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Phytochemicals as Natural Antimicrobials: Prospects and Challenges

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ABSTRACT

Development of multiple drug resistance among pathogens is of global concern today. It is mainly due to non-target use of drugs (poultry, aquaculture, veterinary), over or under use of drugs, prolonged use of an antibiotic or chemotherapeutic agent, use of an antibiotic without the knowledge of the antibiogram pattern of pathogens, and non-completion of drugs doses prescribed. Further, it may be due to pre-existing factors in the microorganisms or may be due to some acquired factors resistance is developed amongst the microorganisms towards the drugs and after resistance is acquired it can spread in the community and among themselves though horizontal gene transfer. It has renewed the interest of researchers and academicians for the development plant based medicines or more precisely herbal medicines more precisely from medicinal and aromatic plants, as the plant products are without any side effects, do not add any physiological pressure on the pathogens for the development of drug resistance, easily degradable, non accumulative in the environment do not cause environmental pollution too. However, the development and use of herbal drugs are lagged behind due to several factors. In this present review, we have made an attempt to discuss various plant derived compounds used as phyto-medicines, their extraction procedures, different screening techniques used to evaluate their potency as antimicrobial compounds with their limitations. Special effort is made to enlist different antimicrobial activity of plant derived drugs described by several workers from time to time in literature. The basic drawbacks of this traditional system are also discussed.

Keywords: Medicinal plants, Phytochemicals, Antimicrobial activity, Test procedures, Herbal drugs.

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Introduction

Antimicrobial Resistance is a Global Problem that Needs Urgent Action

Infectious diseases are world's leading killers after cardiovascular diseases as they account for death of 13.3 million people globally (25 per cent of total global deaths) (WHO, 2000). Microorganism's *viz.*, bacteria, fungi, viruses and protozoa which have the capacity to cause disease are referred to as pathogenic or infectious microorganisms. Pathogenic or infectious microorganisms can be killed or inhibited by agents of biological or non-biological origin commonly referred as antimicrobials. Antimicrobials are used in therapeutically to treat infections.

Drug-resistant infectious microorganisms are those, which are not killed or inhibited by antimicrobial compounds. The increasing incidences of drug resistance and emergence and reemergence of deadly microorganisms are posing a great threat to the society. Drug resistance and emergence of new infectious microorganisms is a set of complex problems driven by a variety of factors ranging from miss use of antimicrobials, interactions of prescriber's and patients, economic incentives, characteristics of a country's health system, and the regulatory environment. Patient's perception of a new drug in the market to be more effective than older drugs leads to self-medication. Prescriber's perceptions regarding patient expectations and demands substantially influences prescribing practice. Physicians can be pressured by patient expectations to prescribe antimicrobials even in the absence of appropriate indications. Patient compliance with recommended treatment is another major problem. Patients forget to take medication, interrupt their treatment when they begin to feel better, or may be unable to afford a full course, thereby creating an ideal environment for microbes to adapt rather than be killed. Hospitals, worldwide are major contributors of the problem of antimicrobial resistance. The combination of highly susceptible patients, intensive and prolonged antimicrobial use and cross-infection have resulted in nosocomial infections with highly resistant bacterial pathogens. Resistant hospital-acquired infections are expensive to control and extremely difficult to eradicate. Around the world, as much as 60 per cent of hospital-acquired infections are caused by drug-resistant microorganisms (World Chiropractic Alliance, 2000). In a nutshell development of drug resistance among pathogens can be attributable to : i) indiscriminate use of antibiotics and chemotherapeutic agent ii) Prolonged use of a particular antibiotic iii) Application of broad spectrum antibiotic without prior knowledge of the antibiogram patterns of the pathogens iv) failure of the complete course of antibiotic v) use of sub optional antibiotics. Further, application of chemotherapeutic agent in poultry and dairy deeds is another major cause of development of drug resistance among pathogens. Furthermore, use of various chemicals in modern aging culture adds a physiological pressure for development of resistance to these compounds.

Developing countries especially, Africa and India suffer significant population losses each year from infectious and parasitic diseases. Approximately 2 million people in India die each year because of these diseases. Thus Africa and India together account for 70 per cent of deaths due to infectious diseases worldwide. Today, 20 per

cent -50 per cent of *Streptococcus pneumoniae* are resistant to widely available antibiotics such as Penicillin, Erythromycin and Sulfamethoxazole. In Vietnam, the majority of *Salmonella typhi* are resistant to all first line antibiotics *e.g.,* Ampicillin, Chloroamphenicol and Sulfamethoxazle. Some microorganisms are showing resistance to second and third line antibiotics as well. In some countries up to 80 per cent of hospital acquired *Staphylococcus aureus* infections are methicillin resistant (MRSA) (WHO, 2002). In India, *S. aureus*, *Enterococcus faecalis*, *Mycobacterium tuberculosis* and *Pseudomonas aeruginosa* have already evaded every antibiotic in the clinician's armamentarium, a stockpile of more than 100 drugs (The Hindu, 2001). Once drug resistance is acquired by the pathogens it can transmit or spread among other pathogens through horizontal gene transfer (Transformation, Transduction and Conjugation). Beside these pathogens more septic ally bacteria develop drug resistance through either of the routes a) the organism may lack the structure of the antibiotics inhibit, for instance, some bacteria such as *Mycoplasma* lack a typical bacterial cell wall and are resistant to penicillin; b) the organism may be impermeable to antibiotics *e.g.,* most Gm-ve bacteria are impermeable to Penicillin; c) the organism may be able to alter the antibiotic to an inactive form such as *Staphylococci* contain ß-lactamase that cleave the ß lactum ring of most of the Penicillin; d) the organism may be able to pump out an antibiotic entering the wall; e) the organism may modify the target of the antibiotic; f) by genetic change alteration may occur in a metabolic pathway that the antimicrobial agents blocks beside the antibiotics and chemotheraptic agents produce a numbers of side effects inside human body. Of the many hundreds of the antibiotics discovered only, few are of wide application in medicine. Pronged use may weak the body's natural defense against invading germs and may have undesirable side effects. Few examples are quite here. Excessive doses damage the kidney in case of Streptomycin, some times causing complete and permanent deafness large doses of Penicillin and Streptomycin have a neurotoxic action. Tetracycline affects the lever, Chloromycetin has toxic effects on haematopoietic (blood cell forming) organs and Chlorotetracycline and Oxytetracycline upon intravenous injection may lead to collapse with lethal outcome. Many times allergic reaction arising during local application of antibiotics too.

Treating resistant infections often requires the use of more expensive or more toxic drugs and can result in longer hospital stays for infected patients and thus impose higher healthcare costs. WHO (2000) in its annual report on infectious diseases, "Overcoming Antimicrobial Resistance", quotes that people throughout the world "may only have a decade or two to make use of many of the medicines presently available to stop infectious diseases". Susceptible microorganisms can replace resistant microorganisms by removing selection pressure. Proposed solutions outlined by the Centre for Disease Control (CDC), USA and World Health Organization (WHO) as a multi-pronged approach includes: prevention, (such as vaccination); improved monitoring; and the development of new treatments. It is this last solution that would encompass the development of new antimicrobials to combat the problems posed by increasing drug resistance as well as emergence and reemergence of deadly infectious diseases (Fauci, 1998). There fore, the human race in a dice need of an alternate. Amongst all medicinal and aromatic plant products are the foremost choice.

As plants are in use for the treatment of various infections since time immemorial. Secondly the products are nature based biodegradable, don't accumulate in the ecosystem causing biomagnification or do not cause any environmental pollution as compared to costly harmful antibiotics and chemotheraptic agent. Most significantly plants have co-evolved in nature along with various pathogens, implies for synthesis of various chemical compounds namely secondary metabolites against these pathogens for self defense.

It is estimated that plant materials are present in, or have provided the models for 50 per cent Western drugs (Robbers, 1996). Many commercially proven drugs used in modern medicine were initially used in crude form in traditional or folk healing practices, or for other purposes that suggested potentially useful biological activity. The primary benefits of using plant derived medicines are that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment. There are essentially two routes of drug discovery, the first one pertains to synthesizing entirely new chemicals and evaluating them for a particular pharmaceutical use and the other approach is identifying the chemical of biological origin (natural product chemistry) and evaluate it for direct or indirect use as a template for development of new drug. 19th century was marked as the golden era for development of synthetic drugs. More and more people became interested in synthetic drugs because of their quick action as compared to traditional medicines and secondly because of their bulk production in industries. Since, 1970$^{'s}$ almost 75 per cent of all standard medicines are of synthetic origin or the product of fermentation. The emerging number of incidences of resistance of microbes towards synthetic drugs and antibiotics of microbial origin has turned the attention of scientists, towards traditional medicines especially herbal drugs or drugs of plant origin.

Plant Derived Antimicrobials

The search for antimicrobial agents has mainly been concentrated on lower plants, fungi and bacteria as sources. Much less research has been conducted on antimicrobials from higher plants (Iwu *et al.,* 1999). Since the advent of antibiotics, in the 1950s, the use of plant derivatives as antimicrobials has been virtually nonexistent. The interest in using plant extracts for treatment of microbial infections has increased in the late 1990s as conventional antibiotics become ineffective (Cowan, 1999). For example, none of the conventional antifungal drugs used to date seems to be ideal in efficacy, safety and antifungal spectrum (Ablordeppey *et al.,* 1999). In addition, many of the antimicrobial drugs in use have undesirable effects or are very toxic, produce recurrence, show drug-drug interactions or lead to the development of resistance (White *et al.,* 1998). Although some new drugs have emerged for the treatment of obstinate fungal infections, such as Allylamines and Caspofungine (Vicente *et al.,* 2003), and combination therapy is sometimes used to make the treatment more effective, there is a real need for a next generation of safer and more potent antifungal drugs (Bartoli *et al.,* 1998). Also, it is increasingly difficult to deliver new antibacterial leads by modifying known antibacterial compounds. Therefore, the focus on much antibacterial research has moved to the identification of new chemical classes and many smaller pharmaceutical companies have taken up this challenge (Boggs and

Miller, 2004). Antimicrobial compounds from plants may inhibit bacteria or fungi through different mechanisms than conventional antibiotics, and could therefore be of clinical value in the treatment of resistant microbes (Eloff, 1998). Phytomedicines derived from plants have shown great promise in the treatment of infectious diseases including opportunistic AIDS infections (Iwu *et al.*, 1999). Investigations on plants used in traditional medicine for skin afflictions might provide new topical antiseptics urgently needed in the third world countries (Taylor *et al.*, 2001). Rapid extinction of some habitats and plant species due to deforestation, especially in the tropical parts of the world, lead to a loss of valuable antimicrobial chemicals (Lewis and Elwin-Lewis, 1995). Thus, many pharmaceutical companies are now intensifying their screening programs on medicinal plants.

Defence Chemicals Produced by Plants

Higher plants produce a great diversity of chemicals that have antimicrobial activity *in vitro* (Van-Etten *et al.*, 1994). Most of these defence molecules are secondary metabolites, of which at least 12, 000 have been isolated (Schultes, 1978). There are two broad categories of plant produced antimicrobials (i) Phytoalexins and (ii) Phytoanticipins. Phytoalexins are low molecular compounds which are produced in response to microbial, herbivorous or environmental stimuli (Van-Etten *et al.*, 1994). These compounds are synthesized *de novo*, and thus require activation of certain genes and enzymes required for their synthesis. Phytoalexins are chemically diverse and include simple phenyl propanoid derivates, flavonoids, isoflavonoids, terpenes and polyketides (Bailey and Mansfield, 1982; Dixon, 1986; Greayer and Harborne, 1994). Phytoanticipins are low molecular compounds which are present in plants before the challenge by microorganisms or are produced from pre-existing constituents after infection (Van-Etten *et al.*, 1994). These phytoanticipin toxins, *e.g.* phenolic and iridoid glycosides, glucosinolates and saponins are normally stored as less toxic glycosides in the vacuoles of plant cells. If the integrity of the cell is broken when penetrated by the microbe, the glycoside comes into contact with hydrolyzing enzymes present in other compartments of the cell, releasing the toxic aglycone (Osbourn, 1996). There is no sharp boundary between phytoalexins and phytoanticipins, and in one plant species a certain chemical can function as a phytoalexin, whereas, it has the function of a phytoanticipin in another species (McMurchy and Higgins, 1984; Higgins and Smith, 1972). The rich diversity of secondary metabolites in plants has partly arisen because of selection for improved defence mechanisms against a broad array of microbes, insects and other plants. Related plant families often make use of similar secondary compounds for defence purposes (isoflavonoids in Leguminosae; sesquiterpenes in Solanaceae). Most antimicrobial secondary metabolites have relatively broad spectrum of activity. The specificity is determined to whether the pathogen has the enzymes necessary to detoxify a particular host product (Van-Etten *et al.*, 1994).

Plant Derived Individual Compounds with Antimicrobial Effects

Phenolic Compounds

Some of the simplest bioactive phytochemicals consist of a single substituted phenolic ring. Cinnamic and caffeic acids are common representatives of a wide

group of phenylpropane-derived compounds which are in the highest oxidation state, known to possess antimicrobial effects (Brantner *et al.*, 1996). Catechol and pyrogallol both are hydroxylated phenols shown to be toxic against micro organisms. Increased hydroxylation of the phenol group has been found to result in increased toxicity to microorganisms (Geissman, 1963). The site(s) and number of hydroxyl groups on the phenol group are thought to be related to their relative toxicity to microorganisms, with evidence that increased hydroxylation results in increased toxicity (Geissman, 1963). On the contrary, it has in some cases been found that highly oxidized phenols are inhibitory (Scalbert, 1991). Phenolic compounds are thought to inhibit microbial enzymes possibly through reaction with sulfohydryl groups (the oxidized phenols) or through non-specific interactions with the proteins (Mason and Wasserman, 1987).

Quinones

The potential range of quinone antimicrobial effects seems to be great. Probable targets for the quinones in the microbial cell are the surface exposed adhesins, cell wall polypeptides and enzymes bound to the membranes. Quinones are known to complex irreversibly with nucleophilic amino acids in proteins (Stern *et al.*, 1996) thus leading to inactivation of the protein and loss of its function. It is also possible that quinones render substrates unavailable to the microorganism (Cowan, 1999). Anthraquinones, the largest group of quinones (Harborne *et al.*, 1999), have been found to possess antibacterial effects by inhibiting nucleic acid synthesis, at least in *Bacillus subtilis* (Levin *et al.*, 1988).

Stilbenoids

Stilbenoids are composed of two benzene rings separated with an ethane or ethene bridge, called bibenzyls and stilbenes, respectively. Phenanthrenes are biosynthetically derived from the bibenzyls and stilbenes. Stilbenes occur as aglycones or glycosides, and sometimes as polymers. Many higher plant families are known to produce stilbenes. Bibenzyls and their derivatives are rare in higher plants but occur in some families including Orchidaceae, Combretaceae and Dioscoreaceae, often alongside the corresponding phenanthrene or stilbene derivates. Many stilbenoids are known for their antifungal and antibacterial properties (Bruneton, 1999). Eloff *et al.* (2005) have found that leaves of the South African *Combretum woodii* contain high concentrations of the antimicrobially active bibenzyl, combretastatin B5.

Flavonoids

Flavonoids are constitutive compounds but are also synthesized by plants in response to microbial infection (Dixon *et al.*, 1983). Nearly half of the 200 phytoalexins characterized up to now belong to the flavonoids (Harborne, 1988). Flavonoids have been found to show *in vitro* antimicrobial activity against a wide range of microorganisms, some showing potent activity against MRSA (Iinuma *et al.*, 1994). Their activity has been attributed to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls (Cowan, 1999). Lipophilic flavonoids may also disrupt microbial membranes (Tsuchiya *et al.*, 1996). There are conflicting findings on the kind of molecular substitutions needed for a flavonoid in

order to recognize antimicrobial activity. Some authors have found that flavonoids lacking hydroxyl groups on their β-rings are more active against microorganisms than flavonoids containing these groups and this finding supports the idea that their microbial target is the membrane specific (Chabot *et al.*, 1992). Several authors have, however, also found the opposite effect; the more hydroxyl groups the greater antimicrobial activity (Sato *et al.*, 1996). The low toxic potential of flavonoids makes them ideal as antimicrobial medicines (Cowan, 1999).

Tannins

Tannins are a large group of polyphenolic compounds which have received attention in recent years due to their claimed ability to cure a variety of diseases (Serafini *et al.*, 1994). Tannins are subdivided into two groups: hydrolysable tannins and proantocyanidins (condensed tannins). Hydrolysable tannins are gallic acid and ellagic acid esters of core molecules that consist of polyols such as sugars. Proantohocyanidins are polymers of flavan-3-ols (for example catechin) and flavan-3, 4-diols linked through an interflavan bond that is not susceptible to hydrolysis (Haslam, 1989). A wide range of anti-infective actions have been assigned to tannins (Haslam, 1996). Tannins have the ability to complex with proteins through nonspecific forces such as hydrogen bonding and hydrophobic effects and also through covalent binding (Stern *et al.*, 1996). The antimicrobial mode of action for tannins may thus be related to their ability to inactivate microbial adhesins, enzymes, cell envelope transport proteins, etc. (Cowan, 1999). There is also evidence that tannins directly inactivate microorganisms, because already low concentrations of tannin (0.063 mg/ml) modify the morphology of germ tubes of*Crinipellis perniciosa* (Brownlee *et al.*, 1990). Tannins have also been found to induce changes in the morphology of several species of ruminal bacteria (Jones *et al.*, 1994). Due to their ability to bind to proteins and metals, tannins also inhibit the growth of microorganisms through substrate and metal ion deprivation (Scalbert, 1991). Hydrolysable and condensed tannins have been found to possess similar antifungal (filamentous fungi) and antibacterial potency, but the hydrolysable tannins were found to be more effective against yeasts (Cowan, 1999). Latté and Kolodziej (2000) found that a panel of different hydrolysable tannins had low antibacterial effects, but that they possessed fairly high anticryptococcal effects. Some research has been performed on the relationship between tannin structure and antimicrobial activity. The presence of a hexahydroxydiphenoyl moiety or its oxidatively modified entities was an important feature for the anticryptococcal activity of the ellagitannins corilagin, pelargoniin B and phyllanthusiin (Latté and Kolodziej, 2000). The pattern of B-ring hydroxylation of monomeric flavonols in condensed tannins has been shown to affect the level of growth inihibition of *Streptococcus sobrinus* and *Streptococcus mutans* (Sakanaka *et al.*, 1989), *Clostridium botulinum* (Hara and Watanabe, 1989), *Proteus vulgaris* and *Staphylococcus* sp. (Mori *et al.*, 1987), and in all cases gallocatechins were inhibitorier than their catechin counterparts. The toxicity of tannins and lower molecular weight phenols has been discussed also in relation to their oxidation state; catechin was found to be devoid of any toxicity against methanogenic bacteria, whereas if oxidized it strongly reduced methane production (Field *et al.*, 1989). The synthesis of red beet β-glucan synthase was found to be strongly inhibited by various oxidized phenols, but

the effect of oxidation was less marked for tannic acid (hydrolysable tannin) than for smaller phenols (Mason *et al.*, 1987). It has also been proposed that tannin toxicity would be related to molecular size since the larger the molecule the more effectively it binds to proteins. This has been observed in many cases; dimeric ellagitannins have been found to be more adstringent than related monomers (McManus *et al.*, 1985). On the other hand, in some cases the toxicity of tannins was found to be no higher than that of catechins (Siwaswamy *et al.*, 1986), although catechins have very poor affinity to proteins. Kakiuchi *et al.* (1986) found that adding BSA to a glucosyl transferase solution before addition of gallotannins failed to remove the inhibition of the enzyme by the tannins and they concluded that inhibition of the enzyme is not necessarily due to the nonspecific binding of tannins to it. In their study of an array of different tannins and their effects on ligand binding to various enzyme receptors, Zhu *et al.* (1997) found that some of the tannins inhibited ligand binding to specific receptors. Thus, this study shows that tannins have specific activity at the receptor level, and that these effects cannot solely be explained in terms of protein binding.

Coumarins

Coumarins are phenolic substances made of fused benzene and a-pyrone rings (O'Kennedy and Thornes, 1997). They are responsible for the characteristic odor of food. As of 1996, at least 1,300 had been identi?ed (Hoult and Paya, 1996). Coumarin was found *in vitro* to inhibit *Candida albicans*. Hydroxycinnamic acids, related to coumarins, seem to be inhibitory to Gram-positive bacteria (Fernandez *et al.*, 1996). Also, phytoalexins, which are hydroxylated derivatives of coumarins, are produced in carrots in response to fungal infection and can be presumed to have antifungal activity (Hoult and Paya, 1996). General antimicrobial activity was documented in *Galium odoratum* extracts (Thomson, 1978). How ever, data about speci?c antibiotic properties of coumarins are scarce, although many reports give reason to believe that some utility may reside in these phytochemicals (Hamburger and Hostettmann, 1991; Scheel, 1972). Recently Smyth *et al.* (2008) studied the antimicrobial activities of 43 naturally occurring and synthetic coumarins using a microtitre assay against both Gram-positive and Gram-negative bacteria, including a hospital isolate of methicillin-resistant *Staphylococcus aureus* (MRSA) and result showed the coumarins exhibiting good bioactivity against clinically isolated MRSA strains.

Terpenoids and Essential Oils

Terpenes are a large group of compounds responsible for the fragrance of plants and comprise the so called essential oil fraction. They are synthesized from isoprenoid units, and share origins with fatty acids. They differ from fatty acids in that they are branched and cyclized. Their general chemical structure is $C_{10}H_{16}$ and they occur as diterpenes, triterpenes, and tetraterpenes (C_{20}, C_{30}, and C_{40}), as well as hemiterpenes (C_5) and sesquiterpenes (C_{15}). When the compounds contain additional elements, usually oxygen, they are termed terpenoids (Cowan, 1999). Terpenoids are synthesized from acetate units, and as such they share their origins with fatty acids. They differ from fatty acids in that they contain extensive branching and are cyclized. Examples of common terpenoids are methanol and camphor (monoterpenes) and farnesol and artemisin (sesquiterpenoids). Terpenes and terpenoids have been found to possess

antibacterial activity (Ahmad *et al.,* 1993; Amaral *et al.,* 1998; Barre *et al.,* 1997; Himejima *et al.,* 1992; Mendoza *et al.,* 1997; Scortichini and Rossi, 1991; Tassou *et al.,* 1995; Taylor *et al.,* 1996), fungi (Ayafor *et al.,* 1994; Hasegawa *et al.,* 1994; Kubo *et al.,* 1993; Rana *et al.,* 1997; Rao *et al.,* 1993; Suresh *et al.,* 1997; Taylor *et al.,* 1996), viruses (Fujioka and Kashiwada, 1994; Hasegawa *et al.,* 1994; Pengsuparp *et al.,* 1994; Sun *et al.,* 1996; Xu *et al.,* 1996), and protozoa (Ghoshal *et al.,* 1996; Viswakarma, 1990). In 1977, it was reported that 60 per cent of essential oil derivatives examined to date were inhibitory to fungi while 30 per cent inhibited bacteria (Chaurasia and Vyas, 1977). The mechanism of action of terpenes is not fully understood but is speculated to involve membrane disruption by the lipophilic compounds. Mendoza *et al.* (1997) found that increasing the hydrophilicity of kaurene diterpenoids by addition of a methyl group drastically reduced their antimicrobial activity. Cichewicz and Thorpe (1996) found that capsaicin might enhance the growth of *Candida albicans* but that it clearly inhibited various bacteria to differing extents. Two diterpenes isolated by Batista *et al.* (1994) were found to be more democratic; they worked well against *Staphylococcus aureus*, *V. cholerae*, *P. aeruginosa*, and *Candida* species.

Alkaloids

Heterocyclic nitrogen compounds are called alkaloids. The ?rst medically useful example of an alkaloid was morphine, isolated in 1805 from the opium poppy *Papaver somniferum*. Diterpenoid alkaloids, commonly isolated from the plants of the Ranunculaceae family (Atta-ur-Rahman and Chaudhary, 1995), are commonly found to have antimicrobial properties (Omulokoli *et al.,* 1997). Solamargine, a glycoalkaloid from the berries of *Solanum khasianum*, and other alkaloids may be useful against HIV infection (McMahon *et al.,* 1995; Sethi, 1979) as well as intestinal infections associated with AIDS (McDevitt *et al.,* 1996). Szlavik *et al.*(2004) reported that lycorine, homolycorine, and acetyllycorine hemanthamine isolated from *Leucojum vernum* possessed high antiretroviral activities with low therapeutic indices, while drymaritin isolated from *Drymaria diandra* had anti-HIV activity (Hsieh *et al.,* 2004). Interestingly the whole extract and harman alkaloid fraction of *Ophiorrhiza nicobarica*, a folklore plant of the little Andaman Islands, completely inhibited the plaque formation and delayed the eclipse phase of HSV replication (Chattopadhyay *et al.,* 2006).

Lectins and Polypeptides

Peptides which are inhibitory to microorganisms were first reported by Balls *et al.* (1942). They are often positively charged and contain disul?de bonds and their mechanism of action may be the formation of ion channels in the microbial membrane (Terras *et al.,* 1993; Zhang *et al.,* 1997) or competitive inhibition of adhesion of microbial proteins to host polysaccharide receptors (Sharon and Ofek, 1986). Recent interest has been focused mostly on studying anti-HIV peptides and lectins, but the inhibition of bacteria and fungi by these macromolecules, such as that from the herbaceous *Amaranthus* sps, has long been known (De Bolle *et al.,* 1996). Thionins are peptides commonly found in barley and wheat and consist of 47 amino acid residues and they are toxic to yeasts and Gram-negative and Gram-positive bacteria (Fernandes de Caleya *et al.,* 1972). Fabatin, a newly identified 47- residue peptide from fava beans, appears to be structurally related to g-thionins from grains and inhibits *E. coli*, *P.*

aeruginosa, and *Enterococcus hirae* but not on any yeast (Zhang and Lewis, 1997). The larger lectin molecules, which include mannose specific lectins are reported from several plants (Balzarin *et al.,* 1991), MAP30 from bitter melon (Lee-Haung *et al.,* 1995), and jacalin (Favero *et al.,* 1993), are inhibitory to viral proliferation (HIV, cytomegalovirus), probably by inhibiting viral interaction with critical host cell components. It is worth emphasizing that molecules and compounds such as these whose mode of action may be to act synergistically.

Other Compounds

Many phytochemicals not mentioned above have been found to exert antimicrobial properties. This review has attempted to focus on reports of chemicals which are found in multiple instances to be active. It should be mentioned, however, that there are reports of antimicrobial properties associated with polyamines (in particular spermidine) (Flayeh and Sulayamen, 1987), isothiocyanates (Donberger *et al.,* 1975, Iwu *et al.,* 1991), thiosul?nates (Tada *et al.,* 1988), and glucosides (Rucker *et al.,* 1992). Polyacetylenes deserve special mention. Estevez-Braun *et al.* (1994) isolated a C17polyacetylene compound from *Bupleurum salicifolium*, a plant native to the Canary Islands. The compound, 8S-heptadeca-2(Z), 9(Z)-diene-4,6-diyne-1,8-diol, was inhibitory to *S. aureus* and *B. subtilis* but not to Gram-negative bacteria or yeasts (Esteevez-Braun *et al.,* 1994). Acetylene compounds and ?avonoids from plants traditionally used in Brazil for treatment of malaria fever and liver disorders have also been associated with antimalarial activity (Brandao *et al.,* 1997). Much has been written about the antimicrobial effects of cranberry juice. Historically, women have been told to drink the juice in order to prevent and even cure urinary tract infections.

An Overview of the Analytical Methods

Nowadays, the interest in study of natural products is growing rapidly, especially as part of drug discovery programs. There are various methods available for the extraction of secondary metabolites from plants. How to extract effectively is influenced of the method selections and suitable solvents.

Extraction Techniques

Solvent Extraction

Solvent extraction is widely used and long-standing methods in studies of natural products.

Maceration

This is simple, and still widely used, procedure involves leaving the pulverized plant to soak in suitable solvent in a closed container at room temperature. To increase the speed of extraction, occasional or constant stirring is added. However, this method also has limitations. Its main disadvantage is a time consumption process. Besides that, to extract exhaustively, a large volume of solvent is used. In addition, some compounds are not be extracted effectively because of insolubility at room temperature.

Percolation

The powdered material is soaked initially in a solvent in a percolator. Additional

solvent is then poured on top of the material and allowed to percolate slowly out of the bottom of the percolator. As maceration, it also takes time and volumes of solvents.

Soxhlet Extraction

This method is convenient and widely used for extraction because of its continuous process, less time and solvent-consumption than maceration and percolation. The powdered plant is placed in a Soxhlet apparatus, which is on top of a collecting flask beneath a reflux condenser. A suitable solvent is added to flask and the set up is heated under reflux. The steam of the solvent, which contacts with material will dissolve metabolites and brings back to flask. Because of the boiling point of the solvent used, the heat may damage the metabolites.

Refluxing Extraction

Material is inundated in solvent in a round bottomed flask, which is connected to a condenser. The solvent is heated until it reaches its boiling point. As the vapor is condensed, the solvent is recycled to the flask. The metabolites may a little damage.

Supercritical Fluid Extraction

SFE (supercritical Fluid Extraction) has long used in industries for extraction of various commercial natural products *viz.*, coffee, hops, spices, flavors and vegetables oils but still it has a limit in natural products extraction. Supercritical fluids (SCFs) are increasingly replacing organic solvents because of a solvent free and environment friendly method of extraction has become the method of choice. The critical point of a pure substance is defined as the highest temperature and pressure, which the substance can exist in vapor-liquid equilibrium. Above this point, a supercritical fluid is formed. It is heavy like a liquid and has the penetration of gas. These qualities make SCFs effective and selective solvent. The choice of the SFE solvent is similar to the regular extraction. Principle considerations are the followings:

- ☆ Good solvent properties
- ☆ Inert to the product
- ☆ Easy separation from the product
- ☆ Among SCFs, *e.g.*, ethane, butane, pentane, N_2O, CHF_3 and water, Carbon dioxide is the most commonly used SCF, due primarily to its low critical parameters (31.1°C, 73.8 bar), low cost and non toxicity. However, several other SCFs have been used in both commercial and new processes.

Advantages

- ☆ Dissolving power of the SCF is controlled by pressure and/or temperature.
- ☆ SCF is easily recoverable from the extract due to its volatility.
- ☆ Non toxic solvents leave no harmful residue.
- ☆ High boiling components are extracted at relatively low temperatures.
- ☆ Separations not possible by processes that are more traditional can sometimes are effected.

- ☆ Thermally labile compounds can be extracted with minimal damage as low temperatures can be employed by the extraction.

Disadvantages

- ☆ Elevated pressure required.
- ☆ Compression of solvent requires elaborate recycling measures to reduce energy costs.
- ☆ High capital investment for equipment.

Chromatographic Methods

Chromatography is the method of choice in separating the problem of isolation of a compound of interest from a complex natural mixture. There are various methods from basic to advance just supporting for isolation and separation compounds effectively.

Thin Layer Chromatography (TLC)

TLC is an easy, cheap, rapid, and basic method for the analysis and isolation of organic natural and synthetic compounds. TLC involves the use of a particulate sorbent spread on an inert sheet of glass, plastic, or metal as a stationary phase. The mobile phase is allowed to travel up the plate carrying the sample that was initially spotted on the sorbent just above the solvent. Depending on the nature of the stationary phase, the separation can be either partition (C18 reversed phase) or adsorption chromatography (Silica gel, alumina, cellulose, and polyamide). The advantage of TLC is that the samples do not have to undergo the extensive cleanup steps, and the ability to detect a wide range of compounds, using reactive spray reagents. Non-destructive detection (fluorescent indicators in the plates, examination under a UV lamp) also makes it possible for purified samples to be scraped off the plate and be analyzed by other techniques.

Preparative TLC (PTLC)

Preparative TLC has long been a popular method as a primary or final purification step in an isolation procedure. Separation can be effected rapidly and the amount of material isolated is from 1mg to 1g. The sorbent thickness of PTLC is 0.5-4 mm is compared with analytical TLC (0.1-0.2mm sorbent thickness). In commercial available PTLC plates, sorbents silica, alumina, C18 and cellulose are usually of thickness 0.5, 1.0, and 2.0 mm. Nevertheless, there are also having advantages and disadvantages.

Advantages

- ☆ Simple technique.
- ☆ Low cost than the others instrument, for example, HPLC or CC.
- ☆ Isolate compounds quickly from milligram to gram.
- ☆ Almost any separation can be achieved with the correct stationary phase and mobile phase.

Disadvantages

- ☆ Poor control of detection and elution compared to HPLC.
- ☆ Manual operation.

TLC Bioassays

In addition, the simplicity, and the ability of TLC to separate mixtures quickly with little expense, it can be readily used to detect biological activity of separated components. Currently, TLC bioassays are used more and more widely. TLC bioassays against fungi and bacteria have proved exceptionally popular owing to their ease of use, low cost, rapidity and ability to be scaled up to assess antimicrobial activity of a large number of samples. Generally, TLC plates are running and then the microorganism is applied to the plate, as a spray (in case of direct bioautography) or plate is cover with a growth medium containing the microorganism in dish or tray (overlay assay). These simple bioassays will continue to prove useful in antimicrobial activity of natural product extracts.

Column Chromatography (CC)

CC consists of a column of particulate material such as silica or alumina that has a solvent passed through it at atmospheric, medium, or low pressure. The separation can be liquid/solid (adsorption) or liquid/liquid (partition). Most systems rely on gravity to push the solvent through, but medium pressure pumps are commonly used in flash CC. The sample is dissolved in a solvent and applied to the front of the column (wet packing), or alternatively adsorbed on a coarse silica gel (dry packing). The solvent elutes the sample through the column, allowing the components to separate. Normally, the solvent is non-polar and the surface polar, although there are a wide range of packing including chemically bound phase systems. The solvent is usually changed stepwise, and fractions are collected according to the separation required, with the eluting products usually monitored by TLC. The solvent system is developed using TLC. The technique is not efficient, with relatively large volumes of solvent being used, and particle size is constrained by the need to have a flow of several ml/min. The advantage is that no expensive equipment is required, and the technique can be scaled up to handle sample sizes approaching gram amounts.

Gas Chromatography (GC)

This is very useful to analysis violate compounds in natural products. The mobile phase in GC is a carrier gas to convey the sample in a vapor state through stationary phase. The columns of stationary phase are capillary or packed of silica. Nevertheless, capillary column is more used. The column is installed in an oven that has temperature control, and the column can be slowly heated up to 350-450°C starting from ambient temperature to provide separation of a wide range of compounds. The carrier gas is usually hydrogen or helium under pressure, and the eluting compounds can be detected in several ways a) "universal" including flames (flame ionization detector-FID), by mass spectrometry (MS), or by changes in properties of the carrier (thermal conductivity detector-TCD). Among them, FID and MS is very common applied in organic compounds and is the appropriate tool to investigate essential oils, the other is only used to analysis gases; b) "selective" (Electron Capture (ECD), Nitrogen-

Phosphorus (NPD), FID etc.) detection for substances which are having negative electric atoms or function groups, such as Halogen, N, P, etc.

Advantages

- ☆ Low viscosity of gas allows for the use of long columns (up to 60m).
- ☆ Requires thermally stable compounds that are also volatile (B.P. < 300°C). If a compound does not have these attributes it may be possible to derivative it to a compound that does.
- ☆ High gas flow rate allows for fast analysis and can be automated.
- ☆ Many different detection methods allow for analysis of molecules containing specific functional groups *e.g.*, halogens or nitrogen.

Disadvantages

- ☆ Not applicable to non volatile compounds.
- ☆ Requires the use of relatively expensive equipment.
- ☆ Requires skilled operators.

High Pressure Liquid Chromatography (HPLC)

Currently, HPLC plays an important role not only in science research field but also in many application areas such as the pharmaceutical industry. HPLC is a development of column chromatography. To improve resolution, HPLC columns are packed with small sized particles (3, 5, 10µm) with a narrow size distribution. Flow rate and column dimensions can be adjusted to minimize band broadening. The required pressures are supplied by pumps that could withstand the involved chemicals. The selection of solvents and eluent condition (gradient or isocratic) are upon to the mixture components and the interested compounds. The commonly used detector in HPLC systems are Ultraviolet/Visible (UV/Vis), Refractive index (RI), Evaporative light scattering (ELS), MS, and Fluorescence detector.

UV detectors are not only places constraints on the solvents that can be used but also is limited to absorbing compounds. RI detectors considered as universal but cannot easily be used with solvent gradients. However, recently, the ELS have emerged as a universal detector. ELS works by passing the eluate through a heated nebulizer to volatilize the eluate and evaporate the solvent. The solvent is carried away as a gas but the solute form is a stream of fine particles, which passes between a light source and detector and scatters the light. The detector measures this scattering effect. The advantages of ELS that it is applied for detection of non volatile and semi-volatile samples and the unprocessed of chromophore compounds. In addition, it can be used with both the isocratic and gradient eluent conditions. But this type of detector can be used for all solutes having a lower volatility than the mobile phase. If any compounds are having the boiling point close to mobile phase, they cannot be detected because of the misapprehension to the background.

Analytical HPLC is used just for separation and identification of a small amount mixture of samples but the pure isolated compounds cannot be collected. However, crude extracts consist of a mixture of numerous components. Therefore, to isolation

or purification fast and efficiently a large amount, preparative HPLC is developed. Preparative HPLC uses one of these kinds: normal phase, reversed phase, gel permeation, and ion exchange chromatography. Nevertheless, reversed phase with C8 and C18 is preferred for isolation most classes of natural products.

Spectroscopic Techniques

Nuclear Magnetic Resonance Spectroscopy (NMR)

NMR has become a very important spectroscopic method and the premier organic spectroscopy available to chemists to determine the detailed chemical structure of the chemicals they were isolating from natural products. NMR spectroscopy is routinely used by chemists to study chemical structure of simple molecules using simple one dimensional techniques (1D-NMR). Two-dimensional techniques (2D-NMR) are used to determine the structure of more complicated molecules.

Mass Spectrometry (MS)

Mass spectrometry is an analytical technique used to measure the mass-to-charge ratio of ions. This is a powerful, sensitive, and highly selective method to identify compounds. It provides both molecular weight and fragmentation pattern of the compound. It relies of production of ions from a parent compound and the subsequent characterization of the pattern that are produced. Mass spectrometers can be divided into three fundamental parts, namely the ionization source, the analyzer, and the detector. The sample has to be introduced into the ionization source of the instrument. Once inside the ionization source, the parent compound is bombarded by high energy electrons stream then converted to ions, because ions are easier to manipulate than neutral molecules. These ions are extracted into the analyzer region of the mass spectrometer where they are separated according to their mass-to-charge ratios (m/z) in a magnetic or electric fields. The separated ions are detected by a detector and this signal sent to a data system where the m/z ratios are stored together with their relative abundance for presentation in the format of an m/z spectrum.

Antibacterial Assays

Perhaps the most common *in vitro* assay used for plant extracts is the assessment of antibacterial activity, with the majority of researchers using one of the three following assays: disk diffusion, agar dilution, or broth dilution or micro dilution. These methods are based on those described for standardized testing of antibiotics (Andrew, 2001a; 2001b; 2004; 2005; 2006; 2007; Brown, 2001; King and Brown, 2001; Livermore *et al.*, 2001; Livermore and Brown, 2001; MacGowan and Wise, 2001; Wheat, 2001), however several factors may affect the suitability of these methods for use with plant extracts. These factors include the type of organism being tested, concentration of inoculum, type of media and nature of the extract being tested (pH, solubility etc.) (Griffin, 2000; Hood *et al.*, 2004). The methods can be used to simply determine whether or not antibacterial activity is present or can be used to calculate a minimum inhibitory concentration (MIC). Table 9.1 summarizes the limitations and advantages of these various methods. All these methods are those most widely used for *in vitro* testing of plant extracts for antibacterial activity, while some other methods are also have been used. For example, Garedew *et al.* (2004) report on the use of a flow calorimetric

Table 9.1: Comparison of strengths and limitations of various assays for antimicrobial activity.

Method	*Strength*	*Limitation*
Disk well diffusion	☆ Low cost ☆ Results available in within 1–2 days. ☆ Does not require specialized laboratory facilities. ☆ Uses equipment and reagents readily available in a microbiology laboratory. ☆ Can be performed by most laboratory staff. ☆ Data is only collected at one or two time points. ☆ Large numbers of samples can be screened. ☆ Results are quantifiable and can be compared statistically.	☆ Differential diffusion of extract components due to partitioning in the aqueous media. ☆ Inoculums size, presence of solubilizing agents, and incubation temperature can affect zone of inhibition. ☆ Volatile compounds can affect bacterial and fungal growth in closed environments.
Agar dilution	☆ Low cost ☆ Does not require specialized laboratory facilities. ☆ Uses equipment and reagents readily available in a microbiology laboratory. ☆ Can be performed by most laboratory staff.	☆ Hydrophobic extracts may separate out from the agar. ☆ Inoculum size, presence of solubilizing agents and incubation temperature can affect zone of inhibition. ☆ Volatile compounds can affect bacterial and fungal growth in closed environments. ☆ Data is only collected at one or two time points. ☆ Use of scoring systems is open to subjectivity of the observer. ☆ Some fungi are very slow growing.
Broth dilution	☆ Allows monitoring of activity over the duration. ☆ More accurate representation of antibacterial activity. ☆ Micro-broth methods can be used to screen large numbers of samples in a cost-effective manner.	☆ Essential oils may not remain in solution for the duration of the assay, emulsifier and solvent may interfere with accuracy of results. ☆ Labor and time intensive if serial dilution are used to determine cell count ☆ Highly colored extracts can interfere with colorimetric endpoints in micro broth methods.
TLC bioautograpy	P Simultaneously fractionation and determination of bioactivity.	☆ Unsuitable where activity is due to component synergy ☆ Dependent on the extraction methods and TLC solvent used.

Contd...

Table 7.1–*Contd...*

Compound Type Chemical Name	*Place (Part Used)*	*References*	
Antiviral assay	☆ Allow simultaneously assessment of cell toxicity with antiviral assay. ☆ Few methods available therefore comparability across studies is high.	☆ Labor, time and cost intensive. ☆ Requires access to cell culture and viral containment facility. ☆ Essential oils may not remain in the solution for the duration of the assay.	
Antiparasitic assay	☆ Methods are well documented. ☆ Some assay, allow simultaneously assessment of cell toxicity.	☆ Labor, time and cost intensive. ☆ May require access to cell culture facility. ☆ Essential oils may not remain in the solution for the duration of the assay.	

method to assess antibacterial activity of honey and demonstrated better sensitivity than other methods and Pitner *et al.* (2000) propose the use of high throughput systems that measure bacterial respiration via a fluorescent signal. However, the practicality of these methods for screening of plant extracts is yet to be determined. An additional method TLC–bioautography allows for identification of bioactive fractions of extracts within a single assay.

Disk Diffusion Method

The disk diffusion method (also known the zone of inhibition method) is probably the most widely used of all methods used for testing antibacterial activity. It uses only small amounts of the test substance (10–30 µL), can be completed by research staff with minimal training, and as such may be useful in field situations. The method involves the preparation of a Petri dish containing 15–25 mL agar, bacteria at a known concentration are then spread across the agar surface and allowed to establish. A paper disk (6 or 8 mm) containing a known volume of the test substance is then placed in the center of the agar and the dish incubated for 24 h or more. At this time the "cleared" zone (zone of inhibition) surrounding the disk is measured and compared with zones for standard antibiotics or literature values of isolated chemicals or similar extracts. Where the extract is viscous or a semi-solid (*e.g.* honey) a well can be created in the agar and the substance allowed diffusing out of the well rather than away from a disk. Data from these assays are typically presented as mean size of zone of inhibition (with or without standard deviation), although some authors employ a ranking system of "+", "++", and "+++" to indicate levels of activity. Few authors provide any statistical analysis of their data and levels of activity (slight, moderate, strong) are used without any reference to standardized criteria.

One of the major criticisms of this method is that it relies on the ability of the extract to diffuse through agar and any component of the extract that does diffuse away from the disk will create a concentration gradient, potentially creating a gradient of active antibacterial compounds. All of the antibacterial testing methods use an aqueous base for dispersion of the test substance, either via diffusion in agar or dispersion within nutrient broth, consequently assays using extracts with limited solubility in aqueous media (*e.g.,* essential oils) may not reflect the true antibacterial activity. There is also no consensus on the best agar to use for these assays. A further limitation that has not been directly addressed in the literature, but for which evidence exists, is inference in the assay from vapours liberated from the extract during incubation. This is unlikely to be a major consideration in aqueous or solvent extracts but may be a significant confounder in assays of essential oils.

Agar Dilution Method

The agar dilution method is another relatively quick method that does not involve the use of sophisticated equipment. Any laboratory with facilities for basic microbiological work can use this method. In this method the test substance is incorporated at known concentrations into the agar and, once set, bacteria are applied to its surface. Replicate dishes can be set up with a range of concentrations of the test substance and by dividing the surface of the agar into wedges or squares, a number of bacterial species may be applied to a single dish. In this way, a large number of

bacteria may be screened within a single assay run. The dishes are incubated for 24 h or more and the growth of the bacteria on the extract/agar mix is scored either as present/absent or a proportion of the control (*e.g.*, 0, 25 per cent, 50 per cent, 75 per cent, 100 per cent). A criticism of this method is that when a scoring system is used it is difficult to guarantee objectivity and to therefore compare one set of results with another. This method suffers from several other limitations, including many that have been discussed previously: (a) use of larger volumes of test substance than in other methods, (b) confounding antibacterial actions from volatiles, (c) difficulty of achieving stable emulsions of essential oils in agar and (d) restriction on the maximum concentration that can be used before the agar becomes too dilute to solidify properly. Perhaps the most frustrating of these is the difficulty of stably incorporating essential oils and other hydrophobic extracts into aqueous environments. This problem occurs not just in agar dilution assays but also in broth dilution and other antimicrobial assays. Many researchers has thought they had incorporated their essential oil into nutrient broth or other media only to find that, on return to the experiment after an hour or so, the oil had separated out and was floating on top of the media. Griffin (2000) in their work on tea tree oil found that at concentrations above 2 per cent v/v the oil separated from the agar substrate and was seen as droplets on the agar surface. The most commonly utilized method to overcome this problem is the use of surfactants such as Tween-20, Tween-80, and alkyl dimethyl betaine (ADB). Several authors have described the use of these products and the effect on antibacterial activity. The results of their studies show that surfactants can interfere with calculation of MIC values and the growth of some test organisms (Hammer *et al.*, 1999) however it has also been demonstrated that it is possible to use very small quantities of Tween (<0.5 per cent v/v) to emulsify the essential oil in media and thus avoid the effects on organism growth (Griffin, 2000; Hood *et al.*, 2004). Hammer *et al*. (1999) also showed that inclusion of organic matter such as bovine serum albumin in the agar also affected the antibacterial activity of tea tree oil.

Broth Dilution Methods

Difficulties with partitioning of hydrophobic compounds in agar and a desire to more accurately monitor antibacterial activity over time has resulted in a move to broth dilution methods for testing of plant extracts. In this method, bacteria are grown in test-tubes in a liquid media in the presence of the test substance. At regular time intervals (*e.g.*, every 10 min or every hour) a sample is removed and the bacterial count determined by serial dilution of the sample, subsequent incubation on agar and counting of colony forming units (CFU). In contrast to the single data point (*e.g.*, 24 h incubation) utilized in disk diffusion and agar dilution assays, the broth dilution method allows much finer evaluation of the antibacterial events over time and features such as recovery from the effects of the test substance and proportion of organisms killed at a given time point can be determined. However, the method is also time and resource intensive and can be impractical where very large numbers of test substances are to be screened. As with other testing methods incorporation of hydrophobic compounds and essential oils into the aqueous media is problematic, and as there is no solid phase to trap these compounds they rapidly separate from the media and form a layer across the surface of the media. For organisms sensitive to oxygen tension

in the media this can present an additional problem as the oil can inhibit gaseous exchange. Tween or ethanol may be used to enhance incorporation into the aqueous media, however as previously discussed these compounds may interfere with the assay results.

Micro broth methods have also been developed, which utilize microtiter plates, thus reducing the volume of extract needed, and have endpoints that can be determined spectrophotmetrically, either a measure of turbidity or use of a cell viability indicator (*e.g.*, resazurin, methylthiazoldiphenyltetrazolium (MTT)) (Mann and Markham, 1998). They also propose that the cell viability indicator is the best method of endpoint determination for essential oils as the oil/water interface may interfere with turbidity measures. While these micro-broth methods generally work well for plant extracts, problems arise when the extract is heavily colored as this can interfere with the measurement of the indicator chemical. Further, as these methods use plastic microtiter plates, essential oils that have a solvent action on plastics (*e.g.*, *Letospermum petersonii*, *Backhousia citriodora*) cannot be used. Also the addition of essential oils to media, changes its pH and this might be expected to be more significant in small volumes, like the micro broth method (Hood *et al.*, 2004). Whether other plant extracts will also have the effect is unknown. Micro broth methods are also less time and resources intensive than other broth methods as the need for multiple serial dilutions to determine bacterial count is eliminated.

TLC-Bioautography

While the methods above are used to test whole extracts or extracts fractionated at another time there is an increasing interest in bioassay guided fractionation, where the separation of extracts into fractions is completed simultaneously with identification of bioactivity. In this method TLC is performed using crude extracts, extract fractions, or whole essential oils. The developed TLC plate is then sprayed with, or dipped into, a bacterial or fungal suspension (direct bioautography) or overlain with agar and the agar seeded with the microorganism (overlay bioautography) (Hamburger and Cordell, 1987; Homans and Fuchs, 1970; Rahalison *et al.*, 1991) The later method has been particularly used for determining the activity of extract against yeasts such as *Candida albicans*, however Masoko and Eloff (2005) suggest that use of fresh cultures of yeasts and shorter incubation times eliminated the previously reported difficulties of using the direct method with yeasts. This method has been used to screen a range of crude and solvent prepared extracts with the activity observed dependent on both the method of extraction and solvents used in the TLC process (Diallo *et al.*, 2001; Nakamura *et al.*, 1999; Nostro *et al.*, 2000; Sridhar *et al.*,2003). While this method has the advantage of combining both separation of extract constituents and simultaneous identification of those fractions with bioactivity, it is not a suitable method for detecting activity that is a product of synergy between two or more compounds. Further, the results will be affected by the breakdown or alteration of compounds during the fractionation phase.

Antifungal Assays

Antifungal assays are regularly used to determine whether plants extracts will have potential to treat human fungal infections (*e.g.*, tinea) or have use in agricultural/

horticultural applications. In general these assays are quick, low cost, and do not involve access to specialized equipments. Activity of plant extracts against the yeast *Candida* is typically assessed using the disk or well diffusion methods described above, and many studies report anti-candida activity with antibacterial activity rather than with activity against fungi for this reason (Haraguchi *et al.*, 1999; Iskan *et al.*, 2002; Rahua *et al.*,2000; Wilkinson and Cavanagh, 2005). Activity against filamentous fungi can be evaluated in well diffusion, agar dilution, and broth/microbroth methods with many of the same limitations and advantages as previously discussed for antibacterial assays (Inouye *et al.*, 2001). When the well diffusion and disk diffusion techniques are used, fungal plugs are removed from an actively growing colony and placed at a predetermined distance (typically 2 cm) from the centre of an agar dish. A well is then bored in the centre of the agar and test substance added to the well, or the test substance is added to a paper disk and the disk placed in the centre of the agar (The specific agar to be used, and temperature and time of incubation, will be determined by the fungi to be used). The growth of the fungi is monitored and any inhibition of mycelia growth noted. This inhibition of growth is then expressed as a percentage of the growth of control colonies. In the agar dilution method (also known as the poison food technique) the test substance is incorporated into the agar substrate and then a sample of actively growing fungus is placed at the centre of the plate. The radial growth of the fungus after an appropriate time, depending on the growth characteristics of the fungus, is then measured and compared with control samples. Sridhar *et al.* (2003) used this method to show the activity of essential oils against a range of fungi of agricultural and medical importance. Alternatively a fungal cell suspension may be inoculated onto the plate and the MIC determined by the lowest concentration of test substance that prevents visible fungal growth de Aquino Lemos *et al.* (2005). Antisporulation activity can be assessed by using scanning electron microscopy (Inouye *et al.*, 1999), while effects on conidium germination can be evaluated by exposing the conidia to the test substance and subsequently counting the number of conidia with germ tubes equal to 1-1.5 times conidium length (Antonov *et al.*, 1997). Additional observations of germinated conidia over a set period will also allow evaluation of the effect of the plant extract on germ tube growth. All the methods have their own advantages and disadvantages as describe above in testing of antibacterial activity. In addition to these Inouye *et al.* (2001) showed that the inclusion of Tween-80 resulted in weaker bioactivity in agar dilution assays and the size of the original fungal inoculum had a significant effect with larger inoculums being more resistant to antifungal effects. Shahi *et al.* (1999) in their study of the antifungal activity of essential oils found that the antifungal response was altered by modifying the pH of the fungal growth media. As the media pH become more alkaline the eucalyptus essential oils had a greater inhibitory effect on the fungi (*Trichophyton* spp., *Microsporum* spp., and *Epidermophyton* spp.).

In vivo Assessment of Antibacterial and Antifungal Activity

The preceding discussion clearly demonstrates the similarity in methods used for *in vitro* antibacterial and antifungal assays of plant extracts and there are many papers in the literature using one of more of the methods. A smaller number of research

groups have moved beyond the *in vitro* environment and are investigating the *in vivo* efficacy of those extracts that show promise in the laboratory. This is a more complex and costly activity as not only does the activity against the microorganisms need to be evaluated, there must also be consideration of mammalian cell toxicity and allergic reactions (Matura *et al.,* 2005). To date most *in vivo* testing of plant extracts has involved the use of essential oils against human skin infections, particularly fungal infections, and testing of extracts follow standard clinical trial protocols. Perhaps the plant extract best known for its *in vivo* antibacterial activity is honey, with a large number of studies demonstrating *in vivo* activity (Dunford *et al.,* 2000; Moore *et al.,* 2001). It is important to note here that demonstrated activity *in vitro* does not always translate to activity *in vivo*. The best example of this is tea tree oil, which has been shown to have excellent activity *in vitro* against the fungi responsible for various tinea's (MIC 0.004–0.06 per cent) (Hammer *et al.,* 2002) yet the results from clinical trials have been far from conclusive (Satchell *et al.,* 2000). This illustrates the caution with which researchers should view results from *in vitro* assays and reinforces the need for clinical trials of plant extracts that show therapeutic promise.

Methods for Assessing Antiviral Activity

In addition to antibacterial and antifungal activity, researchers are also investigating the use of plant extracts for antiviral activity; of particular interest is activity against herpes simplex virus (HSV), human immunodeficiency virus (HIV), and hepatitis C virus (HCV). Standard cytopathic assays are used to determine antiviral activity with activity both pre- and post-infection evaluated. As these assays are performed in an aqueous environment the problems of solubility that have been discussed at length previously are also an issue in these assays. These assays also require expertise in cell culture and appropriate laboratory containment facilities for working with viruses; these two features make these assays more expensive and labor intensive than other assays. However as viruses require a cell host this assay has the added benefit of being able to assess cell toxicity of the test substance as part of the antiviral assay protocol. This means that those extracts with significant cell toxicity, and therefore little potential for use, can be eliminated from investigations prior to *in vivo* testing. Abad *et al.* (2000) tested 10 extracts (both aqueous and ethanol) and demonstrated that aqueous extracts of five plants showed activity against HSV-1 and vesicular stomatitis virus (VSV) with one extract showing activity against poliovirus. These authors suggest that antiviral activity is more likely to be found in aqueous rather than ethanol extracts; this is in contrast to antibacterial and antifungal assays where activity is more commonly seen in solvent extracts and essential oils. However, other studies have identified activity in both aqueous and solvent (ethanol or methanol) extracts of a wide range of plants against the hepatitis C virus (Hussain *et al.,* 2000), VSV (Abad *et al.,* 1999) and human parainfluenza virus type 2 (HPIV-2) (Karagaz *et al.,* 2003). Few plant ex- tracts/essential oils have been shown to demonstrate antiviral activity *in vivo* (Abad *et al.,* 1999) with work by Nawawi *et al.* (1999) showing that, as with other *in vitro* assays, activity *in vitro* is not always matched by a similar level of activity *in vivo*.

Table 9.2: Antimicrobial screening of different plants.

Sl.No.	Plant	Family	Part*	Activity Against**	References
1.	*Abrus precatorius* L.	Fabaceae	Lf	Sa	Valsaraj *et al.,* 1997
2.	*Acacia catechu* Willd.	Mimosaceae	St	Bs Ec Pa Sa An Ca	Valsaraj *et al.,* 1997
3.	*Achillea millefolium* L.	Compositae	Ap Rh	Bc Sa	Kokoska *et al.,* 2002
4.	*Achyranthes aspera* L.	Amaranthaceae	Lf St	Bs Sa	Valsaraj *et al.,* 1997
5.	*Acorus calamus* L.	Araceae	Rh Rt	Bs Sa	Mc Gaw *et al.,* 2000; Valsaraj *et al.,* 1997
6.	*Adhatoda vasica* Ness	Acanthaceae	Lf	Bs Pa Sa	Valsaraj *et al.,* 1997
7.	*Aegle marmelos* (L.) Corr.	Rutaceae	Rt	Bs Ec Sa Samr Stf Pa Par Mp	Taylor *et al.,* 1996; Valsaraj *et al.,* 1997
8.	*Aframomum melegueta* K. Schum.	Zingiberaceae	Sd	Bs Ec Pa Sa An Ca	Konning *et al.,* 2004
9.	*Ageratum conyzoides* L.	Asteraceae	Wp	Bc Pa	Wiart *et al.,* 2004
10.	*Alangium salviifolium* Wang.	Alangiaceae	Rt	Bs Ec Pa Sa An Ca	Valsaraj *et al.,* 1997
11.	*Allium cepa* L.	Alliaceae	Lf	Bs Ec Ml Se	Rauha *et al.,* 2000
12.	*Aloe vera* L.	Liliaeeae	Lf	Sa	Martinez *et al.,* 1996
13.	*Alstonia scholaris* R. Br.	Apocynaceae	Rb	Bs Ec Pa Sa	Valsaraj *et al.,* 1997
14.	*Amaranthus blitum* L.	Amaranthaceae	Wp	Bc	Wiart *et al.,* 2004
15.	*Anchusa strigosa* Lab.	Boranginaceae	Rt	Sa Pv Ca	Ali-shtayeh *et al.,* 1998
16.	*Andrographis paniculata* Ness	Acanthaceae	Rt	Bs Ec Pa Sa	Valsaraj *et al.,* 1997
17.	*Anisomeles malabarica* (Linn.) R. Br. ex Sims	Lamiaceae	Lf	Bs Ec Pa Sa	Valsaraj *et al.,* 1997
18.	*Artemisia vulgaris* L.	Asteraceae	Lf	Bs Ec Pa Sa	Valsaraj *et al.,* 1997
19.	*Asphodeline lutea* (L.) Rehb.	Liliaceae	Wp	Ec Kp Pa Pv Sa Ca	Ali-shtayeh *et al.,* 1998
20.	*Asteracantha longifolia* L.	Acanthaceae	Lf	Ea Bp Sa	PerumalSamy, 2005
21.	*Avena sativa* L.	Poaceae	Lf	Bs Ec Ml Sa Se An Ca	Rauha *et al.,* 2000

Contd...

Table 9.2–*Contd...*

Sl.No.	Plant	Family	Part*	Activity Against**	References
22.	*Baccharis glutinosa* Pers	Compositiae	Wp	Mc Mg Tt Ef Ss Na Nb Sd Cf Ya Lm Pv Cp	Verastegui *et al.,* 1996
23.	*Bauhinia vahlii* Wight and Arnott	Fabaceae	Rt	Bs Samr Sf Pa Par Mp	Taylor *et al.,* 1996
24.	*Boswellia ameero* Balf. f.	Burseraceae	Bk	Bc Mf Sa Se Sh Sa-NGR	Mothana *et al.,* 2005
25.	*Boswellia elongata* Balf. f.	Burseraceae	Bk	Bc Mf Sa Se Sh Sa-NGR	Mothana *et al.,* 2005
26.	*Brassaiopsis palmata* Kurz	Araliaceae	Lf Bk	Bc Ca Ec Sa	Wiart *et al.,* 2004
27.	*Buxus hildebrandtii* Baill.	Buxaceae	Lf	Bc Mf Sa Se Sh Sa-NGR	Mothana *et al.,* 2005
28.	*Calluna vulgaris* L. Hull	Ericaceae	Lf NM	Bs Ml Se Sa Sh	KumarSamy *et al.,* 2002; Rauha *et al.,* 2000
29.	*Calophyllum inophyllum* L.	Clusiaceae	Sb Lf	Bs Ec Pa Sa	Valsaraj *et al.,* 1997
30.	*Calotropis gigantea* L.	Asclepiadaceae	Lf	Bs Ec Pa Sa	Valsaraj *et al.,* 1997
31.	*Capparis spinosa* L.	Capparidaceae	Rt Fl Fr	Pv Sa	Ali-shtayeh *et al.,* 1998
32.	*Cardiospermum halicacabum*	Sapindaceae	Lf St	Bs Ec Pa Sa	Valsaraj *et al.,* 1997
33.	*Carica papaya* L.	Caesalpiniaceae	Sd	Bs Sa	Valsaraj *et al.,* 1997
34.	*Cassia tora* L.	Rubiaceae	Lf	Bc Mf Sa Se Sh Sa-NGR	Mothana *et al.,* 2005
35.	*Cassia fistula* L.	Caesalpiniaceae	Sd	Bs Ec Pa Sa An	Valsaraj *et al.,* 1997
36.	*Carphalea obovata* (Balf. f.) Verdcourt	Caesalpiniaceae	Rt	Bs Ec Pa Sa	Valsaraj *et al.,* 1997
37.	*Catha edulis* (Vahl.) Endl.	Celastraceae	Bk	Bs Sa	Mc Gaw *et al.,* 2000
38.	*Celosia argentea* L.	Celastraceae	Rt	Bs Sa	Mc Gaw *et al.,* 2000
39.	*Centaurea appendicigera* L.	Amaranthaceae	Wp	Bc Ca Ec Pa Sa	Wiart *et al.,* 2004
40.	*Centaurium erythraea* Rafn	Asteraceae	NM	Sa Samr Sh	KumarSamy *et al.,* 2002
41.	*Centella asiatica Urban*	Apiaceae	Wp	Bs Sa	Valsaraj *et al.,* 1997
42.	*Chelidonium majus* L.	Papaveraceae	Ap Rt	Bc Ca Sa	Kokoska *et al.,* 2002

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Table 9.2–*Contd...*

Sl.No.	Plant	Family	Part*	Activity Against**	References
43.	*Cichorium intybus* L.	Compositae	Ap Rt	Bc Sa	Kokoska *et al.,* 2002
44.	*Cinnamomum iners* Reinw. Ex B.	Lauraceae	Lf	Bc Ca Ec Pa	Wiart *et al.,* 2004
45.	*Cissampelos pareira* L.	Menispermaceae	Lf St	Bs Ec Pa Sa	Valsaraj *et al.,* 1997
46.	*Cissus quandrangularis* L.	Vitaceae	Rt St	Bc Bcg Bmt Bp Bst Bs Ssp Sb Sf Sa Se St Sp	Lin *et al.,* 1999
47.	*Citrus acida* Roxb. Hook. f.	Rutaceae	Lf	Bp Sa	PerumalSamy, 2005
48.	*Citrus aurantifolia* (Chrism.) Swingle	Rutaceae	F r	Af Ag Bc Bco Bs Ec Ml Mp Mr Mro Ms Pf Pv Sa Sm	Melendez *et al.,* 2006
49.	*Citrus aurantium* L.	Rutaceae	F r	Af Ag Bc Bco Bs Ec Ml Mp Mro Ms Pf Pv Sa Sm	Melendez *et al.,* 2006
50.	*Clausena excavata* Burm. f.	Rutaceae	Lf Bk	Bc Bs Sa	Wiart *et al.,* 2004
51.	*Clematis cirrhosa* L.	Ranunculaceae	Ap	Ec Kp Pa Pv	Ali-shtayeh *et al.,* 1998
52.	*Cleome socotrana* Balf. f.	Capparaceae	Lf	Bc Mf Sa Se Sh Sa NGR	Mothana *et al.,* 2005
53.	*Clerodendrum indicum* (L.) Kuntze.	Verbenaceae	Ap	Bs Samr Sf Pa Par Mp	Taylor *et al.,* 1996
54.	*Clerodendrum infortunatum* L.	Verbenaceae	Lf Rt	Bs Ec Pa Sa	Valsaraj *et al.,* 1997
55.	*Clerodendrum serratum* (L.) Moon	Verbenaceae	Lf	Bs Ec Pa Sa	Valsaraj *et al.,* 1997
56.	*Clidemia hirta* (L.) D. Don	Melastomataceae	Lf	Ec Ml Mp Mro Ms Pf Pv Sa	Melendez *et al.,* 2006
57.	*Cola greenwayi* Brenan	Staphyleaceae	Lf Tw	Bs Ec Kp Sa	Reid *et al.,* 2005
58.	*Combretum apiculatum* Loefl.	Combretaceae	Lf	Bs Sa	Mc Gaw *et al.,* 2000
59.	*Commelina communis* L.	Commelinaceae	Wp	Ca	Wiart *et al.,* 2004
60.	*Commiphora parvifolia* Engl.	Burseraceae	Bk	Bc Mf Sa Se Sh Sa NGR	Mothana *et al.,* 2005
61.	*Crescentia cujete* L.	Bignoniaceae	Lf	Af Ag Bc Bco Bs Mp Mr Mro Ms Pf Sa	Melendez *et al.,* 2006

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Table 9.2–*Contd...*

Sl.No.	Plant	Family	Part*	Activity Against**	References
62.	*Crithmum maritimum* L.	Apiaceae	NM	Bc Ec	KumarSamy *et al.,* 2002
63.	*Croton hirtus* L Her	Euphorbiaceae	Wp	Bc Bs Sa	Wiart *et al.,* 2004
64.	*Curtisia dentata* (Burm.f.) C.A.Sm.	Coranaceae	Bk	Bs	Mc Gaw *et al.,* 2000
65.	*Cuscuta reftexa* Roxb.	Convolvulaceae	Wp	Bs Ec Pa Sa	Valsaraj *et al.,* 1997
66.	*Cussonia spicata* Thunb.	Araliacae	Lf	Bs Ec Kp Sa	Mc Gaw *et al.,* 2000
67.	*Cyclea pehata* Hook. f. et Thorns;	Menispermaceae	Rt	Bs Pa Sa	Valsaraj *et al.,* 1997
68.	*Cyperus rotundus* L.	Cyperaceae	Rt Bb	Bs Ec Pa Sa	Valsaraj *et al.,* 1997
69.	*Cyphostemma flaviflorum* (Sprague) Descoings	Vitaceae	Lf Rt St	Af Bcg Bmt Bp Bst Bs Ca Kp Ml Pm Pmg Ps Psr Sf Sa Se Sf Sp	Lin *et al.,* 1999
70.	*Cyphostemma lanigerum* (Harv.) Descoings ex Wild and Drum	Vitaceae	Lf Rt St	Af Bcg Bmt Bp Bst Bs Ca Kp Ml mp Ms Pm Pmg Pv Ps Psr Sa Se St Sp	Lin *et al.,* 1999
71.	*Cyphostemma natalitium* (Szyszyl.) J.V.D. Merwe	Vitaceae	Lf Rt St	Af Bc Bcg Bmt Bp Bst Bs Ca Ea Kp Ml Mp Ms Pm Pmg Pv Ps Psr Ssp Sb Sf Sa Se St Sp	Lin *et al.,* 1999
72.	*Cyphostemma* sp.	Vitaceae	Lf Rt St	Bc Bp Bst Bs Ca Kp Ml Ms Ps Psr Sc S Sb	Lin *et al.,* 1999
73.	*Cystostemon socotranus* Balf. f.	Boraginaceae	Lf	Bc Mf Sa Se Sh Sa-NGR	Mothana *et al.,* 2005
74.	*Datura stramonium* L.	Solanaceae	Sd	Ec Sa	Uzum *et al.,* 2004
75.	*Daucus carota* L.	Apiaceae	NM	Bc	KumarSamy *et al.,* 2002
76.	*Delphinium formosum*	Ranunculaceae	Lf Fl	Bc Bs Hp Sa Tr	Buruk *et al.,* 2006
77.	*Desmos dumosus* (Roxburgh) Safford	Annonaceae	Lf Bk	Bc BsSa	Wiart *et al.,* 2004
78.	*Didymocarpus crinita* Jack	Gesneriaceae	Wp	Bc	Wiart *et al.,* 2004

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Table 9.2–*Contd...*

Sl.No.	*Plant*	*Family*	*Part**	*Activity Against***	*References*
79.	*Dillenia suffruticosa* (Griff.) Martelli	Dilleniaceae	Lf	Bc Bs Ca Pa	Wiart *et al.,* 2004
80.	*Dombeya burgessiae* Gerr. ex Harv.	Sterculiaceae	Lf	Bs Ec Kp Sa	Reid *et al.,* 2005
81.	*Dombeya cymosa*	Sterculiaceae	Lf Tw	Bs Ec Kp Sa	Reid *et al.,* 2005
82.	*Dombeya rotundifolia* (Hochst.) Planch.	Sterculiaceae	Lf	Bs Sa	Mc Gaw *et al.,* 2000
83.	*Drynaria quercifolia* (L.) Sm.	Polypodiaceae	Wp	Bs Pa Sa	Valsaraj *et al.,* 1997
84.	*Eclipta alba* Hassk.	Asteraceae	Lf	Bs Ec Pa Sa Ca	Valsaraj *et al.,* 1997
85.	*Eclipta prostrata* L.	Asteraceae	Wp	Bc Bs Ca Sa	Wiart *et al.,* 2004
86.	*Elaeocarpus tuberculatus* Roxb.	Elaeocarpaceae	Sb	Bs Ec Pa Sa	Valsaraj *et al.,* 1997
87.	Elaeodendron transvaalense (Burtt Davy) R.H. Archer	Celastraceae	NM	Bc Bp Bs Sa	Tshikalange *et al.,* 2005
88.	*Elephantopus scaber* L.	Asteraceae	Lf St Wp	Bc Bs Ec Pa Sa	Valsaraj *et al.,* 1997; Wiart *et al.,* 2004
89.	*Elephantorrhiza burkei* Benth.	Fabaceae	NM	Bp Bs Sa	Tshikalange *et al.,* 2005
90.	*Eleusine indica* Gaertn.	Poaceae	Wp	Ca	Wiart *et al.,* 2004
91.	*Emilia sonchifolia* L. DC	Asteraceae	Wp	Bc	Wiart *et al.,* 2004
92.	*Empetrum nigrum* L.	Ericales	Lf	Bs Ml	Rauha *et al.,* 2000
93.	*Enicostema littorale* Blume	Gentianaceae	Lf	Bs Sa	Valsaraj *et al.,* 1997
94.	*Epilobium angustifolium* L.	Onagraceae	Lf	Bs Ec Ml Sa Se	Rauha *et al.,* 2000
95.	*Equisetum telmateia* Ehrh.	Equisetaceae	Ap	Ec Sa Ca	Uzum *et al.,* 2004
96.	*Eryngium creticum* Lam.	Umbilliferae	Lf St Rt	Kp Pa Pv	Ali-shtayeh *et al.,* 1998
97.	*Erythrophleum lasianthum* Corbishley	Fabaceae	Lf	Bs	Mc Gaw *et al.,* 2000
98.	*Eupatorium odoratum* L.	Asteraceae	Ap	Bs Sams Samr	Taylor *et al.,* 1996
99.	*Euphorbia hirta* L.	Euphorbiaceae	Wp	Bc Bs Ca Sa	Wiart *et al.,* 2004

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Table 9.2–*Contd...*

Sl.No.	*Plant*	*Family*	*Part**	*Activity Against***	*References*
100.	*Euryops arabicus* Steud.ex Jaub.&Spach	Asteraceae	Lf	Sa-NGR	Mothana *et al.,* 2005
101.	*Fagonia luntii* Baker	Zygophyllaceae	Lf	Bc Mf Sa	Mothana *et al.,* 2005
102.	*Ficus benghalensis* L.	Moraceae	Ap	Bs Sa	Valsaraj *et al.,* 1997
103.	*Ficus religiosa* L.	Moraceae	Lf	Bs Ec Pa Sa	Valsaraj *et al.,* 1997
104.	*Filipendula ulmaria* (L.) Maxim.	Rosaceae	Lf	Bs Ec Ml Sa Se	Rauha *et al.,* 2000
105.	*Geranium asphodeloides* Burm. f.	Geraniaceae	Ap	Sa Se	Uzum *et al.,* 2004
106.	*Glycyrrhiza uralensis* Fischer	Leguminosae	Ap Rt	Bc Sa	Kokoska *et al.,* 2002
107.	*Gunnera perpensa* L.	Gunneraceae	Rt Rh	Sa	Mc Gaw *et al.,* 2000
108.	*Gymnantes lucida* Sw.	Euphorbiaceae	Lf	Bs Sa	Martinez *et al.,* 1996
109.	*Harpephyllum caffrum* Bernh.	Anacardiaceae	Bk	Bs Ec Kp Sa	Mc Gaw *et al.,* 2000
110.	*Hedyotis capitellata* Wall. ex G. Don	Rubiaceae	Lf Bk	Bs Ca	Wiart *et al.,* 2004
111.	*Hedyotis congesta* Wall	Rubiaceae	Lf Bk	Ca Pa	Wiart *et al.,* 2004
112.	*Hemidesmus indicus* R.Br.	Asclepiadaceae	Lf Rt	Bs Ec Pa Sa	Valsaraj *et al.,* 1997
113.	*Heracleum platytaenium*	Umbelliferae	Lf Fl	Bc Bs Hp Sa Ca Tr	Buruk *et al.,* 2006
114.	*Heteromorpha trifoliata* (Spreng.) Cham. and Schltdl.	Apiaceae	Lf	Bs	Mc Gaw *et al.,* 2000
115.	*Hippophae rhamnoides* L.	Elaeagnaceae	Lf Rt Fr	Bc Ca Pa Sa	Kokoska *et al.,* 2002
116.	*Holarrhena antidysenterica* Wall.	Apocynaceae	Rb	Bs Ec Pa Sa	Valsaraj *et al.,* 1997
117.	*Hyptis suaveolens* Poit.	Lamiaceae	Wp	Ca	Wiart *et al.,* 2004
118.	*Inula viscosa* (L.) Ait.	Compositae	Wp	Kp Pa Pv Sa	Ali-shtayeh *et al.,* 1998
119.	*Jatropha unicostata* Balf. f.	Euphorbiaceae	Bk Lf	Bc Mf Sa Se Sh Sa-NGR	Mothana *et al.,* 2005
120.	*Juglans regia* L.	Juglandaceae	Fl Fr	Kp Pa Pv Sa	Ali-shtayeh *et al.,* 1998

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Table 9.2–*Contd...*

Sl.No.	Plant	Family	Part*	Activity Against**	References
121.	*Juniperus lucayana* (L) Britt.	Cupressaceae	St Br	Sa	Martinez *et al.,* 1996
122.	*Kalanchoe farinacea* Balf. f.	Crassulaceae	Lf Fr	Bc Mf Sa Se Sh Sa-NGR	Mothana *et al.,* 2005
123.	*Kalanchoe pinnata* Pers.	Crassulaceae	Lf	Bc Bs	Wiart *et al.,* 2004
124.	*Knema malayana* Warb.	Myristicaceae	Lf Bk	Bc Bs Ca Pa Sa	Wiart *et al.,* 2004
125.	*Lagerstroemia speciosa* (L.) Pers.	Lythraceae	Lf	Bs Sa	Melendez *et al.,* 2006
126.	*Lamium album* L.	Labiatae	Ap Rh	Bc Sa	Kokoska *et al.,* 2002
127.	*Lantana camara* L.	Verbenaceae	lf	Bs Ec Pa Sa	Valsaraj *et al.,* 1997
128.	*Larrea tridentata* (DC.)Cov.	Zygophyllaceae	Wp	Mc Mg Tt Ef S Na Nb Sd Lm Cp Pv	Verastegui *et al.,* 1996
129.	*Leucas aspera* Link	Lamiaceae	Lf	Bs Sa	Valsaraj *et al.,* 1997
130.	*Lippia nodiflora* (L.) Riche.	Verbenaceae	Ap	Bs Samr Sf Pa Par Mp	Taylor *et al.,* 1996
131.	*Lithraea molleoides* Hook et Arn.	Anacardiaceae	NM	Bs Ml Msp Sa	Penna *et al.,* 2001
132.	*Lycium europeum* L.	Solanaceae	Wp	Kp Pa Pv Sa	Ali-shtayeh *et al.,* 1998
133.	*Lycopodium cernuum* L.	Lycopodaceae	Wp	Bc Ca	Wiart *et al.,* 2004
134.	*Lythrum salicaria* L.	Lythraceae	Lf	Bs Ec Ca Ml Se	Rauha *et al.,* 2000
135.	*Mallotus phillppensis* (Lam.) Muell.-Arg.	Euphorbiaceae	Bk	Bs Sams Mp	Taylor *et al.,* 1996
136.	*Malva moschata* L.	Malvaceae	NM	Sa Se Ec Pm	KumarSamy *et al.,* 2002
137.	*Mangifera indica* L.	Anacardiaceae	Sb	Sa	Valsaraj *et al.,* 1997
138.	*Mapania cuspidata* (Miq) Uitt	Cyperaceae	Rt Bk Lf	Bc Pa	Wiart *et al.,* 2004
139.	*Maranta arundinaceae* L.	Marantaceae	Rt	Bs Ec Pa Sa	Valsaraj *et al.,* 1997
140.	*Matricaria chamomilla*	Compositae	Lf	Bs Ec Ml Se	Rauha *et al.,* 2000
141.	*Melastoma malabathricum* L.	Melastomataceae	Lf	Bs	Wiart *et al.,* 2004

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Table 9.2–*Contd...*

Sl.No.	Plant	Family	Part*	Activity Against**	References
142.	*Melicoccus bijugatus* Jacq.	Sapindaceae	Lf	Af Ag Bc Bs Ml Mp Pv Sa	Melendez *et al.,* 2006
143.	*Memecylon excelsum* Bl.	Melastomataceae	Lf Bk	Bc Bs Sa	Wiart *et al.,* 2004
144.	*Micromeria nervosa* (Desf.) Benth.	Labiatae	Lf	Ec Kp Pa Pv Sa Ca	Ali-shtayeh *et al.,* 1998
145.	*Millettia extensa* (Bentham) Baker	Fabaceae	Rt	Mp	Taylor *et al.,* 1996
146.	*Mimosa pigra* L	Mimosaceae	Lf	Bs Sa	Martinez *et al.,* 1996
147.	*Momordica charantia* L.	Cucurbitaceae	Lf	Sa	Martinez *et al.,* 1996
148.	*Monochordia vaginalis* (Burm. f.) Prels.	Pontideraceae	Wp	Bc Bs Sa	Wiart *et al.,* 2004
149.	*Moringa oleifera* Lam.	Moringaceae	Sb	Bs Pa Sa	Valsaraj *et al.,* 1997
150.	*Murraya exotica* L.	Rutaceae	lf	Bs Ec Pa Sa	Valsaraj *et al.,* 1997
151.	*Murraya koenigii* Spreng.	Rutaceae	Lf	Bs Ec Pa Sa	Valsaraj *et al.,* 1997
152.	*Myrcianthes cisplatensis* (Camb.) Berg	Myrtaceae	NM	Sa	Penna *et al.,* 2001
153.	*Neonauclea pallida* (Reinw. ex Havil.) Bakh. f.	Rubiaceae	Lf Bk	Bs Ca	Wiart *et al.,* 2004
154.	*Ocimum sanctum* L.	Lamiaceae	Wp	Pa Sa	Wiart *et al.,* 2004
155.	*Oldenlandia corymbosa* L.	Rubiaceae	Wp	Bs Ec Pa Sa	Valsaraj *et al.,* 1997
156.	*Onobrychis armena* L.	Labiatae	Lf	Bc Bs Sa Tr	Buruk *et al.,* 2006
157.	*Oroxylum indicum* Kurz	Bignoniaceae	Fr	Bs Ec Pa Sa	Valsaraj *et al.,* 1997
158.	*Oxalis corniculata* L.	Oxalidaceae	Lf	Bs Ec Pa Sa	Valsaraj *et al.,* 1997
159.	*Papaver lateritium* K. Koch	Papaveraceae	Lf Fl	Bc Bs Hp Sa Tr	Buruk *et al.,* 2006
160.	*Parietaria diffusa* (Mert. And Koch)	Urticacaceae	Ap	Ec Kp Pa Pv Sa Ca	Ali-shtayeh *et al.,* 1998
161.	*Pergularia daemia* Chiov.	Asclepiadaceae	Lf St	Bs Ec Pa Sa	Valsaraj *et al.,* 1997
162.	*Peristrophe tinctoria* Nees	Acanthaceae	Wp	Bc Bs Ca Ec Pa Sa	Wiart *et al.,* 2004

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Table 9.2–*Contd...*

Sl.No.	Plant	Family	Part*	Activity Against**	References
163.	*Petitia domingensis* Jacq.	Verbenaceae	Lf	Af Ag Bc Bco Bs Ec Ml Mp Mro Ms Pf Pv Sa Sm	Melendez *et al.,* 2006
164.	*Phaganalon rupestre* (L.) DC.	Compositae)	Wp	Ec Kp Pa Pv Sa	Ali-shtayeh *et al.,* 1998
165.	*Phyllanthus acidus* (L.) Skeels	Euphorbiaceae	F r	Af Ag Bc Bco Bs Ec Ml Mp Mr Mro Ms Pf Pv Sa Sm	Melendez *et al.,* 2006
166.	*Phyllanthus emhlica* L.	Euphorbiaceae	F r	Bs Ec Pa Sa	Valsaraj *et al.,* 1997
167.	*Picea abies* (L.) H.Karst.	Pinaceae	Lf	Bs Ml Se	Rauha *et al.,* 2000
168.	*Pinus sylvestris* L.	Pinaceae	Lf	Bs Ml Sa Se	Rauha *et al.,* 2000
169.	*Piper guineense* L.	Piperaceae	Sd	Bs Ec Pa Sa An Ca	Konning *et al.,* 2004
170.	*Piper longum* L.	Piperaceae	Fl	Bs Ec Pa Sa	Valsaraj *et al.,* 1997
171.	*Piper nigrum* L.	Piperaceae	Lf	Bs Pa Sa	Valsaraj *et al.,* 1997
172.	*Piper porphyrophyllum* N E Br	Piperaceae	Wp	Ca	Wiart *et al.,* 2004
173.	*Piper stylosum* Miq	Piperaceae	Wp	Bc Bs Ca Sa	Wiart *et al.,* 2004
174.	*Pistacia lentiscus L.*	Anacardiaceae	Lf	Ec Kp Pa Pv Sa Ca	Ali-shtayeh *et al.,* 1998
175.	*Pittosporum viridiflorum* Sims	Pittosporaceae	Bk	Bs Sa	Mc Gaw *et al.,* 2000
176.	*Plantago intermedia* L.	Plantaginaceae	Lf	Ec	Uzum *et al.,* 2004
177.	*Plumbago indica* L.	Plumbaginaceae	Lf	Bs Ec Pa Sa An Ca	Valsaraj *et al.,* 1997
178.	*Podocarpus* sp.	Podoearpaceae	Lf	Bs Sa	Martinez *et al.,* 1996
179.	*Polyalthia lateriflora* King	Annonaceae	Lf	Bc Bs Ca Pa Sa	Wiart *et al.,* 2004
180.	*Polyalthia longijblia* Thw.	Annonaceae	Lf	Bs Ec Pa Sa	Valsaraj *et al.,* 1997
181.	*Polygonum punctatum* Elliot var. aquatile (Martins)	Polygonaceae	NM	Bs Ml Msp Sa An	Penna *et al.,* 2001
182.	*Primula longipes*	Primulaceae	Lf Fl Fr	Bc Bs Hp Sa Ca Tr	Buruk *et al.,* 2006

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Table 9.2–*Contd...*

Sl.No.	Plant	Family	Part*	Activity Against**	References
183.	*Prunus padus* L.	Rosaceae	NM	Sa Samr Sh Lp Pm	KumarSamy *et al.,* 2002
184.	*Psoralea corylifolia* L.	Fabaceae	Sd	Bs Pa Sa	Valsaraj *et al.,* 1997
185.	*Psychotria capensis* (Eckl.) Vatke	Rubiaceae	Rt	Sa	Mc Gaw *et al.,* 2000
186.	*Psychotria nervosa* Sw.	Rubiaceae	Lf	Ag Bc Ml Mp Mro Ms Pf Pv Sa	Melendez *et al.,* 2006
187.	*Pulicaria stephanocarpa* Balf. f	Asteraceae	Lf	Bc Mf Sa Se Sh Sa-NGR	Mothana *et al.,* 2005
188.	*Punica granatum* L.	Punicaceae	Lf	Af Ag Bc Bco Bs Ec Ml Mp Mr Mro Pf Pv Sa Sm	Melendez *et al.,* 2006
189.	*Punica protopunica* Balf. f	Punicaceae	Lf	Bc Mf Sa Se Sh Sa-NGR	Mothana *et al.,* 2005
190.	*Quercus macranthera* sp. *syspirensis*	Fagaceae	Lf Fl	Bc Bs Hp Sa Tr	Buruk *et al.,* 2006
191.	*Quercus pontica*	Fagaceae	Lf	Bc Bs Hp Sa Tr	Buruk *et al.,* 2006
192.	*Rafflesia hasseltii* Suring	Rafflesiaceae	Wp	Bc Bs Pa Sa	Wiart *et al.,* 2004
193.	*Randia spinosa* Poir.	Rubiaceae	Fr	Bs Pa Sa	Valsaraj *et al.,* 1997
194.	*Rauvolfia caffra*	Apocynaceae	Lf	Sa	Mc Gaw *et al.,* 2000
195.	Rauvolfia caffra Sond.	Apocynaceae	NM	Ecl	Tshikalange *et al.,* 2005
196.	*Reseda lutea* L.	Resedaceae	NM	Sa Se Sh Sm	KumarSamy *et al.,* 2002
197.	*Rhaponticum carthamoides* (Willd.) Iljin	Compositae	Ap Rt	Bc Sa	Kokoska *et al.,* 2002
198.	*Rhodamnia cinerea* Jack	Myrtaceae	Lf	Bc Bs Sa	Wiart *et al.,* 2004
199.	*Rhododendron ponticum* sp. *ponticum* var. *heterophyllum*	Ericaceae	Lf Fl	Bc Bs Hp Sa Tr	Buruk *et al.,* 2006
200.	*Rhoicissus digitata* (L.F.) Gilg and Brandt	Vitaceae	Lf Rt St	Af Bc Bcg Bmt Bp Bst Bs Ca Kp Ml Mp Ms Pm Pmg Pv Ps Psr Ssp Sf Sa Se St Sp	Lin *et al.,* 1999

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Table 9.2–*Contd...*

Sl.No.	*Plant*	*Family*	*Part**	*Activity Against***	*References*
201.	*Rhoicissus rhomboidea* (E. Mey. Ex Harv.) Planch	Vitaceae	Lf Rt St	Af Bc Bcg Bmt Bp Bst Bs Ca Ea Kp Ml Mp Ms Pm Pmg Pv Ps Psr Sc Ssp Sb Sa Se St Sp	Lin *et al.,* 1999
202.	*Rhoicissus tomentosa* (Lam.) Wild and Drum	Vitaceae	Lf Rt St	Af Bc Bcg Bmt Bp Bst Bs Ca Kp Ml mp Ms Pm Pmg Pv Ps Psr Sc Ssp Sa Se St Sp	Lin *et al.,* 1999
203.	*Rhoicissus tridentata* (L.F.) Wild and Drum	Vitaceae	Lf Rt St	Af Bc Bcg Bp Bst Ca Ml Ms Pm Pmg Sb Pv Ps Psr Ssp Sa Se St Sp	Lin *et al.,* 1999
204.	*Rhoicissus tridentata* (L.F.) Wild and Drum subsp. *cuneifolia* (Eckl. and Zeyh.) N.R. Urton	Vitaceae	Rt	Af Bc Bcg Bp Bst Ml Ms Pm Pmg Pv Ps Psr Ssp Sa Se St sp	Lin *et al.,* 1999
205.	*Ribes nigrum* L.	Grossulariaceae	Lf	Ml	Rauha *et al.,* 2000
206.	*Rosa canina* L.	Rosaceae	NM	Ec	KumarSamy *et al.,* 2002
207.	*Rosa pisiformis*	Rosaceae	Lf	Bc Bs Hp Sa Ca Tr	Buruk *et al.,* 2006
208.	*Rosmarinus officinalis* L.	Lamiaceae	Lf	Af Ag Bco Ml Mp Mr Mro Ms Pf Sa	Melendez *et al.,* 2006
209.	*Rubus chamaemorusa*	Rosaceae	Be Lf	Bs Ec Ml Se	Rauha *et al.,* 2000
210.	*Rubus idaeus*	Rosaceae	Lf	Bs	Rauha *et al.,* 2000
211.	*Rumex hastatus* D. Don	Polygonaceae	Rt	Bs Samr Sf Pa Par Mp	Taylor *et al.,* 1996
212.	*Rungia parviflora* (Retz.) Nees	Acanthaceae	Ap	Mp	Taylor *et al.,* 1996
213.	*Ruscus aculeatus* L.	Liliaceae	Rt	Ec Pa Pv Sa Ca	Ali-shtayeh *et al.,* 1998
214.	*Ruta chalepensis* L.	Rutaceae	Wp	Ec Kp Pv Sa Ca	Ali-shtayeh *et al.,* 1998
215.	*Ruta graveolens* L.	Rutaceae	Lf	Bs Pa Sa	Valsaraj *et al.,* 1997
216.	*Salacia microsperma* L.	Acanthaceae	Lf	Bp Sa Pv	perumalSamy, 2005

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Table 9.2–*Contd...*

Sl.No.	*Plant*	*Family*	*Part**	*Activity Against***	*References*
217.	*Salix caprea*	Silicaceae	Lf	Bs Ml Se	Rauha *et al.,* 2000
218.	*Salix rizeensis*	Salicaceae	Lf Fr	Bc Bs Hp Sa Tr	Buruk *et al.,* 2006
219.	*Salvia fruticosa* (L.) Mill.	Labiateae	Lf	Kp Pa Pv Sa Ca	Ali-shtayeh *et al.,* 1998
220.	*Sanguisorba officinalis* L.	Rosaceae	Ap Rh	Bc Ca Ec Pa Sa	Kokoska *et al.,* 2002
221.	*Sansevieria hyacinthoides* Thunb	Ruscaceae	Lf	Bs	Mc Gaw *et al.,* 2000
222.	*Sarcopoterium spinosum* (L.) Spach	Rasaceae	Lf St Fr	Ec Kp Pa Pv Sa Ca	Ali-shtayeh *et al.,* 1998
223.	*Schefflera heterophylla* Harms	Araliaceae	Wp	Bc Bs Ca Sa	Wiart *et al.,* 2004
224.	*Schefflera oxyphylla* Miq. Vig.	Araliaceae	Lf Bk	Bc Bs	Wiart *et al.,* 2004
225.	*Schinus terebinthifolius* Raddi	Anacardiaceae	Lf	Bs Ec Pa Sa	Martinez *et al.,* 1996
226.	*Schotia brachypetala* Sond.	Fabaceae	Lf	Bs Sa	Mc Gaw *et al.,* 2000
227.	*Scindapsus officinals* (Roxb.) Schott	Araceae	Fr	Tm	Taylor *et al.,* 1996
228.	*Sclerocarya birrea* (A. Rich.) Hochst.	Anacardiaceae	Bk	Bs Sa	Mc Gaw *et al.,* 2000
229.	*Sebastiania brasiliensis* Spreng.	Euphorbiaceae	NM	Ml Msp Sa	Penna *et al.,* 2001
230.	*Sebastiania klotszchiana* Muell. Arg.	Euphorbiaceae	NM	Msp Sa	Penna *et al.,* 2001
231.	*Secale cereale* M. Biebe	Poaceae	Lf	Bs Ml Se	Rauha *et al.,* 2000
232.	*Senecio vulgaris* L.	Asteraceae	Rt	Ec	Uzum *et al.,* 2004
233.	*Sennapetersiana* (Bolle) Lock	Fabaceae	NM	Bc Bp Bs Ecl Sa Sm	Tshikalange *et al.,* 2005
234.	*Sida cordata* (Bruin. f.) Borss.	Malvaceae	Rt	Mp	Taylor *et al.,* 1996
235.	*Sida rhomb*(*Iblia* L.	Malvaceae	Sb	Bs Ec Sa	Valsaraj *et al.,* 1997
236.	*Smila leucophylla* Bl.	Smilacaceae	Lf	Bc	Wiart *et al.,* 2004
237.	*Solanum torvum* Sw.	Solanaceae	Lf	Bs Ec Pa Sa An	Valsaraj *et al.,* 1997

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Table 9.2–*Contd...*

Sl.No.	Plant	Family	Part*	Activity Against**	References
238.	*Solanum torvum* Swartz	Solanaceae	Lf	Bc Bs Ca Pa Sa	Wiart *et al.,* 2004
239.	*Solanum tuberosum* L.	Solanaceae	Lf	Bs Ml Sa Se	Rauha *et al.,* 2000
240.	*Sonerila begoniaefolia* Ridl.	Melastomataceae	Lf	Ca Sa	Wiart *et al.,* 2004
241.	*Spirostachys africana* Sond.	Euphorbiaceae	Rt St	Sa	Mc Gaw *et al.,* 2000
242.	*Spondias pinnata* Kurz	Anacardiaceae	Rb	Pa Sa	Valsaraj *et al.,* 1997
243.	*Stachytarpheta indica* L. Vahl	Verbinaceae	Wp	Pa	Wiart *et al.,* 2004
244.	*Stellaria holostea*	Caryophyllaceae	NM	Pa	KumarSamy *et al.,* 2002
245.	*Tachyspermum ammi* L.	Apiaceae	F r	Bs Ec Pa Sa An Ca	Valsaraj *et al.,* 1997
246.	*Tamarindus indica* L.	Caesalpiniaceae	Lf	Af Ag Bc Bco Bs Ml Mr Mp Ms Pf Sa	Melendez *et al.,* 2006
247.	*Tecomaria capensis* Cape Honeysuckle	Bignoniaceae	Bk	Sa	Mc Gaw *et al.,* 2000
248.	*Terminalia alata* Heyne ex Roth	Combretaceae	Bk	Samr Sf Pa Par Mp	Taylor *et al.,* 1996
249.	*Terminalia bellerica* Roxb.	Combretaceae	Ec	Bs Ec Pa Sa An Ca	Valsaraj *et al.,* 1997
250.	*Terminalia catappa* L.	Combretaceae	Lf	Bco Bs Ea Ml Ms Pf Pv Sa	Melendez *et al.,* 2006
251.	*Terminalia chebula* Retz.	Combretaceae	Ec	Bs Ec Pa Sa	Valsaraj *et al.,* 1997
252.	*Terminalia sericea* Burch. ex DC.	Combretaceae	NM	Bc Bp Bs Sa	Tshikalange *et al.,* 2005
253.	*Thespesia lampas* Dalz.	Malvaceae	Lf Sb	Bs Sa	Valsaraj *et al.,* 1997
254.	*Thespesia populnea*	Malvaceae	Sb	Ec Pa Sa	Valsaraj *et al.,* 1997
255.	*Thottea siliquosa* Lam.	Aristolochiaceae	Rt	Bs Ec Pa Sa	Valsaraj *et al.,* 1997
256.	*Thymus vulgaris*	Lameaceae	Lf	Bs Ec Ml Se	Rauha *et al.,* 2000
257.	*Tinospora cordifolia* (Willd.) Hook. f. et Thomas	Menispermaceae	St Br	Bp Ea Pv Sa	PerumalSamy, 2005

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Table 9.2–*Contd...*

Sl.No.	*Plant*	*Family*	*Part**	*Activity Against***	*References*
258.	*Tinospora cordifolia* Miers.	Menispermaceae	lf St	Bs Ec Pa Sa	Valsaraj *et al.,* 1997
259.	*Trachystemon orientalis* (L.) G. Don.	Boraginaceae	Wp	Ec	Uzum *et al.,* 2004
260.	*Trevesia burckii Boerlage*	Araliaceae	Lf Bk	Bc Ca Sa	Wiart *et al.,* 2004
261.	*Trichocalyx obovatus* Balf. f.	Acanthaceae	Lf Fr	Se Sa NGR	Mothana *et al.,* 2005
262.	*Trichopus zeylanicus* Gaertn.	Dioscoreaceae	Lf	Bs Sa	Valsaraj *et al.,* 1997
263.	*Tussilago farfara* L.	Compositae	Ap Rh	Bc Sa	Kokoska *et al.,* 2002
264.	*Typha capensis* (Rohrb.) N.E.Br.	Typhaceae	Rh	Bs	Mc Gaw *et al.,* 2000
265.	*Urena lobata* L. ssp. *lobata*	Malvaceae	Lf	Sa	Melendez *et al.,* 2006
266.	*Vaccinium myrtillus* L.	Ericaceae	Lf	Ml Ec Pa	Rauha *et al.,* 2000; Valsaraj *et al.,* 1997
267.	*Vaccinium oxycoccus*	Ericaceae	Lf	Ec Sa	Rauha *et al.,* 2000
268.	*Verbascum varians var. trapezunticum*	Scrophulariaceae	Lf St	Bc Ca Tr	Buruk *et al.,* 2006
269.	*Vernonia cinerea* Less.	Asteraceae	lf st	Bs Ec Pa Sa	Valsaraj *et al.,* 1997
270.	*Vitex leucoxyhm* Schau.	Verbenaceae	Sb	Bs Ec Pa Sa	Valsaraj *et al.,* 1997
271.	*Vitex negundo* L.	Verbenaceae	lf	Bs Ec Pa Sa	Valsaraj *et al.,* 1997
272.	*Withania adunensis* Vierh	Solanaceae	Lf	Bc Mf Sa Se Sh Sa-NGR	Mothana *et al.,* 2005
273.	*Withania riebeckii* Schweinf.ex Balf.f.	Solanaceae	Lf	Bc Mf Sa Se Sh Sa-NGR	Mothana *et al.,* 2005
274.	*Woodfrrdia fruticosa* Kurz	Lythraceae	Fl	Bs Ec Pa Sa An	Valsaraj *et al.,* 1997
275.	*Xylopia aethiopica* (Dun.) A. Rich.	Annonaceae	F r	Bs Ec Pa Sa An Ca	Konning *et al.,* 2004
276.	*Zingiber officinale* L.	Zingiberaceae	Rh	Bs Ec Pa Sa An Ca	Konning *et al.,* 2004
277.	*Zingiber officinale* Rosc.	Zingiberaceae	Rh	Bp Ea Sa	PerumalSamy, 2005

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Table 9.2–*Contd...*

Sl.No.	*Plant*	*Family*	*Part**	*Activity Against***	*References*
278.	*Zizyphus jujuba* Lam.	Rhamnaceae	Fr	Bs Ec Pa Sa	Valsaraj *et al.,* 1997
279.	*Zizyphus spinachristi*	Rhamnaceae	Wp	Pa	Ali-shtayeh *et al.,* 1998
280.	*Zygophyllum quatarense* M. N. Hadidi	Zygophyllaceae	Lf	Bc Mf Sa	Mothana *et al.,* 2005

***Parts used:** Ar: Aerial root; Bb: Bulb; Ec: Exocarp; Fl: Flower; Fr: Fruit; Lf: Leaf; Rb: Root bark; Rt: Root; Rz: Rhizome; Sb: Stem bark; Sd: Seeds; St: Stem; Wp: Whole plant.

**** Activity Against-Bacteria:** Af: *A. Faecalis;* Bcl: *Bacillus coagulens;* Bp: *B. Pumilu;* Bce: *Bacillus cereus;* Bcc: *Branhamella catarrhalis* ATCC 25238*;* Cm: *Candida maltosa* SBUG; Kp: *Klebesiella pneumonia;* Lp: *Lactobacillus plantarum;* MSA: multiresistant *Staphylococcus aureus*; MSE: multiresistant *Staphylococcus epidermidis*; Mrsh-multiresistant *Staphylococcus haemolyticus*; Mp: *Mycobacterium phlei*; Sa NGR: *North German reference strain*, Pm: *Proteus mirabilis;* Pmg: *Proteus morganii;* Pv: *Proteus vulgaris;* Pa*: Pseudomonas aeruginosa;* Ps: *Pseudomonas solanaceaeum;* Psr: *Pseudomonas syringae;* Sc: *Saccharomyces cervisiae;* Sm: *Salmonella marcescens;* Ss: *Salmonella sps.;* St: *Salmonella typhimurium;* Smr: *Serratia marcescens;* Sb: *Shigella boydii;* Sf: *Shigella flexneri;* Samr: *Staphylococcus aureus-methicilin* resistant, Sams: *Staphylococcus aureus-methicilin sensitive;* Sh: *Staphylococcus hominis;* Sf: *Streptococcus faecalis;* Sp: *Streptococcus pyrogenes.*

Fungi: An: *Aspergillus niger;* Ca: *Candida albicans;* Cab: *Cryptococcus albidus;* Ck: *Candida krusei;* Cl: *Cryptococcus laurenti;* Cn: *Cryptococcus neoformans;* Cr: *Candida rugosa;* Ef: *Epidermophyton floccosum;* Hp: *Helicobacter pylori ATCC 49503;* Mc: *Microsporum canis;* Mg: *Microsporum gypseum;* Msp: *Mucor sp.;* Sch: *Sporotrix schenckii;* Tm: *Trichophyton mentagrophytes;* Tr: *Trichophyton rubrum.*

Screening of Plant Extracts for Antiparasitic Activity

Parasitic infections are a major public health issue in many parts of the world, causing significant morbidity and mortality, and increasing resistance to the standard treatments for these infections has led to interest in the identification of plant extracts with antiparasitic activity (Rossignol, 1998; Upcroft and Upcroft, 2001). Upcroft and Upcroft, (2001) describe the main drug susceptibility methods: essentially the parasite is incubated in the presence of test substance in either a test tube or microtiter plate and cell counts determined at preset time intervals. Results are then reported as 50 per cent inhibitory concentration (IC50), minimum lethal concentration (MLC), or graphed as a percentage of controls over the length of the incubation period. As with other antimicrobial assays the aqueous environment used in assays for antiparasitic activity can pose difficulties and the need for repeated cell counts makes the assay labour intensive. Microtiter plate methods are less time consuming but have high variability in terms of the gaseous environment in each well, important for anaerobic protozoa, and they cannot be used with essential oils that "eat" plastic. Evaluation of extracts against intracellular parasites (*e.g., Leishmania* and *Plasmodium*) also requires access to an appropriate host cell line, cell culture facilities, and staff with expertise in cell culture. Despite these difficulties, a large number of plant extracts have been tested against *Leishmania*, *Giardia lamblia*, *Trypanosoma* sp., and *Plasmodium* species (Asres *et al.,* 2001; Tripathi *et al.,* 1999; Waechter *et al.,* 1999). Interestingly, most of the work on antiparasitic activity of plant extracts, and also antiviral activity, has used aqueous and ethanol/methanol extracts of plant parts, with few studies involving essential oils. Why this is the case is unknown, but it may be related to difficulties associated with solubility or to the types of plant products traditionally used for parasitic and viral infections. Perhaps this traditional use reflects the fact that viral and parasitic infections tend to be internal and therefore require an ingestible, easily produced remedy (essential oils are rarely used internally due to toxicity and are produced via steam distillation).

Antimicrobial Effects of Plant Extracts

In traditional and alternative medicine it is common to use medicinal plants as such, without isolating the active ingredients from them. Using crude extracts might be a more important way to use medicinal plants than has been realized in Western medicine, since plants contain numerous secondary metabolites, and pathogens in nature interact with many chemicals simultaneously (Izhaki, 2002). Traditional plant remedies or phytomedicines, include crude vegetable drugs (herbs) as well as galenical preparations (extracts, fluids, tinctures, infusions) prepared from them. Although a number of studies of the antimicrobial effects of plant extracts have been performed, many plants used in different traditional medicinal systems have never been evaluated for their antimicrobial effects. For example, in Africa, over 5000 plants are known to be used for medicinal purposes, but only a small percentage have been described or studied scientifically, and different combinations of plant species used in traditional medicines have been studied even to a lesser extent (Taylor *et al.,* 2001). The major problem in investigations on the biological activities of plant extracts and phytomedicines lies in the fact that a variety of plants may be used in a single

traditional medicine preparation, and in the possibility of synergistic effects resulting from the interactions of the compounds in the extract. This can even result in a loss of activity as the extract is purified (Couzinier and Mamatas, 1986). Eloff and McGaw (2006) point out that biologically active extracts can be extremely useful in their entirety, taking into account synergistic and other effects, and according to them an approval of standardized and formulated plant extracts as drugs might be the starting point in developing countries for a successful pharmaceutical industry to be able to compete with Western pharmaceutical companies.

Conclusions

Herbal medicines make an enormous contribution to primary health care and have shown great potential in modern phytomedicine against numerous ailments and the complex diseases and ailments of the modern world. Scientists from divergent fields are investigating plants anew with an eye to their antimicrobial utility. All over the world thousands of phytochemicals have found which have inhibitory effects on all types of microorganisms *in vitro*. There is still a need for more scientific evaluation of Asian herbal medicines including their active constituents, synergistic interactions, formulation strategies, herb drug interactions, standardization, pharmacological and clinical evaluation, toxicity, safety and efficacy evaluation and quality assurance. Furthermore, more of these compounds should be subjected to animal and human studies to determine their effectiveness in whole organism systems, including in particular toxicity studies as well as an examination of their effects on beneficial normal microbiota. It would be advantageous to standardize methods of extraction and *in vitro* testing so that the search could be more systematic and interpretation of results would be facilitated. Also, alternative mechanisms of infection prevention and treatment should be included in initial activity screenings. Attention to these issues could accompany in a poorly needed new era of chemotherapeutic treatment of infection by using plant derived principles.

This review outline the main methods used in the evaluation of antimicrobial activity of plant extracts; each method has advantages and limitations and all have been widely cited in the literature. The question of which is the best one to use is essentially unanswerable as preferred methods depend on a variety factors including access to specialized equipment and facilities, the number of samples to be screened and the nature of the plant extract (*e.g.,* volume, extract versus essential oil, chemical composition). For large-scale screening of extracts for antibacterial and antifungal activity disk and agar diffusion methods offer a fast, cost effective, low tech, and generally reliable method of sorting those extracts worthy of further investigation from those unlikely to be of value. Broth dilution methods provide more information but are more time and labour intensive and are best used as a follow up to a large scale screening of plant extracts. Antiviral and antiparasitic assays are the most time and labour intensive of the *in vitro* antimicrobial testing methods and often require access to cell culture or other specialized laboratory facilities. These are used less frequently than antibacterial and antifungal assays. Despite the limitations of many of the assay techniques, there is a vast amount of good data demonstrating that some plant extracts possess strong to excellent antimicrobial activity. The next step is to

continue this work into the *in vivo* environment and to evaluate the activity of these extracts in the treatment of infectious disease.

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