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Anti-fungal and anti-bacterial activities of ethanol extracts of selected traditional Chinese medicinal herbs

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ABSTRACT

Objective: To evaluate *in vitro* antimicrobial activities of selected 58 ethno-medicinal plant extracts with a view to assess their therapeutic potential. **Methods:** A total of 58 traditional Chinese medicinal plants were carefully selected based on the literature review and their traditional use. The antimicrobial activities of ethanol extracts of these medicinal plants were tested against fungi (*Aspergillus fumigatus*), yeast (*Candida albicans*), gram-negative (*Acinetobacter baumannii* and *Pseudomonas aeruginosa*) and gram-positive bacteria (*Staphylococcus aureus*). The activities were tested at three different concentrations of 1.00, 0.10 and 0.01 mg/mL. The data was analysed using Gene data Screener program. **Results:** The measured antimicrobial activities indicated that out of the 58 plant extracts, 15 extracts showed anti-fungal activity and 23 extracts exhibited anti-bacterial activity. Eight plant extracts have exhibited both anti-bacterial and anti-fungal activities. For instance, *Eucommia ulmoides*, *Polygonum cuspidatum*, *Poria cocos* and *Uncaria rhyncophylla* showed activity against both bacterial and fungal strains, indicating their broad spectrum of activity. **Conclusions:** The results revealed that the ethanol extracts of 30 plants out of the selected 58 possess significant antimicrobial activities. It is interesting to note that the findings from the current study are consistent with the traditional use. A clear correlation has also been found between the antimicrobial activity and the flavonoid content of the plant extracts which is in agreement with the literature. Hence, the results presented here can be used to guide the selection of potential plant species for the isolation and structure elucidation of novel antimicrobial compounds in order to establish the structure-activity relationship. This in turn is expected to lead the way to the discovery of novel antimicrobial agents for therapeutic use.

1. Introduction

As per World Health Organization (WHO) reports, infectious diseases are responsible for over 50% deaths worldwide, occurring mainly in tropical and developing countries[1]. Socioeconomic conditions and unavailability of modern medicine are the main reasons for the people in these areas to rely on traditional medicine[1,2]. Many recent studies showed significant agreement between the traditional use of the plants by indigenous people in the treatment of specific symptoms and experimental anti-bacterial, anti-fungal, anti-cancer and anti-viral activities in the laboratory[3]. Furthermore, medicinal plant derived modern drugs such as quinine, vincristine, digoxin and digitoxin, emetine and artemisinine are considered as novel pharmaceutical compounds. A report by Gen showed that out of the 104 compounds that are used globally as drugs over 37 years,

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60 of them originated from traditional Chinese medicinal plants[4]. More recent reports reveal that some of the plant-derived flavonoids are extremely active at nanomolar levels against certain bacteria and are more active than well established antibiotics such as vancomycin and tetracycline[1-5]. Hence, screening of medicinal plants for their bioactivity is extremely important to identify promising candidates that are sources of potential therapeutic agents[1].

The epidemiology of invasive infections has significantly changed in the last 30 years. As a result, mycoses and bacterial illnesses are currently considered as emerging diseases. This is an important area that demands the discovery of new and effective anti-microbial agents to tackle antibiotic resistant strains of pathogens. Amongst several such pathogenic species, the most significant ones are (i) *Aspergillus fumigatus* (*A. fumigatus*), because of which mortality rate has steadily risen due to invasive aspergillosis, with a 357% increase from 1980-1997[6]; (ii) *Candida albicans* (*C. albicans*) is seen in almost all of the 17% of patients treated in the intensive care units who develop invasive fungal infections which are associated with significant morbidity and mortality[6,7]; (iii) *Staphylococcus aureus* (*S. aureus*) is a facultative anaerobic gram-positive coccal bacterium and it is one of the five most common causes of nosocomial postsurgical wound infections[8,9]; (iv) *Pseudomonas aeruginosa* (*P. aeruginosa*), a gram-negative bacteria, occasionally associated with opportunistic diseases of humans[10]; and (v) *Acinetobacter baumannii* (*A. baumannii*), which is also a gram-negative bacterium that is resistant to most antibiotics[11,12] and the most relevant human pathogen within the *Acinetobacter* genus. Medicinal plant based anti-microbial agents are increasingly showing their importance to tackle these invasive infections[5], and systematic screening programs will be extremely useful in this direction.

Therefore, the search for new anti-microbial agents is a high priority, especially in the tropical countries and other developing nations where infectious diseases are rampant[13]. Hence, the identification of new sources of anti-microbial compounds is an important task for the drug development and the innovative strategies that will contribute to the improvement of screening protocols are constantly evolving[14].

The approach for selecting plants varies from random selection to more guided strategies such as ethnopharmacological experience derived from traditional practice. In the current study, some plants are carefully selected on the basis of their ethnopharmacological and traditional medicinal use[15,16]. In this study, the ethanol extracts of 58 traditional medicinal plants were tested against five microbial strains, i.e., *A. fumigatus*, *C. albicans*, *A. baumannii*, *P. aeruginosa* and *S. aureus* using standard anti-microbial assays.

2. Materials and methods

2.1. Collection of medicinal plants

The dried plant materials were purchased from Beijing Tong Ren Tang Chinese Herbal Medicine shop, Sydney, Australia. The scientific names and family names are given in Tables 1-3. The plant materials were ground to a fine powder in a grinder before extraction.

2.2. Preparation of the ethanol extract

Powdered samples were extracted with 95% (v/v) ethanol

Table 1
List of plant species which showed activity in this study and also have known/reported anti-microbial activity.

S. No	Plant species	Family	Traditional use
1	*Alpinae officinarum*	Zingaberaceae	Anti-fungal[34]
2	*Eucommia ulmoides* Oliv.	Eucommiaceae Engler.	Anti-fungal[28]
3	*Hedyotis diffusa* Willd.	Rubiaceae	Anti-microbial[35]
4	*Ligustrum lucidum* Ait.	Moraceae	Anti-microbial[35]
5	*Mahonia fortunei* (Lindl.) Fedde	Berberidaceae	Anti-fungal[32]
6	*Paeouis suffuticosa* Sndr.	Ranunculaceae	Anti-microbial[35]
7	*Paris polyphylla*	Trilliaceae	Anti-fungal[33]
8	*Pleione bulbocadioides* (Franch.) Rolfe.	Orchidaceae	Anti-bacterial[36]
9	*Ploygala tenuifolia* Willd.	Polygalaceae	Anti-microbial[35]
10	*Polygonum aviculare* L.	Polygonaceae	Anti-microbial[37]
11	*Polygonum cuspidatum*	Polygonaceae	Anti-viral[35]
12	*Pseudostellaria heterophylla* (Miq.) Pax ex Pax et Hoffm.	Caryophyllaceae	Anti-fungal[30]
13	*Sarcandra glabra* (Thunb.) Nakai	Chloranthaceae	Anti-microbial[34]
14	*Schizandra chinensis* (Turcz.) Baill.	Schisandraceae	Anti-viral[34]
15	*Scutellaria barbata* Don.	Labiatae	Anti-microbial[29]
16	*Solanum nigrum* L.	Solanaceae	Anti-microbial[35]
17	*Codonopsis pilosula* Franch.	Cam-panulaceae	Anti-microbial, anti-oxidant[38]
18	*Akebia quinata* (Houtt.) Decne.	Lardizabalaceae	Anti-bacterial[35]

Table 2
List of plant species which showed activity in this study, but without reported antimicrobial activity.

S. No	Plant species	Family	Traditional use
1	*Acanltopanax senticosus*	Araliaceae	No appropriate literature
2	*Artemisia vulgaris* L.	Asteraceae	Emmenagogue[39]
3	*Aster tataricus* L.	Asteraceae	Anti-tumour[35]
4	*Atractylodes macrocephala* Koidz.	Compositae	Anti-tumour[35]
5	*Cynanchum paniculatum* (Bge.) Kitag	Asclepiadaceae	Anti-proliferative properties[40]
6	*Lysinachia christinae* Hance.	Lysimachia	Anti-cancer[35]
7	*Poria cocos* (Schw.) Wolf	Polyporaceae	Anti-cancer[35]
8	*Saposhnikovia divaricata* (Turcz.) Schischk	Apiaceae	Anti-cancer[41]
9	*Semen coicis* L.	Poaceae	Anti-cancer[35]
10	*Smilax glabra* Roxb	Smilacaceae	Anti-cancer[35]
11	*Tussilago farfara* L.	Asteraceae	Antiseptic; antiphlogistic[42]
12	*Uncaria rhyncophylla*	Rubiaceae	Antipyretic, anti-hypertensive and anticonvulsant[43]

Table 3
List of plant species which did not show activity in this study.

S. No	Plant species	Family	Traditional use
1	*Actinidia arguta* (Sieb.et Zucc.) Flarich.ex Miq.	Actinidiaceae	Anti-lipase[44]
2	*Andrographis paniculata* (Burm.f.) Wall. ex Nees	Acanthaceae	Anti-inflammatory and anti-pyretic effect[45]
3	*Asparagus cochinchinensis* (Lour.) Merr.	Asparagaceae	Anti-cancer[35]
4	*Corydalis yanhusuo* W.	Papaveraceae	Anti-cancer[35]
5	*Curcuma aromatica*	Zingiberaceae	Anti-cancer[35]
6	*Curcuma zedoaria* (Christm.) Roscoe	Zingiberaceae	Anti-bacterial[35]
7	*Cyperus rotundus* L.	Cyperaceae	Anti-bacterial[35]
8	*Ducheshea indica* (Andr.) Focke.	Rosaceae	Anti-cancer[35]
9	*Leonurus japonicus* Houtt.	Labiatae	Anti-tumour[35]
10	*Lobelia chinensis* Lour.	Campanulaceae	Anti-cancer[46]
11	*Paeonia lactiflora* Pall.	Paeoniaceae	Anti-inflammatory[35]
12	*Pinellia ternate* (Thunb.) Breit.	Araceae	Anti-tumour[35]
13	*Plantago asiatica* L.	Plantaginaceae	Anti-inflammatory[35]
14	*Platycodon grandiflorus* (Jacq.) A. DC.	Campanulaceae	Anti-inflammatory[35]
15	*Pogostemon cablin* Benth.	Asteraceae	Anti-fungal[35]
16	*Prunella vulgaris* L.	Lamiaceae	Anti-bacterial[35]
17	*Rabdosia rubescens* (Hamst) Hara.	Labiatae	Anti-inflammatory[35]
18	*Rehmannia glutinosa* (Gaertn.) Steud.	Phrymaceae	Anti-tumour[35]
19	*Rheum officinale* L.	Polygonaceae	Anti-tumour[35]
20	*Salvia miltiorrhiza* Bunge.	Caspase	Anti-tumour[35]
21	*Sanguisorba officinalis* L.	Rosaceae	Antimicrobial[42]
22	*Scutellaria baicalensis* Georgi.	Labiatae	Anti-tumour[35]
23	*Solanum lyratum* Thunb.	Solanaceae	Anti-tumour[34]
24	*Sophora flavescens* Ait.	Fabaceae	Anti-inflammatory[35]
25	*Sophora japonica* (L.) Schott.	Fabaceae	Anti-tumour[35]
26	*Spatholobus suberectus* Dunn.	Leguminosae	Anti-bacterial[35]
27	*Taxillus chinensis* (DC.) Danser	Loranthaceae	Anti-virus[35]
28	*Viscum coloratum* (Komar.) Nakai	Viscaceae	Anti-tumour[47]

on water bath at 70 °C for 6 h. The extracted samples were centrifuged and the supernatant was transferred into a 50 mL volumetric flask and adjusted the volume to 50 mL with 95% (v/v) ethanol. The samples were stored at −4 °C until testing. All extracts were filtered and dried to remove the solvent prior to the analysis. Dried extracts have been re-dissolved into appropriate sterile solvent before testing. Three different concentrations (1.00, 0.10 and 0.01 mg/mL) of each plant extract was prepared for screening anti-microbial activities.

2.3. Test microorganisms

In the present study, three bacterial strains and two fungal strains were used for assay. Anti-bacterial susceptibility was tested with the strains *S. aureus* MSSA (methicillin susceptible) EPI167[17], *A. baumannii*, a clinical isolate from MEDINA's Culture Collection, and *P. aeruginosa* PAO-1[18]. Anti-fungal susceptibility was tested with the strains *C. albicans*, clinical isolate from MEDINA's Culture Collection, *A. fumigatus* ATCC 46645 (wild-type strain) and *A. fumigatus* $\Delta akuB^{KU80}$ (lacking the non-homologous end joining pathway)[19].

2.4. Anti-infective assay on A. baumannii

Thawed stock inoculum suspension from cryovial were streaked onto Luria-Bertani agar plates (LBA, 40 g/L) and incubated at 37 °C overnight to obtain isolated colonies. Single colonies of *A. baumannii* were selected to inoculate an overnight culture to be used as assay plate inoculums. The single colonies were inoculated into Erlenmeyer flasks containing 10 mL Luria-Bertani broth medium (LB, 25 g/L in 250 mL). The flasks were incubated overnight at 37 °C with shaking at 220 r/min and were suspended until the absorbance at OD_{612} was adjusted to 0.35 in LB media and then diluted to 1:10 000 in order to obtain assay inoculums of approximately $5-6\times10^5$ CFU/mL. For the screening assay, 90 μL of the diluted inoculums were mixed with 10 μL of herbal extracts as well as ciprofloxacin and amphotericin B were included as internal plate controls. Pipetting and mixing were performed in a Tecan AQUARIUS pipetting station (Tecan, Durham, USA) and the plates used were microtiter 96-plates Costar 3370 (Corning, NY, USA). The absorbance at OD_{612} was measured with a Tecan UltraEvolution spectrophotometer (Tecan, Durham, USA) at T_0 (zero time) and immediately after that, plates were statically incubated at 37 °C for 18-20 h. After incubation, the test plates were shaken using the DPC Micromix-5 and once more the absorbance at OD_{612} was measured at T_f (final time). Percentage inhibition of growth was calculated using the following normalization equation:

$$\text{Percentage inhibition (\%)} = 100\times\{1-[(T_{f\ Sample}-T_{0\ Sample})-(T_{f\ Blank}-T_{0\ Blank})]/[(T_{f\ Growth}-T_{0\ Growth})-(T_{f\ Blank}-T_{0\ Blank})]\}. \quad (1)$$

Where, $T_{0\ Sample}$ = the absorbance of the strain growth in the presence of sample measured at zero time (before incubation);

$T_{f\ Sample}$ = the absorbance of the strain growth in the presence of sample measured at final time (after incubation);

$T_{0\ Growth}$ = the absorbance of the strain growth in the absence of sample measured at zero time (before incubation);

$T_{f\ Growth}$ = the absorbance of the strain growth in the absence of sample measured at final time (after incubation);

$T_{0\ Blank}$ = the absorbance of the broth medium (blank) measured at zero time (before incubation);

$T_{f\ Blank}$ = the absorbance of the broth medium (blank) measured at final time (after incubation).

An extract was considered to have activity when its percentage of inhibition was more than 60%.

2.5. Anti-infective assay on S. aureus

Frozen stocks were used to inoculate LB agar plates containing 15 μg/mL of chloramphenicol for confluent growth. Plates were incubated for 18-20 h at 37 °C. The grown colonies of *S. aureus* were harvested from the LB agar plates and suspended in LB broth medium until the absorbance at OD_{600} was adjusted to 0.3. This inoculum was used to make a second dilution of 1:100 (containing 34 μg/mL of chloramphenicol) of which the concentration was approximately 6×10^5 CFU/mL. Screening assay was performed as described above for *A. baumannii*. Amphotericin B and penicillin G were used respectively as negative and positive controls at each plate. Percentage of growth inhibition was calculated using the same normalization equation as that for *A. baumannii*. An extract was considered to have activity when its percentage of inhibition was more than 60%.

2.6. Anti-infective assay on P. aeruginosa

Thawed stock inoculum suspension from cryovials was streaked onto LB agar plates and incubated at 37 °C overnight to obtain isolated colonies. Single colonies of *P. aeruginosa* were selected to inoculate an overnight culture to be used as assay plate inoculums. The single colonies were inoculated into 10 mL LB medium in 250 mL Erlenmeyer flasks. The flasks were incubated overnight at 37 °C with shaking at 220 r/min. The overnight culture is adjusted to obtain an assay inoculum of approximately 5×10^5 CFU/mL. As reference, the OD_{600} of a 1:20 dilution of the overnight culture is 0.35. Screening assay was performed as described above for *A. baumannii*. Ciprofloxacin and amphotericin B were used as internal controls at each plate. Percentage of growth inhibition was calculated using the same normalization equation as that for *A. baumannii*. An extract was considered to have activity when its percentage of inhibition was more than 60%.

2.7. Anti-infective assay on C. albicans

Thawed stock inoculums suspensions from cryovials were streaked on Sabouraud Dextrose Agar (SDA, 65 g/L) plates for confluent growth. Plates were incubated for 18 h at 37 °C. The *C. albicans* colonies were harvested from the SDA plates and suspended in RPMI-1640 modified medium in order to

prepare an inoculum adjusted to an optical density of 0.25 at 660 nm. Modified medium RPMI-1640 was prepared as follows: 20.8 g of RPMI powder (Sigma) was poured into a 2 L flask, together with 13.4 g of YNB, 1.8 L of Milli-Q water, 80 mL of 1 mol/L Hepes and 72 mL of 500 g/L glucose. The volume was adjusted at 2 L and filtered.

The adjusted suspension was diluted 1:10 and kept on ice until being used to inoculate 96-well microtiter plates. Screening assay was performed as described above for *A. baumannii*. Amphotericin B and penicillin G were used respectively as positive and negative controls at each plate. Percentage of growth inhibition was calculated using the same normalization equation as that for *A. baumannii*. An extract was considered to have activity when its percentage of inhibition was more than 60%.

2.8. Anti-infective assay on A. fumigatus

The high throughput *A. fumigatus* susceptibility assay was applied on two stains, *A. fumigatus* ATCC 46645 (wild-type strain) and *A. fumigatus* $\Delta akuB^{KU80}$, generally used in genetic manipulation experiments. Anti-fungal activity was scored using resazurin, a non-fluorescent blue dye that reduction is converted to the pink colored highly fluorescent. Resazurin is an oxidation-reduction indicator of eukaryotic cell viability and its utility for assessing cell viabilities has been demonstrated[20,21].

For testing of *A. fumigatus* isolates, a conidial suspension was prepared after subculture on PDA medium. The colonies were harvested from the PDA plates and suspended in RPMI-1640 modified medium[22]. Modified medium RPMI-1640 was prepared as describes above for *C. albicans*. The inoculum concentration size was ~2.5×10^4 CFU/mL (determined by counting in a Neubauer chamber) and resazurin was at final concentration of 0.02 g/L. For the screening assay, 7 μL/well of the samples and 150 μL/well of the inoculum were dispensed in 96-well microtiter plates. Amphotericin B was used as positive control. After dispensing the plates were statically incubated at 37 °C for 25-30 h.

After incubation, the plates were read on VICTOR2 multilabel counter (Perkin Elmer™) using wavelength settings for resazurin (excitation 570 nm, emission 600 nm). The percentage of resazurin reduction and growth inhibition was calculated using the following normalization equations:

Percentage of reduction (%) = (Fluorescent intensity of test agent-Fluorescent intensity of untreated control)/(Fluorescent intensity of reduced resazurin-Fluorescent intensity of untreated control)×100 (2)

Percentage of inhibition (%) = 100-Percentage of reduction (3)

The extract was considered to have activity when its percentage of inhibition was more than 60%.

2.9. Statistical analysis

The anti-microbial screening data were analysed using the Genedata Screener program (Genedata AG, Switzerland). The Z′ factor predicts the robustness of an assay by taking into account the mean and standard deviation of both positive and negative controls. The Z′ factors were calculated using the equation:

$$Z' = 1-[3\times(\sigma p-\sigma n)/(\mu p-\mu n)]. \quad (4)$$

Where, p is the measured parameter with positive control, n is the measured parameter with negative control, σ is the standard deviation and μ in the mean.

In general, a Z′ factor value between 0.5-1.0 are considered an excellent assay[23]. The Robust Z′ factor (RZ′ factor) is based on the same formula as the Z′ factor, but standard deviations and means are replaced by the robust standard deviations and medians, respectively. In all experiments performed in this work, the RZ′ factor obtained was between 0.85-0.95. The comparative analysis of various data sets was done using statistical correlation.

3. Results

In this study, the ethanol extracts of 58 plants belonging to 45 families were examined for their activity against fungi, yeast, gram-negative and gram-positive bacteria (*A. fumigatus*, *C. albicans*, *A. bumanni*, *P. aeruginosa* and *S. aureus*). A total of 30 plant extracts showed significant anti-microbial activities against these test microbial strains.

3.1. Anti-fungal activity of selected plant extracts

The anti-fungal activities of the selected plant extracts were tested against two kinds of fungal species, namely, *C. albicans* representing the yeast model and *A. fumigatus* representating filamentous fungus. The activities of these extracts were evaluated in a dose-response curve with 1.00, 0.10 and 0.01 mg/mL concentrations. These results are presented in Table 4. These results revealed that 13 plant extracts, namely, *Solanum nigrum*, *Poria cocos* (*P. cocos*), *Eucommia ulmoides* (*E. ulmoides*), *Atractylodes macrocephala*, *Polygonum cuspidatum* (*P. cuspidatum*), *Ligustrum lucidum*, *Ploygala tenuifolia*, *Saposhnikovia divaricata*, *Mahonia fortunei*, *Cynanchum paniculatum*, *Lobelia chinensis*, *Aster tataricus* and *Uncaria rhyncophylla* (*U. rhyncophylla*), showed high inhibition activity against *A. fumigatus* (that represents filamentous fungus). However, only two plant extracts, namely, *Codonopsis pilosula* and *Tussilago farfara*, showed high inhibition effect against *C. albicans* (that represents the yeast model). These anti-fungal activities suggest that different plant extracts may contain very selective target compounds that have specific selectivity towards *A. fumigatus* and *C. albicans*. The results are very significant as most of the plants showed an inhibitory effect not only at the concentration of 1.00 mg/mL but also at 0.10 mg/mL.

3.2. Anti-bacterial activity of selected plant extracts against gram-negative bacteria

The strains used for the gram-negative assay were *A. baumannii* and *P. aeruginosa* PAO-1 from MEDINA's Culture

Table 4
Anti-fungal activities of medicinal plant extracts towards *Aspergillus fumigatus* and *Candida albicans* (%).

S. No	Plant species	*A. fumigatus* ATCC 46645 and Δaku^{BKU80} strains			*C. albicans* MEDINA collection strain		
		1.00 mg/mL	0.10 mg/mL	0.01 mg/mL	1.00 mg/mL	0.10 mg/mL	0.01 mg/mL
1	*Solanum nigrum*	66±4	30±5	10±3	14±10	13±2	4±3
2	*Poria cocos*	88±1	66±6	22±8	17±12	20±2	9±2
3	*Codonopsis pilosula*	55±2	11±5	4±4	100±0	100±0	4±1
4	*Eucommia ulmoides*	64±10	40±13	8±6	12±10	10±1	2±2
5	*Atractylodes macrocephala*	86±1	56±3	3±4	22±13	10±0	4±2
6	*Polygonum cuspidatum*	63±6	3±5	16±1	1±13	6±1	2±2
7	*Ligustrum lucidum*	71±4	33±5	5±7	5±14	10±1	3±2
8	*Ploygala tenuifolia*	69±13	24±6	2±5	0±13	13±1	3±2
9	*Saposhnikovia divaricata*	70±3	18±8	5±2	1±15	10±1	3±2
10	*Mahonia fortunei*	93±0	65±1	12±1	7±14	9±2	6±2
11	*Cynanchum paniculatum*	73±1	50±2	17±4	8±15	5±0	1±2
12	*Lobelia chinensis* Lour	84±1	55±3	17±6	3±13	7±0	4±3
13	*Tussilago farfara*	2±2	6±5	15±6	76±3	27±4	5±3
14	*Aster tataricus*	65±1	36±7	18±7	4±15	9±1	2±3
15	*Uncaria rhyncophylla*	68±9	42±7	9±6	2±14	12±1	2±2

All data are expressed as mean±SEM. Mean: Mean value of % inhibition of growth.

Collection. The inhibition effects of the plant extracts against these two strains are tabulated in Table 5. The dose response curve was constructed with three dilution points at 1.00, 0.10 and 0.01 mg/mL. The plant extracts of *Artemisia vulgaris* (*A. vulgaris*), *Sarcandra glabre*, *Polygonum aviculare*, *Akebia quinata* and *Scutellaria barbata* (*S. barbata*) exhibited high inhibitory activity against both of the gram-negative strains tested (*A. baumannii* and *P. aeruginosa*). On the other hand, the extracts of *P. cocos*, *E. ulmoides*, *P. cuspidatum*, *Acanltopanax senticosus*, *U. rhyncophylla* and *Hedyotis diffusa* showed significant inhibitory activity against only *A. baumannii* bacteria. High anti-bacterial activity (towards gram-negative bacteria) of tweleve plant extracts out of 58, against *A. baumannii* compare to *P. aeruginosa* bacteria is an extremely important finding.

3.3. Anti-bacterial activity of selected plant extracts against gram-positive bacteria

S. aureus EPI-167 was used in this study as representative gram-positive bacteria. The inhibitory effects of plant extracts against this strain are presented in Table 6. Several plant extracts were found to inhibite the proliferation of this gram-positive bacterium at the concentration of 1.00 mg/mL. Twenty-three plant extracts out of 58 showed a significant inhibitory effect against *S. aureus*. Noticeably, all of the extracts that showed inhibitory effect in gram-negative bacteria also exhibited their activity against gram-positive bacteria indicating that these plant extracts may have a broad spectrum of anti-microbial activities.

Table 5
Anti-bacterial activities of medicinal plant extracts towards *Acinetobact baumannii* and *Pseudomon aeruginosa* (gram-negative bacteria) (%).

S. No	Plant species	*A. baumannii* MEDINA collection strain			*P. aeruginosa* PAO-1 strain		
		1.00 mg/mL	0.10 mg/mL	0.01 mg/mL	1.00 mg/mL	0.10 mg/mL	0.01 mg/mL
1	*Artemisia vulgaris*	98±1	48±2	15±3	65±5	8±0	4±1
2	*Sarcandra glabra*	73±2	37±6	4±3	53±2	5±5	2±3
3	*Poria cocos*	126±8	66±3	23±8	7±9	3±1	6±1
4	*Eucommia ulmoides*	134±2	60±3	5±8	37±33	3±2	5±1
5	*Polygonum cuspidatum*	60±6	1±10	8±4	24±11	4±2	10±0
6	*Polygonum aviculare*	81±3	45±4	6±7	74±6	9±0	4±1
7	*Akebia quinata*	71±5	47±7	5±10	66±2	11±2	8±1
8	*Alpinae officinarum*	88±7	23±2	6±11	32±9	2±2	1±0
9	*Scutellaria barbata*	90±5	32±3	18±4	49±5	6±1	7±1
10	*Acanltopanax senticosus*	94±25	45±4	1±2	31±14	1±1	2±1
11	*Uncaria rhyncophylla*	62±11	20±14	6±3	2±8	6±1	8±2
12	*Hedyotis diffusa*	94±21	45±8	17±5	26±1	2±1	4±0

All data are expressed as mean±SEM. Mean: Mean value of % inhibition of growth.

Table 6
Anti-bacterial activities of medicinal plant extracts towards *Staphylococcus aureus* (gram positive bacteria) (%).

S. No	Plant species	*S. aureus* EPI-167 strain		
		1.00 mg/mL	0.10 mg/mL	0.01 mg/mL
1	*Artemisia vulgaris*	97±1	26±1	0±1
2	*Sarcandra glabra*	98±1	57±3	2±1
3	*Poria cocos*	130±7	49±7	0±2
4	*Pseudostellaria heterophylla*	101±1	8±1	0±2
5	*Paeouis suffruticosa*	103±3	10±1	1±1
6	*Eucommia ulmoides*	117±3	85±17	2±1
7	*Polygonum cuspidatum*	101±2	1±1	1±2
8	*Ligustrum lucidum*	104±3	8±2	0±1
9	*Ploygala tenuifolia*	99±1	8±2	4±2
10	*Polygonum aviculare*	100±0	25±2	2±1
11	*Akebia quinata*	68±0	38±4	2±2
12	*Alpinae officinarum*	103±2	16±14	2±1
13	*Scutellaria barbata*	94±2	14±3	1±3
14	*Schizandra chinensis*	102±2	11±1	1±1
15	*Acanltopanax senticosus*	103±1	6±4	1±1
16	*Paris polyphylla*	99±4	6±2	2±2
17	*Smilax glabra*	97±0	7±1	1±1
18	*Pleione bulbocadioides*	99±4	36±11	2±1
19	*Tussilago farfara*	105±1	6±2	1±2
20	*Aster tataricus*	97±0	9±1	1±1
21	*Uncaria rhyncophylla*	99±1	12±2	1±1
22	*Hedyotis diffusa*	61±13	16±1	11±3
23	*Semen coicis*	97±3	17±2	5±2

All data are expressed as mean±SEM. Mean: Mean value of % inhibition of growth.

4. Discussion

Infective diseases due to microbes such as *A. fumigatus*, *C. albicans*, *A. baumannii*, *P. aeruginosa* and *S. aureus*, are extremely prevalent and responsible for mortality around the world[1-9]. The mortality rate may continue to increase in the future for two reasons: (i) lack of sufficient supply of antibiotics in developing countries and (ii) non-availability of anti-microbial agents to tackle antibiotic resistant strains of pathogens. Medicinal plants have received a significant attention as new sources of anti-microbial agents to address these issues. The synergistic effect of plant based flavonoids in combination with conventional antibiotics is proving to be an important new direction in complementary medicine research[5]. In this respect, it is important to note that less than 10% of higher plant species are analysed for their bioactivity[24] and hence present great potential.

In the current study, 30 out of the carefully selected 58 plant species displayed anti-microbial properties against one or more microbial strains, while remaining were found to be inactive. Of all the 30 active species, 23 plants were found to inhibit gram-positive bacterial strains (*S. aureus*) and 12 of these plants also showed inhibition against the gram-negative bacteria (*A. baumannii* and *P. aeruginosa*). On the other hand, 15 plant extracts showed anti-fungal properties by inhibiting either *A. fumigatus* or *C. albicans*. The results suggest that more plants possess anti-bacterial properties than anti-fungal properties, while eight plant species showed both anti-bacterial and anti-fungal properties. Literature strongly support that plants with high polyphenols and flavonoids contents display anti-microbial properties[5,25]. This further supports the anti-microbial activities of some of the plant extracts in the present study are likely to be due to their high flavonoid content[26].

It is interesting to note that some of the plant extracts showed their broad spectrum of anti-bacterial activities. For instance, the extracts of *A. vulgaris*, *P. aviculare* and *A. quinata* showed activity against both gram-positive (*S. aureus*) and gram-negative bacteria (*P. aeruginosa* and *A. baumannii*). Some plants, namely, *E. ulmoides*, *P. cuspidatum*, *P. cocos* and *U. rhyncophylla* showed activity towards both bacterial strains (*A. baumannii*, and *S. aureus*) and fungal strains (*A. fumigatus*).

Some plants exhibited specificity against specific microbial strains. For instance, the anti-fungal activity of many plants is more towards the filamentous fungus *A. fumigatus* while the activity is less towards the yeast *C. albicans*. Seven plants, namely, *P. heterophylla*, *P. suffuticosa*, *S. chinensis*, *A. senticosus*, *P. polyphylla*, *P. bulbocadioides* and *S. coicis*, showed their specificity against only gram-positive bacteria (*S. aureus*). Plants produce several secondary metabolites that include phenolics, flavones, flavonoids, flavonols, tannins, coumarins, alkaloids, lectins, polypeptides and other compounds and these classes of bioactive compounds

support the plant defence against the predators such as microorganisms, insects and herbivores[25]. These compounds have also been confirmed in the literature to possess anti-oxidant, anti-inflammatory and anti-infectious properties[5,25,26]. Plants with high concentrations of flavonoids have been established in previous studies to possess anti-microbial activity[5,25,26]. Consistent with the literature, some of the active plants reported in this paper have significant levels of flavonoid content[26]. It can be assumed that the varied activities of the plants studied here could be mainly due to the presence of differing quantities of lead anti-microbial compounds[1]. Further bioassay guided fractionation; isolation and characterization of major bioactive constituents is likely to provide an answer to this synergism.

The results presented in this paper strongly support anti-microbial properties of many of the plants studied. Most of the extracts of plants were used traditionally in the form of formulations/individual extracts to treat viral, bacterial and fungal infectious diseases or related symptoms. Interestingly, these plants displayed significant anti-bacterial or anti-fungal properties, showing concordance with traditional use. For example, *P. cuspidatum*, *Sarcandra glabra*, *Pleione bulbocodioides* and *Alpinia officinarum* were traditionally used to treat different infectious diseases, and these plants showed significant activity against different microbial strains.

It should be noted that anti-microbial agents have been previously isolated from some of the plants studied here[27-33]. Two anti-fungal peptides, EAFP1 and EAFP2, that were reported from *E. ulmoides* showed broad spectra of anti-fungal activity against eight fungal stains[28]. In this study, *E. ulmoides* has shown significant anti-fungal activity against filamentous fungus *A. fumigatus* and also anti-bacterial activity against *A. baumannii* and *S. aureus* strains. These observed activities may therefore be attributed to the presence of active peptides in this plant. Essential oils including hexahydro-farnesylacetone, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, menthol and 1-octen-3-ol, which have been isolated from *S. barbata* showed anti-microbial activity against 17 microorganisms[29]. Interestingly, the studies carried out by Yu *et al.*[29] and also the results of present study demonstrate that *S. aureus* is highly sensitive to the extract of *S. barbata*. Lectin and a Kunitz-type trypsin inhibitor isolated from *P. heterophylla* exhibited anti-fungal activity towards *Fusarium oxysporum*[30], while this plant showed anti-bacterial activity against *S. aureus* in the present study.

Some plants were used as anti-microbial agents in the traditional medicine, while these plants did not display activity against any of the selected strains. However, the plants displayed significant anti-infective properties against different microbial strains studied here. To the best of our knowledge, the anti-microbial activities of these twelve plants are reported for the first time in this study.

In summary, amongst the carefully selected 58 traditional Chinese medicinal herbs studied in this work, 30 of them showed significant anti-microbial activity against different microbial strains. Sixteen of active plants confirm their promising anti-microbial activity based on the previous reports and their traditional use, which is in agreement with the current findings. In addition, a good correlation has been found between the anti-microbial activity and the flavonoid content of some of the plant extracts[26]. Hence, the extracts of these 16 plants could be considered as possible candidates for anti-microbial drug discovery. The anti-microbial activities of the twelve plants are reported for the first time in this study. Some of the extracts showed no activity against any of the microbial strains studied here, which might be due to the lack of anti-microbial agents in these plants or they may be selectively active against other pathogens that are not investigated in this paper. However, more studies directed towards the isolation of bioactive compounds from active plant extracts, determination of their structures, establishing structure-activity relationship, evaluation of the mechanism of action and *in vivo* studies including toxicity measurements are expected to lead the way towards new anti-microbial drug development. Currently, isolation and characterisation of anti-microbial agents from *U. rhyncophylla* and *P. cuspidatum* is in progress in our laboratory.

Conflict of interest statement

We declare we have no conflict of interest.

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