharmacol., **66**:103-106.

Hamburger, H and Hostettmann, K. (1991). The link between phytochemistry and medicine. Phytochemistry, **30**:3864-3874.

Hammer, K. A.; Carson ,C. F. and Riley, T. V. (1999). Antimicrobial activity of essential oils and other plant extracts. J. Appl. Microbiol., **86**(6):985.

Harborne, J. B. and Baxter, H. (1999). The handbook of natural flavonoids, Vols 1 and 2. Chichester, UK: John Wiley and Sons.

Harborne, J. B. and Williams, C. A. (2000). Advances in flavonoid research. Phytochemistry, **55**:481-504.

Harmala, P.; Vuorela, H.; Tornquist, K. and Hiltunen, R. (1992). Choice of solvent in the extraction of *Angelica archangelica* roots with reference to calcium blocking activity. Plant Med., **58**(2):176-183.

Havsteen, B. (1983). Flavonoids, a class of natural products of high pharmacological potency. Biochem Pharmacol., **32**:1141-8.

Hufford, C. D., Jia, E. M.; Croom, Jr.; Muhammed, A. L.; Okunade, A. M.; Clark, and Rogers, R. D. (1993). Antimicrobial compounds from *Petalostemum purpureum*. J. Nat. Prod., **56**:1878-1889.

Hunter, M. D. and Hull, L. A. (1993). Variation in concentrations of phloridzin and phloretin in apple foliage. Phytochemistry, **34**:1251-1254.

Infectious Diseases Society of America (2004). Statement of the IDSA concerning 'Bioshield II: Responding to an ever-changing threat'.Alexandria, VA: IDSA.

Interactive European Network for Industrial Crops and their Applications (2000-2005). "Summary Report for the European Union".

Iwasa, K.; Moriyasu, M.; Yamori, T.; Turuo, T.; Lee, D. and Wiegrebe, V. (2001). *In vitro* cytotoxicity of the protoberberine-type alkaloids. J. Nat. Prod., **64**:896-898.

Jones, G. A.; McAllister, T. A.; Muir, A. D. and Cheng, K. J. (1994). Effects of sainfoin (*Onobrychis viciifolia* scop.) condensed tannins on growth and proteolysis by four strains of ruminal bacteria. Appl. Environ. Microbiol., **60**:1374-1378.

Jones, S.B.; Jr., and Luchsinger, A.E. (1986). Plant systematics. McGraw-Hill Book Co., New York, N.Y.

Kadota, S., Basnet, P.; Ishii, E.; Tamura, T and Namba, T. (1997). Antibacterial activity of trichorabdal from *Rabdosia trichocarpa* against *Helicobacter pylori*. Zentbl. Bakteriol., **286**: 63-67.

Karamana, I.F.; Sahin, M.; Gulluce, H.; Ogutcu, Sengul, M. and Adiguzel, A. (2003). Antimicrobial activity of aqueous and methanol extract of *Juniperus oxycedrus* L. Journal of Ethnopharmacology, **85**:231-235.

Keating, G. J., and Kennedy, R. O. (1997). The chemistry and occurrence of coumarins, p. 348. ***In***: R. O'Kennedy and R. D. Thornes (ed.), Coumarins: biology, applications and mode of action. John Wiley & Sons, Inc., New York, N.Y.

Kianbakht, S. and Jahaniani, F. (2003). Evaluation of antibacterial activity of *Tribulus terrestris* L. growing in Iran. Iran J. Pharmacol. Ther., **2**:22-24.

Kim, S. H.; Lee, S. J.; Lee, J. H.; Sun, W. S. and Kim, J. H. (2002). Antimicrobial activity of 9-*O*-acyl and 9-*O*-alkylberberrubine derivatives. Planta Med., **68**:277-281.

Kiranmayee, R.; Bhuvaneswari, Ch.; Lakshmi Narasu, M. and Archana, G. (2010). Antibacterial activity of *Alpinia galanga* (L) Willd crude extracts. Appl. Biochem. Biotechnol., **162**:871-884.

Kubo, A.; Lunde, C. S and Kubo, I. (1995). Antimicrobial activity of the olive oil flavor compounds. J. Agric. Food Chem., **43**:1629-1633.

Kurek, A.; Grudniak, A. M.; Kraczkiewicz- Dowjat, A. and Wolska, K. I. (2011). New antibacterial therapeutics and strategies. Pol. J. Microbiol., **60**:3-12.

Lambert, R.; Skandamis, P.; Coote, P. and Nychas, G .(2001). A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. J. Appl. Microbiol., **91** (3):453-462.

Lamb Andrew, J. and Tim Cushnie, T. P. (2005). Antimicrobial activity of flaavonoids. In. J. of Antimicrobial Agents., **26**:346.

Langfield, R. D.; Scarano, F. J.; Heitzman, M. E,.; Kondo, M.; Hammond, G. B. and Neto, C. C. (2004). Use of a modified microplate bioassay method to investigate antibacterial activity in the Peruvian medicinal plant *Peperomia galiodes*. J. Ethnopharmacol., **94**(2-3): 279-281.

Lazareviè, J. S.; Dordevic, A. S.; Zlatkoviè, B. K.; Raduloviè, N. S. and Paliè, R. M. (2011). Chemical composition and antioxidant and antimicrobial activities of essential oil of *Allium sphaerocephalon* L. subsp. s*phaerocephalon* (Liliaceae) inflorescences. J. Sci. Food Agric., **91**:322-329.

Leite, S. P.; Vieira, J. R. C.; de Madeiros, P. L.; Leite, R. M. P.; Lima, V. L.; Xavier, H.S. and Lima, E. (2006). Antimicrobial activity of *Indigofera suffruticosa*. Evid. Complement. and Alternate Med., **2**(3):261-265.

Longer, M. A. and Robinson, F. R. (1985). History of pharmacy. ***In***: Generand, A.R. (Ed.), Remington's Pharmaceutical Sciences, 17th Edition. Marck Publishing, Easton, PA, pp:8.

Lourens, A. C. U.; Reddy, D.; Baser, K. H. C.; Viljoen, A. M. and Van Vuuren, S. F. (2004). *In vitro* biological activity and essential oil composition of four indigenous South African *Helichrysum* species. J. Ethnopharmacol., **9**:253-258.

Masokoa, P.; Picardb, J. and Eloffa, J. N. (2007). The antifungal activity of twenty-four southern African *Combretum* species (Combretaceae). South African Journal of Botany, **73**(2):173-183.

Mathkega, A.D.M. (2001). Antimicrobial activity of *Helichrysum* species and the isolation of a new phloroglucinol from *H. caespititium*. Ph.D. Thesis. Botany Department. University of Pretoria. South Africa.

Matsumoto, M.; Ishida, K.; Konagai, A.; Maebashi, K. and Asaoka, T. (2001). Strong antifungal activity of SS750, a New Triazole Derivative is based on its Selective Binding Affinity to Cytochrome P450 of fungi. Antimicrob. Agents Chemother., **46**(2):308-314.

Mbata, T. I.; Debiao, L. and Saikia, A. (2006). Antibacterial activity of the crude extract of Chinese Green Tea (*Camellia sinensis*) on *Listeria monocytogenes*. Internet J. Microbiol., **2**(2).

McDevitt, J. T.; Schneider, D. M.; Katiyar, S. K and Edlind, T. D. (1996). Berberine: a candidate for the treatment of diarrhea in AIDS patients, Abstr. 175. *In* Program and Abstracts of the 36th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.

Mendoza, M. T. (1998). What's new in Antimicrobial Susceptibility Testing. Philipp. J. Microbiol. Infect. Dis., **27**(3):113-115.

Meyer, J.J.M. and Afolayan, A. J. (1995). Antibacterial activity of *Helichrysum aureonitens* (Asteraceae). J. Ethnopharmacol., **47**:109-111.

Meyer, J.J.M. and Dilika, F. (1996). Antibacterial activity of *Helichrysum pedunctulatum* used in circumcision rites. J. Ethnopharmacol., **53**(1):51-54.

Middleton Jr, E.; Kandaswami, C. and Theoharides, T. C. (1986). The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacol. Rev., **52**:673-751.

Moreno, S.; Scheyer, T.; Romano, C. and Vojnov, A .(2006). Antioxidant and antimicrobial activities of rosemary extracts linked to their polyphenol composition. Free Radic. Res., **40**(2):223-231.

Muziol, T.; Cody, V. and Wojtczak, A. (2001). Comparison of binding interactions of dibromoflavonoids with transthyretin. Acta Biochim. Pol., **48**:885-92.

Naganawa, R.; Iwata, N.; Ishikawa, K.; Fukuda, H.; Fujino, T. and Suzuki, A. (1996). Inhibition of microbial growth by ajoene, a sulfur-containing compound derived from garlic. Appl. Environ. Microbiol., **62**:4238-4242.

Newman, D.J. (2008). Natural products as leads to potential drugs: an old process or the new hope for drug discovery? J. Med. Chem., **51**: 2589-2599.

Nostro, A.; Germano, M. P.; D'Angelo, V.; Marino, A. and Cannatelli, M. A. (2000). Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Lett. Microbiol., **30**(1): 379-384.

Ofek, I.; Goldhar, J. and Sharon, N. (1996). Anti-*Escherichia coli* adhesin activity of cranberry and blueberry juices. Adv. Exp. Med. Biol., **408**:179-183.

Ohemeng, K.A.; Schwender, C. F.; Fu, K. P. and Barrett, J. F. (1993). DNA gyrase inhibitory and antibacterial activity of some flavones (1). Bioorg. Med. Chem. Lett., **3**:225-30.

Omulokoli, E.; Khan, B. and Chhabra, S.C. (1997). Antiplasmodial activity of four Kenyan medicinal plants. J. Ethnopharmacol., **56**:133-137.

Osato, J. A., Santiago, L. A.; Remo, G. E.; Cuadra, M. S and Mori, A.(1993). Antimicrobial and antioxidant activities of unripe papaya. Life Sci., **53**:1383-1389.

Osbourn, A. E. (1996). Preformed antimicrobial compounds and plant defense against fungal attack. Plant Cell., **8**:1821-1831.

Oussalah, M.; Caillet, S. and Saucier, L. (2006). Inhibitory effects of selected plant essential oils on the growth of four pathogenic bacteria: *Escherichia coli* 0157: H7, *Salmonella typhimurium*, *Staphylococcus aureus* and *listeria monocytogenes*. Meat Science, **73**:236-244.

Ozgena, U.; Houghton, P. J.; Ogundipe, Y. and Coskun, M. (2003). Antioxidant and antimicrobial activities of *Onosma argentatum* and *Rubia peregrina*. Fitoterapia, **74**(7-8):682-685.

Parekh, J.; Jadeja, D. and Chanda, S. (2005). Efficacy of Aqueous and Methanol Extracts of Some Medicinal Plants for Potential Antibacterial Activity. Turk. J. Biol., **29**:203-210.

Perrett, S., Whitfield, P. J.; Sanderson, L and Bartlett, A. (1995). The plant molluscicide *Millettia thonningii* as a topical antischistosomal agent. J. Ethnopharmacol., **47**:49-54.

Peter, G.; Mwitari, M.; Peter, A.; Ayeka, J. O.; Esther, N.; Matu.; Christine, C. and Bii (2013). Antimicrobial Activity and Probable Mechanisms of Action of Medicinal Plants of Kenya: *Withania somnifera*, *Warbugia ugandensis*, *Prunus africana* and *Plectrunthus barbatus*. PLoS ONE., **8**(6):e65619.

Ramezani, M.; Fazli-Bazzaz, B.S.; Saghafi-Khadem, F. and Dabaghian, A.(2004). Antimicrobial activity of four *Artemisia species* of Iran. Fitoterapia, **75**(2):201-3.

Ramos-e-Silva, Marcia (1999). Saint Hildegard Von Bingen (1098–1179): The Light Of Her People And Of Her Time. International Journal of Dermatology., **38**(4):315-320.

Ramkumar, K.M.; Rajaguru, P. and Ananthan, R. (2007). Antimicrobial properties and phytochemical constituents of an antidiabetic plant *Gymnema montanum*. Advances in Biological Research, **1**(1-2):67-71.

Raybaudi-Massilia, R.; Mosqueda-Melgar, J.; Soliva-Fortuny, R. and Martin-Belloso, O. (2009). Control of Pathogenic and Spoilage Microorganisms in Fresh-cut Fruits and Fruit Juices by Traditional and Alternative Natural Antimicrobials. Compr. Rev. Food Sci. Food Saf., **8**(3):157-180.

Rojas, J. J.; Ochoa, V. J.; Ocampo, S. A. and Monoz, J. F. (2006). Screening for antimicrobial activity of ten medicinal plants used in Colombian folkloric medicine: a possible alternative in treatment of **non-nosocomial** infections. BMC Complement. Alternat. Med., **6**:2.

Ross, J. and Kasum, C .(2002). Dietary Flavonoids: Bioavailability, Metabolic Effects, and Safety. Annual Review of Nutrition., **22**(1): 19-34.

Runyoro, D. K. B.; Matee, M. I. N.; Ngassapa, O. D.; Joseph ,C. C. and Mbwambo, Z. H. (2006). Screening of Tanzanian medicinal plants for anti-candida activity. BMC Complem. Alternat. Med., **6**:11.

Salie, F.; Eagles, P. F. K. and Lens, H. M. J. (1996). Preliminary antimicrobial screening of four South African Asteraceae species. J. Ethnopharmacol., **52**(1):27-33.

San-Blas, G.; Marino, L.; San-Blas, F. and Apitz-Castro, R. (1993). Effect of ajoene on dimorphism of *Paracoccidioides brasiliensis*. J. Med. Vet. Mycol., **31**:133-141.

San-Blas, G.; San-Blas, F.; Gil, F.; Marino, L and Apitz-Castro,R. (1989). Inhibition of growth of the dimorphic fungus *Paracoccidioides brasiliensis* by ajoene. Antimicrob. Agents Chemother., **33**:1641-1644.

Satrija, F.; Nansen, P.; Murtini, S and He, S. (1995). Anthelmintic activity of papaya latex against patent *Heligmosomoides polygyrus* infections in mice. J. Ethnopharmacol., **48**:161-164.

Scheel, L. D. (1972). The biological action of the coumarins. Microbiol. Toxins, **8**:47-66.

Schmourlo, G.; Mendonca-Filho, R. R.; Alviano, C. S. and Costa, S. S. (2004). Screening of antifungal agents using ethanol precipitation and bioautography of medicinal food plants. J. Ethnopharmacol., **96** (3): 563-568.

Schultes, R.E. (1978). The Kingdom of Plants. ***In***: W. A. R. Thomson (ed.), Medicines from the Earth. McGraw-Hill Book Co., New York, N.Y., pp:208.

Setzer, W. N.; Vogler, B.; Schmidt, J. M.; Leahy, J. G. and Rives, R. (2004). Antimicrobial activity of *Artemisia douglasiana* leaf essential oil. Fitoterapia, **75**(2):192-200.

Silva, M. T. G.; Simas, S. M.; Batista, T. G. F. M.; Cardarelli, P. and Tomassini, T. C. B. (2005). Studies on antimicrobial activity, *in vitro*, of *Physalis angulata* L. fraction and physalin B bringing out the importance of assay determination. Memorias do Instituto Oswaldo Cruz., **100**(7):779-782.

Silva, M. S. A.; Silva, M. A. R.; Higino, J. S.; Pereira, M. S. V. and Carvalho, A. A. T. (2008). *In vitro* antimicrobial activity and antiadherence of *Rosmarinus officinal* is Linn. against oral planktonic bacteria. Rev. Bras. Pharmacogn., **18**(2):236-40.

Singh, B.; Sahu, P. M. and Singh, S. (2002a). Anti-inflammatory and antimicrobial activities of treterpenoids from *Strobilanthes callosus* Nees. Phytomedicine, **9**:355-359.

Singh, B.; Sahu, P. M. and Singh, S. (2002b). Antimicrobial activity of pyrrolizidine alkaloids from *Heliotropium subulatum*. Fitoterapia, **73**(2):153-5.

Singh, G. and Kumar, P. (2011). Evaluation of antimicrobial efficacy of flavonoids of *Withania somnifera* L. Indian J. Pharm. Sci., **73**(4): 473-8.

Skandamis, P.; Tassou, C. C. and Nychas, G. J. E. (1999). Potential use of essential oils as food preservatives. ***In***: Proceedings of the 17th international conference on committee food microbiology and hygiene. Veldhoven, pp:300-307.

Somaroy, Kiranmayee, R.; Bhuvaneswarai, ch.; Archna, G. and Lasshmi Narsu, M. (2010). Phytochemical analysis of *Andrographis paniculata* extract and its antimicrobial activity. World Journal of Microbiology and Biotechnology, **26**(1):85-91.

Somchit, M.N.; Reezal, I.; Nur, I.E. and Mutalib, A.R.(2003). *In vitro* antimicrobial activity of ethanol and water extracts of *Cassia alata*. J. Ethnopharmacol., **84**(1):1-4.

Stange, R. R., Jr., Midland, S. L.; Eckert, J. W and Sims, J. J. (1993). An antifungal compound produced by grapefruit and Valencia orange after wounding of the peel. J. Nat. Prod., **56**: 1627-1629.

Sumner and Judith (2000). The Natural History of Medicinal Plants. Timber Press. pp:17.

Suryakala, G. and Archana, G. (2013). Production and quantification of Asiatic acid from *in vitro* raised shoots and callus cultures of *Centella asiatica* (L.) urban. Ann. Phytomed., **2**(1):95-101.

Suresh, B.; Sriram, S.; Dhanaraj, S. A.; Elango, K and Chinnaswamy, K.(1997). Anticandidal activity of *Santolina chamaecyparissus* volatile oil. J. Ethnopharmacol., **55**:151-159.

Tenover, F. C.; Swenson, J. M.; O'Hara, C. M. and Stocker, S. A. (1995). Ability of Commercial and Reference Antimicrobial Susceptibility Testing methods to detect vancomycin resistance in *Enterococci*. J. Clin. Microbiol., **33**(6):1524-1527

Tepe, B.; Donmez, E.; Unlu, G.; Polissiou, M. and Sokmen, A. (2004). Antimicrobial and antioxidative activities of the essential oils and methanol extracts of *Salvia cryptantha* (Montbret et Aucher ex Bench.) and *Salvia multicaulis* (Vahl). Food Chem., **84**(4):519-525.

Tereschuk, M. L.; Riera, M. V.; Castro, G. R. and Abdala, L. R. (1997). Antimicrobial activity of flavonoids from leaves of *Tagetes minuta*. J Ethnopharmacol., **56**:227-32.

Thiem, B. and Olga, G. (2002). Antimicrobial activity of *Solidago virgaurea* L. from *in vitro* cultures. Fitoterapia,**73**:514-516.

Tim Cushnie, T. P.; Andrew, J. and Lamb (2005). Antimicrobial activity of flavonoids. International Journal of Antimicrobial Agents, **26**: 343-356.

Trease, G and Evans, W. (1972). Pharmacognosy, Univ. Press, Aberdeen, Great Britain.

Trease, G. E. and Evans, W. C. (1985). Pharmacognosy, 17th Edition. Bahive Tinal, London, pp:419.

Umeh, E. U.; Oluma, H. O. A. and Igoli, O. (2005). Antibacterial screening of four local plants using an indicator-based microdilution technique. Afr. J. Tradit. Complement. Alternat. Med., **2**(3):238-243.

Uzel, A.;Guvensen, A. and Cetin, E.(2004). Chemical composition and antimicrobial activity of the essential oils of *Anthemis xylopoda* O. Schwarz from Turkey. J. Ethnopharmacol., **95**(2-3):151-4.

Villemin, D.; Martin, B. and Bar, N. (1998). Application of microwave in organic synthesis; dry synthesis of 2-arylmethylene-3(2)-naphthofuranones. Molecules, **3**:88-93.

Vohora, S. B.; Rizwan, M and Khan, J. A. (1973). Medicinal uses of common Indian vegetables. Planta Med., **23**:381-393.

Wan, J.; Wilcock, A and Coventry, M. J. (1998). The effect of essential oils of basil on the growth of *Aeromonas hydrophila* and *Pseudomonas fluorescens*. J. Appl. Microbiol., **84**:152-158.

WHO. (1993). Summar WHO guidelines for the assessment of herbal medicines. Herbal Grom., **28**:13-14.

Witkowska, B. E.; Wiesyawa, B.; Irena, M.; Olga, G. and Zygmunt, M. (2005). Antimicrobial activity of *Viola tricolor.* Fitoterapia, **76**:458-461.

Wild, R. (1994). The complete book of natural and medicinal cures. Rodale Press, Inc., Emmaus.

Xu, H. X. and Lee, S. F. (2001). Activity of plant flavonoids against antibiotic resistant bacteria. Phytother. Res., **15**:39-43.

Yu, J.; Jiachuan, L.; Huaidong, Yu.; Xuan, C. and Guolin, Z. (2004). Chemical composition and antimicrobial activity of the essential oil of *Scutellaria barbala*. Phytochemistry, **65**:881-884.