

## Review

# Therapeutic Potential of Plants as Anti-microbials for Drug Discovery

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The uses of traditional medicinal plants for primary health care have steadily increased worldwide in recent years. Scientists are in search of new phytochemicals that could be developed as useful anti-microbials for treatment of infectious diseases. Currently, out of 80% of pharmaceuticals derived from plants, very few are now being used as anti-microbials. Plants are rich in a wide variety of secondary metabolites that have found anti-microbial properties. This review highlights the current status of traditional medicine, its contribution to modern medicine, recent trends in the evaluation of anti-microbials with a special emphasis upon some tribal medicine, *in vitro* and *in vivo* experimental design for screening, and therapeutic efficacy in safety and human clinical trails for commercial outlet. Many of these commercially available compounds are crude preparations administered without performing human clinical trials. Recent methods are useful to standardize the extraction for scientific investigation of new phytochemicals and anti-microbials of traditionally used plants. It is concluded that once the local ethnomedical preparations of traditional sources are scientifically evaluated before dispensing they should replace existing drugs commonly used for the therapeutic treatment of infection. This method should be put into practice for future investigations in the field of ethnopharmacology, phytochemistry, ethnobotany and other biological fields for drug discovery.

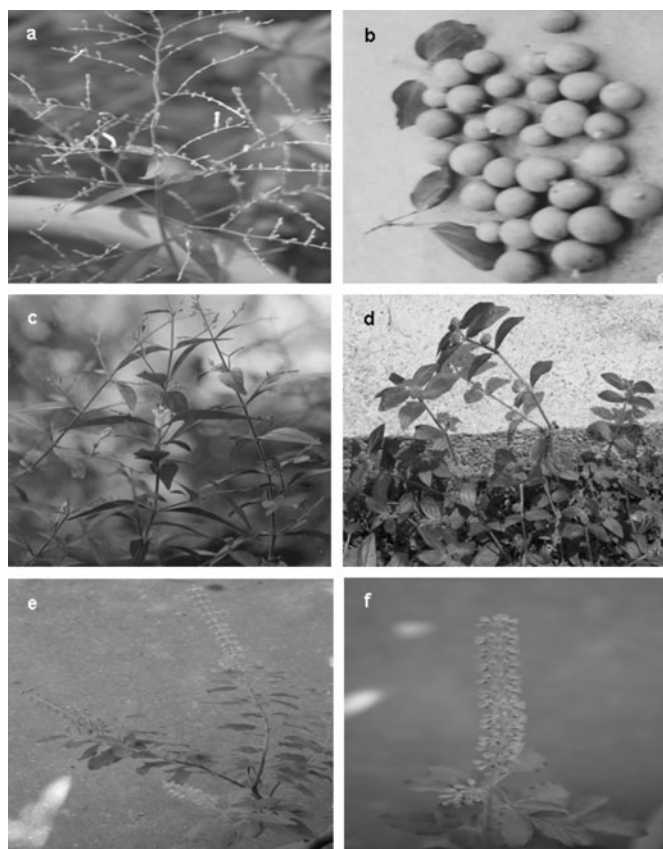
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## Introduction

Primitive people have used plants to cure a variety of human ailments. Even today, 85% of Indians use higher plants as effective anti-microbials for the treatment of various diseases (1). A large number of anti-microbial agents derived from traditional medicinal plants are available for treating various diseases caused by micro-organisms (2). They are used to eliminate the infecting micro-organisms. The therapeutically useful novel agents

should inhibit the germs and exhibit greater selective toxicity towards the infecting germ than the host cells (3). The mode of action for plant-derived agent should target biochemical features of the invading pathogens that are not possessed by the normal host cell. Some of the factors important for anti-microbial treatment include methods such as sensitivity of the infecting micro-organism to a particular agent (4). Side-effects of the plant-derived agent can be tested relative to direct toxicity upon animal cells because of their close association with human tissues or cells. So, we attempt to summarize information linked to plant extracts/chemical substances for the effective treatment of certain bacterial and fungal diseases. We also discuss the obvious necessity for new anti-microbial agents in various therapy regimens.

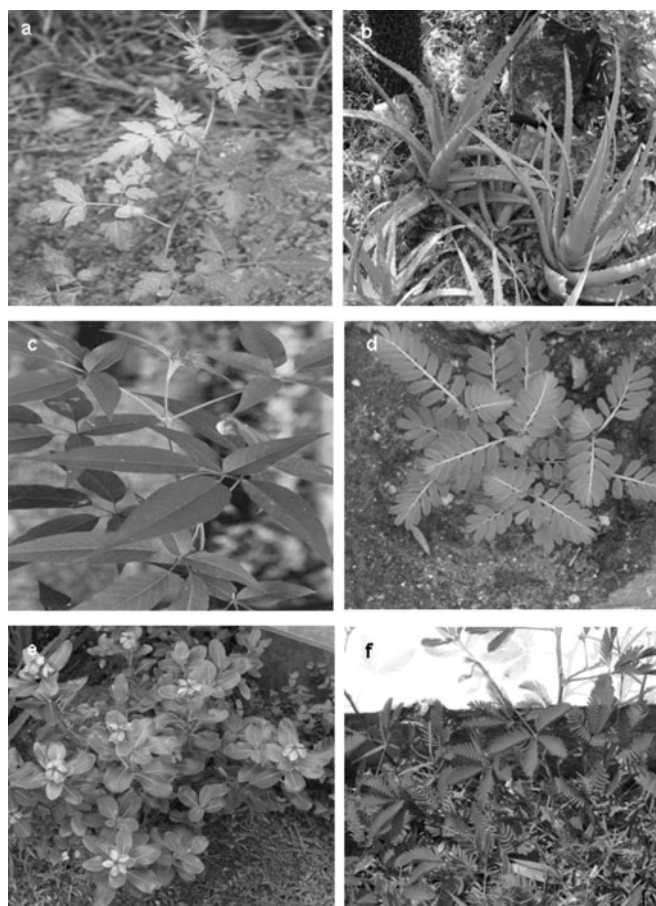
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**Figure 1.** Some of the folk medicinal plants and its various parts used as therapeutic potential in Southern Tamil nadu, Western Ghats of India. (a and c) Seed with flower of *Andrographis paniculata* Wallich ex Nees (Acanthaceae), (b) fruit of *Strychnos nux-vomica* L. (Loganiaceae), (d) *Euphorbia hirta* L. (Euphorbiaceae), (e) whole plant of *Ocimum sanctum* L. (Lamiaceae) and (f) inflorescence of *Ocimum sanctum* L.

### Recent Trends in the Evaluation of Anti-microbials

Anti-bacterial screening of traditional medicinal plants has been the source of innumerable therapeutic agents (Figs 1 and 2). In the area of antibiotics, random screening as a tool of discovering new biologically active molecules has been most productive. Chemotaxonomic considerations and target-directed screening also play a crucial role. For a successful outcome the main requirement is access to a large number of compounds/extracts that must be well screened (5). Ethanol extracts of 78 traditional medicinal plants from India are used for treating infectious diseases and show bacterial and fungal activity at 1.6 mg/ml (6). The 50% ethanol extracts of 285 plant materials were screened for 61 biological activities and revealed effective anti-bacterial, and a wide range of pharmacological, activities (7). Anti-microbial and phytochemical studies revealed 45 Indian medicinal plants effective against multi-drug-resistant bacteria (8). These results suggest the presence of either good anti-bacterial potency or the high concentration of an active principle in the extract. Plant extracts were



**Figure 2.** Some of the folk medicinal plants and its various parts used as therapeutic potential in Southern Tamil nadu, Western Ghats of India. (a) *Cardiospermum halicaccabum* L. (Sapindaceae), (b) *Aloe vera* Mill. (Liliaceae), (c) *Vitex negundo* L. (Verbenaceae), (d) *Phyllanthus amarus* (Euphorbiaceae) (e) *Cathranthus roseus* (L) and (f) *Mimosa pudica* L. (Mimosaceae).

screened phytochemically and 20% of the species yielded positive reactions for alkaloids, 25% species contained steroids/triterpenoids and 45% of species possessed saponins (9). Those plants with anti-bacterial effects are rich in polyphenolic substances such as tannins, catechins, alkaloids, sterioids and polyphenolic acids. The anti-bacterial activity also could be due to various chemical components and the presence of essential oils in adequate concentrations, which damage microorganisms (10). The insolubility of essential oils and non-polar extracts make it very difficult for them to be used in an aqueous medium during the study of anti-microbial activity (4). A great number of factors can influence the results such as the extraction method, volume of media, culture composition and incubation temperature. However, the recent advanced method of bioautographic TLC assays makes it possible to localize anti-microbial activity on a chromatogram (11); while bioassay-guided fractions led to the isolation of compounds (12).

## Anti-microbial Compounds from Plants

Traditional medicinal plants have an almost maximum ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives (13). Most of these are secondary metabolites, of which 12 000 plant-derived agents have been isolated in the recent past. Many of these substances serve as plant defense mechanisms against invasion by micro-organisms (Table 1), insects and herbivores. Some of the plant substances such as terpenoids are responsible for odor (quinones and tannins) plus pigment of the plant. Many compounds are responsible for plant flavor (e.g. the terpenoid capsaicin from chili peppers), and some of the same herbs and spices used by humans to season food yield useful medicinal compounds. The useful major groups of anti-microbial phytochemicals can be divided into several categories that include alkaloids, flavones (flavonoids, flavonols, Quinones), essential oils, lectins, polypeptides, phenolics, polyphenols, tannins and terpenoids.

### Alkaloids

Heterocyclic nitrogen compounds are called alkaloids. The first medically useful alkaloid was morphine, isolated in 1805 from *Paver somniferum* (opium poppy) (27). The name morphine comes from the Greek word Morpheus, which means 'god of dreams'. Codeine and heroin are both derivatives of morphine. Diterpenoid alkaloids, isolated from the plants of the Ranunculaceae family, are commonly found to have anti-microbial properties. Bioassay-guided isolation studies done on the root extract of *Polyalthia longifolia* shows that it possesses significant anti-bacterial activity led to the isolation of three new alkaloids pendulamine A, pendulamine B and penduline along with stigmasterol 3-*O*-beta-D-glucoside, allantoin, the known diterpenoid kolavenic acid and the azafluorene alkaloid isoursuline. Compound pendulamine A and pendulamine B were found to be active. Micro-organism inhibition concentrations, abbreviated as MICs, are ~0.02–20 µg against bacteria (12). The seed pods of *Erythrina latissima* yielded erysotrine, erysodine, syringaresinol, vanillic acid and a new erythrina alkaloid, (+)-10,11-dioxoerysotrine that was lethal to brine shrimp. 2-(5'-Hydroxy-3'-methoxy phenyl)-6-hydroxy-5-methoxybenzofuran has strong anti-microbial activity against yeast spores (28). Ethanol extracts of the *Guatteria multivenia* root have furnished known alkaloids such as liriodenine, lysicamine, lanuginosine, guadiscine and *O*-methylpallidine. Lanuginosine possesses weak inhibitory effects against fungi and liriodenine was found to have anti-microbial activity against both bacteria and *Candida albicans* (29). Pyrrolizidine alkaloids (*Heliotropium subulatum*) extracts showed anti-microbial activity against both fungal and bacterial species (3). Alkaloids isolated (*Schizozygia coffaeoides*)

using bioassay-guided fractionation was isoschizogaline, schizogynine and indoline that were subsequently shown to be the most active anti-fungal compounds (30). The anti-microbial berberine alkaloid isolated from *Mahonia aquifolium* was active against bacteria (31). For the anti-microbial components of berberin from *Hydrastis canadensis*, change in the lipophilicity of protoberberinium salts caused by modification of the substituents appears to influence the anti-bacterial activity. Both berberine and palmatine exhibited the greatest anti-bacterial activity (32). Biologically active carbazole alkaloids (from *Murraya koenigii*) showed mosquitocidal and anti-microbial activities, as well as exhibited topoisomerase I and II inhibition activities (33).

### Flavones, Flavonoids and Flavonols

Flavones are phenolic structures containing one carbonyl group. They are hydroxylated phenolic substances that occur as C6–C3 units linked to an aromatic ring. Flavonoids are known to be synthesized by plants in response to microbial infection (34) and are effective anti-microbial substances against a wide array of micro-organisms. Anti-microbial flavonoids have been reported from *E. latissima* (28). Dimethoxyflavone and bonducellin were isolated from the aerial parts of *Caesalpinia pulcherrima*. Isobonducellin was found to be a homoisoflavanoid containing a *cis* (*Z*)-double bond possessing anti-microbial activity (35). Compounds of *C. pulcherrima* with anti-viral activities were derived from the flavonoid of quercetin (36). Moreover, the flavonoids, acacetin-7-*o*-β-D-galactopyranoside of *C. morifolium* was found to be active as towards HIV (37). A wide variety of flavonoids, sesquiterpenoid alcohols, triterpenoids and quinic acid caffeates product from plants may also be useful as anti-microbials (38). The activity is probably due to their ability to form a complex with extra-cellular and soluble proteins, which then binds to bacterial cell wall. More lipophilic flavonoids may also disrupt microbial membranes (39). Flavonoids lacking hydroxyl groups on their β-rings are more active against micro-organisms and the microbial target is the membrane with –OH groups (40).

### Essential Oils and Terpenoids

The anti-microbial properties of aromatic volatile oils from medicinal, as well as other edible, plants have been recognized since antiquity. Essential oil, which is used as a food flavoring agent, possesses a broad spectrum of anti-microbial activities attributed to the high content of phenolic derivatives such as carvacrol and thymol. Some essential oils are used for systemic and superficial fungal infections and further exploration reveals a broad spectrum effect against other pathogenic manifestations that include malignancy (41). Moreover, fragrance of

Table 1. Some of the traditional medicinal plants and their class of anti-microbial compounds

Scientific names	Parts/solvents	Class	Compounds	Mechanism of traditional medicine	References
<i>Baccharis grisebachii</i> Hieron (Asteraceae)	Resinous exudate	Diterpenes, <i>p</i> -coumaric acid derivatives, flavones	3-Prenyl- <i>p</i> -coumaric acid and 3,5-diprenyl- <i>p</i> -coumaric acid	Argentinian traditional medicine showed activity towards dermatophytes and bacteria (MICs 50,100 and 125 µg/ml).	Feresin <i>et al.</i> (14)
<i>Cassia podocarpa</i> Guill et Perr. (Caesalpinaceae)	Leaf and flower	Glycosides	Antraquinone glycosides, anthraquinones, free aglycone	Optimum laxative activity and reduced toxicity.	Abo and Adeyemi (15)
<i>Chrysanthemum morifolium</i> Ramat. (Compositae)	Flowers	Flavonoids	Apigenin 7- <i>O</i> -β-D-glucuronide	Glucuronide showed strong HIV-1 integrase inhibitory activity in a cell culture assay using HIV-1 <sub>IIIB</sub> -infected MT-4 cells.	Lee-Huang <i>et al.</i> (16)
<i>Curcuma longa</i> L. (Zingiberaceae)	Rhizome	Flavonoids	Curcumin and curminoids	A number of different molecules involved in inflammation that are inhibited by curcumin including lipo-oxygenase, phospholipase and elastase.	Chaimani-Wu (17)
<i>Origanum virens</i> L. (Lamiaceae)	Aerial parts	Essential oil	Carvacrol (68.1%), γ-terpinene (9.9%) and <i>p</i> -cymene (4.5%)	Anti-fungal activity against <i>Candida</i> species. The fungicidal effect is primarily due to an extensive lesion of the membrane.	Salgueiro <i>et al.</i> (18)
<i>Osmitopsis asteriscoides</i> L. (Asteraceae)	–	Essential oil	Cineole and camphor, Camphor and 1,8-cineole	Anti-microbial activity (0.5–2% v/v) synergistic effect is presented as a possible explanation for the traditional use for chest pain in South Africa.	Viljoen <i>et al.</i> (19)
<i>Santiria trimera</i> (Oliv.) Aubrev. (Bursaceae)	Bark	Essential oil monoterpenes	β-Pinene (20.0%), α-pinene (66.6%)	Plant widely used by the traditional healers, anti-microbial activity (MIC 1.11–0.71 µg/ml).	Martins <i>et al.</i> (20)
<i>Stephania dinklagei</i> Engl. (Menispermaceae)	Ethanol extract	Aporphine alkaloids	Liriodenine, corydine, isocorydine, atherospermidine, stephalagine and dehydrostephalagine	Liriodenine showed strong cytotoxic activity while corydine and atherospermidine showed DNA damaging activity.	Gören <i>et al.</i> (21)
<i>Warburgia ugandensis</i> Sprague (Canellaceae) <i>Zanthoxylum chalybeum</i> Engl. (Rutaceae)	Seed	Alkaloid	Skimmianine	Ugandan plants showed anti-microbial activity by agar well assay and anti-fungal activity. Its mode of action is unclear from these results.	Olila <i>et al.</i> (22)
<i>Hydrastis canadensis</i> L. (Ranunculaceae)	Whole plant	Alkaloid	Berberine-etrahydroberberine and 8-oxoberberine	Chinese herb-exhibited vasodilator activity has been attributed to multiple cellular mechanisms. And its derivatives are attributed to the blockade of K <sup>+</sup> channels [delayed rectifier and K <sup>+</sup> (ATP)] and stimulation of Na <sup>+</sup> –Ca <sup>2+</sup> exchanger.	Lau <i>et al.</i> (23)
<i>Zingiber officinale</i> Rose (Gingiberaceae)	Rhizome	Polyphenolics	6-,8-,10-gingerol and 6-shogaol	The gingerols inhibit the growth of <i>H. pylori</i> CagA <sup>+</sup> strains <i>in vitro</i> at 6.25–50 µg/ml.	Mahady <i>et al.</i> (24)
<i>Boswellia serrata</i> Roxb. (Bursaceae)	Gum resin	Pentacyclic triterpenes	Boswellic acids	Boswellic acids inhibit the leukotriene biosynthesis in neutrophilic granulocytes by a non-redox, non-competitive inhibition of 5-lipoxygenase.	Ammon (25)
<i>Bougainvillea xbutitiana</i> (Nyctaginaceae)	Leaf	Proteins	Lysine	The inhibitor showed <i>N</i> -glycosidase activity on 25S rRNA of tobacco ribosomes, which interfered with virus multiplication through ribosome interaction.	Narwal <i>et al.</i> (26)

plants is associated with essential oils. This oil consists of secondary metabolites which are highly enriched in compounds based on an isoprene structure. They are called terpenes and occur as diterpenes, triterpenes, tetraterpenes as well as hemiterpenes and sesquiterpenes. When the compounds contain additional elements, usually oxygen, they are termed as terpenes. Terpenes or terpenoids are active against bacteria (29). Nearly 60% of all essential oil derivatives possess inhibitory effects upon fungi while 39% inhibited bacteria (40). The seeds of *Nigella sativa* Linn. (Ranunculaceae) contain active constituents, e.g. volatile oil and thymoquinone showed protection against nephrotoxicity and hepatotoxicity induced by either disease or chemicals. The seed oil has anti-inflammatory, analgesic, anti-pyretic, anti-microbial and anti-neoplastic activity (42). Petroleum ether extract of *Melicope indica* afforded two unusual pentacyclic triterpenes and the ubiquitous steroids, stigmasterol and sitosterol (43). Pentacyclic triterpenes were isolated from *Combretum imberbe* that are novel glycosidic derivatives (hydroxyimberbic acid). *Terminalia stuhlmannii* Engl. stem bark yielded two glycosides of hydroxyimberbic acid, several of which had anti-bacterial activity. Imberbic acid showed potent activity against *Mycobacterium fortuitum* and *Staphylococcus aureus* (44). New cycloartane-type triterpenes isolated from the aerial parts of *Acalypha communis* exhibited moderate anti-microbial activity (MIC 8 and 32 µg/ml) against vancomycin-resistant enterococci. Compounds tested in an *in vivo* model did not provide protection to mice infected with *S. aureus* (45). Friedelin, epifriedelinol, β-amyrin, β-sitosterol, β-sitosterol 3-β-D-glucopyranoside and naringin isolated from the methanol extract of dried rhizome from *Drynaria quercifolia* showed concentration-dependent broad spectrum of anti-bacterial activity (46). Andrographolide, neoandrographolide and andrographiside (Fig. 1) are the diterpene lactone of *Andrographis paniculata* (king of bitter) possesses liver protection under various experimental conditions (47). It showed weak anti-microbial activity against bacteria and viruses. Asiaticoside and hypaphorine (Fig. 1) are the mixture of pentacyclic triterpene of *Centella asiatica*. Topical and oral applications of asiaticoside improved wound healing in guinea pigs (1 mg/kg dose) (48). Tinosporaside and columbin are diterpenes and cordifolioside (Fig. 1) is a sesquiterpene glucoside of *Tinospora cordifolia* as reported (49). An ether extract of the aerial part of *T. cordifolia* inhibited the growth of *M. tuberculosis* at 1:50 000 dilutions (50). The mechanism of action of terpenes is not fully understood but it is speculated to involve membrane disruption by the lipophilic compounds.

### Lectins and Polypeptides

The active compounds can be grouped into two major classes: anti-microbial proteins and a wide variety of non-protein compounds. Their distribution is often tissue

specific (51) and they are usually found in cells located at the external layers of plant tissues, thus suggesting that these compounds would be the first line of defense against a pathogen attack. An anti-microbial compound of 316 Da, present in the soluble fraction of strawberry (*Fragaria ananassa*) leaves shows *in vitro* activity against bacterial and fungal plant pathogens (52). An anti-microbial protein (WjAMP-1) purified from leaves of *Wasabia japonica* showed anti-microbial activity against both fungi and bacteria (53). Oleanolic acid (*C. paraguayensis* Burk, Fabaceae) from an Argentinean legume was found active against *Bacillus subtilis* and *S. aureus* with MICs 64 µg/ml (54). Peptides that are inhibitory to micro-organisms were first reported in 1942. Peptides called cathelicidins represent an important native component of innate host defense in mice and provide protection against necrotic skin infection caused by *Streptococcus* (55). They are often positively charged and contain disulfide bonds (56). The mechanism of action may be ion channel formation in the microbial membrane or competitive inhibition of adhesion of microbial proteins to host polysaccharide receptors (57). Diverse application has been demonstrated for anti-microbial peptides as anti-infective agents. The broad spectrum activity displayed by anti-microbial peptides is considered a 'chemical condom' against HIV infection and *Herpes simplex* virus (58).

### Polyphenols and Phenolics

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 that are in the highest oxidation state. The common traditional medicinal plants have such compounds that are effective against bacteria (59). Phenolic compounds possessing a C3 side chain at a lower level of oxidation and containing no oxygen are classified as an essential oil and reported as anti-microbials. Coumarins are phenolic substances made of fused benzene and α-pyrone rings (60). Several coumarins have anti-microbial properties and anti-viral effects reported in 1954 (61). Anti-microbial properties of phenolic compounds (Finnish berries) active against pathogenic bacteria exhibited different sensitivities towards phenolics. These properties can be utilized in functional food development and for food preservation (62). Phenols are toxic to micro-organisms because of the sites and numbers of hydroxyl groups on the phenol groups, which is all related to their relative toxicity of micro-organism. There is evidence that highly oxidized phenols possess more inhibitory action (63). The mechanism responsible for phenolic toxicity to micro-organisms includes enzyme inhibition by the oxidized compounds, possibly through reaction with sulfhydryl groups or through more non-specific interactions with proteins (64).

Polyphenols which can form heavy soluble complexes with proteins may bind to bacterial adhesions thereby disturbing the availability of receptor on the cell surface.

### Tannins

Tannins are generally descriptive of a group of polymeric phenolic substances capable of tanning leather or precipitating gelatin from solutions, the property known as astringency. Their molecular weights range from 500 to 3000 Da (55) and are found in almost every plant parts: bark, leaf, root, wood and fruit. They form two groups, hydrolysable and condensed tannins based on gallic acid. The first group is usually found as multiple esters with D-glucose, while the more numerous condensed tannins are derived from flavonoid monomers. Tannins (tannic acid) are water-soluble polyphenols that are present in many plant foods (65). Polyphenols (Tea) and many tannin components have been suggested to be anti-carcinogenic. The anti-microbial activities of tannins are well documented. The growth of many fungi, yeasts, bacteria and viruses were inhibited by tannins. Tannic acid and propyl gallate inhibit food borne, aquatic and off-flavor-producing micro-organisms. Their anti-microbial properties seemed to be associated with the hydrolysis of an ester linkage between gallic acid and polyols hydrolyzed after the ripening of many edible fruits. Tannins in these fruits thus serve as a natural defense mechanism against microbial infections. The anti-microbial property of tannic acid can also be used in food processing to increase the shelf-life of certain foods, such as catfish fillets. Tannin components of epicatechin and catechin (*Vaccinium vitis-idaea* L.) showed strong anti-microbial activity against bacteria and fungi. Such anti-microbial activity could potentially be used as a possible alternative for the treatment of periodontal diseases (66). Eucaglobulin is a new complex of gallotannin and monoterpene of leaves of *Eucalyptus globulus* possessing anti-bacterial effects (67). Methanol extracts of *T. citrina* fruit yielded known tannins such as corilagin, punicalagin and chebulagic acid that were tested for anti-microbial action (68). Arjunolic acid, ethyl gallate, flavone, ellagic acid and gallic acid are the active constituents of *T. arjuna* (Fig. 1). Macrocyclic structures of bioactive ellagiannins (gluconic acid core) and oligomeric ellagitannins have been found in species of Myrtaceae and Elaeagnaceae and they too possess anti-bacterial activity against *Helicobacter pylori* (69). Proanthocyanidins (condensed tannins) and hydrolyzable tannins are the two major classes of tannins. Proanthocyanidins are flavonoid polymers, the most common type of tannin found in forage legumes (70). Hydrolyzable tannins are polymers of gallic and ellagic acid esterified to a core molecule of *Phyllanthus emblica* (Fig. 1). Phyllanthin and hypophyllanthin are lignans of *P. niruri* (Fig. 1) that enhance cytotoxic response against

multi-drug-resistant cells (71). The mode of anti-microbial action of tannins and their ability to inactivate microbial adhesins, enzymes and cell envelop transport proteins also have been studied.

### Mixtures

The neem (*Azadirachta indica*), verasingam pattai (*Zanthoxylum limonella*), Indian babool (*Acacia nilotica*) stick are widely used as tooth brushes by various tribes throughout India, Africa and Nigeria. The neem (*A. indica*), traditionally used as medicine, and in particular the stem bark extract showed activity against various *Candida* species. The minimum fungicidal concentrations ranged from 0.06 to >8 mg/ml (72). Anti-bacterial effects of neem mouthwash have been tested over a period of 2 months against salivary levels of *Streptococcus mutans*, *Lactobacillus species* and *S. mutans*. They were all inhibited by neem-based mouth washes (73). The active component of the Nigerian chewing stick (*Fagara zanthoxyloides*) was found to consist of various alkaloids (74). Ayurvedic practitioners rely on plant extracts, both single and mixed combination, for the preparation. The preparations are used to treat a wide range of human, as well as animal, ailments (75). *Cleistanthus collinus* is known as oduvanthalai (Nillipalai) in India and all parts of this plant are highly poisonous. Various extracts of this plant yield a multitude of compounds that include glycosides, arylmethoxy lignan lactones such as cleistanthin A and B, collinusin and oduvin found to have anti-microbial activities (76). Strawberry extracts were strong inhibitors of *Salmonella* bacteria. The dried flower-heads of *Chrysanthemum morifolium* are an oriental drug as well as a popular herbal tea in China, which has been used for the treatment of eye diseases in Japan. They have been found to possess anti-bacterial, anti-fungal, anti-viral and anti-inflammatory activities (77).

### Experimental Designs for Extraction

Traditional healers prepare a wide range of healing juices, crude extracts, paste and tincture from various herbs by using a water extract. Water or alcohol (methanol/ethanol) are mainly used for a large number of crude extract/library preparations (dry powder soaking or suspension, mechanical shaker, distillation of essential oils), sequential grinding (alkaloids, steroids, triterpenoids), gradual centrifugation (lectins and polypeptides) and acid hydrolysis (phenols) for a specific time frame. A variety of extractants are used for their ability to solubilize anti-microbials and also other factors from plants. This particular study provided a more standardized extraction method for a wide variety of plants (78). The crude extracts or mixtures of compound-rich residues

are used for the initial screening of plants for anti-microbial activities. In many reports, methanol or ethanol are used for alkaloid extraction; acetone for flavonoids and steroids, hexane, diethyl ether and chloroform for fat soluble oils, wax, lipids and esters; dichloromethane for terpenoids, ethyl acetate for esters, ethanol for sterols, polyphenols, tannins and water for the water soluble components like glycosides, polysaccharides, polypeptides and lectins, which are most effective against pathogens. TLC, other chromatography separations (79) and several solvent systems are used for the elution of enormous water and organic solvent (Fig. 3) soluble anti-microbial compounds (Table 2). Diverse analytical spectral devices are often used for the identification and structural characterization of active components from plants (Fig. 4). However, the water medium is the most suitable for the treatment point of view in humans/animals.

### **Efficacy for *In vitro* Studies**

Several plants used for the treatment of various infections have some *in vitro* activity against pathogenic Gram-positive bacteria. In our findings, the popular use of *Tragia involucrata* as a traditional medicine for the treatment of scabies and skin infection was documented in India. The most promising *T. involucrata* and its extracts (organic and water) exhibited marked and/or broad spectrum activity especially towards Gram-negative and -positive bacteria. These results suggested the presence of good anti-microbial potency and a high concentration of active principles that were proven useful for wound healing in a rat model. Anti-inflammatory properties and safety of extracts were also evaluated. Effective inhibition is due to the high content of active principles in the extract. Whereas, among catechins (a constituent of green tea) and pyrogallol (*Holmskioldia sanguinea* Retz) (89), as well as other anti-fungal compounds like ginger (*Zingiber officinale* Roscoe) were found active against human pathogens (90). The use of natural remedies for the treatment of viral diseases has a long history in traditional systems of medicines. An extract of *Ribes nigrum* has been used as an ingredient in a variety of food and folk medicines in Japan. The extract has activity which inhibits virus replication in cells, due to the inhibition of protein synthesis among infected cells from the very early stage of infection (91). Anti-protozoal and cytotoxic activities were also reported (92).

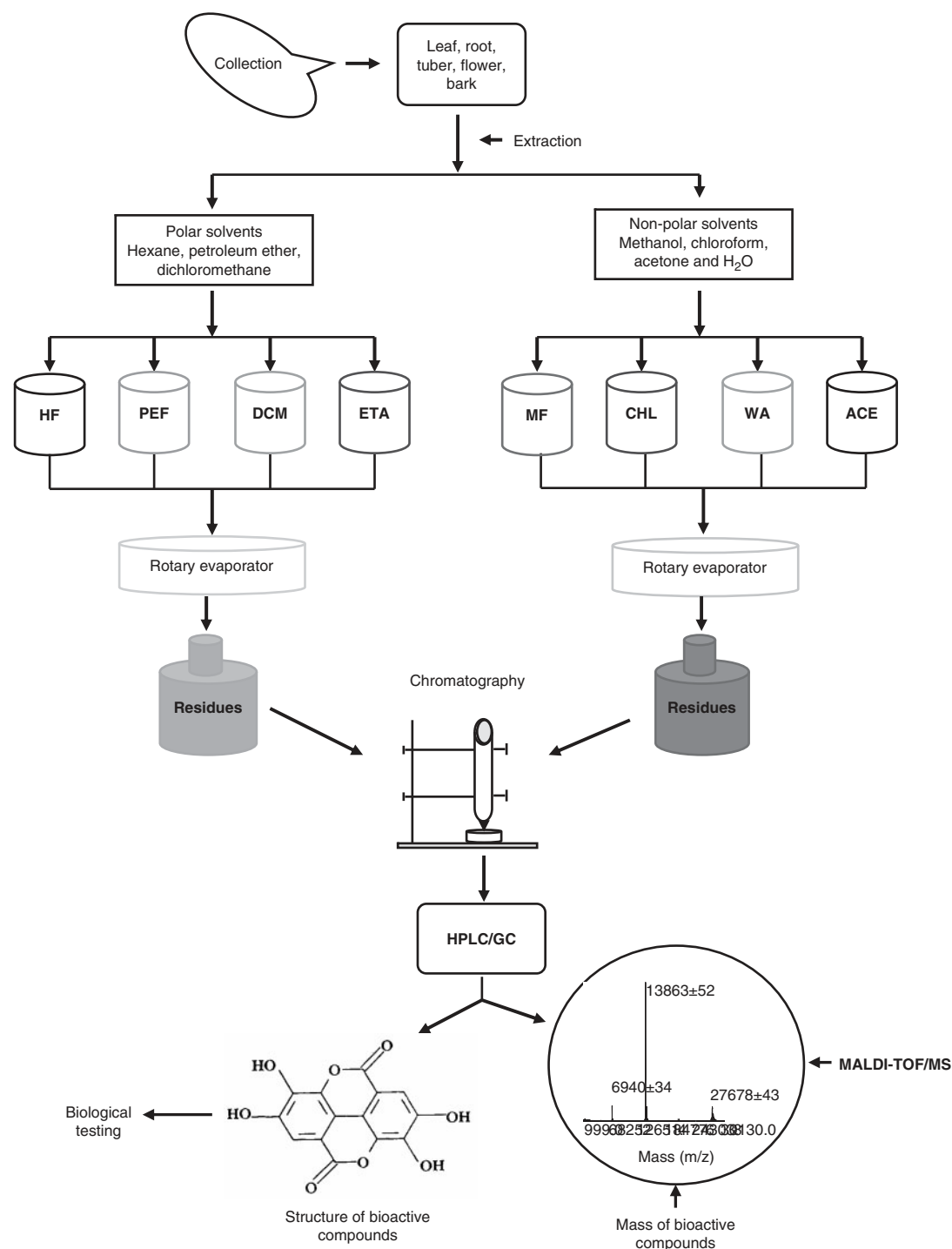
### **Need to Explore New Anti-microbials**

This review highlights some of the important medicinal plants used for traditional medicine and the medicinal information was obtained from various traditional healers or medical practitioners in the Hilly areas in

India. Tribal healers in most countries, where traditional ethnomedical treatment is frequently used, provide instructions to local people as how to prepare medicine from the plants (93). They keep no records and the information is mainly passed on verbally from generation to generation (94). World Health Organization (WHO) has shown great interest in documenting the use of medicinal plants from tribes in different parts of the world (95). Many developing countries have intensified their efforts in documenting the ethnomedical data on medicinal plants. Moreover, an unexplored reservoir of phytochemical information is being rapidly destroyed by deforestation. About 10–20% of all plant species become extinct and above all, there is a continuing threat and pressure upon the existing plants by fast-growing industrialization and commercial exploitation of pharmaceutical industry (96). However, the medicinal plants are an important health resource in all the regions of India and particularly among the primitive communities (97). Though a vast number of plants have not been studied for their anti-microbial properties these may become new sources of plants for anti-microbial activity. There is a need for further collaborative biological screening of plant extracts in single, or combination, form. Phytochemicals derived from plant products are quite effective in controlling the growth of micro-organism. The recent approaches of bioautography assay (11) and high-throughput screening methods are the most suitable method to detect the anti-microbial component present in the extract. With the current trend on increasing awareness in traditional medicine, the plant-derived agents have been attracting much interest as natural alternatives to synthetic compounds because microbes slowly develop resistance against antibiotics. Scientists are trying to tap the pharmaceutical and food values of these many unidentified plants. It is believed that the plants (traditional medicine) will be a major source of new chemicals and raw materials for the pharmaceutical industry.

### **Conclusions**

From the above studies, it is concluded that the traditional plants may represent new sources of anti-microbials with stable, biologically active components that can establish a scientific base for the use of plants in modern medicine. These local ethnomedical preparations and prescriptions of plant sources should be scientifically evaluated and then disseminated properly. The knowledge that primitive people used plants gives a clear idea about the unclear botanical preparation of traditional sources of medicinal plants. These can be extended for future investigation into the field of pharmacology, phytochemistry, ethnobotany and other biological actions for drug discovery.



**Figure 3.** Flow chart for the various sequential protocols involved for the purification, characterization and structural derivation of medicinal plants and their bioactive compounds. Various fractions, i.e. HF: hexane fraction; PEF: petroleum ether fraction; DCM: dichloromethane fraction; ETA: ethyl acetate fraction; MF: methanol fraction; CHL: chloroform fraction; WA: water and ACE: acetone fraction.

**Table 2.** Different types of organic solvents are used for the extraction of active compounds

Acetone	Chloroform	Diethyl ether	Ethanol	Hexane	Methanol	Petroleum ether	Water
Flavonols (80), alkaloids (33), naphthol, glucoside (81),	Terpenoids (82), flavonoids (83), steroids (74), ursolic acid	Alkaloids, terpenoids, fatty acids (84)	Alkaloid (22), tannins, flavonol, terpenoid, sesquiterpenes (85), sterols, polyphenols, aporphine alkaloids (21)	Carvacrol (86), carvacrol (86)	Terpenoids, flavonnes, polyphenols, tannins (68)	Proanthocyanidin (69), tannins (70)	Anthrocyanins (87), tannins (61), saponins (88), glycosides (74)



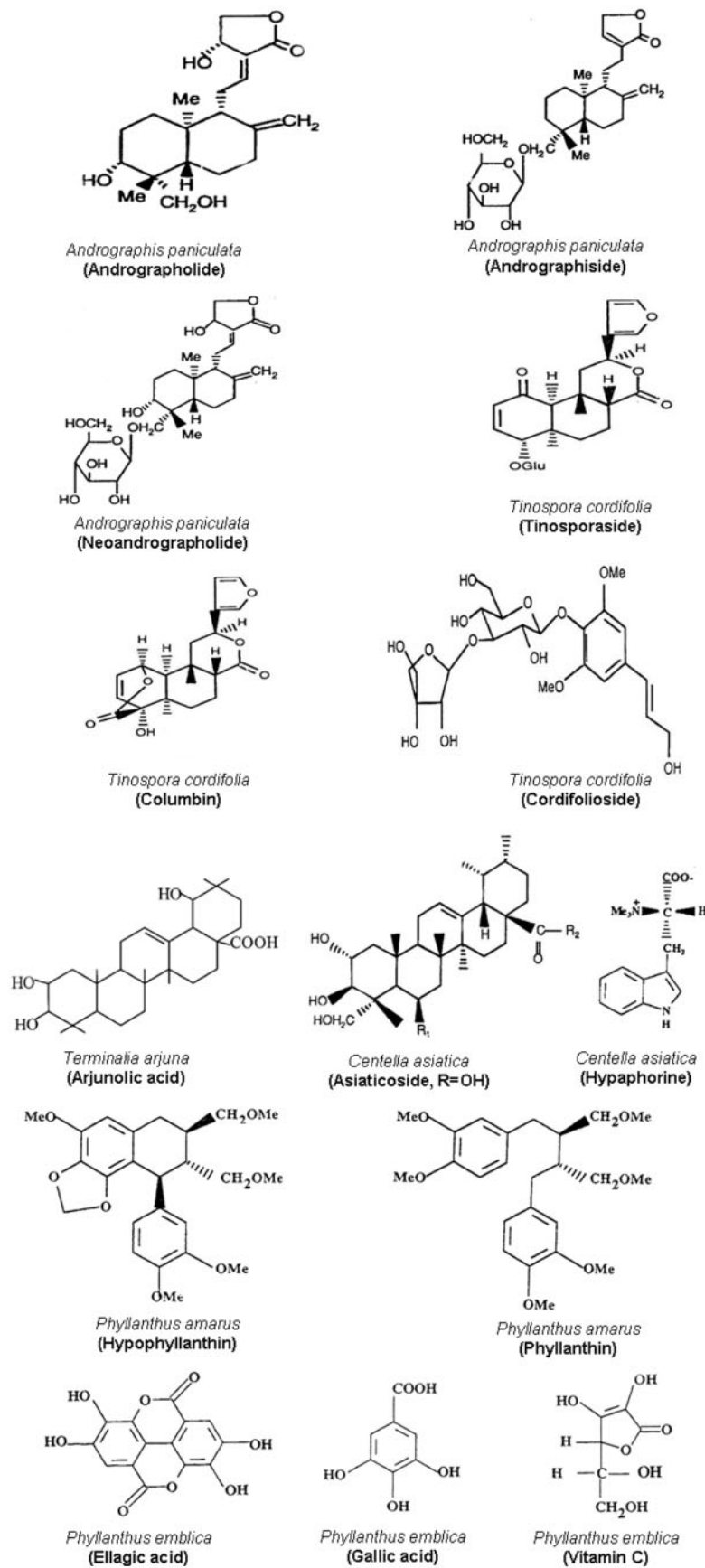


Figure 4. Structure of common anti-microbial compounds from the popularly used traditional medicinal plants.

In addition, folklorist, pharmacologist, phytochemists and ethnobotanists are investigating plants for anti-microbials. Several plant-based chemicals have shown potential inhibitory action on a wide range of micro-organism *in vitro* experiment. Although tannins, poly-phenols (98), oils (99) and others were identified as the effective component for the bacterial damaging or killing action in the *in vitro* system, many of these compounds failed in human clinical trails to determine their therapeutic effectiveness.

We concluded that well-designed bioassay-guided isolation and studying anti-microbial effect should be able to complete this task. In the past 20 years, several studies proving the extract or chemicals derived from plant extracts were reported. These studies indicated that extracts contain interesting biopharmaceutical substances (anti-microbials) that have attracted significant scientific attention. More detailed investigation at molecular, cellular levels, suitable animal models and human clinical studies are necessary to elucidate anti-microbial and other biological activities.

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