Screening and characterization of selected drugs having antibacterial potential

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Abstract: Due to ever increasing antibiotic resistance offered by pathogenic bacterial strains and side effects of synthetic antibiotics, thereof, there is a need to explore the effective phytochemicals from natural resources. In order to help overcoming the problem of effective natural drug and the side effects posed by the use of the synthetic drugs, five different plants namely *Thymus vulgaris, Lavandula angustifolia, Rosmarinus officinalis*, *Cymbopogon citratus and Achillea millefolium* were selected to study their antibacterial potential. Antibacterial activity and minimum inhibitory concentration (MIC) checked against the selected bacterial strains. As compared to other test plants, ethanolic extract of *Rosmarinus officinalis* leaves showed the most promising inhibitory effect i.e: inhibition zone (18.17± 0.44mm) against *Klebsiella pneumoniae* and the lowest inhibition (15.5±0.29mm) against *Pseudomonas aeruginosa* and *Escherichia coli* ($p<0.05$). The MIC values were recorded in the range of 1 to 20mg/ml. Screening of the selected extracts for the test plants additionally indicate some unique variations. Results were further confirmed through TLC for alkaloids and terpenoids (15% sulphuric acid and Dragedroff's reagent) in ethanolic extract. Characterization of *Rosmarinus officinalis* of ethanolic extract was carried out using column chromatography. The appearance of orange crystals may indicate the presence of alkaloidal bioactive compounds which need to be further investigated. The tested plants may have a potential for fighting against some infectious diseases caused by selected human pathogenic bacterial strains. This knowledge may incite a gateway to effective drug search and so on.

Keywords: Medicinal plants, antibacterial effects, phytochemical analysis.

INTRODUCTION

Before the introduction of chemical medicines, man relied on the healing properties of medicinal plants. Some people value these plants due to an ancient belief that plants are a source for food, medical treatment, and other effects. The World Health Organization estimates that about 80% of the people rely (almost) exclusively on traditional medicines for their primary healthcare needs. Medicinal plants constitute the richest bioresource of drugs for traditional systems of medicine, nutraceuticals, food supplements, modern medicines, pharmaceutical intermediates, folk medicines and chemical entities for synthetic drugs (Koehn and Carter, 2005). Medicinal plants are extensively used by all sector of people either indirectly in the pharmaceutical preparations of modern medicine or directly as folk remedie or in different indigenous medicine systems. In modern medicine, natural products have been used to treat a variety of diseases e.g; Hippocrates, the father of modern medicine, noted that a powder derived from the bark of the willow tree helped reduce pain from headaches (Jones *et al.*, 2006). Importance of natural products for use in medicine is due of their diversity of structure and analogs which can be synthesized, curing a varied type of ailments and their frequent. Similarly, extensive synthetic pathways, high cost and low productivity of synthetic products continue to enhance the importance of natural products. Although, medicinal plants provide slow recovery, their therapeutic use is becoming popular because of their low side effects and less resistance offered by microorganisms (Seyyednejad and Motamedi, 2010). Plants have a long history of usage for treatment of different human diseases. Plant-derived medicines have been the part of traditional health care in most parts of the world for thousands of years and interest has been increase in plants as a source to fight microbial diseases. For instance, a few plants have historic record to be used in 2600 B.C. that includes pooppy capsule latex (*Papver somniferum*), myrrh (*Commiphora* species) and licorice (Glycyrrhiza glabra). These plants are still used in medication either as herbal preparations or as part of drug (Newman and Cragg, 2007). Among higher plants, many species have been reported to have phytochemicals of medicinal importance in their leaves, stems, flowers, seeds and roots. Phytochemical components such as tannins, alkaloids, terpenoids, phenolic compounds, steroids and flavonoids are responsible for the medicinal values of the plants which induce specific physiological response in human body (Vasu *et al.,* 2009). Because of biological and

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structural variety of plant constituents, it is considered that for the discovery of new biological entities and potential drugs, terrestrial plants tender an inimitable and renewable resource. Since plant secondary metabolites have been elaborated within living systems, they are presumed showing more inclination towards drugs and biological friendliness than the synthetic molecules. For synthesis of complex chemical compounds knowledge of the chemical constituents of plants is desirable. In 2001, infectious diseases accounted for about 26% economy and health care system of deaths worldwide (Kindhauser, 2003). The emerging infectious diseases and their (basic) causes pose a threat to instability of the nation's worldwide. The spread of antimicrobial resistance is another emerging global public health issue. New pathogens and antimicrobial-resistant strains of the pathogens continue to emerge, some with the potential for a rapid and a global spread, with high morbidity and mortality (Gupta and Muralidhara, 2012). Keeping in view the above plant products and availability of techniques to explore new and design novel drugs, some of the selected plants were investigated in this study to evaluate their potential against the selected pathogenic bacterial strains and to identify active components accordingly.

MATERIALS AND METHODS

Collection of plant material

Five different test plants (*Thymus vulgaris, Lavandula angustifolia, Rosmarinus officinalis, Cymbopogon citratus* and *Achillea millefolium*) were studied with their passport data listed in table 1 and were processed in the month of August, 2012 from the living repository of Plant Genetic Resource Institute (PGRI), National Agricultural Research Centre (NARC), Islamabad, Pakistan.

Collection of bacterial strains

The bacterial strains used in this study included: *Staphylococcus aureus*, *Pseudomonas aeruginosa, Escherichia coli, Proteus vulgaris* and *Klebsiella pneumoniae* collected from Microbiology Lab of Quaid-e-Azam international hospital, Rawalpindi, Pakistan. The bacterial strains were maintained on nutrient agar at 4°C and sub-cultured 24 hours before testing.

Chemicals

All the chemicals including ethanol, hexane, chloroform, hydrochloric acid (HCL) and glacial acetic acid (of Analytical Grade) were purchased from Scharlau. TLC plates (Silica gel 60 F254) Merck, Silica gel 60, 0.06-0.2 mm for column chromatography (70-230 mesh) and Nutrient agar (NA, Oxoid).

Preparation of crude extracts

Plants were first washed and dried under shade (at room temperature) for two weeks. Leaves of plants were separated and pulverized in electric grinder and stored in airtight polyethylene bags separately until further use. Crude extract of selected plants were prepared by maceration method (Reddy and Mishra, 2012). Thirty grams of each plant (dried powder) were soaked in 150ml ethanol and hexane separately and kept on shaker for 48 hours. Then filtered through muslin cloth and again filtered through filter paper (Whatman No.1) under fume hood. The filtrate was then dried in a rotary evaporator until all the solvent got evaporated and only dry extract was left behind. After thorough evaporation of the solvent, plant extracts were stored at 4 C in air tight bottles after accurately weighed for further experiments.

Screening of the extract for antibacterial activity

Antibacterial activity of selected plants leaf extracts was determined by the dics diffusion method (Bakht *et al.*, 2011). Each extract was dissolved in 99.9% dimethyl sulfoxide (DMSO) to get 100mg/ml concentration. Fresh inoculams of each bacterial strain were streaked on each plate separately. Sterilized filter paper disc (6mm) were placed on each plate (separately for each extract). Using micropipette, 50µl of crude plant extracts with concentration of 100mg/ml was applied on disks. In a separate plate, 2 disks were placed and in one DMSO used as negative control and Amoxicillin (10µg ml^{-1}) in second disk was used as positive control. Plates were incubated at $37^{\circ}C$ for 24 hours. Thereafter, diameter of clear zones, showing no bacterial growth around each disk was measured in millimeter. The experiments were performed in triplicate. The data were analyzed by ANOVA analysis of variance by using Statistix 8.1 software.

Minimum inhibitory concentration

The minimum inhibitory concentration (MIC) was determined for the three highly active plant species that showed antibacterial activity against test microorganisms. This procedure was also repeated through disk diffusion (method) with some modifications (Bakht *et al*., 2011). A 40µl volume of extracts with different concentrations (20mg/ml, 10mg/ml, 5mg/ml and 1mg/ml) were placed separately on 6mm filter paper disk with the help of micropipette. Four filter paper disks with different concentrations of the same extract were placed in a single Petri plate. The disk soaked in DMSO acted as negative control in center of plate was placed. The plates were then incubated for 24 hours at $37^{o}C$ after which zone of inhibition were measured. The minimum inhibitory concentrations of the extract that inhibited bacterial growth were recorded.

Preliminary screening of secondary metabolites

Identification by chemical test

The crude ethanolic and hexane extracts were screened to identify the phytochemical for the presence of flavonoids, coumarins, terpenoids, quinones, saponins, alkaloids, phenols and tannins. All these contents were qualitatively screened according to the standard procedure (Sofowora, 1931; Harborne, 1973; Trease and Evans, 1989).

Separation of secondary metabolites by thin layer chromatography

The extracts of ethanolic and hexane extracts were transferred to thin layer chromatography (TLC).Plates (GF 254 60; Merck) 250µm thick were developed with solvent system chloroform: Methanol (20:1) after standardization which separated components into wide range of RF values. The plates were visualized under visible and UV light (254nm and 365nm) and sprayed with Dragendorff's reagent for alkaloids and 15% sulphuric acid for terpenes (Harborne, 1984).

Column chromatography

On the basis of preliminary assay results, rosemary extract was fractionated through silica gel column chromatography. Plant extract was dissolved in a ratio of hexane: chloroform (1:1) and adsorbed on silica gel and allowed to dry it to a fine powder. After column packing, solvent system for mobile phase was chosen. Column was eluted with hexane, chloroform and methanol mixtures with increasing polarity. Fractions were collected and dried in vacuum hood. Fractions were subjected to TLC plates. Similar Rf value fractions were then pooled and different groups were formed (Sasidharan *et al.*, 2011)

RESULTS

Extraction of crude extracts

In present study, five different plants namely *Thymus vulgaris, Lavandula angustifolia, Rosmarinus officinalis, Cymbopogon citratus and Achillea millefolium* were collected from clonal repository of IABGR, NARC, Islamabad. Ethanolic plants extracts yield ranged from 9 to 12%. Whereas in hexane extracts, highest (7.3%) yield was obtained with *Achillea millefolium* (table 2).

Antibacterial activity of the selected plants

The antibacterial activity results were recorded individually for studying the effect of solvents as well as plants against the tested strains. For the two solvents, ethanolic extract of *Rosmarinus officinalis* (test plant 1) proved most active against all the tested strains. Overall, negative control (DMSO) showed no activity where as positive control (Amoxicillin 10µg ml^{-1}) showed different inhibition zones against tested strains. Maximum activity was shown against *K. pneumoniae* with zone of inhibition 18.17±0.44mm and minimum zone of inhibition was exhibited against *E. coli* and *P. aeruginosa* with 15.5± 0.29mm zone of inhibition. *S. aureus* growth was arrested an inhibition zone of 17.5±0.29mm while *P. vulgaris* showed 16.83±0.17mm zone of inhibition (table 3). Over all *Rosmarinus officinalis* ethanolic extract showed significant activity [($p<0.05$) (table. 3)]. Whereas, hexane extract of the same plant inhibited 80% of the tested strains (table 4).

Test plant 2, *Thymus vulgaris* ethanolic extract showed maximum inhibition (16.5±0.29mm) against *E. coli* whereas its hexane extract appreciatly inhibited *K. pneumoniae* and *S. aureus* with 11.8±0.4 and 12.8 ±0.6mm zone of inhibition respectively. *P. vulgaris* offered highest resistance against the tested extracts (ethanol and hexane) of *Thymus vulgaris* with minimum inhibition zones range of 9.67±0.17 to 10.3±0.3mm (table 3& 4).

For *Achillea millefolium* (test plant 3), both extracts (9.3±0.3 to 13.167±0.6mm) showed maximum inhibition against *E. coli* and *K. pneumonia*. Minimum activity of extracts was observed against *P. aeruginosa* and *S. aureus* respectively (table 3 & 4).

The ethanolic extract of *Lavandula angustifolia* (test plant 4) exhibited maximum inhibition measuring 15.3±0.44 mm and 15±0.58mm respectively against *E. coli* and *K. pneumoniae* and minimum inhibition zone measuring 12.7±0.67mm was exhibited against *P. vulgaris*. In case of hexane extract, *S. aureus* (7.67±0.33mm) and *E.coli* (7.5±0.29mm) exhibited positive activity except *P. aeruginosa*, *K. pneumonia* and *P. vulgaris* (table 3 & 4).

Ethanol and hexane extracts of test plant 5, *Cymbopogon citratus* showed different behavior. Hexane extract did not show any inhibition against all the tested bacterial strains whereas positive activity (7±0.58 to 15.17±0.44mm) against *K. pneumoniae* and *P. vulgaris* was manifested (table 3 & 4).

Minimum inhibitory concentration

Minimum Inhibitory Concentration (MIC) was also determined for selected plants. For the test plant 1, *Rosmarinus officinalis*, ethanolic and hexane extracts showed MIC at 20mg/ml against *P. vulgaris* while both were also active against *K. pneumoniae* at 10mg/ml. MIC for ethanol extract of test plant 1 against *P. aeruginosa* was 5mg/ml whereas no activity was observed for the same test strain with hexane extract. *E. coli* and *S. aureus* had similar MIC value for hexane extract (table 5).

Test plant 2, *Thymus vulgaris* ethanolic extract showed 20mg/ml MIC value for *P. aeruginosa* and *P. vulgaris*, it decrease up to 10mg/ml (for *E. coli* and *K. pneumoniae*) finally lowered down to 5mg/ml for *S. aureus*, but was 20mg/ml for hexane extract (table 5).

Test plant 3, *Achillea millefolium* ethanolic and hexane extract showed 10mg/ml and 20mg/ml MICs against *K. pneumonia*. Similar MIC (20mg/ml) was recorded for *E. coli* and *P. aeruginosa* but lowest for *S. aureus* (5mg/ml) as shown in table 5.

Test plant 4, *Lavandula angustifolia* ethanolic extract exhibited 5mg/ml MIC against *P. aeruginosa* and *S. aureus* it was 10 mg/ml for *E. coli*, *K. pneumoniae* and 20 mg/ml for *P. vulgaris* as indicated in table 5.

Table 1: Selected medicinal plants and their properties.

Scientific Name	Common Name	Passport Data	Traditional uses
Rosmarinus officinalis	Rosemary	Quetta	Used as a diaphoretic, digestant, diuretic, emmenagogue, cholagogue, laxative, menstrual disorders, defective memory, asthma and tiredness.
Thymus vulgaris	Thyme	Canada	Used to prevent hardening of the arteries, treatment of toothache, urinary tract infection, dyspepsia and depression.
Achillea millefolium	Yarrow	Kashmir	Used to treat wounds, urinary infections, digestive disorders, hemorrhages, inflammation, pain, spasmodic diseases.
Lavandula angustifolia	Lavender	Syria	Used as antiseptic, bronchitis, problem of colon, cough, urinary infection, wounds, burns and rheumatic.
Cymbopogon citratus	Lemongrass	China	Used to treat nervous, gastrointestinal disorders, fever, cough, menstrual disorder and hypertension.

Table 2: Percentage yield of ethanol and hexane extracts of selected plants

Plants/solvents	Dry weight (grams)		Crude weight (grams)		% yield w/w	
	Ethanol	Heaxane	Ethanol	Heaxane	Ethanol	Heaxane
Rosmarinus officinalis	30	30	3.6	1.8	12	6
Thymus vulgaris	30	30	2.3	1.9	7.6	6.3
Achillea millefolium	30	30	2.6	2.2	8.8	7.3
Lavandula angustifolia	30	30	2.7	2.1	9	7
Cymbopogon citratus	30	30	2.7	2	9	6.6

Table 3: Antibacterial activity of ethanol extracts

Plants/strains	Mean value for zone of inhibition(mm)± SD				
	E.c	K.p	P.v	P.a	S.a
Rosmarinus officinalis	15.5±0.29	18.17±0.44	16.83±0.17	15.5±0.29	17.5±0.29
Thymus vulgaris	16.5±0.29	15.17±0.6	9.67±0.17	11.5±0.29	15.67±0.33
Achillea millefolium	17.5±0.29	17±0.58	0±0	13.16±0.60	15.16±0.44
Lavandulaangustifolia	15.3±0.44	15±0.58	12.7±0.67	14.5±0.29	14.33±0.33
Cymbopogon citratus	14.3±0.33	15.17±0.44	7±0.58	0±0	14.33±0.33

Table 4: Antibacterial activity of hexane extracts

Plants/strains	Mean value of zone of inhibition(mm) ±SD				
	E.c	K.p	P.v	P.a	S. a
Rosmarinus officinalis	11.3±0.44	13.5±0.29	11.5±0.29	0±0	12.2±0.44
Thymus vulgaris	11.8±0.4	9.5±0.3	10.3±0.3	0±0	12.8±0.6
Achillea millefolium	11.5±0.29	11.167±0.6	10.67±0.3	0±0	9.3±0.3
Lavandula angustifolia	7.67±0.33	0±0	0±0	0±0	7.5±0.29
Cymbopogon citratus	0	0±0	0±0	0±0	2.3±2.3

Table 5: MIC for ethanol and hexane extract against tested strains

Solvents Plants/strains	MIC mg/ml (Ethanol Extract)					MIC mg/ml (Hexane Extract)				
	P.a	E.c	P.v	K.p	S.a	P.a	E.c	P.v	K.p	S.a
Rosmarinus officinalis	5	10	20	10	5	NT	20	20	10	20
Thymus vulgaris	20	10	20	10	5	NT	ND	ND	ND	20
Achillea millefolium	20	20	ND	10	5	NT	ND	20	20	ND
Lavandula angustifolia	5	10	20	10	5	NT	NT	NT	NT	NT
Cymbopogon citratus	NT	10	ND	1	20	NT	NT	NT	NT	NT

Keys: E.c=*Escherichia coli*, K.p=*Klebsiella pneumoniae*, P.v=*Proteus vulgaris*, P.a=*Pseudomonas aeruginosa*, S.a= *Staphylococcus aureus.*

Table 6: Qualitative analysis of the phytochemicals of the tested plants

Solvents test/plants	(Ethanol extract)					(Hexane extract)				
	R	T	Y	L	Lg	R	T	Y	L	Lg
Alkaloid	+++	-	-	++	+	-	++	-	+	-
Flavonoids	++	-	-	+	-	++	+	-	++	-
Coumarins	++	+	+	-	-	-	+	+	-	-
Tannins	+++	-	+++	+	+	+++	+	+++	++	-
Phenols	+++	+++	+++	+++	+++	+++	++	-	++	+++
Quinones	-	++	-	-	-	-	-	++	-	-
Saponins	+++	++	+	++	+	-	-	-	-	-
Terpenoids	-	-	-	-	-	-	+++	++	+++	-

Key: R= *Rosmarinus officinalis*, T=*Thymus vulgaris*, Y= *Achillea millefolium*, L=*Lavandula angustifolia* and Lg=*Cymbopogon citratus*.

Test plant 5, C*ymbopogon citratus* ethanolic extracts have shown striking activity against the test strains i.e., *K. pneumonia* (1mg/ml) < *E. coli* (10mg/ml) < *S. aureus* (20mg/ml) as shown in table 5. Screening activity of hexane extract of test plant 5 exhibited no inhibition. ANOVA results of hexane extracts showed that variation in zone is significant at p<0.05. It is evident from the present study that both the solvents have remarkable effect on the solubility of bioactive compounds present in the test plants as it is confirmed by their antimicrobial activity.

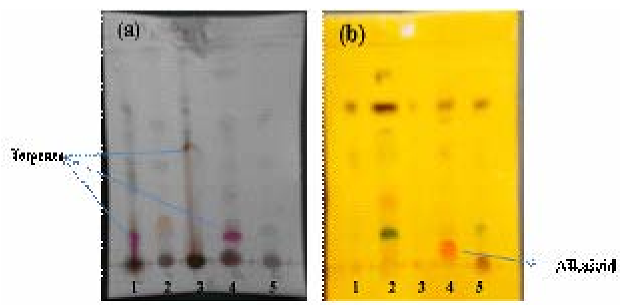

Fig. 1: TLC Plates of ethanolic extracts (a) sprayed with 15% sulphuric acid and (b) dragendorffs reagent lines respresenting 1. Lavender 2. Thyme 3. Lemongrass, 4. Rosemary and 5. Achillea.

Phytochemical analysis

Crude extracts were subjected to preliminary phytochemical analysis by using simple chemical tests. A noteworthy observation was the positive results of alkaloids with ethanol and hexane extracts of *Lavandula angustifolia* but it was negative for *Achillea millefolium*. Additionally, effect of the solvent was clear shown by *Thymus vulgaris*, *Rosmarinus officinalis* and *Cymbopogon* C*itratus* for alkaloids test. In case of ethanol extracts, coumarins are detected in *Thymus vulgaris, Rosmarinus officinalis* and *Achillea millefolium,* whereas, in hexane extracts of *Thymus vulgaris* and *Achillea millefolium* showed presence of coumarins and was absent in rest of the tested plants. Flavonoids were detected in both extracts of *Rosmarinus officinalis* and *Lavandula angustifolia*. Only *Thymus vulgaris* showed positive result with hexane extract. Test for phenols in both solvents was positive except *Achillea millefolium* hexane extracts. All ethanolic extracts of the tested plants were positive for saponins except hexane extracts. Ethanolic and hexane extracts were positive for tannins except *Thymus vulgaris* and *Cymbopogon citratus*, respectively. Terpenoids were positive in *Thymus vulgaris*, *Lavandula angustifolia* and *Achillea millefolium,* hexane extracts only. Results of quinones test were positive for *Thymus vulgaris* and *Achillea millefolium* in ethanolic and hexane extracts respectively. All the phytochemical analysis results are summarized in table 6.

Thin layer chromatography

Bioactive compounds from the test plants were also confirmed with different staining procedure on TLC plate. Separation of compounds varies with different combinations of organic solvents as evident from the observations. Precised separation of bioactive compounds was witnessed with chloroform: methanol (20:1).

Detection of alkaloids & terpenoids through TLC

Plant extracts with both solvents showed different results for alkaloid detection i.e., only positive for *Rosmarinus officinalis* ethanolic extract and negative for hexane extracts of the all tested plants (fig. 1). Staining with 15% sulphuric acid also displayed variation for the presence of terpenoids.

Column chromatography

In the present study, *Rosmarinus officinalis* ethanolic extract showed the maximum inhibitory zone, hence it was selected for column chromatography (purification procedure). During column chromatography, 135 fractions were collected. Crystalline form was observed in some fractions

DISCUSSION

Infectious diseases are the leading cause of death world-wide. Antibiotic resistance has become a global concern. The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant bacterial strains. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases. In order to confirm ethnobotanical importance of plants screening for the presence of bioactive compounds and bioassays (e.g., antimicrobial activity) of taxonomically identified plants needs to be conducted according to the standard procedure (Hussain *et al.*, 2007). In the present study, crude extracts of selected medicinal plants were screened for the presence of bioactive compounds and their therapeutic potential as relied upon their antimicrobial activity.

The antibacterial activity results of *Rosmarinus officinalis* hexane extract are in agreement with previous findings (Witkowska *et al.,* 2013). This might be due to the fact that plant extract compounds have the ability to damage bacterial cell membrane and thus, rendering permeable with an ultimate death due to leakage of ions and molecules. Similar observations for *Thymus vulgaris* ethanolic extract against pathogenic strains for *S. aureus*, *E. coli* and *P. aeruginosa* were reported earlier (Yazdi *et al.,* 2013; Dababneh, 2007). It is also reported that antimicrobial activity of *Thymus vulgaris* mainly depends on its main components thymol and carvacrol, which are well known for their antimicrobial potential. Results similar to those out of the present study of ethanolic extract of *Lavandula angustifolia* were supported earlier (Al-Hussaini and Mahasneh, 2011). This is indicative of the presence of similar compounds present in either of the samples. In contrast, findings of another study contradicted our results particularly for hexane extract that might be due to the reason extract activities vary nor only with active ingredients but also with plant parts (Shafaghat *et al.,* 2012). Results of *Cymbopogon citratus* of as present study are in accordance with earlier observations (Danlami *et al.,* 2011; Ewansiha *et al.,* 2012). Lower MIC values indicate toward better efficiency of extracts against the tested strains. It can be concluded from the present results that choice of the solvents for extraction has significant effect on the antibacterial activity as well as for exploring the bioactive compounds from the test plants. The presence of these compounds could provide a rationale for the ethanomedicinal use of these plants for the treatment of infectious diseases caused by different bacterial strains.

The presence of phytochemicals has an important role in biological activities of medicinal plants. Presence of alkaloids in this study gives us the clue for their involvement in cytotoxicity, antibacterial, analgesic and antispasmodic activities (Yadav and Agarwala, 2011). As reported earlier, the presence of coumarins in plants exhibit antimicrobial, antitumor and cytotoxic activities (Cao *et al.,* 1998). These plants have potential be used against treating different microbial diseases. Similarly, presence of flavonoids is indicative for their applications for controlling microbial infections, cancer and tumours (Lopez-Lazaro, 2002). It is already reported that existence of phenols in plant is responsible for different biological activities such as antibacterial, anti-allergic, anti-inflammatory, antitumor, antioxitadive and antiviral activities (Stefani *et al.,* 1999). Saponins are part of more than 90 families of plant kingdom and they possess antimicrobial, antitumor as well as antidiabetic properties and also used in treating immunoregulatory and heart diseases (Yang *et al.,* 2006). Presence of tannins in plants was documented for various biological activities e.g., antibacterial as is also confirmed by the present study (Doss *et al*., 2009). The presence of terpenoids in our test plants supports for their utilization as anticancerous, antimicrobial, antiviral, antifungal, antiparasitic, anti-allergenic, antispasmodic and anti-inflammatory properties (Rabi and Bishayee, 2009).

Previous studies support our methodology used for the detection of alkaloids. These fractions were found different with reference to their color shades. Orange colored crystals were observed in 2 vials that might be the indication of the presence of alkaloid bioactive compounds present in *Rosmarinus officinalis* like carnosic acid, rosmarinic acid, carnosol and ursolic acid as reported earlier (Abou-Donia *et al.,* 2013; Ibarra *et al*., 2010; Jang *et al*., 2010).

CONCLUSIONS

The present study revealed that tested plant extracts may have effective antibacterial activity against the selected human pathogenic bacterial strains. As bacterial resistance against broad range of antibiotics is a great challenge for medical scientists, the results of this study may provide an insight to further characterize the bioactive compounds to explore bacterial growth inhibitor against wide range of infectious disease. The diversity of extracted compounds in different solvents also provides the clue for broad based utilization of these plants for industrial purposes. Further biological activities and structure determination studies (FTIR) need to be conducted for the identification of lead the compound(s).

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