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

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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 chloramphanicol) 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 IC_{50} 14.3±0.23 and 5.225±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 (Efferth *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.

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

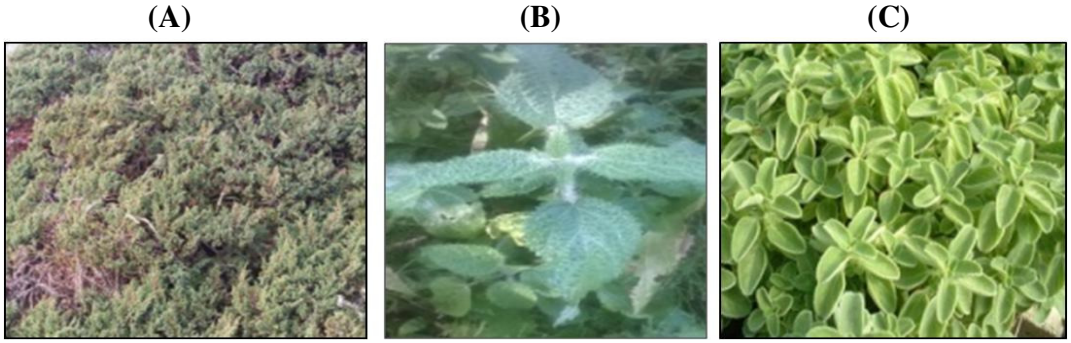

Fig. 1: Medicinal plants in their natural habitat. *J. communis* (A), *U. dioica* (B) and *C. forskohlii*(C).

Materials and Methods

Collection, processing and preparation of extracts of selected medicinal plants

Leaves of *J. communis* were collected from Chanshal Valley (3,755m above the sea level), *U. dioica* and *C. forskohlii* were collected from Anand Parvat (2086-2350m above the sea level), and Shatul Valley (3500m above the sea level) respectively of district Shimla, Himachal Pradesh, India in the month of July and August, 2018. The collected leaves were thoroughly washed with running tap water and surface sterilized using 70% ethanol for 2 min, followed by washing with sterilized distilled water. After washing, the samples were dried completely in hot air oven at 40 ºC and then ground to fine powder with the help of electric grinder.

About 50 g of dried powder of leaves of *J. communis, U. dioica* and *C. forskohlii* were subjected to methanol (500 ml) extraction using cold maceration method. The extract was filtered through Whatmann filter paper no. 1 and the collected filtrate was dried at 40 ºC and stored at 4 ºC till further use.

Qualitative analysis of phytochemicals

Methanolic leaves extracts of *J. communis, U. dioica* and *C. forskohlii* were tested for the presence of various secondary metabolites such as phenolics, tannins, flavonoids, saponins, alkaloids, glycosides and phytosteriods using methods described by Khandelwal (2008) and Guleria *et al.* (2016). For the detection of alkaloids and glycosides, 50 mg of dried extracts were dissolved in 5 ml of HCl (1%) and filtered through Whatmann filter no 1. The filtrates were used for the detection of alkaloids and glycosides. On the other hand, 50 mg of extracts were dissolved in 5 ml of distilled water and filtered to remove insoluble matter and the filtrates were used for the detection of phenolics, tannins, phytosterols, phytosteriods, carbohydrate, flavonoids, proteins and amino acids.

Quantitative analysis of total phenolic contents (TPC) and flavonoids (TFC)

TPC and TFC of methanolic extract of *J. communis, U. dioica* and *C. forskohlii* were quantified using Folin-ciocalteau reagent method (Singleton *et al*., 1999) and aluminium chloride method (Zhishen *et al*., 1999) respectively. Amount of phenolic and flavonoid was calculated from calibration curve of gallic acid (5-100 µg ml^{-1}) and rutin (5-100 µg ml^{-1}) and expressed in terms of gallic acid equivalents (GAE)/ rutin equivalents (RE) per gram of the extract using the following equation:

$$C = \frac{c \times V}{m}$$

Where 'C' is total content of phenolic compounds in mg g^{-1} plant extract in GAE, 'c' is the concentration of gallic acid/rutin estimated from the calibration curve (mg ml^{-1}), 'V' is the volume of extract in milliliter and 'm' is the weight of crude plant extract in grams.

In-vitro* antioxidant activity of methanolic extract of leaves of *J. communis, U. dioica* and *C. forskohlii

Methanolic extract of leaves of *J. communis, U. dioica* and *C. forskohlii* were dissolved at a concentration of 1 mg/ml in methanol and diluted to prepare a gradient of different concentrations ranged from 5-40 µg ml^{-1}. The antioxidant potency of the extracts was expressed in term of IC_{50} value (half maximal inhibitory concentration), which was measured by using 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assays. Ascorbic acid was used as standard antioxidant compound for comparative analysis in both *in vitro* assays.

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

DPPH radical scavenging activity of extract of *J. communis, U. dioica* and *C. forskohlii* were measured by the method described by Barros *et al*. (2007) and Kumar *et al*. (2018). The capability of scavenging DPPH radical was calculated using the following equation:

% DPPH radical scavenging activity = $[A_{(control)}\text{-}A_{(sample)}] / A_{(control)}] \times 100$

where A $_{(control)-}$ Absorbance of control and A $_{(sample)}$ - 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-

ABTS radical scavenging activity (%)

$$= [(A_{control} - A_{sample})]/(A_{control})] \times 100$$

where $A_{control}$ is the absorbance of ABTS radical + methanol; A_{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* MTCC39 and *E. coli* ATCC25922) and two fungal strains (*Candida albicans* ATCC90028 and MTCC277) 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 ºC, while, fungal strains were grown in yeast peptone dextrose (YPD) broth at 30 ºC with shaking at 200 rpm. The cell turbidity of the culture was adjusted to 0.5 McFarland standards, which is approximately equivalent to 2×10^8 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 pates. 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, chloramphanicol and tetracycline) and antifungal (fluconazole and amphotericin B) antibiotics was measured by broth dilution method described under CLSI guidelines using 5-tripheny 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-tripheny 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 descried previously by Rolta *et al*. (2018a).

Synergistic enhancement of antibacterial activity of antibacterial antibiotics (erythromycin, chloramphanicol, 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, chloramphanicol, 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:

$$FICI = FIC_{(Antibiotics)} + FIC_{(Extract)}$$

Where, $FIC_{(Antibiotics)}$ = MIC of antibiotic in combination/ MIC of antibiotic alone; $FIC_{(Extract)}$ = 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. dioca*, *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*

Phytoconstituents	Tests	*J. Communis*	*C. forskohlii*	*U. dioica*
Phenolics and Tannins	Ferric chloride test	+	+	+
	Gelatin test	+	+	+
Flavonoids	Lead acetate test	+	+	+
Carbohydrates	Fehling solution test	-	-	+
Glycosides	Borntrager test	+	-	-
Alkaloids	Dragendorff test	+	+	+
	Mayer test	+	+	+
Saponin	Foam test	-	+	+

'+' 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, R^2 =0.953); whereas the estimation flavonoid content was done using standard curve of rutin (Y=0.012x+0.080, R^2 =0.944). Amount of TPC was higher in *C. forskohlii* (250±4.54 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. forskohli* (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 linoloal 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*. Kurkric *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*

Medicinal plants	Plant part used	Total phenolic content, TPC (µg ml^{-1} GAE)	Total flavonoid content, TFC (µg ml^{-1} RE)
J. communis	Leaves	20.81±2.21	112±1.2
U. dioica	Leaves	220±2.14	178.56±2.56
C. forskohlii	Leaves	250±4.51	270±2.2

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* (IC_{50}-14.3±0.23 µg ml^{-1}, 5.225±0.1 µg ml^{-1}) followed by *J. communis* (IC_{50}-14.56±0.38 µg ml^{-1}, 5.715±0.091 µg ml^{-1}) and *U. dioica* (IC_{50}-18.21±0.763 µg ml^{-1}, 19.8±4 µg ml^{-1}). Ascorbic acid showed IC_{50} 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 Fierascu *et al.* (2018). Similarly, Pandey *et al*. (2018) also reported the free radicals scavenging activity of essential oil of *J. communis* barry (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 (IC_{50}=245.65±10.2µg ml^{-1}) and water extract of leaves of *U. dioica*. (IC_{50} = 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 (IC_{50}-0.75µM).

Table 4: Antioxidant analysis of methanolic extract of selected medicinal plants using DPPH and ABTS assay.

Assay	Half of inhibitory contraction (IC_{50} µg ml^{-1})			
	Ascorbic acid	***J. communis***	***U. dioica***	***C. forskohlii***
DPPH	8.13±1.23	14.56±0.38	18.21±0.76	14.3±0.23
ABTS	5.05±0.15	5.715±0.091	19.8±4	5.225±0.1

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).

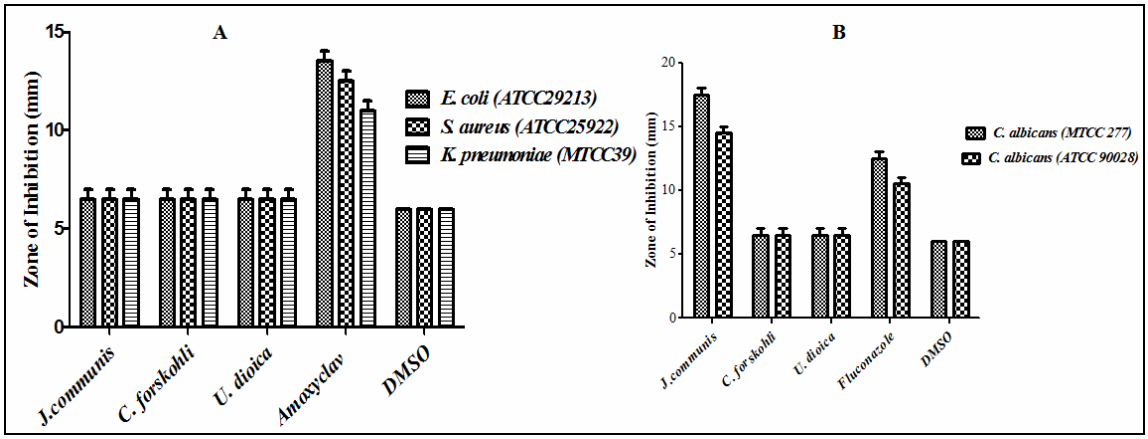

Fig. 2: Antimicrobial analysis of methanolic extract of medicinal plants from North West Himalayas against tested bacterial strains (A) and fungal strains (B).

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. froshkolii* 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).

Table 5: Summary showing MIC of selected medicinal plants against bacterial and fungal strains.

Bacterial strains	Minimum inhibitory concentration (MIC) in µg/ml							
	J. communis	***C. forskohlii***	***U. dioica***	**Ab1**	**Ab2**	**Ab3**	**Ab4**	**Ab5**
E. coli (ATCC25922)	250	125	125	500	7.81	31.25	-	-
S. aureus (ATCC 29213)	250	500	250	500	125	250	-	-
K. pneumoniae (MTCC39)	250	250	62.5	250	125	250	-	-
C. albicans (MTCC277)	31.25	125	62.5	-	-	-	62.5	62.5
C. albicans (ATCC90028)	62.5	62.5	62.5	-	-	-	62.5	15.625

Ab1- Erythromycin; Ab2- Chloramphanicol; Ab3- Tetracycline; Ab4- Fluconazole; Ab5-Amphotericin B

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=0.25, fold 8), chloramphanicol against *E. coli* ATCC25922 (FIC =0.25, fold 8), *S. aureus* ATCC 29213 (FIC =0.5, fold 4), erythromycin against *E. coli* ATCC25922 (FIC=0.124, fold 4), *S. aureus* ATCC 29213 (FIC=0.125, fold 16), *K. pneumoniae* MTCC39 (FIC=0.030, fold 67). Combination of methanolic extract of *C. forskohlii* was found indifferent with tetracycline against *S. aureus* ATCC 29213 (FIC=1), *K. pneumoniae* MTCC39 (FIC =0.93) with chloramphanicol 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), *K. pneumoniae* MTCC39 (FIC=0.19, fold 16), chloramphanicol against *E. coli* ATCC25922 (FIC=0.25, fold 8), *S. aureus* ATCC29213 (FIC=0.5, fold 4), *K. pneumoniae* MTCC39 (FIC =0.5, fold 4), erythromycin against *E. coli* ATCC25922 (FIC=0.5, fold 8), *S. aureus* ATCC29213 (FIC=0.38, fold 8), *K. pneumoniae* MTCC39 (FIC=0.5, fold 4) as shown in table 6.

Table 6: Synergistic potential of methanolic extract of *J. communis, U. dioica, C. forskohlii* in combination with antibacterial antibiotics against bacterial strains

Bacterial Strains	FIC Index								
	J. communis			***U. dioica***			***C. forskohlii***		
	Ab1	**Ab2**	**Ab3**	**Ab1**	**Ab2**	**Ab3**	**Ab1**	**Ab2**	**Ab3**
E. coli (ATCC25922)	0.125 (*16)	0.5 (*4)	0.5 (*4)	0.062 (*8)	0.25 (*8)	0.5 (*16)	0.25 (*515)	0.25 (*8)	0.124 (*8)
S. aureus (ATCC29213)	0.5 (*8)	1	1	0.25 (*2)	0.5 (*4)	0.38 (*16)	1	0.5 (*4)	0.125 (*8)
K. pneumonia (MTCC39)	0.5 (*4)	0.5 (*4)	0.5 (*4)	0.19 (*2)	0.5 (*2)	0.5 (*67)	0.934	1	0.030 (*4)

Ab1-Tetracycline, Ab2-Chloromphanicol, Ab3-Erythromycin, *fold decrease

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 16), and amphotericin B against *C. albicans* MTCC277 (FIC=0.25, fold 8), *C. albicans* ATCC90028 (FIC=0.25, fold 8). Methanolic extract of *C. forskohlii* 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 (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.

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

Fungal strains	FIC Index					
	J. communis		*U. dioica*		*C. forskohlii*	
	Ab1	**Ab2**	**Ab1**	**Ab2**	**Ab1**	**Ab2**
C. albicans (MTCC277)	0.5 (*4)	0.25 (*8)	0.25 (*8)	0.5 (*4)	0.25 (*8)	0.5 (*4)
C. albicans (ATCC90028)	0.25 (*8)	0.25 (*8)	0.25 (*8)	0.25 (*8)	0.25 (*8)	1

Ab1-Fluconazole, Ab2-Amphotericin 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, ampiciline, 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 ampicillin 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. 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).

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 $R^2 = 0.976$ (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 R_f 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 0.13% 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.*

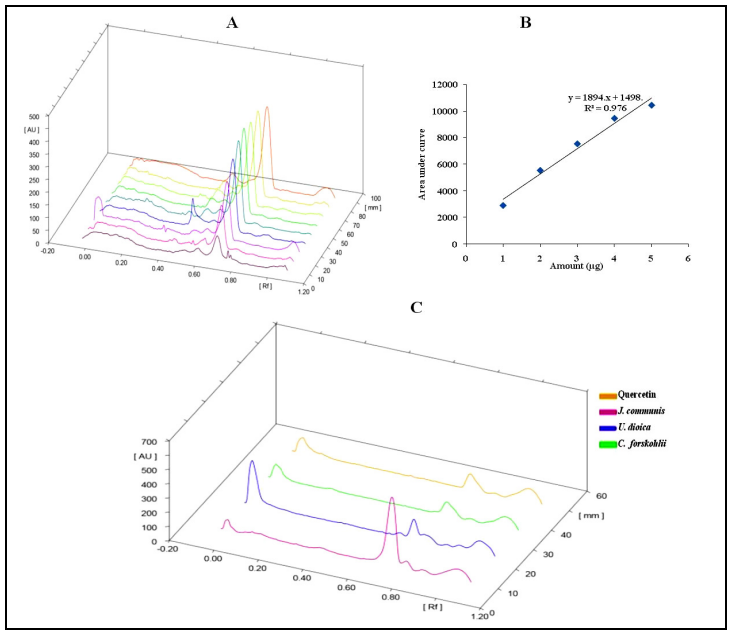

Fig. 3: Quantification of quercetin in methanolic extracts of *J. communis*, *U. dioica*, and *C. forskohlii*. HPTLC fingerprint of different concentrations of quercetin (1-10 µg) (A). Linear regression graph of quercetin (1-5 µg) (B). HPTLC fingerprint of methanolic extract of *J. communis*, *U. dioica*,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

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References

Ahmad, I.F. and Aqil, M.O. (2006). Modern phytomedicine: Turning medicinal plants into drugs. John Wiley & Sons.

Ahmad, I.; Aqil, F. and Owais, M. (2006). Modern phytomedicine: Turning medicinal plants into drugs. John Wiley & Sons.

Aiyegoro, O.A. and Okoh, A.I. (2009). Use of bioactive plant products in combination with standard antibiotics: implications in antimicrobial chemotherapy. Med. Plant Res., 3: 1147-1152.

Alturkar, P. and Singh, P. (2015). Antimicrobial activities of root extracts of coleus forskohlii briq. Int J Recent Sci Res., 6:7732-4.

Kumar, A.; Saklani, S.; Gahlot, M.; Singh, R.; Patial, R. and Kashyap, P. (2011). Antimicrobial Activity of Extracts of the Medicinal Plant *Coleus forskohlii*. Int. J. Drug Res. Tech., 1: 52-59.

Barros, L.; Ferreira, M.J.; Queiros, B.; Ferreira, I.C. and Baptista, P. (2007). Total phenols, ascorbic acid, β-carotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities. Food Chem., 103: 413-419.

CLSI C. M100-S25, (2012). Performance Standards for Antimicrobial Susceptibility Testing. Twenty-Fifth Informational Supplement.

D'Abrosca, B.; Ciaramella, V.; Graziani, V.; Papaccio, F.; Della Corte, C.M.; Potenza, N.; Fiorentino, A.; Ciardiello, F. and Morgillo, F. (2019). *Urtica dioica* L. inhibits proliferation and enhances cisplatin cytotoxicity in NSCLC cells via Endoplasmic Reticulum-stress mediated apoptosis. Sci. Rep., 9: 4986.

Dev, K.; Kumar, V.; Rolta, R. and Sharma, A. (2017). Herbal Pharmaceutical Excipient for Enhancing Antifungal and Antibacterial Properties of Existing Drugs. *Indian Patent* Filing Number: 201711028454.

Efferth, T.; Saeed, M.E.; Mirghani, E.; Alim, A.; Yassin, Z.; Saeed, E.; Khalid, H.E. and Daak, S. (2017). Integration of phytochemicals and phytotherapy into cancer precision medicine. ONCOTARGET. 8: 50284.

Eumkeb, G.; Siriwong, S. and Thumanu, K. (2012). Synergistic activity of luteolin and amoxicillin combination against amoxicillin-resistant Escherichia coli and mode of action. J PHOTOCH PHOTOBIO B. 117: 247-253.

Fattahi, S.; Ardekani, A.M.; Zabihi, E.; Abedian, Z.; Mostafazadeh, A.; Pourbagher, R. and Akhavan-Niaki, H. (2013). Antioxidant and apoptotic effects of an aqueous extract of *Urtica dioica* on the MCF-7 human breast cancer cell line. Asian Pac J Cancer Prev., 14: 5317-5323.

Fierascu, I.; Ungureanu, C.; Avramescu, S.M.; Cimpeanu, C.; Georgescu, M.I.; Fierascu, R.C.; Ortan, A.; Sutan, A.N.; Anuta, V.; Zanfirescu, A. and Dinu-Pirvu, C.E. (2018). Genoprotective, antioxidant, antifungal and anti-inflammatory evaluation of hydroalcoholic extract of wild-growing *Juniperus communis* L. (Cupressaceae) native to Romanian southern sub-Carpathian hills. BMC Complement Altern Med., 18: 3.

Garbyal, S.S.; Aggarwal, K.K. and Babu, C.R. (2005). "Traditionaly Used Medicinal Plants in Dharchula Himalayas of Pithoragarh District, Uttaranchal." Indian J. Tradit. Knowl., 4: 199–207.

Gibbons, S. (2005). Plants as a source of bacterial resistance modulators and anti-infective agents. Phytochem Rev., 4: 63-78.

Gülçin, I.; Küfreviolu, Ö.İ.; Oktay, M. and Büyükokurolu, M.E. (2004). Antioxidant, antimicrobial, antiulcer and analgesic activities of nettle (*Urtica dioica* L.). J. Ethnopharmacol., 90: 205-215.

Handzlik, J.; Matys, A. and Kieć-Kononowicz, K. (2013). Recent advances in multi-drug resistance (MDR) efflux pump inhibitors of Gram-positive bacteria S. aureus. Antibiotics., 2: 28-45.

Infectious Diseases Society of America (IDSA) (2011). Combating antimicrobial resistance: policy recommendations to save lives. Clinical Infectious Diseases, 52, S397-S428.

Jyoti, K.; Baunthiyal, M. and Singh, A. (2016). Characterization of silver nanoparticles synthesized using *Urtica dioica* Linn. leaves and their synergistic effects with antibiotics. J. Radiat. Res. Appl. Sc., 9: 217-227.

Khandelwal, K. (2008). Practical pharmacognosy. Pragati Books Pvt. Ltd..

Khatun, S.; Chatterjee, N.C. and Cakilcioglu, U. (2011). Antioxidant activity of the medicinal plant *Coleus forskohlii* Briq. Afr. J. Biotechnol., 10: 2530-2535.

Kukrić, Z.Z.; Topalić-Trivunović, L.N.; Kukavica, B.M.; Matoš, S.B.; Pavičić, S.S.; Boroja, M.M. and Savić, A.V. (2012). Characterization of antioxidant and antimicrobial activities of nettle leaves (*Urtica dioica* L.). Apteff., 43: 1-342.

Kumar, V.; Sharma, N.; Sourirajan, A.; Khosla, P.K. and Dev, K. (2018). Comparative evaluation of antimicrobial and antioxidant potential of ethanolic extract and its fractions of bark and leaves of *Terminalia arjuna* from north-western Himalayas. India. J Tradit Complement Med., 8: 100-106.

Lal, B. and Singh, K.N. (2008). Indigenous herbal remedies to cure skin disorders by the natives of Lahaul-Spiti in Himachal Pradesh. Indian J. Tradit. Knowl., 7: 237–241.

Levy, S.B. (2002). From tragedy the antibiotic era is born. The antibiotic paradox: how the misuse of antibiotics destroys their curative powers, 2nd ed. Cambridge, MA: Perseus Publishing, 1-14.

Meena, A.K.; Verma, S.C.; Rani, R.; Rao, M.M.; Panda, P.; Padhi, M.M. and Devalla, R.B. (2012). Evaluation of preliminary phytochemical and physicochemical studies on *Juniperus communis* L. fruit used in ayurvedic formulations. R. J. Pharma. Tech., 5: 88.

Meghani, N.; Jethra, B.; Shah, R. and Nagendran, S. (2018). Fingerprint analysis for validation and simultaneous quantification of quercetin and kaempferol in *Moringa Oleifera* by RP-HPLC and HPTLC. Int. J. Pharm. Sci., 9: 1499-1510.

Miceli, N.; Marino, A.; Köroğlu, A.; Cacciola, F.; Dugo, P.; Mondello, L. and Taviano, M.F. (2018). Comparative study of the phenolic profile, antioxidant and antimicrobial activities of leaf extracts of five *Juniperus* L. (Cupressaceae) taxa growing in Turkey. NAT PROD RES., 1-6.

Modarresi-Chahardehi, A.D.; Fariza-Sulaiman, S.I. and Mousavi, L. (2012). Screening antimicrobial activity of various extracts of *Urtica dioica*. Rev. Biol. Trop., 60: 1567-76.

Muniandy, K.; Hassan, Z. and Hafez, M. (2014). Antimicrobial Activity of the Ethanolic Extract of *Coleus aromaticus* against Common Wound Pathogens. Res. J. Pharm. Biol. Chem. Sci., 5: 1294.

Pandey, S.; Tiwari, S.; Kumar, A.; Niranjan, A.; Chand, J.; Lehri, A. and Chauhan, P.S. (2018). Antioxidant and anti-aging potential of Juniper berry (*Juniperus communis* L.) essential oil in *Caenorhabditis elegans* model system. IND CROP PROD., 120: 113-122.

Pandith, S.A.; Dhar, N.; Rana, S.; Bhat, W.W.; Kushwaha, M.; Gupta, A.P.; Shah, A.M.; Ram, V. and Lattoo, K. (2016). Functional promiscuity of two divergent paralogs of type III plant polyketide synthases. Plant Physiol., 171: 2599-2619.

Peleg, A.Y. and Hooper, D.C. (2010). Hospital-acquired infections due to gram-negative bacteria. N. Engl. J. Med., 362: 1804-1813.

Perez, C. (1990). Antibiotic assay by agar-well diffusion method. Acta Biol Med Exp., 15: 113-115.

Peruč, D.; Tićac, B.; Abram, M.; Broznić, D.; Štifter, S.; Staver, M.M. and Gobin, I. (2019). Synergistic potential of *Juniperus communis* and *Helichrysum italicum* essential oils against nontuberculous mycobacteria. J. Med. Microbiol., 68: 703-710.

Rajkumar, K. and Malathi, R. (2015). Phytochemical investigation, GC-MS analysis and *in vitro* antimicrobial activity of *Coleus forskohlii*. Bangladesh J Pharmacol., 10: 924-930.

Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M. and Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med., 26: 1231-1237.

Rolta, R.; Sharma, A.; Kumar, V.; Sourirajan, A.; Baumler, D.J. and Dev, K. (2018a). Methanolic Extracts of the Rhizome of *R. emodi* Act as Bioenhancer of Antibiotics against Bacteria and Fungi and Antioxidant Potential. J. Med. Plant Res., 8.

Rolta, R.; Sharma, A.; Kumar, V.; Chauhan, PK.; Sourirajan, A. and Dev, K. (2018b). Traditional Medicinal Plants of Higher Altitude of Himachal Pradesh as Functional Food Ingredient cum Food Preservative and Bioavailability Enhancer of Anifungal Antibiotics. Available at SSRN 3299285.

Saklani, S.; Gahlot, M.; Kumar, A.; Singh, R.; Patial, R. and Kashyap, P. (2017). Antimicrobial activity of extracts of the medicinal plant *Coleus forskohlii*. Int. J. Drug Dev. Res., 1: 8.

Sánchez-Chávez, AC.; Salazar-Gómez, A.; Zepeda-Vallejo, L.G.; Hernández de Jesús, M.D.; Quintos-Escalante, M.; Vargas-Díaz, M.E. and Luna-Herrera, J. (2019). *Trixis angustifolia* hexanic extract displays synergistic antibacterial activity against *M. tuberculosis*. Nat. Prod. Res., 33: 1477-81.

Scandorieiro, S.; de Camargo, L.C.; Lancheros, C.A.; Yamada-Ogatta, S.F.; Nakamura, C.V.; de Oliveira, A.G.; Andrade, C.G.; Duran, N.; Nakazato, G. and Kobayashi, R.K. (2016). Synergistic and additive effect of oregano essential oil and biological silver nanoparticles against multidrug-resistant bacterial strains. Front Microbiol., 7: 760.

Senthilkumar, CS.; Kumar, M.S. and Pandian, M.R. (2010). *In vitro* Antibacterial activity of crude leaf extracts from *Tecoma stans* (L) Juss. et Kunth, *Coleus Forskohlii* and *Pogostemon patchouli* against human pathogenic bacteria. Int. J. Pharm. Tech. Res., 2: 438-42.

Shailajan, S.; Patil, Y.; Joshi, M.; Menon, S. and Mhatre, M. (2019). Simultaneous Quantification of Pharmacological Markers Quercetin and Berberine Using High-Performance Thin-Layer Chromatography (HPTLC) and High-Performance Liquid Chromatography (HPLC) from a Polyherbal Formulation *Pushyanuga Churna*. J. AOAC Int., 102: 1003-1013.

Singh, B.; Mungara, P.; Nivsarkar, M. and Anandjiwala, S. (2009). HPTLC densitometric quantification of glycyrrhizin, glycyrrhetinic acid, apigenin, kaempferol and quercetin from *Glycyrrhiza glabra*. Chromatographia, 70: 1665.

Singh, M. and Sengar, B. (2019). Study on Phytochemical and Antioxidative potential of leaf extract of stinging nettle, *Urtica dioica* in Uttarakhand, India. J. Emerg. Technol. Innov. Res., 6: 291-297.

Singleton, V.L.; Orthofer, R. and Lamuela-Raventós, R.M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In Methods in enzymology, 152-178

Sofowora, A.; Ogunbodede, E. and Onayade, A. (2013). The role and place of medicinal plants in the strategies for disease prevention. Afr. J. Tradit. Complem., 10: 210-229.

Stanković, M.M.; Terzić, J.N. and Stefanović, O.D. (2019). Synergistic antibacterial activity of *Curcuma longa* L. and *Urtica dioica* L. extracts and preservatives. Kragujevac J. Sci., 107-116.

Vidyarthi, S.; Samant, S.S. and Sharma, P. (2013). Traditional and indigenous uses of medicinal plants by local residents in Himachal Pradesh, North Western Himalaya, India. Int. J. Biodivers. Cci. Ecosyst. Serv. Manag., 9: 185-200.

Zhishen, J.; Mengcheng, T. and Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem., 64: 555-559.