INTERNATIONAL JOURNAL OF SCIENCE AND NATURE

www.scienceandnature.org

ANTIBACTERIAL AND ANTICANDIDAL SCREENING OF CERTAIN TRADITIONALLY USED INDIAN MEDICINAL PLANTS AGAINST MULTI-DRUG RESISTANT HUMAN PATHOGENS

[1]Milin K. Agrawal, [2]Surendra K. Goyal, [3]Alok K. Varma & [4]Alka Varma
[1]Division of Microbiology, Department of Botany, St. Mary's P. G. College, Vidisha, M.P. India.
[2] Department of Sericulture, Resham Project Kendra, Sironj, Vidisha, M.P. India.
[3]Department of Zoology, Raja Bhoj Govt. College, Mandideep, Raisen, M.P. India
[4]Department of Botany, Govt. Sarojini Naidu Girls College, Bhopal, M.P. India

ABSTRACT
Ethanolic and aqueous extracts of 20 Indian medicinal plants traditionally used in medicine were studied for their antibacterial and anticandidal activity against five multi – drug resistant bacteria i. e. *Staphylococcus aureus* (NCIM 2079), *Bacillus subtilis* (NCIM 2063), *Escherichia coli* (NCIM 2065), *Salmonella typhimurium* (NCIM 2501), *Streptococcus pneumoniae* (NCIM 5281) and a yeast *Candida albicans* (NCIM 3471). Of these, 76 ethanolic and 64 aqueous extracts showed varied levels of antibacterial activity against test bacterial strains whereas anticandidal activity was detected in 07 ethanolic and 05 aqueous extracts. Overall, broad-spectrum antibacterial activity was observed in 08 plants (ethanolic extracts) and 06 plants (aqueous extracts) against all test bacteria and 07 plants (ethanolic extracts) and 05 plants (aqueous extracts) against *Candida albicans*.

KEYWORDS: Antibacterial screening; Anticandidal screening; Medicinal plants; Multi drug resistance; Human pathogens.

INTRODUCTION

Infectious diseases are the world's leading cause of premature deaths, killing almost 60000 people per day despite remarkable advances in Medical research and treatment during the 20th century, infectious diseases remain among the leading cause of death worldwide. Of these, nosocomial infections comprise about 5 to 10% (Culver *et al*., 1985). It has been estimated that one third of all nosocomial infections may be preventable and are frequently caused by organisms acquired within the hospital environment (Hughes, 1988). In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world (Omololu *et al*., 2011; Robin *et al*., 1998; Mulligen *et al*., 1993; Piddock and Wise, 1989). Today, the situation is alarming in developing as well as developed countries due to indiscriminate use of antibiotics. However, rapid emergence of antimicrobial resistance among pathogenic microorganisms has led to a renewed search for new antimicrobial agents. Severe infections caused by bacteria that are resistant to commonly used antibiotics have become a major global healthcare problem in the 21st century (Alanis, 2005). The most resistant bacteria causing important community acquired infections include methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Staphylococcus aureus* (VRSA), vancomycin-resistant *Enterococcus* (VRE), extended spectrum-lactamase (ESBL) producing bacteria such as *E. coli* and *Klebsiella* sp. and multiple drug resistant *Mycobacterium tuberculosis* (MDR-MTB). In India, medicinal plants are widely used by all sections of people either directly as folk remedies or in different indigenous systems of medicine or indirectly in the pharmaceutical preparations of modern medicines. A survey by UNCTAD has shown that 33 % of total drugs produced by the industrialized nations are plant derived and 60 % of medicinal products are of natural origin (UNCTAD / Gatt, 1974). In the traditional medicinal system of India, Rigveda mentions 67 plants having therapeutic effects, Yajurveda lists 81 plants and Atharveda have 290 plants (Singh and Bisht, 1992) besides this the different systems of medicine practiced in India, Ayurveda, Siddha, Unani, Amchi and local health traditions, utilize a large number of plants for the treatment of human diseases. In the present scenario of emergence of multiple drug resistance to human pathogenic organisms, this has necessitated a search for new antimicrobial substances from other sources including plants. Therefore, it is imperative to search for new, efficacious and safe antibiotics from natural sources to combat the menace of drug-resistant infections. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug-resistant microbial pathogens. In the present study, we have selected 20 Indian medicinal plants to be screened against multi-drug resistant bacteria including *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi* and *Streptococcus pneumoniae* and a yeast *Candida albicans*. The selection of the medicinal plants is based on their traditional uses in India (Chopra *et al*., 1992).

MATERIAL & METHODS

Plant Material

Eleven authenticated plants namely *Azadirachta indica* A. Juss., *Emblica officinalis* Gaerth., *Eucalyptus* sp., *Ficus*

religiosa L., *Lantana camara* L., *Lawsonia inermis* L., *Nyctanthes arbortristis* L., *Ocimum sanctum* L., *Punica granatum* L., *Syzgium cumini* L. and *Terminalia arjuna* W & A samples were collected locally and nine plant samples namely *Beta vulgaris* L., *Camelia sinensis* L., *Hemidesmus indicus* R. Br., *Holarrhena antidysenterica* R., *Morus alba* L., *Saussurea lappa* C. B. Clarke, *Syzgium aromaticum* L., *Terminalia belerica* Roxb. and *Terminalia chebula* Retz. were purchased from the local market of Vidisha. The taxonomic identities of these plants were confirmed by relevant data and were further identified by a senior taxonomist Dr. R. K. Jain, Dept. of Botany, S. S. L. Jain P. G. College, Vidisha and voucher specimens have been deposited in the Department of Botany, St. Mary's P. G. College, Vidisha. The details of medicinal plants along with their voucher specimen number are listed in Table No. 1.

TABLE 1: Ethnobotanical data of 20 Indian Medicinal Plants

S. N.	Botanical Name; Family Voucher Specimen No.	Common Name	Part Used	Traditional Uses (Chopra *et al*., 1992)
01	*Azadirachta indica* A. Juss.; Meliaceae SMCDB-01/15	Neem	Bark	Tonic, astringent, anti periodic, snake periodic bite.
02	*Beta vulgaris* L.; Chenopodiaceae SMCDB-02/15	Chokunder	Roots	Cooling, diaphoretic.
03	*Camelia sinensis* L.; Theaceae SMCDB-03/15	Chai	Leaves	Astringent, diuretic stimulant.
04	*Emblica officinalis* Gaerth.; Euphorbiaceae SMCDB-04/15	Amla	Fruit	Acrid, cooling, refrigerant diuretic, used in diarrhea, dysentery, anemia, jaundice and cough.
05	*Eucalyptus* sp.; Myrtaceae SMCDB-05/15	Eucalyptus	Leaves	Antiseptic, infections of upper respiratory tract, skin diseases, burns, rheumatism.
06	*Ficus religiosa* L.; Moraceae SMCDB-06/15	Pipal	Leaves	Purgative.
07	*Hemidesmus indicus* R. Br.; Asclepiadaceae SMCDB-07/15	Anatmul	Roots	Demulcent, tonic, diaphoretic, skin diseases, syphilis, blood purifier.
08	*Holarrhena antidysenterica* R.; Apocyanaceae SMCDB-08/15	Kurachi	Bark	Dropsy, diarrhea.
09	*Lantana camara* L.; Verbenaceae SMCDB-09/15	Raimunia / Ghaneri	Leaves	Decoction used in malaria, atoxy, rheumatism.
10	*Lawsonia inermis* L.; Lythraceae SMCDB-10/15	Heena / Mehandi	Leaves	Headache, burning of skin, decoction used for sore throat.
11	*Morus alba* L.; Moraceae SMCDB-11/15	Shahtut	Leaves	Decoction used for sore throat.
12	*Nyctanthes arbortristis* L.; Oleaceae SMCDB-12/15	Harsingar	Leaves	Fever, obstrinate sciata, rheumatism.
13	*Ocimum sanctum* L.; Labiatae SMCDB-13/15	Tulsi	Leaves	Gastric disorder, bronchitis, ear ache, antiseptic, diaphoretic, hepatic affections.
14	*Punica granatum* L.; Punicaceae SMCDB-14/15	Anar	Rind	Diarrhea and dysentery.
15	*Saussurea lappa* C. B. Clarke ; Compositae SMCDB-15/15	Kuth	Roots	Tonic, spasmodic in cough, cholera, chronic skin diseases.
16	*Syzgium aromaticum* L.; Myrtaceae SMCDB-16/15	Laung	Bud	Stimulant, carminative, used in dyspepsia.
17	*Syzgium cumini* L.; Myrtaceae SMCDB-17/15	Jamun	Bark	Astringent, used for sore throat, diarrhea.
18	*Terminalia arjuna* W. & A.; Combretaceae SMCDB-18/15	Arjun	Bark	Astringent, bilious affections, heart diseases.
19	*Terminalia belerica* Roxb.; Combretaceae SMCDB-19/15	Bahera	Fruit	Antipyretic, leprosy, diarrhea, dropsy.
20	*Terminalia chebula* Retz.; Combretaceae SMCDB-20/15	Harir	Fruit	Laxative, ulcers, used in curious teeth, piles.

Preparation of Plant Extracts

Plant extracts were prepared by the method of Harborne (1984). Briefly, 100 grams of each powered plant sample was extracted with 200 ml of ethanol and distilled water for ethanolic and aqueous extracts respectively using the Soxhlet apparatus. After extraction, an excess was evaporated under reduced pressure in vacuum evaporator. The dried crude extracts were sterilized overnight by UV radiation and then stored at 4ºC in amber color glass vials until further use.

Preparation of Plants Derived Antibiotic Discs

Both the crude extracts (ethanolic and aqueous) each 100 mg. were dissolved in 1 ml of dimethyle sulphoxide (DMSO) and were filtered by using membrane (pore size 0.47 µm). The discs of 6 mm diameter of Whatman filter paper No. 01 (Sterile blank) were impregnated into the concentration of the each extracts. The final impregnated discs used for the sensitivity test were 100 mg $disc^{-1}$. These impregnated discs were dried in incubator at 37 ºC for 18 – 24 hours and after this stored in an amber colour glass bottle at room temperature until further use.

Microorganisms Used

The test organisms were used *Staphylococcus aureus* (NCIM 2079), *Bacillus subtilis* (NCIM 2063), *Escherichia coli* (NCIM 2065), *Salmonella typhimurium* (NCIM 2501), *Streptococcus pneumoniae* (NCIM 5281) and *Candida albicans* (NCIM 3471). The test microorganisms were obtained from the National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory (NCL), Pune, India.

Antibiotics Resistance of test strains

The antibiotic sensitivity of all the test strains was determined by the standard disk diffusion method of Bauer *et al*. (1966) against a number of antibiotics. The potency of antibiotic discs are: for bacteria, Ciprofloxacin, Levofloxacin, Methicillin (05 µg $disc^{-1}$ each), Gentamycin, Norfloxacin, Streptomycin (10 µg $disc^{-1}$ each), Erythromycin, Linezolid (15 µg $disc^{-1}$ each), Amoxycillin, Chloramphenicol, Doxycycline, Vancomycin (30 µg $disc^{-1}$ each) and for Yeast, Fluconazole, Ketoconazole (10 µg $disc^{-1}$ each), Amphotericin B (20 µg $disc^{-1}$) and Nystatin (50 µg $disc^{-1}$). All antibiotic discs were purchased from the Hi-Media, Bombay, India. The details of antibiotics resistance of test strains are listed in Table No. 2 and 3.

TABLE 2: Antibacterial Screening of some antibiotics

Antibiotics	Antimicrobial activity					
	[a]SA	BS	EC	ST	SP	Total Susceptible Antibiotics
Amoxycillin 30 µg $disc^{-1}$	[b]3+	--	1+	3+	--	03
Chloramphenicol 30 µg $disc^{-1}$	3+	3+	2+	3+	--	04
Ciprofloxacin 05 µg $disc^{-1}$	--	--	3+	2+	--	02
Doxycycline 30 µg $disc^{-1}$	2+	--	--	--	--	01
Erythromycin 15 µg $disc^{-1}$	--	1+	1+	--	2+	03
Gentamycin 10 µg $disc^{-1}$	1+	2+	--	--	--	02
Levofloxacin 05 µg $disc^{-1}$	--	--	2+	1+	--	02
Linezolid 15 µg $disc^{-1}$	--	--	--	1+	--	01
Methicillin 05 µg $disc^{-1}$	--	2+	--	--	--	01
Norfloxacin 10 µg $disc^{-1}$	--	2+	--	--	--	01
Streptomycin 10 µg $disc^{-1}$	2+	--	--	1+	1+	03
Vancomycin 30 µg $disc^{-1}$	--	--	--	2+	1+	02
[c]DMSO blank solvent	--	--	--	--	--	00
Total number of active antibiotics	05	05	05	07	03	25

[a]SA = *Staphylococcus aureus*, BS = *Bacillus subtilis*, EC = *Escherichia coli*, ST = *Salmonella typhi*, SP = *Streptococcus pneumoniae*.
[b]1+ = 6.5 – 10.0 mm; 2+, 10.1 – 15.0 mm; 3+, 15.1 – 20.0 mm; -- , no zone of inhibition.
[c]DMSO blank solvent used as a control.

TABLE 3: Anticandidal Screening of some antibiotics

Antibiotics	*Candida albicans*
Amphotericin B 20 µg $disc^{-1}$	[a]--
Fluconazole 10 µg $disc^{-1}$	1+
Ketoconazole 10 µg $disc^{-1}$	--
Nystatin 50 µg $disc^{-1}$	--
[b]DMSO blank solvent	--
Total number of active antibiotics	01

[a]1+ = 6.5 – 10.0 mm; 2+, 10.1 – 15.0 mm; 3+, 15.1 – 20.0 mm; -- , no zone of inhibition.
[b]DMSO blank solvent used as a control.

Antimicrobial Assay of Plants Derived Antibiotic Discs

For this, the standard disk diffusion method of Bauer *et al*. (1966) was used. 0.1 ml of diluted inoculum (10^5 CFU / ml) of test organisms was spread on Muller-Hinton agar and Sabouraud Dextrose agar plates. The plant derived antibiotics discs were placed on the agar plates. DMSO was used as a control. The plates were incubated at 37 ºC for 18 – 24 hours for bacteria and for yeast, incubated at 48 ºC for 36 – 48 hours. The antimicrobial activity was evaluated by measuring the zone of inhibition (ZOI) in mm against the test organisms. All the experiments were done at triplicate and the mean values of inhibition zone are presented in Table No. 4 and 5.

TABLE 4: Antibacterial Screening of Ethanolic and Aqueous Extracts of 20 Indian Medicinal Plants

Plant name	Antimicrobial activity											
	Ethanolic extract						Aqueous Extract					
	[a]SA	BS	EC	ST	SP	[c]TSE	SA	BS	EC	ST	SP	TSE
01. *Azadirachta indica* A. Juss.	[b]2+	2+	2+	4+	4+	05	1+	1+	1+	2+	2+	05
02. *Beta vulgaris* L.	2+	--	2+	--	--	02	--	--	2+	--	--	01
03. *Camelia sinensis* L.	3+	2+	3+	--	--	03	2+	--	2+	--	--	02
04. *Emblica officinalis* Gaerth.	3+	2+	--	3+	2+	04	3+	2+	--	2+	1+	04
05. *Eucalyptus* sp.	3+	2+	2+	3+	3+	05	2+	2+	1+	2+	2+	05
06. *Ficus religiosa* L.	--	--	--	2+	2+	02	--	--	--	1+	1+	02
07. *Hemidesmus indicus* R. Br.	2+	2+	2+	3+	3+	05	1+	1+	1+	2+	2+	05
08. *Holarrhena antidysenterica* R.	2+	2+	2+	3+	2+	05	1+	1+	--	2+	1+	04
09. *Lantana camara* L.	2+	2+	2+	3+	2+	05	1+	1+	1+	2+	1+	05

10. *Lawsonia inermis* L.	3+	2+	1+	--	--	03	3+	1+	--	--	--	02
11. *Morus alba* L.	2+	--	--	2+	3+	03	--	--	--	1+	2+	02
12. *Nyctanthes arbortristis* L.	2+	--	--	2+	--	02	1+	--	--	1+	--	02
13. *Ocimum sanctum* L.	2+	--	1+	2+	4+	04	1+	--	1+	--	3+	03
14. *Punica granatum* L.	3+	2+	2+	2+	2+	05	2+	2+	1+	2+	2+	05
15. *Saussurea lappa* C. B. Clarke	2+	--	3+	3+	2+	04	1+	--	--	--	1+	02
16. *Syzgium aromaticum* L.	3+	2+	3+	3+	3+	05	3+	2+	2+	2+	2+	05
17. *Syzgium cumini* L.	--	--	--	--	1+	01	--	--	--	--	1+	01
18. *Terminalia arjuna* W. & A.	3+	--	2+	3+	2+	04	2+	--	--	2+	1+	03
19. *Terminalia belerica* Roxb.	3+	2+	2+	3+	2+	05	2+	--	1+	2+	1+	04
20. *Terminalia chebula* Retz.	3+	2+	--	3+	2+	04	2+	--	--	2+	--	02
[d]DMSO	--	--	--	--	--	00	--	--	--	--	--	00
Total number of active plants	18	12	14	16	16	76	16	09	10	14	15	64

[a] SA = *Staphylococcus aureus*, BS = *Bacillus subtilis*, EC = *Escherichia coli*, ST = *Salmonella typhi*, SP = *Streptococcus pneumoniae*.
[b] 1+ = 6.5 – 10.0 mm; 2+, 10.1 – 15.0 mm; 3+, 15.1 – 20.0 mm; 4+, 20.1 – 25.0 mm, -- , no zone of inhibition
[c]TSE = Total Susceptible Plant Extracts
[d]DMSO blank solvent used as a control.

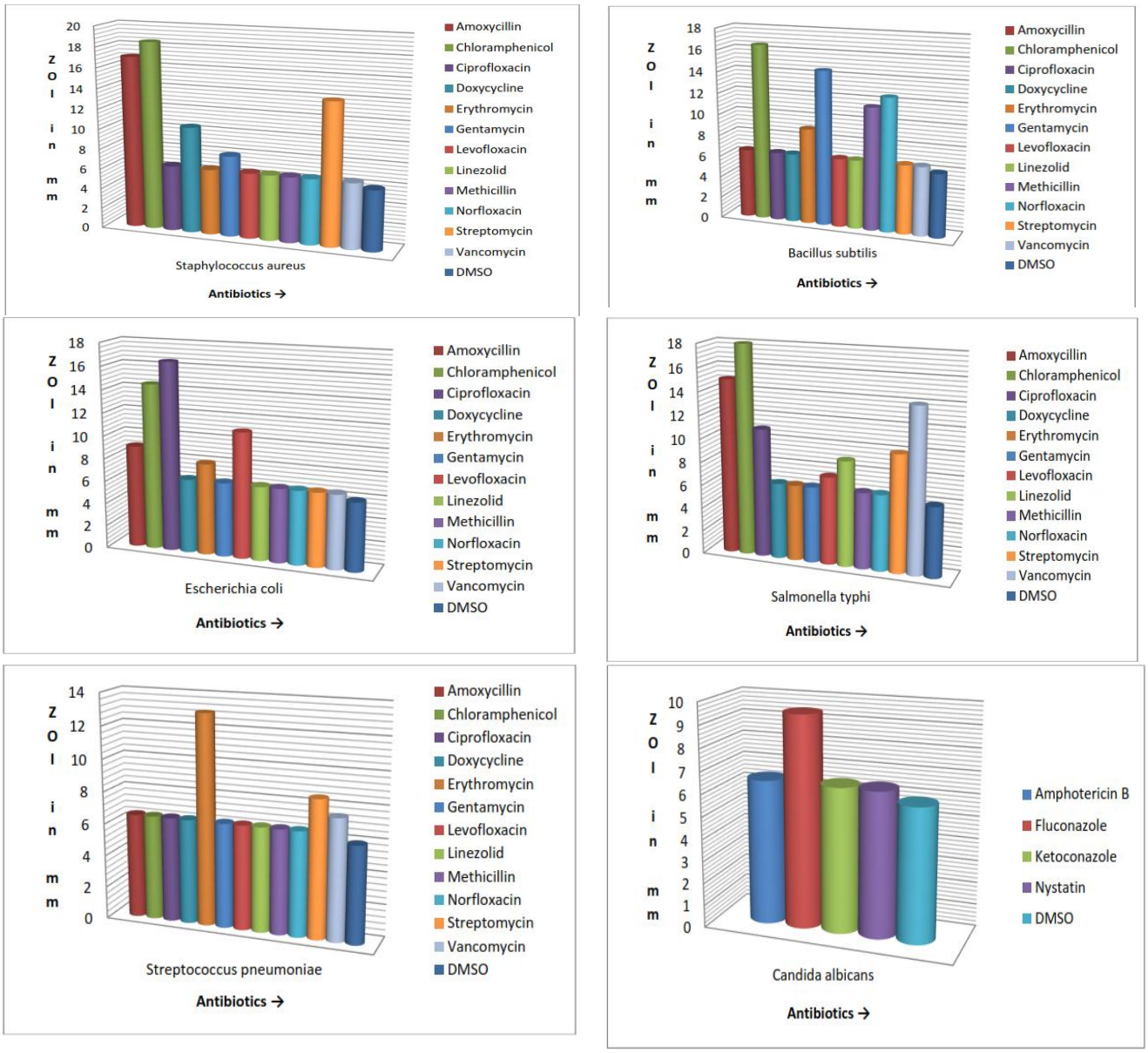
TABLE 5: Anticandidal Screening of Ethanolic and Aqueous Extracts of 20 Indian Medicinal Plants

Plant name	Anticandidal activity	
	Ethanolic extract	Aqueous Extract
	Candida albicans	
01. *Azadirachta indica* A. Juss.	[a]2+	2+
02. *Beta vulgaris* L.	--	--
03. *Camelia sinensis* L.	--	--
04. *Emblica officinalis* Gaerth.	2+	--
05. *Eucalyptus* sp.	--	--
06. *Ficus religiosa* L.	2+	1+
07. *Hemidesmus indicus* R. Br.	--	--
08. *Holarrhena antidysenterica* R.	--	--
09. *Lantana camara* L.	3+	2+
10. *Lawsonia inermis* L.	--	--
11. *Morus alba* L.	--	--
12. *Nyctanthes arbortristis* L.	--	--
13. *Ocimum sanctum* L.	1+	--
14. *Punica granatum* L.	--	--
15. *Saussurea lappa* C. B. Clarke		--
16. *Syzgium aromaticum* L.	2+	1+
17. *Syzgium cumini* L.	--	--
18. *Terminalia arjuna* W. & A.	--	--
19. *Terminalia belerica* Roxb.	2+	1+
20. *Terminalia chebula* Retz.	--	--
[b]DMSO	--	--
Total number of active plants	07	05

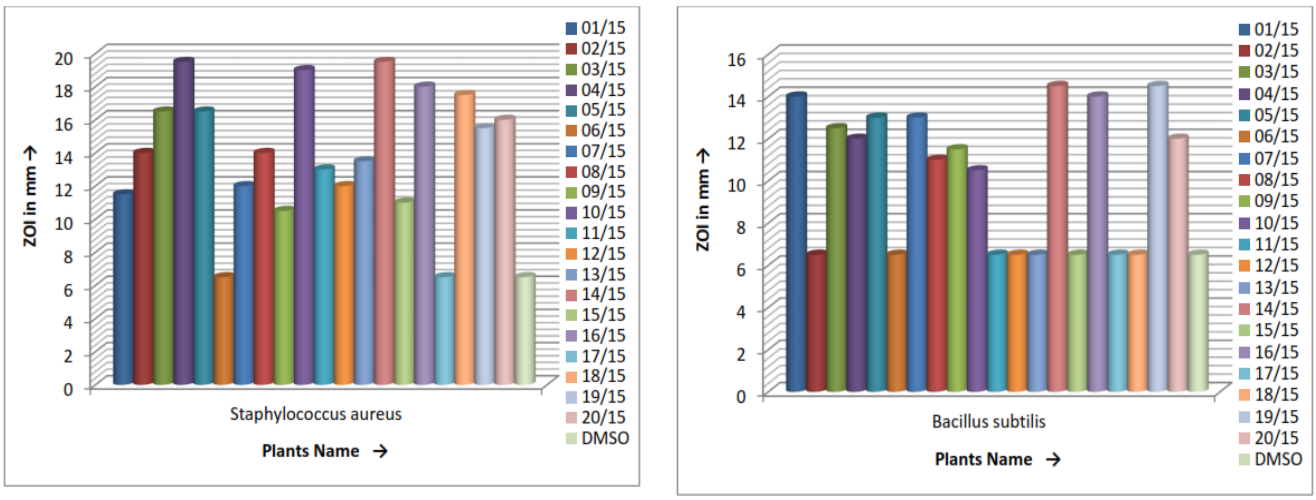
[a]1+ = 6.5 – 10.0 mm; 2+, 10.1 – 15.0 mm; 3+, 15.1 – 20.0 mm; 4+, 20.1 – 25.0 mm, -- , no zone of inhibition.
[b]DMSO blank solvent used as a control.

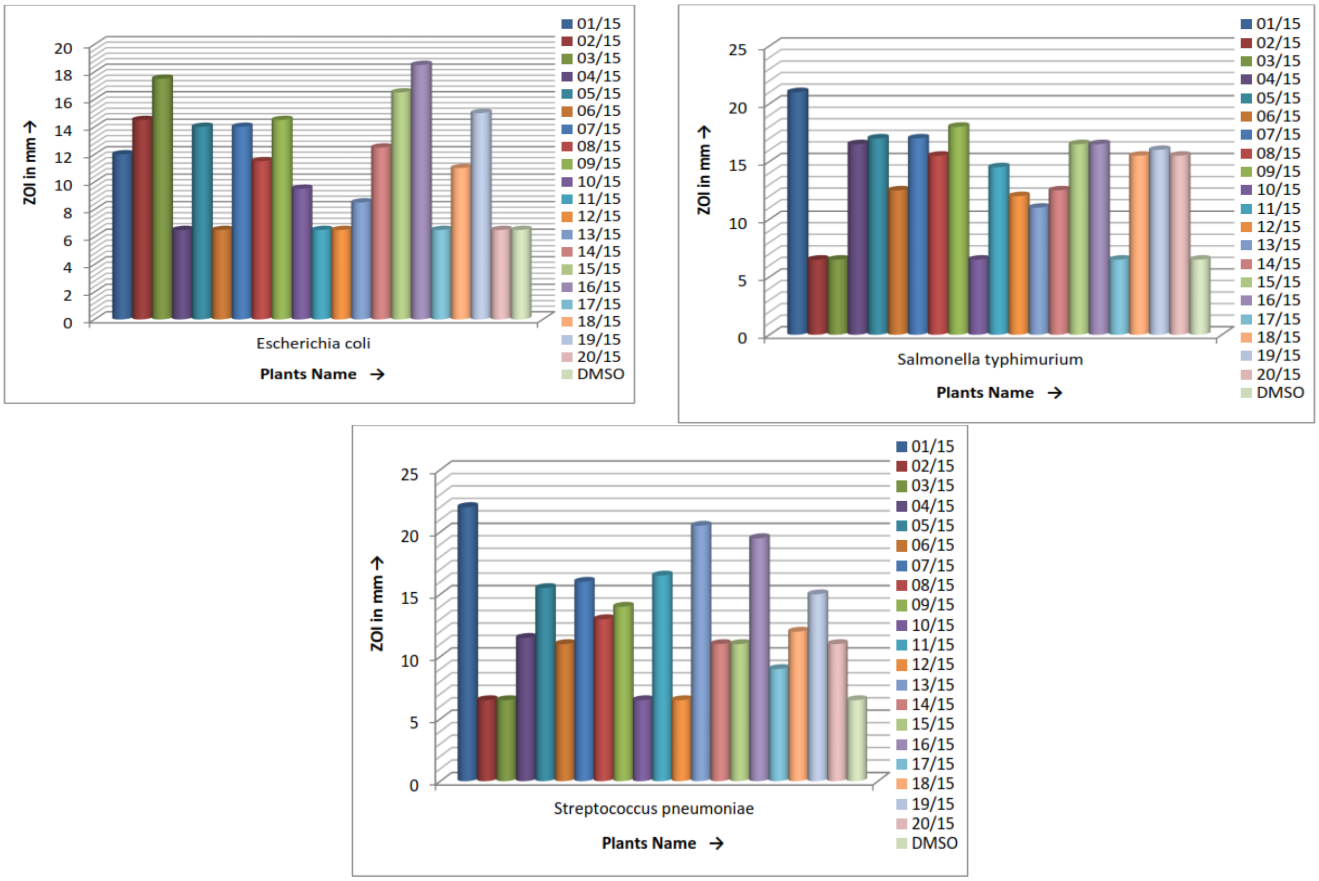
RESULTS

Emergence of multidrug resistance in human and animal pathogenic bacteria as well as undesirable side effects of certain antibiotics has triggered immense interest in the search for new antibiotics or antimicrobial drugs of plant origin. In the present study ethanolic and aqueous extracts of 20 traditionally used Indian medicinal plants have been tested against multi-drug resistant bacteria and a pathogenic yeast, *Candida albicans*. Ethno-botanical data, plant parts used along with their voucher specimen number are given in Table No. 1. The microbial susceptibilities to the tested antibiotics are shown in Table No. 2 and 3; Graph No. 1. Most of the antibiotics were inhibited the growth of at least one bacterial strains accept chloramphenicol that inhibited four bacterial strains. Hence all the bacterial strains are showed multi-drug resistance against the tested antibiotics. The details of multi-drug resistant bacterial strains are listed in Table No. 2. Similarly, only one antibiotic was inhibited the growth of *Candida albicans* (Table No. 3). The antimicrobial screening of the extracts and their potency was quantitatively assessed by the presence or absence of inhibition zone and zone diameter as given in Table No. 4 and 5; Graph No. 2, 3 and 4. The zone of inhibition above 6.5 mm. in diameter was taken as positive results. The results of screening are encouraging as out of the 20 plants, 18 ethanolic and 16 aqueous extracts showed antibacterial activity against *S. aureus*, similarly 16 ethanolic and 15 aqueous extracts inhibited the growth of *S. pneumoniae*. The strain of *S. typhimurium* showed considerable degree of variation in their sensitivity towards 16 ethanolic and 14 aqueous extracts. Out of the 05 bacterial strains, *E. coli* was found to be susceptible to 14 ethanolic and 10 aqueous extracts. Rest of the bacterial strains *B. subtilis* showed antibacterial activity against 12 ethanolic and 09 aqueous extracts of various medicinal plants while 07 ethanolic and 05 aqueous extracts showed anticandidal activity.

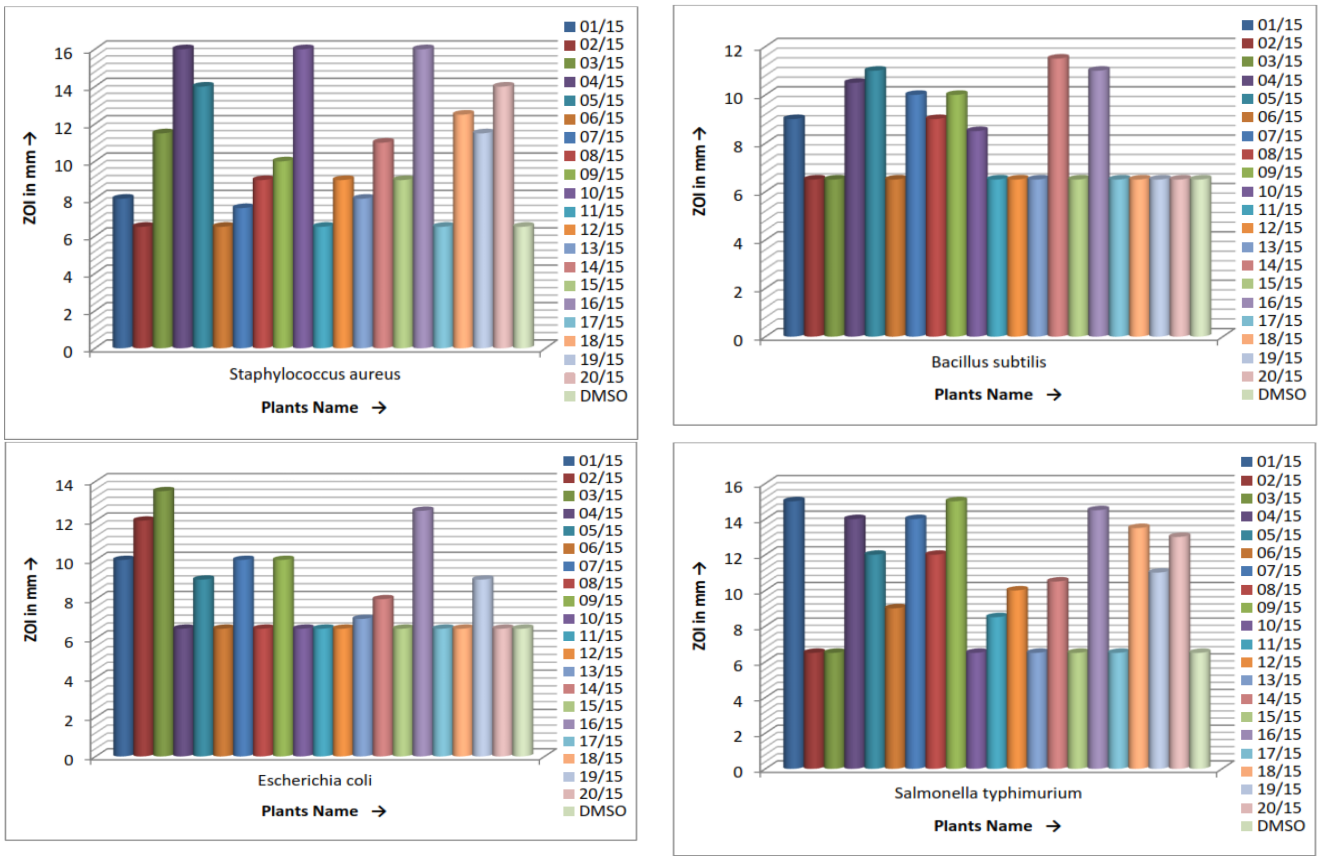


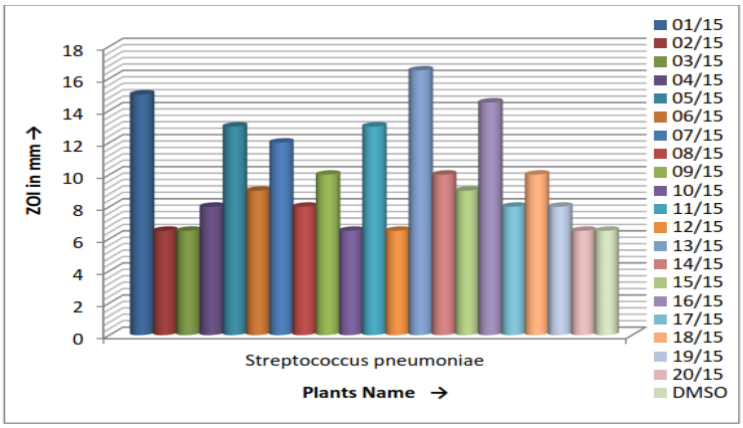
GRAPH 1: Showing the Antimicrobial Screening of various Antibiotics



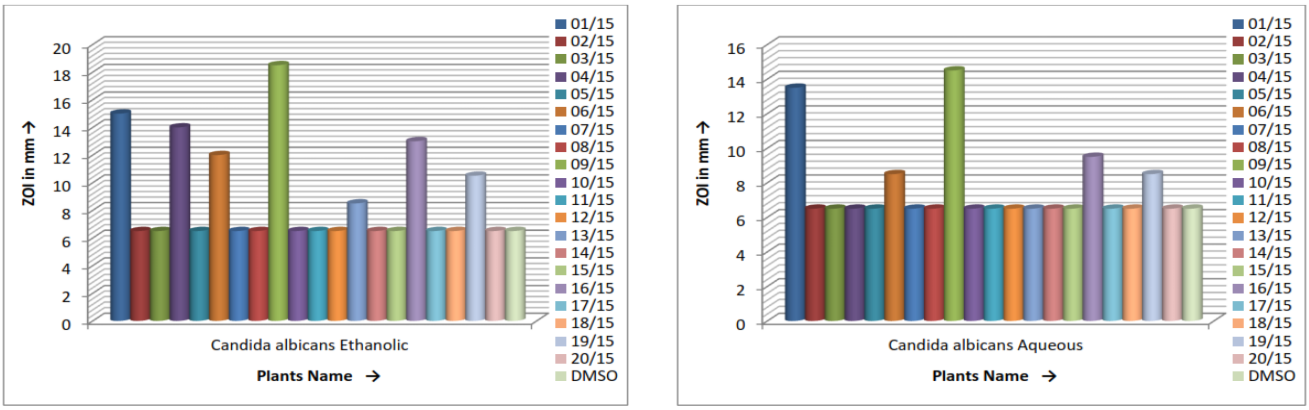


GRAPH 2: Showing the Antibacterial Screening of Ethanolic Extracts of 20 Indian Medicinal Plants





GRAPH 3: Showing the Antibacterial Screening of Aqueous Extracts of 20 Indian Medicinal Plants



GRAPH 4: Showing the Anticandidal Screening of Ethanolic and Aqueous Extracts of 20 Indian Medicinal Plants

Of these, 76 ethanolic and 64 aqueous extracts showed varied levels of antibacterial activity against test bacterial strains (Graph No. 2 and 3) whereas anticandidal activity was detected in 07 ethanolic and 05 aqueous extracts (Graph No. 4). Overall, broad-spectrum antibacterial activity was observed in 08 plants (ethanolic extracts) and 06 plants (aqueous extracts) against all test bacteria and 07 plants (ethanolic extracts) and 05 plants (aqueous extracts) against *Candida albicans*. Ethanolic extracts of 08 medicinal plants namely *A. indica*, *Eucalyptus* sp., *H. indicus*, *H. antidysenterica*, *L. camara*, *P. granatum*, *S. aromaticum* and *T. belerica* inhibited all the test bacteria whereas aqueous extracts of 06 medicinal plants namely *A. indica*, *Eucalyptus* sp., *H. indicus*, *L. camara*, *P. granatum* and *S. aromaticum* inhibited all the test bacteria in broad spectrum. Similarly, ethanolic extracts of 07 medicinal plants namely *A. indica*, *E. officinalis*, *F. religiosa*, *L. camara*, *O. sanctum*, *S. aromaticum* and *T. belerica* inhibited the growth of *Candida albicans* whereas aqueous extracts of 05 medicinal plants namely *A. indica*, *F. religiosa*, *L. camara*, *S. aromaticum* and *T. belerica* inhibited the growth of *Candida albicans*. Similar reports on antibacterial and anticandidal activities of Indian medicinal plants were also reported by other workers (Nimri *et al*., 1999; Geeta *et al*., 2001; Farrukh and Ahmad, 2003).

The sensitivity of all the test strains was, in decreasing order for ethanol and aqueous extracts are:

For Ethanolic Extract: *S. aureus* (18) > *S. pneumoniae* (16) = *S. typhimurium* (16) > *E. coli* (14) > *B. subtilis* (12) > *C. albicans* (07).

For Aqueous Extract: *S. aureus* (16) > *S. pneumoniae* (15) > *S. typhimurium* (14) > *E. coli* (10) > *B. subtilis* (09) > *C. albicans* (05).

In the case of test bacteria, the basis for their differences in susceptibility might be due to the differences in the cell wall composition of Gram + ve and Gram - ve bacteria (Grosvenor *et al*., 1995). *B. subtilis* was least sensitive against ethanolic and aqueous extracts of all screened Indian medicinal plants compared to other test bacteria, which may be due to their ability to form highly resistant resting stages called endospores. Drug-resistant strains of bacteria and *C. albicans* were found to be sensitive to the tested plant extracts. This has clearly indicated that antibiotic resistance does not interfere with the antimicrobial action of plant extracts and these extracts might have different modes of action on test organisms.

DISCUSSION & CONCLUSION

The antibacterial and anticandidal screening of the ethanolic and aqueous extracts of all 20 medicinal plants from the Vidisha district were studies by the disc diffusion method (Bauer *et al*., 1966) against five bacterial strains and yeast. Our results show a remarkable antibacterial and anticandidal efficacy of the ethanolic and aqueous extract of all tested medicinal plants. The alcoholic and aqueous extracts were tested, as alcohol was found to be a better solvent for extraction of antimicrobially active substances compared to water. Our findings of the present study were supported by Ahmad *et al*., 1998.

Major factor limiting the long-term use of antibiotic agents is resistance. Before antibiotics era, many people died of bacterial infections caused by pathogens as *Staphylococcus aureus*, *Salmonella typhimurium* and *Streptococcus pneumonia*. Use, abuse or misuse of antimicrobial agents has encouraged the evolution of bacteria towards resistance that often results in therapeutic failure (Straut *et al.,* 1995). The wide spread and most often use of antimicrobial drugs, inappropriate prescribing of antibiotics and poor infection control strategies have led to a general rise in the emergence of resistant bacteria particularly to ciprofloxacin (Tolun *et al*., 2004). Prescribing practice of specific class of antibiotics to certain organisms has been found to play a critical role in development of resistance against that antibiotic (Costelloe *et al.,* 2010; Metz-Gercek *et al.,* 2009). Thus, antimicrobial resistance findings and understanding are necessary to help minimize the emergence of multi drug resistant organisms by promoting prudent use of antibiotics, for this purpose, the need for general public to be appropriately informed on use of antibiotics has been emphasized (Euro surveillance editorial team, 2010). A wide range of antibiotics, which have been used in different bacterial and candidal practice, show-increasing frequency of resistance. Therefore, today antibiotics can treat most bacterial infections effectively. The indiscriminate use of antibiotics in some parts of the world in both human and veterinary medicine has led to the emergence of resistant strains of bacteria. Thus, the rational use of antibiotics is of utmost importance. This is why, the antibacterial therapy by medicinal plants will focus on a few problem areas (Agrawal *et al*., 2013). In the recent years, the development of resistance of pathogens against antibiotics has become a difficult issue caused by the indiscriminate use of modern antibiotics (Kunin, 1993; Cohen, 1992; Neu, 1992; Kunin, 1983). Therefore, it is important to find out newer, safer and more effective natural or synthetic antibacterial drug molecules. Considering the high cost of the synthetic drugs and their side effects, wide varieties of natural plants can be considered as a vital source for anti-microbial agents (Geyid *et al.,* 2005). Therefore, the demand for new and effective anti-microbial agents with broad-spectrum of activity from natural sources is increasing day-by-day (Mandal *et al*., 2011; Rahman *et al.,* 2008). The antibacterial activity is passively because of the presence of secondary metabolites existed in the plant. Hence, it is difficult to explain the limited spectrum of activity of other extracts compared with the ethanolic extracts since all the extracts had had the secondary metabolites (Le *et al*., 2003). Ruhe *et al.,* (2005), very recently reported that the resistance mechanism developed by the *S. aureus* against tetracycline and methicilline. Hence, the purpose of our present investigation was to evaluate the antibacterial and anticandidal activity of some Indian indigenous plants for the discovery of potential antibacterial and anticandidal agents that might be used for the management of bacterial and candidal infectious diseases.

For thousands of years, natural products have been used in traditional medicine all over the world and predate the introduction of antibiotics and other modern drugs. Plant materials or their extracts have been utilized as drugs since long in many parts of the world; India is the oldest among them (Chopra *et al.,* 1992). Over the last few years a large number of plant species have been evaluated for their antibacterial (Agrawal *et al.,* 2012a; Agrawal *et al.,* 2012b; Agrawal *et al.,* 2007; Indu *et al*., 2006; Srinivasan *et al*., 2001; Perumal *et al.,* 1999; Ahmad *et al*., 1998) as well as anticandidal activity (Parivuguna *et al*., 2013; Nayak *et al*., 2011; Vaijayanthimala *et al*., 2000; Mehmood *et al*., 1999). The difference in potency may be due to the stage of collection of the plant sample, different sensitivity of test strains and method of extraction (Nimri *et al*., 1999). In conclusion, results of the present investigation were to evaluate that the various medicinal plants possess a broad spectrum of antimicrobial activity against a panel of microorganisms responsible for the most common microbial infections of human. Further phytochemical studies are necessary to isolate the active constituents and evaluate the antibacterial and anticandidal effects against a wide range of microbial population.

ACKNOWLEDGEMENT

The authors are very thankful to Father Sabu, Director, St. Mary's P. G. College, Vidisha, M. P., India, for giving laboratory facilities, and Dr. R. K. Jain, Ex HOD, Dept. of Botany, S. S. L. Jain P. G. College, Vidisha, M. P., India for giving the valuable suggestions and authentication of plant. Thanks are also due to Dr. Ramkrishna Agrawal, Ret. Ayurvedic Medical Officer, Vidisha for providing authentic medicinal values of locally available and traditionally used medicinal plants.

REFERENCES

Agrawal, M. K., Rathore, D., Goyal, S., Varma, A. and Varma, A. (2013) Antibacterial efficacy of *Brassica campestris* Root, Stem and Leaves extracts, *International Journal of Advanced Research*. **1 (5),** 131-135.

Agrawal, M. K., Varma, A. and Goyal, S. (2012a) Antibacterial screening of extract of the leaves of the *Lantana camara*. *Indian Journal of Life Sciences*, **1 (2)**, 97-99.

Agrawal, M. K., Varma, A., Varma, A. and Goyal, S. (2012b) *In vitro* and *in vivo* antibacterial activity of *Lantana camara* (leaves) against the pathogen causing skin diseases i. e. *Staphylococcus aureus*. *Indian J. Applied & Pure Bio.*, **27 (2)**, 279-286.

Agrawal, M. K., Varma, A. K., Dwevedi, S. and Saify, T. (2007) The antibacterial activity of the extracts of *Lantana camara* leaves against the skin disease causing bacteria. *Indian Journal of Applied Life Sciences*, **3 (2)**, 15-16.

Ahmad, I., Mehmood, Z. and Mohammad, F. (1998) Screening of some Indian medicinal plants for their antimicrobial properties. *Journal of Ethnopharmacology*, **62**, 183-193.

Alanis, A. J. (2005) Resistance to antibiotics: are we in the post-antibiotic era? *Arch Med Res*., **36,** 697-705.

Bauer, R. W., Kirby, M. D. K., Sherris, J. C. and Turck, M. (1966) Antibiotic susceptibility testing by standard

single disc diffusion method. *Am. J. Cl. Pathol*, **45**, 493-496.

Chopra, R. N., Nayer S. L. and Chopra I. C. (1992) *Glossary of Indian Medicinal Plants*. 3rd edition, Council of Scientific and Industrial Research, New Delhi, 7-246.

Cohen, M. L. (1992) Epidemiology of drug resistance: implications for a post-antibacterial era. *Science*, **257**, 1050-1055.

Costelloe, C., Metcalfe, C., Lovering, A., Mant, D. and Hay, A. D. (2010) Effect of antibiotic prescribing in primary care on antimicrobial resistance in individual patients: systematic review and meta-analysis. *BMJ*., **340**, c2096.

Culver, D. H., Haley, R. W. and White, J. W. (1985) The efficacy of infection surveillance and control programs in preventing nosocomial infection in the hospital. *Am. C. Epidemiol.*, **121,** 181-2005.

Euro Surveillance Editorial Team (2010) Eurobarometer on antimicrobial resistance highlights areas for action. *Euro. Surveill.*, **15** (15): 19540.

Farrukh, A. and Ahmad, I. (2003) Broad-spectrum antibacterial and antifungal properties of certain traditionally used Indian medicinal plants. *World Journal of Microbiology and Biotechnology*. **19 (6),** 653-657.

Geeta, D. M., Vasudevan, R., Kedlaya, S., Deepa and Ballal, M. (2001) Activity of *Ocimum sanctum* (the traditional Indian medicinal plant) against the enteric pathogens. *Indian Journal of Medical Sciences*. **55 (8)**, 434-438.

Geyid, A., Abebe, D., Debella, A., Makonnen, Z., Aberra, F., Teka, F., Kebede, T., Urga, K., Yersaw, K., Biza, T., Mariam, B.H. and Guta, M. (2005) Screening of some medicinal plants of Ethiopia for their anti-microbial properties and chemical profiles. *J. Ethnopharmacol.*, **97,** 421- 422.

Grosvenor, P. W., Supriono, A. and Gray, D. O. (1995) Medicinal plants from Riau Province, Sumatra, Indonesia. Part 2, Antibacterial and antifungal activity. *Journal of Ethnopharmacology*, **45**, 97-111.

Harborne, J. B. (1984) Phytochemical methods - *A guide to modern technique of plant analysis* (II edition). Chapman and Hall, New York, 1-228.

Hughe, J. M. (1988) Study on the efficacy of nosocomial infection control. *Infect. Control Epidemol.*, **34**, 553-561.

Indu M. N., Hatha, A. A. M., Abirose, C., Harsha, U. and Vivekanandan, G. (2006) Antimicrobial activity of some of the South – Indian spices against serotypes of *Escherichia coli*, *Salmonella*, *Listeria monocytogenes* and *Aeromonas hydrophila*. *Brazilian Journal of Microbiology*, **37,** 153-158.

Kunin, C. M. (1983) Antibiotic resistance - a world health problem we cannot ignore (Editorial). *Ann. Intern. Med.*, **99,** 859-860.

Kunin, C. M. (1993) Resistance to Antimicrobial Drugs – A Worldwide calamity. *Ann. Intern. Med.*, **118,** 557-561.

Le, H. T., Schaldach, C. M., Firestone, G. L. and Bjeldanes, L. F. (2003) Plant-derived 3, 3'-Diindolylmethane is a strong androgen antagonist in human prostate cancer cells. *J Biol Chem.*, **278 (23),** 36-45.

Mandal, S., Deb Mandal, M., Saha, K. and Pal, N. K. (2011) In vitro antibacterial activity of three Indian spices against Methicillin-Resistant *Staphylococcus aureus*. *Oman Medical Journal*, **26 (5),** 319-323.

Mehmood, Z., Ahmad, I., Mohammad, F. and Ahmad, S. (1999) Indian medicinal plants: A potential source of anticandidal drugs. *Pharmaceutical Biology*. **37**, 237-242.

Metz-Gercek, S., Maieron, A., Strauss, R., Wieninger, P. Apfalter, P. and Mittermayer, H. (2009) Ten years of antibiotic consumption in ambulatory care: trends in prescribing practice and antibiotic resistance in Austria. *BMC Infect. Dis.*, **9**, 61.

Mulligen, M. E., Murry-Leisure, K. A., Ribner, B. S., Standiford, H. C., John, J. F., Karvick, J. A., Kauffman, C. A. and Yu, V. L. (1993) Methicillin resistant *Staphylococcus aureus*. *American Journal of Medicine*, **94**, 313-328.

Nayak, A., Nayak, R. N., Soumya, B., Bhat K. and Kudalkar, M. (2011) Evaluation of antibacterial and Anticandidal efficacy of aqueous and alcoholic extracts of Neem (Azadirachia India) and in vitro study. *IJPAP*. **1,** 230-235.

Neu, H. C. (1992) The crisis in antibiotic resistance. *Science*, **257,** 1064-1073.

Nimri, L. F., Meqdam, M. M. and Alkofahi, A. (1999) Antibacterial activity of Jordanian medicinal plants. *Pharmaceutical Biology*. **37 (3)**, 196-201.

Omololu-Aso, J., Kolawole1, D. O., Omololu-Aso, O. O. and Ajisebutu, S. O. (2011) Antibiotics sensitivity pattern of *Staphylococcus aureus* from fomites in the Obafemi Awolowo University Teaching Hospital Complex (OAUTHC) Nigeria. *International Journal of Medicine and Medical Sciences*, **3 (2)**, 32-36.

Parivuguna, V., Doss, A. and Abitha, B. (2013) Anti candidal activity of the leaf extracts of *Fragaria virginiana* Duchesne and *Lantana camera* Linn. *International Journal of Bio-Technology and Research*. **3 (2),** 21-26.

Perumal, S. R., Ignacimuthu, S. and Patric, R. D. (1999) Preliminary screening of ethnomedicinal plants from India. *J. Ethnopharmacol.*, **66,** 235-240.

Piddock, K. J. V. and Wise, R. (1989) Mechanisms of resistance to quinolones and clinical perspective. *Journal of Antimicrobial Chemotherapy*. **23**, 475-483.

Rahman, M. S., Rahman, M. Z., Wahab, M. A., Chowdhury, R. and Rashid, M. A. (2008) Antimicrobial Activity of Some Indigenous Plants of Bangladesh. *J. Pharm. Sci.,* **7,** 23-26.

Robin, E. H., Anril, W., Alexander, M., Loeto M. and Keith, K. (1998) Nasopharyngeal carriage and antimicrobial resistance in isolates of *Streptococcus pneumoniae* and *Heamophilus influenza* Type b in children under 5 years of age in Bostwana. *International Journal of Infectious Diseases*. **3 (1)**, 18-25.

Ruhe, J. J., Manson, T., Bradsher R. W. and Menon, A. (2005) Use of long acting tetracyclines for Methicillin-Resistant *Staphylococcus aureus* infections: Case series and Review of literature. *Clinical Infectious Diseases.* **40,** 1429-1434.

Singh, N. C. and Bisht, M. (1992) Medicinal plants and welfare of the mankind. *Journal of Nature Conservation*. **4**, 149-152.

Srinivasan, D., Thirumurugan, N., Hansi, T. and Persumalasamy, P. L. (2001) Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine.

J. Ethnopharmacol. **74,** 217-220.

Straut, M., Surdeanu, M., Oprisan, G., Otelea, D. and Damian, M. (1995) Antibiotics and bacterial resistance. A few elements of generic basis for this relationship. *Roum. Arch. Microbiol. Immunol.*, **54** (4), 241-254.

Tolun, V., Kucukbasmaci, O., Torumkuney-Akbulut, D., Catal, C., Ang-Kucuker, M. and Ang, O. (2004) Relationship between ciprofloxacin resistance and extended spectrum beta-lactamase production in *Echerichia coli* and *Klebsiella pnuemoniae* strains. *Clin Microbiol Infect*., **10,** 72-75.

UNCTAD / Gatt (1974) Markets for selected medicinal plants and their derivatives. UNCTAD, Germany.

Vaijayanthimala, J., Anandi, C., Udhaya, V. and Pugalendi, K. V. (2000) Anticandidal activity of certain South Indian medicinal plants. *Phytother. Res.*, **14 (3),** 207-209.