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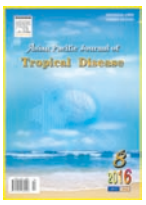

Review article doi: 10.1016/S2222-1808(16)61106-4

In vitro and *in vivo* protocols of antimicrobial bioassay of medicinal herbal extracts: A review

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ARTICLE INFO

Article history:
Received 8 Mar 2016
Received in revised form 21 Mar, 2nd revised form 9 Apr 2016
Accepted 28 May 2016
Available online 19 Jul 2016

Keywords:
Antimicrobial
Herbal extracts
Microorganisms
Antimicrobial protocols

ABSTRACT

Antimicrobial susceptibility testing against pathogenic microorganisms is the most significant task of clinical microbiology laboratory. The present study was therefore designed to review the *in vitro* and *in vivo* protocols of antimicrobial bioassays of various medicinal herbal extracts against a diversity of pathogenic microorganisms. Plants have a broad variety of antimicrobial agents which are extensively used as herbal drugs against different microbes. The review covers the antimicrobial techniques and antimicrobial bioassays of medicinal herbal extracts against different bacterial and fungal strains from 2000 onward. Plants have diverse concentrations of bioactive constituents such as alkaloids, saponins, tannins, terpenoids, steroids, carbohydrates, proteins and lipids. These phytochemicals are used against an extensive range of bacteria (*Escherichia coli, Mycobacterium, Corynebacterium pervum, Bordetella pertusis, Klebsiella pneumoniae, Salmonella typhi*), viruses (simian-virus, retrovirus) and fungi (*Pseudomonas aeruginosa, Aspergillus fumigatus, Aspergillus flavus, Fusarium solani*). A variety of antibiotics (tetracycline, terramycin, ampicillin) has also been isolated from different medicinal plants. This review was therefore intended to explore the techniques used for antimicrobial activities of herbal medicinal extracts.

1. Introduction

Medicinal plants are abundant in phytochemicals such as flavonoids, terpenoids, glycosides and alkaloids, which have remedial antimicrobial potential[1]. Several microbial contaminations are removed by the treatment of plant extracts and their essential oils[2]. *Bryophyllum pinnatum* and *Kalanchoe crenata* which are full of bryophyllin, malate, potassium, ascorbic and citric acids have action against diabetes, inflammation and cancer[3]. *Maytenus boaria* has also been used conventionally for the healing of gastrointestinal diseases, fever, rheumatism and asthma worldwide. *Maytenus boaria* plant (bark) pasted with mustard oil was used against lice in hair[4]. Four plant extracts (*Phyllanthus emblica, Tinospora cordifolia, Eclipta alba and Cassia occidentalis*) were evaluated against pathogenic bacterial strains[5]. Antimicrobial potential was found by the isolated compounds from remedial plants[6]. Current studies have confirmed that some plant species have good results against virus and inflammation. A recent study has reported that methanol/water extracts of *Plantago lanceolata* are rich in phenolic acids, benzoic acid derivative, hydroxyl benzoate and 3, 4, 5-trihydroxybenzoate[7]. Plant extracts from Asteraceae and Lamiaceae family have antimicrobial commotion against *Staphylococcus epidermidis* (*S. epidermidis*)[8]. *Annona mucosa* has acetogenins, alkaloids and lignans that showed antimicrobial commotion[9]. Soft tissues and skin contaminations such as impetigo, folliculitis, furunculosis, cellulitis and abscesses are caused by methicillin-resistant *Staphylococcus aureus* (*S. aureus*). Chemotherapeutic drugs are therefore required to control the development and growth of these pathogenic bacterial strains[10]. Medicinal plants isolated compounds have been preferred over synthetic compounds due to its use in conventional medicine[11]. Therefore, these compounds are considered the substituted source of antimicrobial drugs[12]. The fresh leaves of *Coleus forskohlii* are used to cure asthma and constipation[13]. Modern study shows that extract of *Hypochaeris radicata* possess antifungal activity used against certain pathogens[14]. *Acalypha indicia* Linn. (family Euphorbiaceae) is used as diuretic, anthelmintic and for

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The journal implements double-blind peer review practiced by specially invited international editorial board members.

the treatment of respiratory problems such as bronchitis, asthma and pneumonia[15]. Medicinal plants are used as a substitute to antibiotics being potent antimicrobial agents[16]. These agents are considered significant against pathogenic infections. But immune suppressed patients over the last 30 years have been at risk due to antibiotic resistant microorganisms[17]. Plant extracts can act as a substituent of antibiotics which cause resistance to bacteria that cause disease in aquaculture system[18]. Several infectious diseases such as AIDS is accomplished by the use of medicinal plants remedies. The seeds also have a hypotensive activity. Fatty oil in the essence, as well as aqueous or alcoholic extracts of *Citrullus landaus* var. *Citroide* have been used to paralyze tapeworms and roundworms[19]. Quetico, a plant from the genus *Pterocaulon*, is used to treat fungal infection[20]. Olive leaves extract is now used as nutraceutical in liquid and capsule form. Oleuropein is phenolic constituent of olive leaves extracts that make vinegary taste of olive and are used against large number of pathogens such as *S. aureu*, *Campylobacter jejuni* and *Helicobacter pylori*[21]. Chief furnisher of essential oils is *Eucalyptus globulus*. These oils are used against malaria, cancer, anodyne, antiseptic burn, diarrhea, diphtheria, dysentery, encephalitis, enteritis, erysipelas, diabetes, fever, flu, inflammation, laryngalgia, laryngitis, leprosy and malaria. Sometimes, they are used in industries for the formation of soap and cosmetics *etc. Leptospermum petersonii* (lemon scented tea-tree: family Myrtaceae) essential oils and the complementary volatile (vapour) components have been estimated to exhibit efficient antifungal action. The mechanism of action of *Leptospermum petersonii* is observed by a range of experiments to confirm the effect of oil on both *Candida albicans* (*C. albicans*) and the hyphae of *Aspergillus* spp.[22]. Medicinal plants contain phenolic compounds which can increase lipid oxidation quality and microbial development[23]. Essential oils and medicinal plants are significant due to their potential in animal digestion and antimicrobial activities. Spice antimicrobial efficiency is dependent upon substrate, spice types, food storage methods, structure, types and concentration of pathogens. This plant was also used as food additives, preservatives, flavoring and coloring agent. It is used as anti-parasitic, anti helmintic, analgesic, cough medicine, sedative, antibacterial and anti-diabetic substances worldwide[24]. Brucellosis is a global zoonotic disease in humans as well as other animals. Brucellosis is transmitting to humans by the shortest-contact with the infectious animals or consumption of unpasteurized or inappropriately cooked milk or dairy products. Therefore, a study was performed to trace out the antimicrobial confrontation outline of human *Brucella* isolates from patients[25]. The major cause of urinary tract infection is *Escherichia coli* (*E. coli*), *Klebsiella, Enterobacter, Pseudomonas fluorescens* and *Proteus*. Urinary tract infection and human oxidative stress have been treated by *Vaccinium macrocarpon* (cranberry fruits). *E. coli* association with uro-epithelial cells were treated by these fruits[26]. The research was aimed to estimate the antimicrobial action of some metal complexes and to distinguish their activity in free ligands[27]. Natural products used as antimicrobial and anti-ailments have been encouraged due to the resistance of some microbes against antibiotics and the disinclination of chemicals consumption[28]. Consumers therefore enhanced the demand for medicinal plants use due to its antimicrobial effects[29]. Due to the multi-purpose applications, crude extracts of plants have been considered of great interest by consumers[30]. A large number of artificial derivatives as well as naturally occurring peptides are now in progress. Propanesulfonic acids have diverse structure and composition of amino acids, but they still have some resemblances. The characteristics of peptide having antimicrobial action were studied by bioinformatics tools[31]. Plants are considered as the significant sources of medicines in the world despite the development of medicinal science[32]. Herbal products are getting attention due to the disadvantages and complications of several synthetic and chemical antibiotics. Therefore, herbal medicinal products can be used as a substitute for particular health care needs[33].

2. Antimicrobial techniques

2.1. Agar diffusion technique

Agar diffusion technique was used against *Bacillus subtilis* (*B. subtilis*), *S. aureus, Salmonella choleraesuis, Pseudomonas aeruginosa* (*P. aeruginosa*), *C. albicans, Proteus* spp., *Klebsiella pneumoniae* (*K. pneumoniae*), *Enterobacter aerogenes* (*E. aerogenes*) and *E. coli* (Table 1). Infusion agar and Mueller-Hinton agar of heart and brain were used for this biological activity. Clove and jambolan extracts exhibited activities against microorganisms. *E. coli* was reported resistant against drugs, benzoic acid and cinnamic acid. Development of *P. aeruginosa* was repressed by extracts of cloves, jambolan and thyme. Extracts of jambolan and thyme inhibited growth of bacteria[34,35]. Cloramfenicol, ampicilline and tetracycline were used as standard[36]. Anacardic acid and totarol are phytochemical that are used to inhibit *E. aureus*[37]. Agar well diffusion technique was followed for antimicrobial action by *Alternanthera maritime* extracts. Strains of *P. aeruginosa, Micrococcus luteus, S. aureus, Candida glabrata, Streptococcus mutans, Candida krusei, Trichophyton mentagrophytes, C. albicans,* and *Enterococcus faecalis* (*E. faecalis*) were used. Propylene glycol was used as negative control for which no inhibitory effect could be observed[38,39]. Gentamicin, bacitracin and ketoconazole were used as positive control against mixed bacterial and fungal strains respectively[40,41]. *Bixa orellana, Pyrethrum pulchrum* and *Gliricidia sepium* plants extracts were used against several microorganisms such as beta-hemolytic, *E. coli, Bacillus cereus* (*B. cereus*), *C. albicans, P. aeruginosa* and *S. aureus*[42]. A diverse variety of agar media were used against different microorganism and agar diffusion technique was followed for the assay[43,44]. Stem bark extract of *Jatropha curcas* revealed significant antimicrobial potential[45]. For antifungal assessment, *C. albican, Aspergillus niger* (*A. niger*), *Aspergillus fumigatus* (*A. fumigatus*) and *Aspergillus flavus* (*A. flavus*) were used[46]. The determination of antimicrobial assay of plant crude extract was accomplished by agar well diffusion scheme[47]. Crude, chloroform, *n*-hexane, ethyl acetate and aqueous extracts of *Trigonella foenum* recorded noteworthy antibacterial bioassays against different bacterial species[48]. Agar diffusion scheme was followed with slight modifications[49]. Antibacterial assessment was executed against *S. aureus*, *E. coli*, *Erwinia carotovora*, *P. aeruginosa*, *Agrobacterium tumefaciens* and *Salmonella typhi* (*S. typhi*) by disc diffusion susceptibility technique[50]. Ethyl acetate and chloroform extracts of plant revealed noteworthy activities against all bacterial strains[51].

Table 1

Antimicrobial protocols and therapeutic effects of herbal medicinal extracts of different plant species.

Medicinal plant species	Herbal medicinal extracts	Microbial strains	Anti-microbial protocols	Therapeutic effects	References
Cloves, jambolan	Ethanol (C_2H_6O)	*E. aureus*, *Salmonella choleraesuis*, *P. aeruginosa*, *B. subtilis*, *C. albicans*, *Proteus* spp., *K. pneumoniae*, *P. aeruginosa*, *E. aerogenes* and *E. coli*	Agar diffusion method	Anti-bacterial	[34]
Elsinoë australis, *Acacia tetragonophylla*, *Eremophila alternifolia*, *Eremophila duttonii*	Ethanol (C_2H_6O)	*B. cereus*, *E. faecalis*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. typhimurium*, *S. aureus* and *S. pyogenes*	Plate hole diffusion assay	Anti-bacterial	[52]
Achillea millefolium, *B. crassifolia*	Ethanol (C_2H_6O)	*B. subtilis*, *E. coli*, *E. aureus*, *P. aeruginosa*, *C. albicans*, *B. crassifolia*, *Chelidonium majus*, *Rhaponticum carthamoides*, *Sanguisorba officinalis* and *Tussilago farfara*	Liquid dilution method	Anti-bacterial	[53]
Satureja hortensis L.	Methanol (CH_3OH)	*B. subtilis*, *E. faecalis*, *P. aeruginosa*, *Salmonella enteritidis* and *S. pyogenes*	Clevenger distillation method	Anti-bicrobial	[54]
Abronia maritima	Ethanol (C_2H_6O), Hexane (C_6H_{14})	*E. coli*, *Pyrgulopsis micrococcus*, *Staphylococcus* strain, *Eenterococcus* strain, *Streptococcus* strain and *C. albicans*	Agar well diffusion method	Anti-bacterial, Anti-fungal	[38]
Hypericum mysorense, *Hypericum hookerianum*	Ethanol (C_2H_6O), Aqueous (H_2O)	Herpes simplex virus-1	Plate-hole diffusion method	Anti-viral activity	[55]
Andrographis paniculata, *Vitex negundo*	Aqueous (H_2O)	*S. aureus*, *K. pneumoniae*, *P. aeruginosa* and *E. coli*	Disc diffusion method	Anti-bacterial	[56]
Borbo ferruginea, *Ageratum conyzoides*, *Terminalia avicennioides*, *Ocimum gratissimum*, *Acalypha wilkesiana*	Ethanol (C_2H_6O), aqueous (H_2O)	*S. aureus*	Broth micro-dilution method	Anti-bacterial	[57]
Anacardium occidentale, *Artocarpus integrifolia*	Leaves aqueous (H_2O)	*B. cereus*, *E. coli, P. aeruginosa* and *S. aureus*	Agar well diffusion method	Anti-viral	[42]
Sarcina lutea, *C. pulcherrima*	Methanol (CH_3OH)	*B. cereus*, *S. aureus*, *E. aerogenes*, *E. coli* and *K. pneumoniae*	Disc diffusion method	Anti-bacterial	[58]
C. alata	Aqueous (H_2O)	*A. flavus*	Liquid dilution method	Anti-fungal	[59]
Kalanchoe crenata, *Bryophyllum pinnatum*	Palm wine, methanol (CH_3OH)	*S. aureus*, *E. faecalis*, *B. subtilis*, *K. pneumoniae*, *P. aeruginosa*, *E. coli*, *Shigella flexneri*, *Salmonella paratyphi* and *Citrobacter* species	Agar well diffusion method	Anti-bacterial	[3]
Swertia chirata	Aqueous (H_2O)	DNA viruses and herpes simplex virus-1	Cytotoxic assay method	Anti-viral	[60]
Jatropha curcas	Ethanol (C_2H_6O)	*K. pneumoniae*, *E. coli*, *Citrobacter freundii* and *E. aerogens*	Agar well diffusion method	Anti-bacterial, Anti-fungal	[45]
Apis mellifera	Hexane (C_6H_{14}), methanol (CH_3OH), ethyl acetate ($C_4H_8O_2$)	*Streptococcus pneumoniae*, *S. aureus*, *K. pneumoniae* and *E. coli*	Agar dilution and macro-dilution method	Anti-bacterial	[61]
Cassia fistula	Hydroalcohol crude extract	*S. aureus*, *S. pyogenes*, *E. coli*, *P. aeruginosa*, *A. niger*, *Aspergillus clavatus* and *C. albicans*	Agar disc diffusion and agar cup method	Anti-bacterial, Anti-fungal	[1]
M. emarginata	Methanol (CH_3OH)	*E. coli*, *E. cloacae*, *B. cereus*, *S. aureus*, *P. aeruginosa*, *A. niger*, *A. flavus*, *A. solani*, *Rhizopus stolonifer* and *F. oxisporum*	Micro-dilution method for anti-bacterial	Anti-fungal	[62]
Acacia karoo, *Erythrophleum lasianthum*, *Spirostachys africana*	Methanol (CH_3OH)	*Mycobacterium tuberculosis*, *K. pneumoniae*, *E. aerogenes* and *E. coli*	Micro-plate dilution method	Anti-bacterial	[63]
Rhus coriaria	Ethanol (C_2H_6O)	*K. pneumoniae*	Disc diffusion method	Anti-bacterial	[64]
Picrorhiza kurroa, *Datura metel*, *Acacia catechu*, *Cissus quadrangularis*, *Cassia tora*, *Berberis aristata*, *Pongamia pinnata*	Acetone (C_3H_6O), methanol (CH_3OH), aqueous (H_2O), chloroform ($CHCl_3$)	*E. coli*, *S. typhi*, *S. aureus*, *B. subtilis*, *P. aerguinosa* and *K. pneumoniae*	Dilution assay method	Anti-bacterial	[5]
Trigonella foenum	Ethyl acetate ($C_4H_8O_2$), chloroform ($CHCl_3$)	*E. coli*, *P. aeuroginosa*, *S. aureus*, *S. typhi*, *Erwinia carotovora*, *Agrobacterium tumefaciens*	Agar well diffusion method	Anti-bacterial, Anti-fungal	[48]
P. nodiflora	Methanol (CH_3OH), hexane (C_6H_{14}), ethyl acetate ($C_4H_8O_2$), butanol (C_4H_9OH)	*E. coli*, *P. aeuroginosa*, *K. pneumoniae*, *Salmonella*, *S. epidermidis*, *S. aureus* and *B. subtilis*	Agar well assay method	Anti-bacterial	[65]

(*Continued on next page*)

S. typhimurium: *Salmonella typhimurium*; *S. pyogenes*: *Streptococcus pyogenes*; *B. crassifolia*: *Byrsonima crassifolia*; *E. cloacae*; *Enterobacter cloacae*; *A. solani*: *Alternaria solani*; *C. pulcherrima*: *Caesalpinia pulcherrima*; *C. alata*: *Cassia alata*; *P. nodiflora*: *Phyla nodiflora* Linn.; *M. emarginata*: *Maytenus emarginata; F. oxisporum*: *Fusarium oxisporum*.

Table 1 (*continued*)
Antimicrobial protocols and therapeutic effects of herbal medicinal extracts of different plant species.

Medicinal plant species	Herbal medicinal extracts	Microbial strains	Anti-microbial protocols	Therapeutic effects	References
Ballota nigra	Hexane (C_6H_{14}), ethyl acetate ($C_4H_8O_2$), chloroform ($CHCl_3$), butanol (C_4H_9OH)	*E. faecalis*, *P. mirabilis*, *S. aureus*, *K. pneumoniae*, *E. coli*, *S. typhi*	Agar tube dilution method, well assay method	Anti-bacterial, anti-fungal	[66]
Allium sativum, *Mangifera indica*, *P. guajava*, *Vernonia amygdalina*	Ethanol (C_2H_6O)	*E. coli*, *P. guajava*	Agar slant method	Anti-bacterial	[67]
Azadirachta indica, *Cymbopogon citrates*, *Mentha arvenis*, *Ocimum sanctum*	Acetone (C_3H_6O), diethyl ether $(C_2H_5)_2O$, methanol (CH_3OH)	*E. coli*, *S. typhi*, *Shigella*, *S. aureus*	Agar well diffusion method	Anti-bicrobial	[3]
Adiantum capillus-veneris	Ethanol (C_2H_6O), methanol (CH_3OH), ethyl acetate ($C_4H_8O_2$)	*E. coli*, *P. aeruginosa*, *K. pneumonia*, *S. aureus*, *C. albicans*, *Trichoderma*, *Pythium*, *A. flavus*, *A. niger*	Agar well diffusion method	Anti-bacterial,Anti-fungal	[68]
Heliotropium bacciferum	Methanol (CH_3OH), ethyl acetate ($C_4H_8O_2$), hexane (C_6H_{14})	*S. typhi*, *E. coli*, *P. aeruginosa*, *S. aureus*, *K. pneumoniae*, *B. subtilis*, *Trichoderma longibrachiatum*, *A. flavus*, *A. niger*, *Fusarium solani*, *C. albican*	Disc diffusion susceptibility method and agar tube dilution method	Anti-bacterial, anti-fungal	[69]
Cannabis indica	Methanol (CH_3OH), ethyl acetate ($C_4H_8O_2$), butanol (C_4H_9OH)	*S. aureus*, *B. cereus*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *P. mirabilis*, *A. niger*, *Aspergillus parasiticus*, *Aspergillus oryzae*, *A. fumigatus*, *C. albicans*	Agar well diffusion method	Anti-bacterial, anti-fungal	[70]
Cynodon dactylon	Ethanol (C_2H_6O), chloroform ($CHCl_3$)	*Citrobacter freundii*, *E. cloacae*, *P. aeruginosa*, *Salmonella enteritidis*, *Vibrio cholerae*, *Streptococcus pyogenes*, *S. aureus*, *E. faecalis*, *Streptococcus agalactiae*, methicillin-resistant *S. aureus*, *S. typhi*, *E. coli*, *S. typhimurium*	Agar well and micro-broth dilution method	Anti-bacterial, anti-fungal, anti-viral	[71]
Annona mucosa	Methanol (CH_3OH), hexane (C_6H1_4)	*B. subtilis*, *S. pyogenes*, *S. aureus, P. aeruginosa*	Agar dilution method	Anti-bacterial	[72]
Cistanche tubulosa	Methanol (CH_3OH), chloroform ($CHCl_3$), ethyl acetate ($C_4H_8O_2$)	*Cistanche tubulosa*, *P. mirabilis*, *K. pneumonia*, *E. coli*, *A. fumigatus*, *A. niger*	Agar well diffusion method	Anti-microbial	[73]

P. guajava: *Psidium guajava*; *P. mirabilis*: *Proteus mirabilis*.

2.2. Plate-hole diffusion assay

A large variety of plants (*Sarcostemma viminale*, *Scae ola spinescens*, *Pterocaulon sphacelatum*, *Isotoma petraea* F.Muell, *Centipeda cunninghamii*, *Lepidosperma gladiatum* Labill, *Amyema maidenii*) were collected and extracted with ethanol. The plants extracts were treated for antibacterial examination by plate hole diffusion technique against several bacterial species such as *K. pneumonia*, *S. typhimurium*, *E. coli*[74]. *Eremophila duttonii* antibacterial investigation was made against different bacterial strains. Noteworthy antibacterial potential was recorded by plant extracts against *S. aureus, B. cereus, S. pyogenes* and *E. faecalis*[52]. *Melia dubia* extract and *Cryptostegia grandiflora* extract exhibit partial activity at high concentrations. Plants different parts were collected and macerated in solvent of different concentrations. These extracts were screened for their cytotoxicity against vero cell line by protein estimated[55]. Cytopathic inhibition and the activities of virus yield reduction were used for the antiviral assay of plant extracts[75,76]. *Hypericum mysorense*, *Usnea complanta* and *Hypericum hookerianum* extracts displayed significant anti-viral assays[77].

2.3. Well diffusion technique

Leaves extracts of *Agave bracteosa* Benth, *Ziziphus sativa* and *Calotropis procera* plants were assessed for antimicrobial bioassay by agar well diffusion technique. Methanol and *n*-hexane extracts of plants revealed notable activities against all bacterial strains[78]. Antimicrobial assay of nine flowering plants such as *Boerhaavia diffusa*, *Tribulus terrestris*, *Acacia acuminata, Tinospora cordifolia, Punica granatum, Smilax febrifuga, Azadirachta indica*, *Terminalia chebula* and *Bauhinia variegata* were investigated by agar well diffusion technique[79]. Leaves of *Bougainvillea spectabilis* were examined against different Gram-positive and Gram-negative bacterial species by using agar diffusion methodology. Ethyl acetate, ethanol, chloroform and methanol extracts displayed notable inhibition against *E. coli, B. subtilis, S. typhi, Shigella flexneri, S. aureus, S. faecalis, Proteus vulgaris* and *Micrococcus luteus*[80]. Another report investigated the antimicrobial bioassays of six medicinal plants against several pathogenic microorganisms by using agar well diffusion scheme[81].

2.4. Agar dilution scheme

Plant materials of *Acacia mellifera* (*A. mellifera*) were collected. *S. pneumonia*, *S. aureus*, *K. pneumoniae* and *E. coli* (Gram-negative and Gram-positive) were used for antimicrobial examination[61]. Minimum inhibitory concentration (MIC) of water extract was found by the agar dilution scheme[82]. Treatment of *Aloe vera* gel extracts were investigated for antibacterial action against different bacterial species[83]. The acetate extract of *A. mellifera* has flavonoids that have antioxidant properties. Methanol extract has antimicrobial activity against *Streptococcus pneumoniae* and *K. pneumoniae*. Ethyl acetate extract of *A. mellifera* has antimicrobial activity against *S. aureus* and *E. coli*[84].

2.5. Broth micro dilution method

The extracts of *Acalypha wilkesiana, Phyllanthus discoideus, Borbo ferruginea, Terminalia avicennioides, Ocimum gratissimum* and *Ageratum conyzoides* were administered for antimicrobial assessment. Methicillin defiant-*S. aureus* was treated as control bacterial species. The evaluation of minimum inhibitory concentration was made by broth dilution technique[57,85]. Antimicrobial assessment of four quaternary ammonium compounds was accomplished against zoonotic and food-borne microorganisms by micro-dilution and agar dilution techniques[86]. The antimicrobial potential of dalbavancin (antimicrobial drug) also has been reported by using broth micro-dilution scheme[87]. The antimicrobial assessment of the pathogens of fish such as *Flavobacterium psychrophilum* and *Flavobacterium columnare* were accomplished by using broth micro-dilution methodology[88].

2.6. Disc diffusion technique

Antimicrobial assessment was carried out by disc diffusion technique. *E. coli*, *P. aeruginosa*, *S. aureus*, and *K. pneumonia* were used for this assay[89]. Positive control was used as standard commercial antibiotic disc which was followed by four treated discs. Dimethyl sulfoxide was treated as negative control[56]. Aqueous extract (2 μg/disc) of *Andrographis paniculata* was found to be very active against *P. aeruginosa*[90]. Plant methanol extract revealed antibacterial potential against *K. pneumonia*[91]. *C. pulcherrima* was mainly active against Gram-positive bacteria (*B. cereus*). *C. pulcherrima* extracts revealed significant inhibition against pathogenic microorganisms responsible for different diseases[58]. The collection of *K. pneumoniae* was carried out. Ampicillin, amikacin, chloramphenicol, erythromycin, lincomycin and kanamycin were used as drugs. These antimicrobial agents were tested by disc-diffusion method by streaking the agar plate with bacterial inoculums. MIC was calculated by using serial dilution method. Study revealed that isolates of *K. pneumoniae* were collected. According to the category and number of antimicrobials of *K. pneumoniae*, isolates are multiresistant (resistance to more than two antibiotics). *K. pneumoniae* was cleared from the blood of infectious mice by alcoholic extract. Further, it was very effective than other treatments. The effect of *Rhus coriaria* extract may be due to tannin and other compounds. The ethanol extract exhibited large spectrum activity[64].

2.7. Liquid dilution method

Sanguisorba, Achillea millefolium, and *B. crassifolia* extracts were used as antimicrobial agents. Liquid dilution scheme was followed for antimicrobial assessment[92]. *B. crassifolia* extracts displayed notable activities against *Sanguisorba officinalis, B. crassifolia, Tussilago farfara* and *C. albican*[53]. Flower extracts of *C. alata* were treated against the toxigenic strain of aflatoxin producing fungi (*A. flavus*) and plant pathogenic fungi (*C. albicans*). Each Petri dish contained the medium alone and contained the medium with bavistin, griseofulvin and flower extract mixed with medium. MIC was determined by liquid dilution method. *C. alata* flowers were studied for antifungal activities. The aqueous extract of dried powder of *C. alata* flower has shown good antifungal activity against fungi. The growth of these fungi decreased as the concentration of extract increased and growth was completely inhibited at their MIC. Methanol extract of flower at 4 mg/mL inhibited many type of bacteria but no moulds. The aqueous extract of *C. alata* flower is a significance inhibitor of growth of aflatoxin producing plant and human pathogenic fungi tested during this investigation[59,93].

2.8. Agar slant scheme

Collection of various plant species such as garlic bulb (*Allium sativum*), mango leaf (*Mangifera indica*), guava leaf (*P. guajava*), bitter leaf (*Vernonia amygdalina*) and ginger rhizome (*Zingiber officinale*) was accomplished for antibacterial bioassay[94]. Ciprofloxacin is a antibiotic used in this experiment. It was reported that studied all the rats have negative effects for *E. coli*. Variation was also noticeable in the quantity of *E. coli* shed in feces in different groups of rat. Antimicrobial activity against *E. coli* was exhibited by the plant of ethanol fraction. *P. guajava* was the pathogen in the faeces of the rats. It was affected by antibiotic drugs. The antibiotic drug inhibited the competitive microorganism in the gut more than *E. coli* strain. The result of this study revealed that the rats infected with *E. coli* had symptoms of diarrhea infection by *E. coli* which caused non-bloody diarrhea[67].

2.9. Agar cup diffusion methodology

Screening of antimicrobial assay of plant extracts was accomplished against different microorganisms by using the agar cup diffusion techniques[95]. The antibacterial activities of methanol, ether, ethyl acetate, chloroform and aqueous extracts of *Pimpinella anisum* L. were investigated against *E. coli, B. subtilis, K. pneumoniae, S. aureus* and *P. aeruginosa*. The antifungal activity of plant extracts was also screened against *C. albican* and *A. niger*. The agar cup plate diffusion methodology was used for the activity[96]. The potato dextrose agar and Mueller-Hinton agar media were used for testing bacterial and fungal strain[97].

2.10. Agar well assay procedure

Chloroform, ethyl actetate, *n*-hexane and *n*-butanol extracts of *P. nodiflora* were evaluated for antibacterial assay. Butanol and hexane extracts inhibited the growth of *E. coli* and *P. aeruginosa*. All extracts of plants were found active against *B. subtilis* and *S. epidermidis*[65]. Antibacterial assessments of plant extracts were carried out against *S. epidermidis*, *B. subtilis*, *E. coli*, *K. pneumoniae*, and *S. aureus*. As a control, rifampin was used[98]. *P. nodiflora* extracts were also investigated for antimicrobial potential against different pathogenic microorganisms and agar well assay procedure was followed for the assessment[99].

2.11. Micro-dilution technique

M. emarginata extracts were examined for antimicrobial activities by using micro-dilution technique. Fluconazole and methanol were used as standards for fungal strains, while for bacterial species, ampicillin was used as control[100]. Plant extracts revealed noteworthy activities against *E. coli, S. aureus, E. cloacae, P. aeruginosa, B. cereus, F. oxisporum, A. solani, A. flavus, Rhizopus stolonifer* and *A. niger*[101]. In *M. emarginata,* the maximum antimicrobial potential of root and leaf extract was practiced in opposition to *E. coli*, *F. oxisporum*, *E. cloacae* and *P. aeruginosa* respectively. However, callus extracts showed submissive inhibitory

effects against *S. aureus*, *B. cereus* and *A. solani*[102].

3. Conclusion

Antimicrobial susceptibility testing against pathogenic microorganisms is the most significant task of clinical microbiology laboratory. The major endeavor of the testing is to detect the frequency of pathogenic drug resistance and assurance of susceptibility to particular infection drugs. This information helps in the selection of appropriate antimicrobial agent, careful development of antimicrobial policies and provides data for observation. The present study was therefore designed to review the *in vitro* and *in vivo* protocols of antimicrobial bioassays of various medicinal herbal extracts against a diversity of pathogenic microorganisms. It is concluded from the review that recent testing techniques provides precise discovery of common anti-microbial resistance mechanisms.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

We are thankful to the Department of Biochemistry, Bahauddin Zakariya University, Multan, Pakistan.

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