WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 3, Issue 6, 80-90. Research Article ISSN 2277 – 7105

POTENCY OF ESSENTIAL OILS EXTRACTED FROM SOME SELECTED MEDICINAL PLANTS AGAINST BACTERIAL AND FUNGAL PATHOGENS

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Article Received on
05 June 2014,

Revised on 30 June 2014,
Accepted on 25 July 2014

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ABSTRACT

Medicinal plants have a great importance in biological field. *Mentha longifolia, Angilica glauca, Artemisia meritima,Cerdus deodara, Thymus serypelleum* and *Sassura lappa* are selected medicinal plants used in this study for the sake of extraction of essential oils. The essential oils were extracted by hydro distillation method. Essential oils from these medicinal plants have great impact for antimicrobial (antibacterial and antifungal) activities against different strain of bacteria by using microbiological assay (disc diffusion method) and minimum inhibitory concentration (MIC). The results revealed that essential oils have moderate antibacterial effect.

KEY WORDS: medicinal plants, essential oils, antimicrobial assays.

INTRODUCTION

Bioactive compounds that are extracted from the medicinal plants are used for treatment of different diseases and against the pathogenic microorganisms (Behmanesh, *et al.,* 2007). Use of such bioactive compounds has a great significance since ancient time for the treatment of different ailments (Nostro *et al*., 2000) According to survey only 20% of the plants have been studied and 60% of synthetic medicines owe their origin to plants (Camps, 1985). Like that of

quinine related drugs *Artemisinin* is a sesquiterpene lactone in nature that have peroxide group at its end and therefore play a significant role for its antimicrobial and anti malarial activities(Dorman *et al*.,2000). Due to its gametocytocidal nature of derivatives of *Artemisinin* are responsible for reduction and transmission of malaria (Merlin *et al*., 2000). Ethanolic extract of *Saussurea lappa* effect was studied on adherence, acid production, growth and water-insoluble glucan synthesis of *Streptococcus mutans* (Twarog and Kapoor, 2004).Ethanolic extract of *Saussurea lappa* was known to have inhibitory action against *Streptococcus mutans* (Rani *etal*.,2004), that are responsible for the formation of dental carries and dental plaque (Anderson *et al*., 2005). The extractof *Saussurea lappa* inhibited growth of *Streptococcus mutans* provides some scientific rationales that the local inhabitants used the extracts for treatment of dental diseases (Shouji *et al*., 2000). Pathogenic reactions in plants are restricted by two mechanism, one is responsible for development of the structure and compound synthesized during the plant normal development (constitutive resistance factor) (Sunita and Mahendra, 2008).

The other mechanism is defensive in nature that is activated only after contact with the pathogen (induced resistance factor) (Tepe *et al.,* 2002). These defensive mechanisms have two phases, one is recognition of elicitors and other is signal transduction, which is responsible normally in regulation of expression of genes related to defensive system (Lamidi *et al.,* 2005). This mechanism induced the resistance in plants either by localized or systematic way. When plant is exposed by pathogen or any other external factor then then systematic and localized defensive system become activated and defend the plant body (Greenberg, 1997; Heath, 2000).

Medicinal plants used as a large unexplored source of development and creation of new drugs that have great potential against the resistive diseases and a great source of antibiotics. Medicinal plants plays important role in the basic health needs in developing countries (Tellez *et al.,* 2003). Therefore it is of great interest to reveal the active principle by isolation and characterization of their constituents that have antimicrobial effects. (Ali *et al.,* 2001).

Material and method

The essential oils from some selected medicinal plants were extracted by hydro distillation method. Aerial parts of plants were air dried and ground into fine powder. This powder form is subjected into Clevenger type apparatus for hydro distillation for 3 hours recommended by

British Pharmacopeia (1988). By this procedure essential oils were evaporated together with water vapors and essential oils separated by condenser (Sarah *et al.*,2000).

Antimicrobial Assay

Growth medium for bacteria, cultures and inoculum formation

For the bacterial growth nutrient agar medium used, either prepared in slants or in Petri plates. For the preparation of incoulum nutrient broth was suspended in distilled water and autoclaved for 15 min at 121^0C. A Loop full of a bacterial strain culture was mixed in medium and placed in shaker for 24 hours at 37C.The inocula were stored at 4C.The inocula with 1×10^8 spores/mL were used for further analysis(Suaib *et al.*,2005).

Antibacterial assay by disc diffusion method

Nutrient agar will be suspend in distilled water and mixed well. Sterilized the medium by autoclaving and inoculum will be added to the medium and pour in sterilized Petri plates. After this small wrick paper discs will be laid flat on the growth medium containing sample extract. The Petri plates will be incubated a 37^0C for 24 hours, for growth of medium .The extract having antibacterial activity, inhibited the bacterial growth and clear zone will form (Haung *et al*., 2000; Huynh *et al*., 2001; Rehman *et al*., 2001).

Antifungal assay

Growth medium, culture and inoculum preparation:

Pure culture of the fungi wasobtained on sabouraud dextrose agar medium either in petri plate or in slant hat were sterilized in hot air oven at 180 C for 3 hours. These culture slants were incubated at 28 C for 3-4 days for the multiplication of fungal strains (Shahverdi*et al.*, 2002)

Antifungal assay by disc diffusion method

The growth medium was prepared and transferred to the sterilized Petri plates. The Petri plates were incubated at 28 C for 48 hours, for the growth of fungus. Small filter paper discs were laid flat on growth medium having fungal growth, and 100 µL of extracts was applied on each disc. The Petri plates were again incubated. The extracts having antifungal activity exhibited clear zones around the discs (Haung *et al*., 2000; Huynh *et al.*, 2001; Rehman *et al*., 2001).

Minimum Inhibitory Concentrations (MIC) of plant extracts

In 96 well plates (microdilution plates) poured 100 µL of nutrient broth in all wells then pour 100 µL of sample in first well followed by two fold dilution method. After that add 20 µL given bacterial culture in each well. After that add reducing dye resurizine and incubate the plate at 37C for 24 hour. The color of sample plates well change from purple to pink which indicate the bacterial growth in different dilution wells. The absorbance was measured at 597nm by micro quant (Razilian, 2004). The minimum inhibitory concentration were determined by using the following formula %growth inhibition: Acontrol - Aexperimental/Absorbance of control*100

RESULTS AND DISCUSSION

Antimicrobial Activity of Essential Oils

Antimicrobial activity of these essential oils (*Sassurea lappa, Thymus serypellum, Cedrus deodara, Angilica glauca Mentha longifolia, Artemisia meritima and Skima laureda,*) was performed by using disc diffusion method; against four bacterial and four fungal strains. The results are represented by mathematical signs. Results indicate that different essential oils showed both broad spectrum of activity by forming clear zones of inhibition or negligible zones of inhibition at very poor activity against these strains (Ping *et al.*, 2007). Negative results that indicate that these oils had no active compound or if present it had either very low concentration or it might have lost its activity. The results are presented below:

Table 1: Grading of antimicrobial activity in symbolic and digitalized from along with interpretation for activities

Mathematical sign	Zone size (mm)	Interpretation
-	0	No or poor activity
+	1-15	Activity present
++	16-20	Strong activity
+++	21-35	Very strong activity
++++	35-100	Excellent

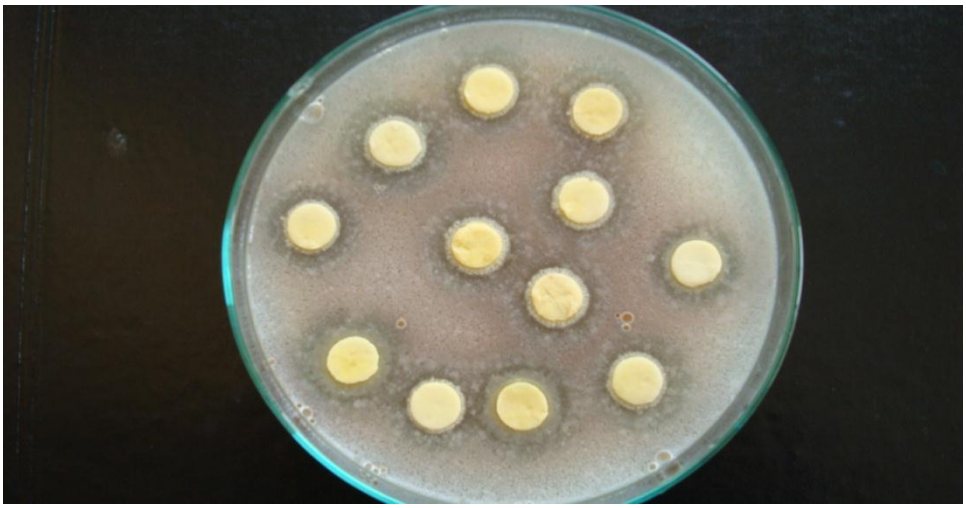
1.1: Antimicrobial Assay of Essential Oil of Some Medicinal Plants (*Sassurea lappa, Cedrus deodara, Mentha longifolia, Angilica glauca, Thymus serypellum, Skima laureda, Artemisia meritima)* Representation of results of antibacterial activity of essential oil of medicinal plants by mathematical signs

Medicinal Plants	Conc. of Sample	*B. subtilis*	*S. aureus*	*E. coli*	*P. multocida*
Angilica glauca	50 μl	+	+	+	-
	100 μl	-	+	+	+
	150 μl	+	++	++	+
Cedrus deodara (fresh)	50 μl	+	+	-	+
	100 μl	++	-	+	+
	150 μl	+	+	++	+
Mentha longifolia	50 μl	++	+	-	-
	100 μl	+	+	+	+
	150 μl	++	+	+++	+
Skima laureda	50 μl	+	+	+	+
	100 μl	+	+	+	+
	150 μl	+	+	+++	++
Cedrus deodara (old)	50 μl	++	++	++	++
	100 μl	+	+	+	+++
	150 μl	+	+	-	-
Artemisia meritimia	50 μl	-	+	+	+
	100 μl	+	+	+	+
	150 μl	+	-	++	++
Sassurea lappa (old)	50 μl	-	+	+	+
	100 μl	++	+	+	-
	150 μl	+	+	++	++
Sassurea lappa (fresh)	50 μl	+	+	++	+
	100 μl	+	+	+	+
	150 μl	++	++	+++	+++
Thymus serypellum (old)	50 μl	+	++	+++	+
	100 μl	+	++	++	++
	150 μl	-	++	++	++
Thymus serypellum (fresh)	50 μl	++	+	++	-
	100 μl	+	+	+	+

1.2: Representation of Results of antibacterial activity of essential oils of medicinal plants

Medicinal Plants	Conc. of Sample	*B. subtils*	*S. aureus*	*E. coli*	*P. multocia*
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Angilica glauca	50 μl	11 ± 0.5	11.9 ± 0.5	11.6 ± 0.7	
	100 μl		11.3 ± 0.28	10.83 ±0.76	11.1 ± 0.28
	150 μl	11.6 ± 0.62	12.8 ± 2.2	13.6 ± 3.65	11.8 ± 0.84
Cedrus deodara (fresh)	50 μl	11.8 ± 1.04	11.9 ± 0.28		11.8 ± 1.04
	100 μl	13.0 ±3.04		11.6 ± 0.76	11.3 ± 0.28
	150 μl	10.8 ± 0.62	12.5 ± 1.77	13.3 ±2.95	11.5 ± 0.40
Mentha longifolia	50 μl	12.8 ± 2.7	12.0 ± 0.76		
	100 μl	11.3 ± 0.28	11.3 ± 0.28	11.5 ± 0.5	11.6 ± 0.76

	150 μl	12.8 ± 2.24	12.0 ± 1.0	14.6 ± 4.86	12.6 ± 2.01
Skima laureda	50 μl	11.1 ± 0.28	12.2 ± 0.5	11.3 ± 0.28	11.3 ± 0.28
	100 μl	12.1 ±1.60	11.6 ± 0.76	12.0 ± 1.32	11.5 ± 0.5
	150 μl	11.3 ± 0.23	11.1 ±0.223	14.8 ± 5.0	12.8 ± 2.2
Cedrus deodara (old)	50 μl	13.8 ± 4.8	12.3 ± 4.19	13.8 ± 4.4	12.8 ± 2.7
	100 μl	10.8 ± 0.76	12.1 ± 1.60	12.6 ± 2.4	14.6 ± 5.92
	150 μl	12.0 ± 1.08	11.8 ± 0.84		
Artemisia meritimia	50 μl		12.1 ± 0.5	11.3 ± 0.28	11.5 ± 0.5
	100 μl	11.5 ± 0.5	11.3 ± 0.28	11.5 ± 0.5	12.3 ± 1.89
	150 μl	11.0 ± 0.40		13.1 ± 2.7	13.0 ± 2.4
Sassurea lappa (old)	50 μl		12.25 ± 0.5	12.3 ± 1.89	12.0 ± 1.32
	100 μl	13.8 ± 4.4	11.6 ± 0.76	12.1 ± 1.60	
	150 μl	12.16 ± 1.3	12.6 ± 2.01	12.8 ± 2.2	14.3 ± 4.3
Sassurea lappa (fresh)	50 μl	12.0 ± 1.3	12.5 ± 1.04	13.8 ± 4.4	11.5 ± 0.5
	100 μl	12.0 ± 1.3	11.3 ± 0.28	12.1 ± 1.60	11.3 ± 0.28
	150 μl	13.3 ± 2.29	13.8 ± 3.65	14.1 ± 4.1	14.8 ± 5.07
Thymus serypellum (old)	50 μl	11.6 ± 0.7	12.8 ± 4.48	12.16 ±5.05	12.5 ± 2.17
	100 μl	11.6 ± 0.76	13.8 ± 4.4	13.6 ± 4.1	13.0 ± 3.0
	150 μl		13.6 ± 3.4	14.3 ± 4.3	14.3 ± 4.36



1.1: Antibacterial activity of *oil extracts* against *Staphylococcus aureus*

The disc diffusion method for antibacterial activity showed significant reduction in bacterial growth in terms of zone of inhibition around the disc. The extract of *Angilica glauca* showed maximum activity against *S. aureus* and minimum against *P.multocia. Cedrus deodara* (fresh) and *Mentha longifolia* showed maximum activity against *S.aureus* and minimum against *E. coli.* Similarly *Skima laureda* showed maximum against *S.aureus* and minimum against *B. subtils. Cedrus deodara* (old) showed maximum activity against *B. subtils* and minimum against *P. multocia.* Similarly *Sassurea lappa* (old) and *Sassurea lappa* (fresh)

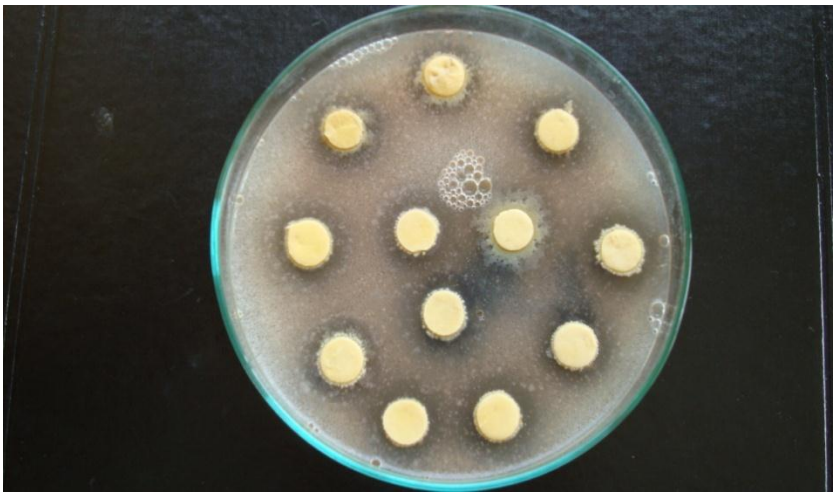
showed maximum activity against *E.coli* and minimum against *P. multocia. Thymus serypellum* (old) and *Thymus serypellum* (fresh) both showed maximum activity against *S. aureus* and minimum against *P. multocia.*

1.3: Representation of Antifungal activity of essential oils of medicinal plants by mathematical signs

Medicinal Plants	Conc.of Sample	*A. Niger*	*A. Flavus*	*R. Solani*	*Fusarium*
Angilica glauca	150 *mM*	+	+	+	+
drus deodara (fresh)	150 *mM*	+	+	+	+
Mentha longifolia	150 *mM*	+	+	+	+
Skima laureda	150 *mM*	+	-	+	+
drus deodara (old)	150 *mM*	+	+	+	+
Artemisia meritimia	150 *mM*	+	+	+	+
Sassurea lappa (old)	150 *mM*	+	+	+	+
surea lappa (fresh)	150 *mM*	+	+	+	+
mus serypellum (old)	150 *mM*	+	+	+	+
mus serypellum (fresh)	150 *mM*	+	+	+	+

1.4: Representation of results of antifungal activity of essential oils of medicinal plants

Medicinal Plants	Conc. of Sample	*A. Niger*	*A. Flavus*	*R.Solani*	*Fusarium*
		Mean±SD	*Mean±SD*	*Mean±SD*	*Mean±SD*
Angilica glauca	150 *mM*	15.3 ± 5.7	12.33 ±1.54	11.33 ±0.23	14.5 ± 4.06
drus deodara (fresh)	150 *mM*	14 ± 3.89	12 ± 1.08	13.33 ±2.95	12.33 ±1.54
Mentha longifolia	150 *mM*	14.16 ±4.12	11.16 ±0.23	15 ± 5.30	14 ± 3.89
Skima laureda	150 *mM*	13.16 ± 2.7		13.66 ±3.42	13.83 ±3.65
drus deodara (old)	150 *mM*	12.16 ±1.31	13 ± 2.48	12.66 ±2.01	13.83 ±3.65
Artemisia meritimia	150 *mM*	12.66 ±2.01	12.5 ± 1.77	13.5 ± 3.18	11.16 ±0.23
Sassurea lappa (old)	150 *mM*	12.66 ±2.01	13.8 ± 3.65	14.16 ±4.12	13.66 ±3.42
surea lappa (fresh)	150 *mM*	13.5 ± 3.1	11.6 ± 0.62	13.8 ± 3.65	13.66 ±3.42
mus serypellum (old)	150 *mM*	13.5 ± 3.1	12.5 ± 1.77	13.8 ± 3.65	12.5 ± 1.77
Thymus serypellum (fresh)	150 *mM*	13.16 ±2.71	11.6 ± 0.62	13.33 ±2.95	11.66 ±0.62



1.2: Antifungal activity of *oil extracts* against *Rhizopus solani*

The extract of oil of different medicinal plants were tested against four fungal strains; *A. Niger, A. Flavus, Fusarium, R. Solani.* The table above showed that of *Angilica glauca* and *Cedrus deodara* (fresh) against *A. Niger* and minimum against *R. Solani* and *A. Flavus* respectively. *Mentha longifolia* showed maximum activity against *R. Solani* and minimum against *A. Flavus. Skima laureda* and *Cedrus deodara* (old) showed maximum activity against *Fusarium* and minimum against *A. Flavus* respectively. *Artemisia meritimia* and *Sassurea lappa* (old) showed maximum activity against *R. Solani* and minimum activity against Fusarium and *A. Niger* respectively. Similarly *Sassurea lappa* (fresh), *Thymus serypellum* (old) and *Thymus serypellum* (fresh) showed maximum activity against *R. Solani* and minimum activity against *A. Flavus* and *Fusarium.* Fungi once thought harmless to humans have emerged as important causes of morbidity and mortality, especially in immuno-compromised patients. The fungi, often resistant to conventional antifungal therapy, include *Aspergillus sp*., and *Fusarium.*

Minimum Inhibitory Concentration

1.5: Minimum Inhibitory Concentration (MIC) of essential oil of medicinal plants against selected fungal strains

Medicinal Plants	Conc. of Sample	Minimum Inhibitory Concentration (MIC) mg/ml
		Mean ± SD
Angilica glauca	100 *µl*	0. 0493 ± 0.00617
Cedrus deodara (fresh)	100 *µl*	0.0637 ± 0.0194
Mentha longifolia	100 *µl*	0.0194 ± 0.00167
Skima laureda	100 *µl*	0.0637 ± 0.00194
Cedrus deodara (old)	100 *µl*	0.092 ± 0.0068
Artemisia meritimia	100 *µl*	0.026 ± 0.0036
Sassurea lappa (old)	100 *µl*	0.0194 ± 0.00167
Sassurea lappa (fresh)	100 *µl*	0.092 ± 0.0068
Thymus serypellum (old)	100 *µl*	0.0637 ± 0.00194
Thymus serypellum (fresh)	100 *µl*	0.052 ± 0. 0036

The MIC of the oil of medicinal plants was determined against fungal strain (*Rhizopus solani).* The results were given in table. Minimum Inhibitory Concentration of oil extracts of medicinal plants, *Angilica glauca* showed least value 0.00493 mg/mL and *Artemisia meritimia* showed highest value 0.026 mg/ mL against *R. Solani*.

1.6: Minimum Inhibitory Concentration of oil extract of medicinal plants against selected bacterial strain

Medicinal Plants	Conc. of Sample	Minimum Inhibitory Concentration (MIC) mg/ml
		Mean ± SD
Angilica glauca	100 *µl*	0.71 ± 0.49
Cedrus deodara (fresh)	100 *µl*	0.87 ± 0.22
Mentha longifolia	100 *µl*	0.93 ± 0.12
Skima laureda	100 *µl*	0.99 ± 0.01
Cedrus deodara (old)	100 *µl*	0.91 ± 0.144
Artemisia meritimia	100 *µl*	0.73 ± 0.46
Sassurea lappa (old)	100 *µl*	0.92 ± 0.13
Sassurea lappa (fresh)	100 *µl*	0.82 ± 0.30
Thymus serypellum (old)	100 *µl*	0.85 ± 0.26
Thymus serypellum (fresh)	100 *µl*	0.96 ± 0.06

In the MIC of the samples against bacteria the strain that used was *Saccharomyces cereviceiae*.Then for results calculation were performed in mg/m. The calculations are given in the table. *Skima laureda* showed highest value 0.99 mg/mL and *Angilica glauca* showed least value 0.71 mg/mL against selected bacterial strain (*Saccharomyces cereviceiae*).

CONCULSION

Due to multifunctional properties, antimicrobial peptides/proteins isolated and purified from natural sources like animals, plants, microorganism etc. are being considered as alternative to synthetic antibiotics against which of the infection causing agents have developed resistance. The present research work was therefore, chalked out to screen medicinal plants for their antimicrobial activity, to find MIC values of these proteins, to see the effect of different environmental condition on these proteins and effect of these protein on different enzymes. From these antimicrobial results, it is suggested that the medicinal plants have antimicrobial peptides/proteins and in future, may be used for industrial scale extraction and isolation of antimicrobial compounds particularly peptide/protein which may find place in medicinal industry as constituent of antibiotics.

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