PHYTOCOMPOUNDS OF THREE MEDICINAL PLANTS (JUNIPERUS COMMUNIS, URTICA DIOICA AND COLEUS FORSKOHLII) OF NORTH WEST HIMALAYAS INCREASES THE POTENCY OF ANTIBACTERIAL AND ANTIFUNGAL ANTIBIOTICS

Rajan Rolta¹, Vikas Kumar¹, Anuradha Sourirajan¹, Navneet Kumar Upadhyay² and Kamal Dev¹*¹
¹Faculty of Applied sciences and Biotechnology, Shoolini University of Biotechnology and Management Sciences, Bajhol, PO- Sultanpur, District Solan-173229, Himachal Pradesh, India.
²School of Pharmaceutical Sciences, Shoolini University of Biotechnology and Management Sciences, Bajhol, PO- Sultanpur, District Solan-173229, Himachal Pradesh, India.
*Corresponding author email : kamaldevbhardwaj1969@gmail.com

Abstract

The present study was designed to test the synergistic potential of three medicinal plants (Juniperus communis, Urtica dioica and Coleus forskohlii) with antibacterial (Tetracycline, erythromycin and chloramphenical) and antifungal (fluconazole) antibiotics. Total phenolic and flavonoid content was highest in C. forskohlii (250±4.54 mg g⁻¹ GAE) and (270±2.2 mg g⁻¹ RE). Among three medicinal plants, methanolic extract of leaves of C. forskohlii showed the highest antioxidant potential (DPPH and ABTS method IC₅₀ 14.3±0.23 and 5.22±0.1 μg ml⁻¹ respectively). Methanolic extract of J. communis, U. dioica and C. forskohlii showed the comparative antimicrobial activity against bacterial (E. coli ATCC25922, S. aureus ATCC 29213 and K. pneumoniae MTCC39) and fungal strains (C. albicans ATCC90028 and MTCC277) as measured by broth dilution method. Methanolic extracts of J. communis, U. dioica and C. forskohlii showed synergistic activity in combination with antibacterial and antifungal antibiotics against bacterial and fungal pathogens and caused reduction in MIC of antibiotics by 4-515 folds. Furthermore, HPTLC quantification showed higher amount of quercetin in the methanolic extracts of C. forskohlii (168.6367μg mg⁻¹) followed by U. dioica (159.3197μg mg⁻¹) and J. communis (142.306 μg mg⁻¹). The present study highlighted the importance of selected medicinal plants in enhancing the activity of antibiotics against pathogenic bacteria and fungi.

Keywords: Medicinal herbs, Phytocompounds, Synergistic activity and HPTLC.

Introduction

Infectious diseases are spreading rapidly in human and animals. Throughout the history of mankind, infectious diseases have remained a major cause of death and disability. The discovery of antibiotics was an essential part in combating bacterial infections that once ravaged humankind. Different antibiotics exercise their inhibitory activity on different pathogenic organisms. The development and spread of resistance to currently available antibiotics is a worldwide concern. An estimation of the exact economic impact of resistant pathogen infections is still a huge global challenge and antibiotic resistance is a substantial economic burden to the whole world (Guidos, 2018). Now a day’s clinically important bacteria and fungi are showing multiple drug resistance it is the legacy of past decades of antimicrobial use and misuse (levy 2002). According to World health organization report (WHO, 2018), about 50,000 people all over the world die every day due to multiple drug resistance. Multidrug resistance (MDR) in bacteria enhances the burden of infection as well as increases the severity of infections both in hospital and community. This ultimately results in inferior treatment by the new generation antibiotics (Handzlik et al., 2013). Gram-negative bacteria are responsible for more than 30% of hospital-acquired infections of which E. coli is responsible for majority of urinary tract infections (Peleg and Hooper, 2010).

Traditionally, all over the world medicinal plants and their compounds have been suggested as potential alternative of antibiotics against infectious diseases (Scandorieiro et al., 2016). Herbal medicine plays a major role in the health systems of many developing nations because of their far-reaching availability and rich traditional knowledge (Effert et al., 2017). The emphasis on the use of medicinal plants had hitherto been placed on the treatment rather than prevention of diseases (Sofowora et al., 2013). Plants have always been known to be a source of drugs because of their compounds work as natural blueprint or may be directly used as phytomedicine (Gibbons, 2005). Structural diversity the secondary metabolite has now placed plants as promising source of natural antimicrobial agents (Aiyegoro and Okoh, 2009).

Synergistic actions developed from the constituents of the plants are vital for treatment and thus medicinal plants are widely used (Jamshidi-Kia et al., 2018). The shift towards traditional ethnopharmacy coupled with increase in preference for natural bioactive compounds in healthcare system encourages exploiting the natural phenolics from unexplored species so as to relieve the tremendous pressure on the natural stock of known medicinal plants. Therefore, the current study was focused on phytochemical, antioxidant, Antimicrobial and synergistic activity of methanolic extract of leaves of Juniperus communis, Urtica dioica and Coleus forskohlii medicinal plants of Himalayan region (Figure 1). The detail of the plants, their family, plant part used and importance in traditional medicine have been listed in table 1.
Table 1: Description of medicinal plants, their common name, family, plant part used and their traditional importances.

<table>
<thead>
<tr>
<th>Medicinal plants</th>
<th>Family</th>
<th>Common names</th>
<th>Plant Part used</th>
<th>Pharmacological properties</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Juniperus communis</em></td>
<td>Cupressaceae</td>
<td>Bethda</td>
<td>Leaves</td>
<td>The heartwood oil is used for massage to cure boils (Lal and Singh, 2008).</td>
</tr>
<tr>
<td><em>Urtica dioica</em></td>
<td>Urticaceae</td>
<td>Bichu</td>
<td>Leaves</td>
<td>The young leaves are used as blood purifier and cure skin diseases (Vidyarthi et al., 2013).</td>
</tr>
<tr>
<td><em>Coleus forskohlii</em></td>
<td>Lamiaceae</td>
<td>Makandi</td>
<td>Leaves</td>
<td>Intestinal Ulcer, wound healing, tumours, cancer (Garbyal et al., 2005).</td>
</tr>
</tbody>
</table>

Fig. 1: Medicinal plants in their natural habitat, *J. communis* (A), *U. dioica* (B) and *C. forskohlii* (C).
where \( A_{\text{control}} \) is the absorbance of control and \( A_{\text{sample}} \) is the absorbance of the test/standard.

**ABTS radical scavenging assay**

ABTS scavenging activity was calculated using the method described by Re et al. (1999). The percentage ABTS radical scavenging activity was calculated using the following equation:

\[
\text{ABTS radical scavenging activity} (\%) = \left( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100
\]

where \( A_{\text{control}} \) is the absorbance of ABTS radical + methanol; \( A_{\text{sample}} \) is the absorbance of ABTS radical + sample extract/standard.

**Growth inhibition activity of methanolic extract of leaves of J. communis, U. dioica and C. forskohlii**

Growth inhibition activity of methanolic extract of leaves of *J. communis*, *U. dioica* and *C. forskohlii* were evaluated using agar well diffusion method and MIC microdilution assay. Three bacterial (*S. aureus* ATCC29213, *K. pneumoniae* ATCC359 and *E. coli* ATCC25922) and two fungal strains (*Candida albicans* ATCC90028 and ATCC277) were used to test the antimicrobial activity of methanolic extract of leaves of *J. communis*, *U. dioica* and *C. forskohlii*. All the bacterial and fungal strains were procured from IMTECH, Chandigarh and maintained at Yeast Biology Lab, Shoolini University, Solan, Himachal Pradesh, India. The bacterial strains were grown in nutrient broth (NB) at 37 °C, while fungal strains were grown in yeast peptone dextrose (YPD) broth at 30 °C with shaking at 200 rpm. The cell turbidity of the culture was adjusted to 0.5 McFarland standards, which is approximately equivalent to 2×10⁸ colony forming units (CFU) ml⁻¹ for antimicrobial assay. Fluconazole (25 µg) and Amoxyclav filter disk (10 µg) purchased from Himedia Biosciences, Mumbai (India) were used as a positive control and dimethyl sulphoxide (DMSO) and methanol alone were used as negative control in the antimicrobial assay.

**Agar well diffusion and broth dilution assays for measuring antimicrobial activity of methanolic extract of rhizome of J. communis, U. dioica and C. forskohlii.**

In agar well diffusion method (Perez et al., 1990), growth inhibition activity of extracts was expressed in terms of diameter of zone of inhibition against the growth of bacteria and fungi on NB/YPD agar plates. Zone of inhibitions (mm) were measured using Hi Antibiotic Zone scale-C (Himedia Biosciences, Mumbai (India)). The tests were performed in triplicate and results were recorded as mean ± SD.

MIC of the methanolic extracts of leaves of *J. communis*, *U. dioica* and *C. forskohlii* and antibacterial (erythromycin, chloramphenicol and tetracycline) and antifungal (fluconazole and amphotericin B) antibiotics was measured by broth dilution method described under CLSI guidelines using 5-triphenyl tetrazolium chloride (CLSI, 2012). The methanolic extracts were dissolved in DMSO and geometric dilutions ranging from 500–0.025 µg/ml were prepared in a 96-well microtiter plate, including one growth control (broth containing DMSO) and a positive control (broth inoculated with bacterial or fungal culture and containing amoxyclav (25 µg) and amphotericin B (25 µg) or fluconazole (25 µg). Following incubation period of 18 h for bacteria and 48h for fungi, 5-triphenyl tetrazolium chloride (5 µg) was added to each well and incubation was continued for another 2h. Change in color from purple to pink or colorless was observed and used as a measure to calculate MIC. The lowest concentration at which color change appeared was considered as the MIC of the test compound or antibiotics described previously by Rolta et al. (2018a).

**Synergistic enhancement of antibacterial activity of antibacterial antibiotics (erythromycin, chloramphenicol, tetracycline) and antifungal antibiotics (fluconazole and amphotericin B) in combination with methanolic extract of leaves of J. communis, U. dioica and C. forskohlii**

Synergistic interaction of phytocompounds present in methanolic extract of leaves of *J. communis*, *U. dioica* and *C. forskohlii* in combination with antibacterial (erythromycin, chloramphenicol, tetracycline) and antifungal antibiotics (fluconazole and amphotericin B) was determined by using checkerboard method (Bonapace et al., 2002; Eumkeb et al., 2012; Dev et al., 2017 and Rolta et al., 2018b). Synergistic potential of all the extracts were expressed in terms of fractional inhibitory concentration index (FICI). The interactions were classified as synergistic when FICI value ≤ 0.5, additive (FICI ≥ 0.5–1.0), indifferent (FICI ≥ 1.0 ≤ 4.0) or antagonistic (FICI > 4.0) (Ahmad et al., 2006).

Briefly, combinations of MIC of methanolic extract of leaves of *J. communis*, *U. dioica* and *C. forskohlii* and antibacterial and antifungal agents were prepared and broth dilution method was performed to determine FIC index. FIC value for each extract was calculated using the following formula:

\[
\text{FICI} = \frac{\text{MIC of Antibiotics}}{\text{MIC of Antibiotic alone}} + \frac{\text{MIC of Extract}}{\text{MIC of Extract alone}}
\]

Where, \( \text{MIC of Antibiotics} = \text{MIC of antibiotic in combination/MIC of antibiotic alone} \); \( \text{MIC of Extract} = \text{MIC of extract in combination/MIC of extract alone} \)

**Quantification of quercetin in methanolic extract of U. dioica, J. communis and C. forskohlii by HPTLC Method**

The amount of quercetin in crude methanolic extract of *J. communis*, *Urtica dioica* and *Coleus forskohlii* was quantified by using CAMAG HP-TLC system consisting of an automatic Linomat V sample applicator, a chamber for developing TLC and a CAMAG TLC scanner for densitometric evaluation of chromatograms. CATS 4 software was used for interpretation of results. Stock solution of quercetin was prepared at concentration of 1 mg ml⁻¹ in HPLC grade methanol. 7 µl of methanolic extracts of *U. dioica*, *J. communis* and *C. forskohlii* were spotted on TLC plate (Silica gel 60 F254 HP-TLC aluminum sheets [10×10] cm with 0.2 mm thickness from E. Merck, USA) using a sample applicator and allowed to separate up to a distance of 9 cm height using toluene: ethyl acetate: formic acid (5:4:1, v/v) as a mobile phase. The amount of quercetin in each extract was calculated from the standard curve of quercetin (1-9 µg). The developed TLC plate was dried and scanned densitometrically using TLC scanner 3 with software WINCATS at 566 nm.

**Results and Discussion**

**Qualitative analysis of phytochemicals of methanolic leaves extracts of J. communis, U. dioica and C. forskohlii**

Results of preliminary screening of methanolic extracts of *J. communis*, *C. forskohlii* and *U. dioica* were summarized...
in Table 2. Screening of phytochemicals revealed the presence of phenolics, tannin, flavonoids, carbohydrates and glycosides in methanolic extracts of J. communis; phenolics, tannin, flavonoids, alkaloids and saponins were detected in C. forskohlii and phenolics, tannin, flavonoids, carbohydrates, glycosides and saponins were detected in U. dioica. In contrast to our study, Meena et al. (2012) reported the presence of coumarins, flavonoids, alkaloids, glycosides, tannins, sugars and terpenoids in n-hexane, chloroform, ethyl-acetate, alcoholic and water extract of J. communis. Rajkumar and Malathi (2015) reported the presence of carbohydrates and glycosides in aqueous, chloroform and ethanolic extracts of leaves of C. forskohlii. Singh and Sengar (2019) reported the presence of proteins, amino acids, carbohydrates, flavonoids, tannins, phenols and alkaloids in the ethanolic extract of leaves of U. dioica collected from Dehradun, Rudraprayag and Puri region of Uttrakhand, India.

Table 2: Qualitative estimation of phytochemicals in methanolic extracts of J. communis, U. dioica and C. forskohlii

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>J. Communis</th>
<th>C. forskohlii</th>
<th>U. dioica</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenolics and Tannins</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloïds</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponin</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

‘+’ indicates the presence of phytoconstituents and ‘-’ sign indicates the absence of phytoconstituents.

Quantification of TPC and TFC in methanolic extracts of J. communis, U. dioica and C. forskohlii

TPC of methanolic extract of J. communis, C. forskohlii and U. dioica was calculated from standard curve of gallic acid (Y = 0.002x + 0.002, R² = 0.953); whereas the estimation flavonoid content was done using standard curve of rutin (Y = 0.012x + 0.080, R² = 0.944). Amount of TPC was higher in C. forskohlii (250 ± 5.44 µg ml⁻¹ GAE), followed by U. dioica (220 ± 2.14 mg g⁻¹ GAE) and J. communis (20.81 ± 2.21 mg g⁻¹ GAE). TFC was found to be higher in C. forskohlii (270 ± 2.2 mg g⁻¹ RE), followed by J. communis (178.56 ± 5.56 mg g⁻¹ RE) and U. dioica (112 ± 1.2 mg g⁻¹ RE) as shown in Table 3. Study from Fierscu et al. (2018) reported 13.44 ± 0.14 mg linoleal equivalent of total terpenoids, 19.23 ± 1.32 mg gallic acid equivalent of phenolic content and 51096 ± 4.7 mg rutin equivalent of flavonoid content in hydro alcoholic extract of J. communis. Pandey et al. (2018) have reported TPC (9.165 mg g⁻¹ GAE equivalent) and TFC (14.03 mg equivalent RE g⁻¹) in essential oil of J. communis. Similarly, Khatun et al. (2011) reported the TPC in ethanolic extracts of tubers (27.05 µg catechol equivalents g⁻¹ dry tissue), roots (24.22 µg catechol equivalents/g dry tissue) and stem (21.26 µg catechol equivalents/g dry tissue). TFC in tubers (4.37 mg rutin equivalents/g dry tissue) was found to be significantly higher (P < 0.05) as compared to other parts of C. forskohlii. Kurkic et al. (2012) reported total phenolics (TPC 208.37 ± 4.38 mg GAE g⁻¹) and flavonoids (TFC 20.29 ± 0.48 quercetin g⁻¹) in ethanolic extract of U. dioica leaves.

Table 3: Quantitative estimation of total phenolics and flavonoids content in methanolic extract of J. communis, U. dioica and C. forskohlii

<table>
<thead>
<tr>
<th>Medicinal plants</th>
<th>Plant part used</th>
<th>Total phenolic content, TPC (µg ml⁻¹ GAE)</th>
<th>Total flavonoid content, TFC (µg ml⁻¹ RE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>J. communis</td>
<td>Leaves</td>
<td>20.81±2.21</td>
<td>112±1.2</td>
</tr>
<tr>
<td>U. dioica</td>
<td>Leaves</td>
<td>220±2.14</td>
<td>178.56±2.56</td>
</tr>
<tr>
<td>C. forskohlii</td>
<td>Leaves</td>
<td>250±4.51</td>
<td>270±2.2</td>
</tr>
</tbody>
</table>

Analysis of antioxidant activity of methanolic extract of leaves of J. communis, C. forskohlii and U. dioica

DPPH (2, 2-diphenyl-1-picrylhydrazyl) and ABTS (2, 2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity of methanolic extracts of J. communis, U. dioica and C. forskohlii followed a dose-dependent pattern. Both DPPH and ABTS radical scavenging activity was found to be higher in the methanolic extract of C. forskohlii (IC₅₀ 14.3±0.23 µg ml⁻¹, 5.225±0.1 µg ml⁻¹) followed by J. communis (IC₅₀ 14.56±0.38 µg ml⁻¹, 5.715±0.091 µg ml⁻¹) and U. dioica (IC₅₀ 18.21±0.763 µg ml⁻¹, 19.8±4 µg ml⁻¹). Ascobic acid showed IC₅₀ value of 8.13±1.23 µg ml⁻¹, and 5.05±0.15 µg ml⁻¹ with DPPH and ABTS assay respectively (Table 4). Antioxidant nature of ethanolic extract of J. communis (81.63±0.38%) was also reported by Fierscu et al. (2018). Similarly, Pandey et al. (2018) also reported the free radicals scavenging activity of essential oil of J. communis berry (86.39%). Similarly, Khatun et al. (2011) evaluated the antioxidant potential of ethanolic extracts of root, stem, leaves and tubers of C. forskohlii and found that the radical-scavenging activity of the tubers (90.32% at 1.0 mg ml⁻¹) was found to be significantly higher (P < 0.05) followed by leaves (87.34% at 1.0 mg ml⁻¹), roots (85.34% at 1.0 mg/ml) and stem (69.40% at 1.0 mg/ml), respectively. Mzid et al. (2017) reported the antioxidant activity of ethanolic extract (IC₅₀=243.65±10.2 µg ml⁻¹) and water extract of leaves of U. dioica. (IC₅₀ = 142.94 ±10.54 µg ml⁻¹). Fattahi et al. (2013) also reported the antioxidant activity of aqueous extract of leaves of U. dioica by FRAP assay (IC₅₀= 0.75 µM).
Table 4: Antioxidant analysis of methanolic extract of selected medicinal plants using DPPH and ABTS assay.

<table>
<thead>
<tr>
<th>Assay</th>
<th>Ascorbic acid</th>
<th>J. communis</th>
<th>U. dioica</th>
<th>C. forskohlii</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8.13±1.23</td>
<td>14.56±0.38</td>
<td>18.21±0.76</td>
<td>14.3±0.23</td>
</tr>
<tr>
<td>ABTS</td>
<td>5.05±0.15</td>
<td>5.71±0.091</td>
<td>19.8±4</td>
<td>5.22±0.1</td>
</tr>
</tbody>
</table>

Antimicrobial analysis of methanolic extract of J. communis, C. forskohlii and U. dioica leaves of medicinal plants of North West Himalayas

Antimicrobial activity by agar well diffusion method

Methanolic extract (50µg) of J. communis, C. forskohlii and U. dioica were found to be ineffective against tested bacterial strains as shown in fig. 1(A). In antifungal analysis only methanolic extract (50µg) of J. communis showed inhibition to the growth of C. albicans (MTCC277), C. albicans (90028), whereas C. forskohlii and U. dioica extracts did not show any antifungal as shown in Fig. 1(B).

Determination of minimum inhibitory concentration by broth dilution method

Broth dilution method was done to determine MIC of methanolic extract of leaves of J. communis, U. dioica and C. forskohlii. It was found that methanolic extracts of leaves of C. forskohlii and U. dioica showed lowest MIC (125 µg ml⁻¹) against E. coli (ATCC25922), whereas J. communis and U. dioica extracts showed lowest MIC (250 µg ml⁻¹) against S. aureus (ATCC 29213). Extracts of U. dioica also showed lowest MIC (62.5 µg ml⁻¹) against K. pneumoniae (MTCC 39) (Table 6). In case of antifungal activity, methanolic extract of J. communis, C. forskohlii and U. dioica showed comparable MIC (62.5 µg ml⁻¹) against C. albicans ATCC90028. However, J. communis extract showed lowest MIC (31.25 µg ml⁻¹) against C. albicans MTCC277 followed by U. dioica (62.5 µg ml⁻¹) and C. forskohlii (125 µg ml⁻¹) (Table 5).

Micelli et al. (2018) reported the antimicrobial activity of methanolic and water extracts of J. communis against S. aureus (MIC 78.12 and 1250.00 µg ml⁻¹ respectively). They also reported that the methanolic extract of J. communis was not active against gram negative bacteria and yeast. Atulkar et al. (2015) evaluated the antimicrobial activity of the ethanol extract root of Coleus forskohlii using agar well diffusion method. They found that ethanol extract showed strongest inhibition zone of 12.03 mm against Staphylococcus aureus, 11.21mm against E. coli, 11.03 mm against B. subtilis, 10.34mm against Pseudomonas aeruginosa and 9.38 mm against Aspergillus niger. Antimicrobial activity of Coleus forskohlii plant was also reported in previous reports (Kumar et al., 2011; Kalesware et al., 2014). Rajkumar and Malathi (2015) investigated the phytochemical constituents, gas chromatography-mass spectrometry (GC-MS) analysis and antimicrobial activity of various extracts of different parts of Coleus forskohlii. They found that among all parts and extracts, ethanol extract of C. forskohlii root showed highest antibacterial activity compared with stem and leaf. The highest antimicrobial activity was observed against Klebsiella pneumonia (19 mm) and Candida albicans (16 mm) in ethanol extract of root. Senthikumar et al. (2015) reported remarkable antibacterial activity of leaves of C. forskohlii against gram-negative bacterial strains than gram-positive. The most effective activity was proven by C. forskohlii with maximum zone of inhibition ranging from 15mm against Salmonella typhi and 14mm with Staphylococcus aureus. In case of U. dioica, Gulcin et al. (2003) reported the antimicrobial activity of water extract showing zone of inhibition (mm) against Escherichia coli (8mm), Proteus mirabilis (8mm), Citrobacter koseri (9mm), Staphylococcus aureus (8mm), Streptococcus pneumoniae (9mm), Enterobacter aerogenes (9mm), Micrococcus luteus (13mm), Staphylococcus epidermidis (11mm) and C. albicans (8mm). Similarly, the antibacterial nature of U. dioica leaves was also reported in previous studies (Modarresi-Chahardehi et al., 2012; Kukrić et al., 2012).
Synergistic potential of methanolic extract of *J. communis*, *U. dioica*, *C. forskohlii* in combination with antibacterial antibiotics against bacterial strains

Methanolic extract of rhizome of *C. forskohlii* showed synergistic potential with decrease in MIC of antibiotics from 4-67 folds in combination with tetracycline against *E. coli* ATCC25922 (FIC=0.062, fold 8), *S. aureus* ATCC29213 (FIC=0.125, fold 16), *K. pneumoniae* MTCC39 (FIC=0.19, fold 16), *C. albicans* MTCC277 (FIC=0.25, fold 8), *C. albicans* ATCC90028 (FIC=0.5, fold 8). Combination of methanolic extract of *C. forskohlii* was found indifferent with tetracycline against *S. aureus* ATCC29213 (FIC=1), *K. pneumoniae* MTCC39 (FIC=0.93) with chloramphenicol against *K. pneumoniae* MTCC39 (FIC=1).

Methanolic extract of rhizome of *U. dioica* showed synergistic potential with decrease in MIC of antibiotics from 4-67 fold in combination with tetracycline against *E. coli* ATCC25922 (FIC=0.062, fold 515), *S. aureus* ATCC29213 (FIC=0.25, fold 8), *C. albicans* MTCC39 (FIC=0.5, fold 4), *C. albicans* ATCC90028 (FIC=0.25, fold 8), *K. pneumoniae* MTCC39 (FIC=0.5, fold 4), *S. aureus* ATCC29213 (FIC=0.38, fold 8), *K. pneumoniae* MTCC39 (FIC=0.5, fold 4) as shown in table 6.

Synergistic potential of methanolic extract of *J. communis*, *U. dioica*, *C. forskohlii* in combination with antifungal antibiotics against fungal strains

Methanolic extract of *J. communis* showed synergistic potential with decrease in MIC of antibiotics from 4-8 folds in combination with fluconazole against *C. albicans* MTCC277 (FIC=0.5, fold 8), *C. albicans* ATCC90028 (FIC=0.25, fold 8), and amphotericin B against (FIC=0.36, fold 4). *C. albicans* MTCC277 (FIC=0.5, fold 8). Combination of methanolic extract of *C. forskohlii* was found indifferent with amphotericin B against *C. albicans* ATCC90028 (FIC=0.5, fold 4). Methanolic extract of *U. dioica* showed synergistic potential with decrease in MIC of antibiotics from 4-8 folds in combination with fluconazole against *C. albicans* MTCC277 (FIC=0.25, fold 8), *C. albicans* ATCC90028 (FIC=0.25, fold 8), and amphotericin B against *C. albicans* MTCC277 (FIC=0.5, fold 4), *C. albicans* ATCC90028 (FIC=0.25, fold 8) as shown in table 7.
isolated compounds. Mazlan compounds, demonstrating the synergistic potency of coincubated with sub-MIC (*8) were reduced between 4 and 8-fold when these strain s were combined with these polyphenolic compounds.

The MICs of oxacillin for each of methicillin-resistant S. aureus when combined with four standard antibiotics (nalidixic acid, ampicillin, tetracycline, and sulfamethoxazole/trimethoprim) resulted in fold increase in area 69.00%, 21.37%, 16.16%, and 4.16%, respectively. Similarly, Sánchez-Chávez et al. (2019) also reported a synergistic antibacterial effect of active fraction from hexane extract of Trixis angustifolia in combination with pebrellin (FIC < 0.5).

### Table 7: Synergistic potential of methanolic extract of *J. communis*, *U. dioica*, *C. forskohlii* in combination with antifungal antibiotics against fungal strains

<table>
<thead>
<tr>
<th>Fungal strains</th>
<th><em>J. communis</em></th>
<th><em>U. dioica</em></th>
<th><em>C. forskohlii</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ab1</td>
<td>Ab2</td>
<td>Ab1</td>
</tr>
<tr>
<td><em>C. albicans</em> (MTCC277)</td>
<td>0.25 (*8)</td>
<td>0.25 (*8)</td>
<td>0.25 (*8)</td>
</tr>
<tr>
<td><em>C. albicans</em> (ATCC90028)</td>
<td>0.25 (*8)</td>
<td>0.25 (*8)</td>
<td>0.25 (*8)</td>
</tr>
</tbody>
</table>

Ab1-Fluconazole, Ab2-Amphotericin B, * b fold decrease

In contrast to our study Peru et al. (2019) reported the synergistic activity of *J. communis* essential oil against *Mycobacterium avium* and *M. intracellulare* by checkerboard synergy method. Stanković et al. (2019) studied the synergistic activity of ethanolic, acetone and ethyl acetate extract of *U. dioica* in combination with preservatives (potassium nitrile, sodium nitrile) against *S. enterica*, *S. typhimurium*, *E. coli* ATCC25922, *E. coli* O157, *K. oxytoca*, *P. mirabilis*, *S. aureus*, *S. aureus* ATCC 25923 and *B. subtilis*.

Abrosca et al. (2019) studied the synergistic anti-proliferative effect of *U. dioica* extract and ciplatin against human lung cancer. Similarly Jyoti et al. (2016) studied the synergistic effect of AgNPs of *U. dioica* in combination with streptomycin, amikacin, kanamycin, tetracycline, ampicillice, cefepime, amoxicillin and cefetaxime against *B. cereus*, *S. epidermidis*, *S. aureus*, *B. subtilis*, *E. coli*, *S. typhimurium*, *K. pneumoniae* and *S. marcescens*. Torres et al. (2019) isolated polyphenols such as Coumaric acid, catechin/epicatechin, and luteolin from *Cuspidaria convoluta* leaves. They showed a synergistic antibacterial activity of antibiotics when they were combined with these polyphenolic compounds. However, the combination of luteolin and ampicillice had the most potent antibacterial activities. The MICs of oxacillin for each of methicillin-resistant *Staphylococcus aureus* strains were reduced between 4 and 8-fold when these strains were coincubated with sub-MIC (≤ ½ MIC) levels of these compounds, demonstrating the synergistic potency of isolated compounds. Mazlan et al. (2019) isolated Mangiferin from *Mangifera indica* leaves and found synergistic antibacterial effect of mangiferin (4mg/disc) on *S.

Quantification of Quercetin in methanolic extract of *J. communis*, *U. dioica* and *C. forskohlii* by HPTLC Method

Quercetin has been quantified from many other plants, but there are no reports of quantification of quercetin in *J. communis* and *C. forskohlii*. The HPTLC analysis showed linear regression equation as Y = 1894.x + 1498 with R² = 0.976 (Fig. 8A, B & C). The amount of quercetin was found highest in methanolic extract of leaves of *C. forskohlii* (168.6367 µg mg⁻¹) followed by *U. dioica* (159.3197 µg mg⁻¹) and *J. communis* (142.3059 µg mg⁻¹) with Rₚ value 0.7 (Figure 3). In contrast to our study Singh et al. (2009) quantified the quercetin in rhizome extract of *Glycyrrhiza glabra* (0.271% w/w). Meghani et al. (2018) quantified quercetin in methanolic extract of leaves, fruits and fruits of *Moringa oleifera* 0.52, 0.62 and 0.15% w/w respectively. Shailajan et al. (2019) quantified quercetin (mg g⁻¹) in different [In house (1.119±0.025), akashala (0.837±0.012), dabur (0.901±0.010), patanjali (1.55±0.004) and dhootpapeshwar (2.014±0.062)] polyherbal formulation Pushyanuga Churna.

**Fig. 3:** Quantification of quercetin in methanolic extracts of *J. communis*, *U. dioica*, and *C. forskohlii*. HPTLC fingerprint of different concentrations of quercetin (1-10 µg) (A). Linear regression graph of quercetin (1-5 µg) (B). HPTLC fingerprint of methanolic extract of *J. communis*, *U. dioica*, and *C. forskohlii* (Track 1- quercetin, Track 2 - *J. communis*, Track 3 - *U. dioica* and Track 4 - *C. forskohlii*) (C).
Conclusion

Based on bioavailability data, methanolic extracts of leaves of Juniperus communis, Urtica dioica and Coleus forskohlii have a huge potential to develop more potent antibiotic formulations to treat drug resistant. Based on antimicrobial data, the extracts of these medicinal plants could also be used in food preservations against bacterial and fungal contaminations. The high antioxidant potential of the extracts provides valuable information to develop the extracts as functional food ingredients. In summary, the current study proposed the role these extracts in managing multiple drug resistance in bacterial and fungal pathogens, and importance in developing plant based formulations.

Acknowledgements

The authors acknowledge Shoolini University, Solan, for providing infrastructure support to conduct the research work. Authors also acknowledge the support provided by Yeast Biology Laboratory, School of Biotechnology, Shoolini University, Solan, India.

References


