Antimicrobial Activity of Darchini (*Cinnamomum zeylanicum*) and Sheesham (*Dalbergia sissoo*) on Some Common Human Pathogens

[1]*Aziz ur Rahman,
[2]Tariq Rahim Malik
and
[1]Tajuddin

[1]Department of Saidla,
[2]Department of Ilmul Advia,
A.K. Tibbiya College,
Aligarh Muslim University,
Aligarh-202002

Abstract

The alcoholic and aqueous extracts of cinnamon bark and sissoo wood were evaluated for antibacterial and antifungal activity by disc-agar diffusion technique at three concentration levels (50, 25 and 12.5µg/disc). For evaluation of antibacterial activity different gram positive and gram negative bacterial strains were used viz. *Staphylococcus aureus, Streptococcus pyogenes E. coli, Klebsiella sp. Pseudomonas aerugenosa, Proteus sp. Bacillus sp., Shigella sp.* and *Salmonella typhimurium* and to assess antifungal activity the species used are namely *Aspergillus flavus, Aspergillus fumigates, Aspergillus niger, Candida albicans, Candida keyfr, Candida kruisei, Candida parapsilosis and Candida tropicalis.* The reference drugs used for comparison are Ciprofloxacin (5 µg/disc) for antibacterial potential and Fluconazole (10 µg/disc), Nystatin (100 µg/disc), Amphotericin (100 µg/disc) and Clotrimazole (10 µg/disc) for antifungal activity. Zone of Inhibition was taken as the parameter for the assessment of antimicrobial activity. The alcoholic extract of *Cinnamomum zeylanicum* showed significant antibacterial effect against *Streptococcus pyogenes E. coli and Pseudomonas aerugenosa* while the alcoholic and aqueous extracts of *Dalbergia sissoo* exhibited significant effect against all the tested bacterial strains at the different doses. The extract of both the test drugs also gives significant effect against different fungal strains used. Furthermore, to confirm the originality and quality of the test drugs the samples were also screened on the basis of certain physico-chemical parameters.

Keywords: *Cinnamomum zeylanicum, Dalbergia sissoo,* Antibacterial, Antifungal, Physico-chemical

Introduction

With the increase in bacterial resistance to antibiotics, antimicrobials plant products have gained attention in the scientific research. The use of natural antimicrobial compounds is important not only in food preservation, but also in the control of human diseases and plant microbial origin (Choi, 2010). The use of natural products with therapeutic properties, whether mineral, vegetable and animal origin, were the main sources of important therapeutic agents as well as important raw materials for the manufacture of traditional and modern medicines (Ishrat et al., 2011). Now medicinal plants are considered an important source of new chemical substances with potential therapeutic effects (Blumenthal., 2000). They contain a wide range of substances that can be used to treat chronic and infectious diseases. Oils are also a very important group of secondary metabolites that are potentially

[1]*Author for correspondence; Email: rahman.mdi@gmail.com,
[2]Present Address: Medical Officer, Marizgam, District Kupwara-193224(J&K)

useful sources of antimicrobial compounds. Many studies have been published on the antimicrobial activity of oils (Moussaoui and Alaoui 2016).

In last decades there has been a growing interest in traditional systems of medicine in developing and developed countries. Now greater attention has been given to the plant and their products for use in therapy due to hazards and side effects of synthetic drugs. Several modern drugs have been isolated from natural resources and many of these isolations were based on the use of the agents in traditional medicine (Owolabi *et al*., 2007). The antibiotics in allopathic system have tremendous effect in controlling the infectious diseases (Finland, M. 1978). However, the advent of escape mechanism adopted by most of the pathogens certainly needs a suitable replacement of the presently available antibiotics (Conly *et al.,* 1992; Threlfall *et al.,* 1996). Therefore, there is a constant requirement to find new, safe and effective therapeutic agents for the treatment of various infectious diseases. Unani system of medicine also shows good potential to treat and manage various infectious diseases by a number of single and compound preparations that are used since ancient time (Rahman *et al.,* 2013).

Therefore, present study was conducted to evaluate the anti-microbial potential of aqueous and alcoholic extract of Darchini (*Cinnamomum zeylanicum*) – bark and Sheesham (*Dalbergia sissoo*) – wood and oil. These Unani medicinal plants were selected on the basis of their actions mentioned in classical as well as modern medico-ethno-botanical literatures as antiseptic, anti-microbial, anti-fungal, or are in clinical practices of Unani physicians for treatment of various infectious diseases. Furthermore, before evaluating for antimicrobial activity these test drugs were also standardized for their originality, to make it less controversial and more efficacious to use, by certain physico-chemical parameters. It appears that such type of study has not been done so far. Hence the present work.

Materials and Methods

Collection of plants

Darchini and Sheesham both were procured from Dawakhana Tibbiya College, Aligarh Muslim University, Aligarh. The samples were authenticated in Pharmacognosy section of the Department of Ilmul Advia and found within the range of standards (Anonymous, 2007, Khory and Katrak, 1985). Both test drugs were dried in shade and then powdered for study. This study was conducted at the department of Ilmul Advia in the year 2004.

Physicochemical parameters

It includes extractive values, ash values (total ash, acid insoluble ash, water soluble

ash), moisture content, loss of weight on drying, pH (1% and 10% solution), water and alcohol soluble contents in the test drugs (Anonymous, 1968, Jenkins *et al.,*1967, Anonymous, 1987), chemical treatment of powdered drugs, fluorescence analysis (Anonymous, 1992) and qualitative estimation of Darchini and Sheesham (Bhattacharjee and Das, 1969).

Anti-microbial studies

Extracts preparation

Alcoholic and aqueous extracts of both the test drugs alongwith oil of *Dalbergia sissoo* were used for the anti-microbial studies. Alcoholic extract of the coarsely powdered drug was prepared by the method of Ibrahim and Osman (1995) with some modifications by using a Soxhlet's apparatus with 95% ethanol as solvent, at 55°C for 06 hours. The resultant extract was then concentrated on a water bath, at 40°C. Same procedure was carried out to prepare aqueous extracts using distilled water as solvent at 60°C and concentrated at 50°C.

Strict aseptic precautions were followed throughout the process and the heat wherever needed was kept as low as possible to prevent the damage of thermolabile substances present in the drugs.

Bacterial and fungal strains used in the study

Various Gram positive and Gram negative bacterial and fungal strains were used for detection of the anti-microbial activity of the extracts (Table 1). Standard and clinical strains were obtained from the Department of Microbiology, Jawaharlal Nehru Medical College and Hospital, Aligarh.

Table 1: Bacterial and fungal strains used for the detection of antimicrobial activity of the drug

Gram Positive bacterial strains	**Gram Negative bacterial strains**	**Fungal Strains**
Bacillus sp.	*E. Coli*	*Aspergillus flavus*
Staphylococcus aureus	*Klebsiella sp*	*Aspergillus fumigatus*
Streptococcus pyogenes	*Proteus sp.*	*Aspergillus nigur*
	Pseudomonas aerugenosa	*Candida albicans*
	Salmonella typhimurium	*Candida keyfr*
	Shigella sp.	*Candida krusei*
		Candida parapsilosis
		Candida tropicalis

Antibacterial and antifungal susceptibility screening

Serial dilutions of the alcoholic extracts were prepared in ethyl alcohol so that 01 ml contained 100 times the amount of the extracts required per disc. Aliquots (01 ml) of these dilutions were transferred to bottles containing batches of 100 discs (06mm in diameter) of filter paper (Whatmann No.1, Whatmann, England). Bottles were placed in a water bath at 50°C with occasional shaking to allow an even distribution of the extracts between discs until complete evaporation of the alcohol had been achieved (Malik *et al.,* 2016).

Antibacterial and antifungal activity was determined using the disc-agar diffusion technique according to the method described by Finegold and Martin, 1982. The whole experiment was performed in triplicate and diameters of zones of inhibition were recorded.

Results

Physicochemical parameters

Extractive values of the powdered test drugs in different solvents viz. petroleum ether, diethyl ether, chloroform, benzene, alcohol and distilled water were also determined with Soxhlet's apparatus. The extractive values were expressed in percentage and depicted in table 2.

The analytical values of different physicochemical parameters such as Solubility, Moisture content, Loss of weight on drying, Ash values and pH values of the Darchini and Sheesham were determined and are depicted in Table 3.

Qualitative estimation

The therapeutic properties of the crude drugs are mainly due to presence of physiologically active chemical constituents present in the drugs. The qualitative

Table 2: Successive extractive values of the test drugs

Solvent	Extractive values in % (Mean±SE)	
	Darchini	Sheesham
Petroleum Ether	1.55±0.00	0.30±0.00
Diethyl Ether	0.95±0.00	0.64±0.01
Chloroform	0.54±0.00	0.38±0.00
Benzene	0.11±0.00	0.08±0.00
Alcohol	8.91±0.01	5.60±0.01
Distilled Water	6.19±0.00	2.11±0.00

phytochemical analysis of all the three test drug extracts was done systematically and the presence or absence of active compounds is shown in table 4.

Table 3: Physico-chemical values

Physico-chemical parameters	Darchini (Mean±SE)	Sheesham (Mean±SE)
Water soluble contents (1% solution)	3.53±0.04	2.13±0.05
Water soluble contents (10% solution)	1.75±0.02	0.65±0.01
Alcohol soluble contents (1% solution)	8.12±0.05	2.42±0.19
Alcohol soluble contents (10% solution)	3.24±0.02	0.92±0.02
Moisture content (%)	14.00±0.24	12.00±0.24
Loss on drying at 105^0C (%)	14.89±0.09	13.60±0.25
Total Ash (%)	2.22±0.05	1.75±0.01
Acid Insoluble Ash (%)	1.56±0.05	1.29±0.00
Water Soluble Ash (%)	0.60±0.02	1.49±0.01
pH (1% solution)	1.10±0.05	1.10±0.00
pH (10% solution)	1.30±0.03	1.10±0.03

Table 4: Qualitative analysis of chemical constituents

S.No.	Tested for	Darchini	Sheesham
1.	Alkaloids	+ve	+ve
2.	Amino acids	+ve	+ve
3.	Flavonoids	+ve	+ve
4.	Phenols	+ve	+ve
5.	Proteins	+ve	+ve
6.	Resins	-ve	-ve
7.	Saponins	-ve	-ve
8.	Sterols / Terpenes	+ve	+ve
9.	Sugar (Reducing)	+ve	+ve
10.	Sugars (Non-reducing)	+ve	+ve
11.	Tannins	+ve	+ve

Chemical treatment and fluorescence analysis

Powder of Darchini and Sheesham gives different colour in day light when they were treated with different chemicals as shown in table 5. For fluorescence analysis powder of these two drugs were treated with different reagents and change in color was observed in Ultra-violet (UV) radiation alongwith day light, results are depicted in Table 6.

Table 5: Treatment of test drugs with chemicals

Chemical Treatment	**Darchini Change in color**	**Sheesham Change in color**
Concentrated HNO_3	Brown	Orange
Concentrated HCL	Dark Brown	Light Brown
Concentrated H_2SO_4	Dark Brown	Black
2% Iodine Solution	Golden Brown	Yellow
Acetic Acid	Brown	Light Brown
10% NaOH Solution	Brown	Dark Brown
Acetic Acid + H_2SO_4	Red	Brown
10% NaOH + few drops of $CuSO_4$ sol.	Green	Green
10% NaOH + few drops of 5% lead acetate	Reddish Black	Brown
Acetic Acid + few drops of 5% ferric chloride + Concentrated H_2SO_4	Yellowish Brown	Brown
5% ferric chloride	Fluorescent Green	Fluorescent Yellow

Table 6: Fluorescence analysis of test drugs

Reagents	**Darchini Change in color**		**Sheesham Change in color**	
	Day light	**UV Light**	**Day light**	**UV Light**
1N HCL	Brown	Reddish Green	Light Brown	Yellow
Concentrated HNO_3	Brown	Green	Orange	Green
Concentrated H_2SO_4	Dark Brown	Reddish Black	Black	Black
Water	Brown	Green	Light Brown	Yellow
Acetic Acid	Brown	Black	Light Brown	Yellow
5% Ferric Chloride	Fluorescent Green	Blackish Green	Fluorescent Yellow	Green
10% NaOH	Brown	Black	Dark Brown	Green
2N HCL	Golden Brown	Dull Green	Light Brown	Green
1N H_2SO_4	Golden Brown	Black	Light Brown	Cream
4N H_2SO_4	Light Brown	Brown	Dull Pink	Brown

Anti-microbial studies

Aqueous and alcoholic extract of Darchini and Sheesham alongwith oil of the Sheesham were screened against gram positive and gram negative bacterial strains and fungal strains. The activity of both the test drugs were recorded and shown in table 7, 8, 9 and 10.

Table 7: Antibacterial potential of Darchini (*Cinnamomum zeylanicum*) (P value ≤ 0.05 was considered statistically significant)

Bacterial Strains	Test Drug Concentration (µg/disc)	Zone of inhibition (ZOI) in mm (Mean ± S.E.)		
		Alcoholic extract	Aqueous extract	Ciprofloxacin (5µg/disc)
Staphylococcus aureus	50	No Effect	No Effect	25.16±0.02
	25	No Effect	No Effect	
	12.50	No Tested	Not Tested	
Streptococcus pyogenes	50	12.33±0.11 p<0.001	No Effect	10.13±0.01
	25	10.26±0.02 p<0.005	No Effect	
	12.50	08.16±0.02 p<0.001	Not Tested	
E. Coli	50	12.16±0.01 p<0.001	No Effect	19.06±0.00
	25	08.36±0.11 p<0.01	No Effect	
	12.50	08.26±0.14 p<0.01	Not Tested	
Klebsiella sp	50	No Effect	No Effect	25.03±0.08
	25	No Effect	No Effect	
	12.50	Not Tested	Not Tested	
Pseudomonas aerugenosa	50	09.36±0.11 p<0.01	No Effect	23.16±0.17
	25	No Effect	No Effect	
	12.50	Not Tested	Not Tested	
Proteus sp.	50	No Effect	No Effect	13.16±0.02
	25	No Effect	No Effect	
	12.50	Not Tested	Not Tested	
Bacillus sp.	50	No Effect	No Effect	17.10±0.01
	25	No Effect	No Effect	
	12.50	Not Tested	Not Tested	
Salmonella typhimurium	50	No Effect	No Effect	10.13±0.02
	25	No Effect	No Effect	
	12.50	Not Tested	Not Tested	

Table 8: Antifungal activity of Darchini (*Cinnamomum zeylanicum*) (P value ≤ 0.05 was considered statistically significant)

Fungal Strains	Test Drug Concentration (µg/disc)	Zone of inhibition (ZOI) in mm (Mean ± S.E.)					
		Cinnamomum zeylanicum		Standard Drugs			
		Alcoholic extract	Aqueous extract	Fluconazole (10µg/disc)	Nystatin (100µg/disc)	Amphotericin B(100µg/disc)	Clotrimazole (10µg/disc)
Aspergillus flavus	50	11.15±0.10 p<0.001	10.20±0.11 p<0.001	No Effect	15.40±0.12	12.16±0.07	18.10±0.01
Aspergillus fumigatus	50	22.33±0.20 p<0.003	14.06±0.11 p<0.003	No Effect	14.24±0.20	08.16±0.07	12.46±0.07
Candida albicans	50	32.33±0.17 p<0.001	21.23±0.11 p<0.001	26.46±0.17	22.10±0.04	22.10±0.04	16.06±0.03
Candida keyfr	50	22.21±0.10 p<0.001	10.34±0.11 p<0.001	32.33±0.01	16.30±0.04	14.16±0.03	28.20±0.05
Candida krusei	50	No Significant Effect	No Significant Effect	28.10±0.03	18.10±0.05	22.13±0.03	16.06±0.03
Candida parapsilosis	50	No Significant Effect	No Significant Effect	10.30±0.04	10.20±0.04	13.20±0.05	14.16±0.03
Candida tropicalis	50	20.33±0.20 p<0.003	11.20±0.14 p<0.001	30.20±0.10	18.06±0.03	14.16±0.03	16.06±0.03

Table 9: Antibacterial potential of Sheesham (*Dalbergia sissoo*) (P value ≤ 0.05 was considered statistically significant)

Bacterial Strains	Test Drug Concentration (µg/disc)	Zone of inhibition (ZOI) in mm (Mean ± S.E.)			
		Alcoholic extract	Aqueous extract	Oil	Ciprofloxacin (5µg/disc)
Staphylococcus aureus	50	10.13±0.02 p<0.001	09.26±0.16 p<0.003	14.20±0.02 p<0.001	25.16±0.02
	25	07.26±0.02 p<0.001	No Effect	No Effect	
	12.50	07.23±0.10 p<0.001	Not Tested	Not Tested	
Streptococcus pyogenes	50	No Effect	09.26±0.02 p<0.001	12.26±0.13 p<0.001	10.13±0.01
	25	No Effect	No Effect	No Effect	
	12.50	Not Tested	Not Tested	Not Tested	
E. Coli	50	No Effect	12.20±0.12 p<0.001	No Effect	19.06±0.00
	25	No Effect	No Effect	No Effect	
	12.50	Not Tested	Not Tested	Not Tested	
Klebsiella sp	50	No Effect	No Effect	16.53±0.02 p<0.001	25.03±0.08
	25	No Effect	No Effect	No Effect	
	12.50	Not Tested	Not Tested	Not Tested	
Pseudomonas aerugenosa	50	07.23±0.17 p<0.001	No Effect	No Effect	23.16±0.17
	25	No Effect	No Effect	No Effect	
	12.50	Not Tested	Not Tested	Not Tested	
Proteus sp.	50	10.33±0.02 p<0.001	No Effect	No Effect	13.16±0.02
	25	08.36±0.11 p<0.001	No Effect	No Effect	
	12.50	06.46±0.15 p<0.001	Not Tested	Not Tested	
Bacillus sp.	50	08.30±0.02 p<0.002	No Effect	No Effect	17.10±0.01
	25	No Effect	No Effect	No Effect	
	12.50	Not Tested	Not Tested	Not Tested	
Salmonella typhimurium	50	No Effect	No Effect	No Effect	10.13±0.02
	25	No Effect	No Effect	No Effect	
	12.50	Not Tested	Not Tested	Not Tested	
Shigella Sp.	50	No Effect	11.36±0.23 p<0.002	No Effect	16.10±0.01
	25	No Effect	07.20±0.01 p<0.001	No Effect	
	12.50	Not Tested	No Effect	Not Tested	

Table 10: Antifungal activity of Sheesham (*Dalbergia sissoo*) (P value d" 0.05 was considered statistically significant)

Fungal Strains	Test Drug Concentration (µg/disc)	Zone of inhibition (ZOI) in mm (Mean ± S.E.)					
		Dalbergia sissoo		Standard Drugs			
		Alcoholic extract	Aqueous extract	Fluconazole (10µg/disc)	Nystatin (100µg/disc)	Amphotericin B (100µg/disc)	Clotrimazole (10µg/disc)
Aspergillus flavus	50	14.41±0.06 p<0.009	12.30±0.16 p<0.003	No Effect	15.40±0.12	12.46±0.07	18.06±0.03
Aspergillus fumigatus	50	14.24±0.16 p<0.004	12.22±0.02 p<0.002	No Effect	14.24±0.20	08.16±0.05	12.16±0.07
Aspergillus niger	50	14.20±0.20 p<0.001	12.33±0.15 p<0.001	26.46±0.17	22.10±0.04	22.10±0.05	16.10±0.05
Candida albicans	50	32.30±0.31 p<0.003	28.26±0.15 p<0.006	32.33±0.01	16.06±0.03	14.16±0.03	28.10±0.03
Candida krusei	50	No Effect	No Effect	28.10±0.03	18.10±0.05	22.26±0.09	16.10±0.05
Candida parapsilosis	50	No Effect	No Effect	10.30±0.04	10.20±0.05	13.20±0.05	14.40±0.05
Candida tropicalis	50	No Effect	No Effect	30.20±0.10	18.10±0.05	14.10±0.03	16.10±0.05

Discussion and Conclusion

In the present study, antimicrobial activity of Darchini (*Cinnamomum zeylanicum*) and Sheesham (*Dalbergia sissoo)* was screened against different fungal and bacterial strains and efficacy was evaluated by agar well diffusion method. The aqueous and alcoholic extracts of the plant product were prepared and the extracts were poured in the well. The agar plate swabbed with microbes showed the zone of inhibition which is the indication of antimicrobial activity shown by the plant. The positive and negative controls were used for the comparative study. Standards were used as positive controls and Dimethyl Sulphoxide (DMSO) was used as a negative control. Darchini were screened at the doses of 12.5µg/disc, 25µg/disc and 50µg/disc for antibacterial activity and for antifungal activity the dose was 50µg/disc. The alcoholic extract showed significant effect against *Streptococcus pyogenes* and *E. coli* at all the test doses and against *Pseudomonas aerugenosa* at the dose of 50µg/disc, however, aqueous extract of Darchini does not show any response to any tested bacteria. While both the extracts of Darchinin showed significant effect against different fungi named as *Aspergillus flavus, Aspergillus fumigates*, *Candida albicans,Candida keyfr* and *Candida tropicalis.* The antifungal activity may be due to the essential oil present in the Darchini along with their chemical constituents (Tiwari *et al.*, 1994, Quale *et al.,* 1996). Alcoholic and aqueous extract and oil of Sheesham were screened against wide range of bacterial species at the doses of 12.5µg/disc, 25µg/disc and 50µg/disc. Alcoholic extract exhibited significant activity at all the doses used against *S. aureus* and *Proteus Sp.* and at the dose of 50µg/disc it shows significant effect against *P. aeruginosa* and *Bacillus Sp.* The aqueous extract of the test drug shows significant activity against *S. aureus, S. pyogenes, E Coli.* and *Shigella Sp.* at the dose of 50µg/disc and at the dose of 25µg/disc it shows significant effect against *Shigella Sp.* While the Oil of Sheesham at the dose of 50µg/disc exhibited significant activity against *S. aureus, S. pyogenes,* and *Klebsiella Sp*. While for antifungal activity both the extracts depicted significant effect against *Aspergillus flavus, Aspergillus fumigates, Aspergillus niger* and *Candida albicans*.

Further to say that, it is quite basic thing that to standardize the plant drugs for their rational therapeutic use. Because many medicinally active constituents have been isolated from plant sources based on the uses of the agents in traditional system of medicine (Rahman *et al.,* 2011). As the uses of herbal medicines increases day by day around the globe, the safety and quality of medicinal plant materials and finished goods have become a major concern. It is quite difficult to maintain quality of plant drug materials because its collection and storage is mostly done by untrained person causes deterioration in the efficacy of drugs. Physico-chemical parameters such as solubility, extractive values, moisture content, pH, ash values and fluorescence analysis etc. are considered as tools of checking

quality, identity, purity and strength of the Unani drugs. Therefore, for establishing the standards of any drug these parameters plays an important role, as the adulterated or exhausted drug material will give different values rather than the genuine one (Jenkins *et al.,*1967; Jacob *et al*., 2006; Chase and Pratt, 1949). Active chemical constituents present in plant drugs are responsible for their actions. Therefore, qualitative analysis of the test drugs was also done. Alkaloids, tannins, sterols, phenols, flavonoids, oils etc were present which may possess antibacterial property. (Said, 1996; Katzung, 2001; Anuradha and Goyal, 1995; Evans, 2003).

In this study both the tested drugs shows significant antibacterial as well as antifungal effect. It revealed the broad spectrum activity of the crude extract of plant originated drugs and this may be due to the presence of active chemical constituents present in the drugs. Therefore, further evaluation of the active constituents is required to ascertain the antimicrobial activity of the test drugs. However, the alcoholic and aqueous extract of *Darchini and Sheesham alongwith oil of Sheesham* could be a possible source of antimicrobial herbal medicines to treat the infections and its complications.

References

Anonymous, 1968. British Pharmacopoea. General Medicine Council. Pharmaceutical Press, Bloomsbury square, London, pp. 27-28, 1276-1277, 1286-1288, 982-985.

Anonymous, 1992. Standardization of Single Drugs of Unani Medicine, CCRUM, New Delhi, Part-2: pp.1-6, 148-152.

Anonymous, 2007. The Unani Pharmacopoeia of India, Part 1 & 5. Ministry of Health and Family Welfare, Deptt. of AYUSH, Govt. of India, New Delhi, pp. 26, 92.

Anonymous. 1987. Physiochemical Standards of Unani Formulations, Part 1 & 2. Central Council for Research in Unani Medicine, New Delhi, pp. 274-278.

Anuradha, V. and Goyal, M.M., 1995. Phytochemical study on the leaves of *Alstonis scholaris* and their effects on pathogenic organism. *Ancient science of life* 15 (1): 30-34.

Bhattacharjee, A.K. and Das, A.K., 1969**.** Phyto-chemical Screening of Some Indian Plants. *Quart. Jour. Crude Drug. Res.* 9: 1408-1412

Blumenthal, M., 2000. Herbal medicines: expanded commission E monographs. Integrative Medicine Communications, Boston, pp. 419-423.

Chase, C.R. and Pratt, R., 1949. Fluorescence of powdered vegetable drugs with particular reference to develop of a system of identification. *J. Amer. Pharm. Asso*. 38: 324.

Choi, H.S., 2010. Antioxidative activity. In: Sawamura M. (Editor) Citrus essential oils: flavor and fragrance. John Wiley & Sons, Inc, New Jersey, pp. 231-43.

Conly, J., Rennie, R., Johnson, J., Farah, S. and Hellman, L, 1992. Disseminated candidiasis due to Amphotericin – B resistant *Candida albicans. J. Infect. Dis*. 165: 761-764.

Evans, W.C., 2003. Trease and Evan's pharmacognosy, WB Saunders Company Ltd. London, UK pp. 23-38, 251,257.

Finegold, S.M., and Martin, H.J., 1982. Diagnostic Microbiology, 6th ed. C.V. Mosby Company, London, pp. 532-557.

Finland, M., 1978. The new development in antibiotics. *Ann. Intern.Med*. 89: 849-853.

Ibrahim, D., and Osman, H., 1995. Antimicrobial activity of *Cassia alata* from Malaysia. *J. Ethnopharmacology* 45: 151-156.

Ishrat, J.B., Laizuman N., Farhana A.R., Obaydul H., 2011. Antibacterial, cytotoxic and antioxidant activity of chloroform, n-hexane and ethyl acetate extract of plant Amaranthus spinosus. *Int. J. Pharm. Tech. Res.* 3(3): 1675-1680.

Jacob, S., Shirwaikar, A.A. and Srinivasan, K.K. *et al*., 2006. Stability of Proteins in Aqueous Solution and Solid State. *Indian Journal of Pharmaceutical Sciences* 68 (2): 154-163.

Jenkins, G.L., Knevel, A.M. and Digangi, F.E., 1967. Quantitative Pharmaceutical Chemistry. The McGraw Hill Book Company Limited, London, pp. 225, 229-235, 276-277, 336.

Katzung, B.G., 2001. Basic and clinical pharmacology, McGraw-Hill Company, USA p. 850.

Khory, R.N. and Katrak, N.N., 1985. Materia Medica of India and Their Therapeutics. Neeraj Publishing House, Delhi, pp. 527-528.

Malik, T.R., Tajuddin and Rahman, A. 2016. Standardization and evaluation of antibacterial activity of some Unani drugs. *Hippocratic Journal of Unani Medicine* 11(1): 61-77.

Moussaoui, F., Alaoui T., 2016 Evaluation of antibacterial activity and synergistic effect between antibiotic and the essential oils of some medicinal plants. *Asian Pacific Journal of Tropical Biomedicine* 6(1): 32-37

Owolabi, J., Omogbai, E.K.I. and Obasuyi, O., 2007. Antifungal and antibacterial activities of the ethanolic and aqueous extract of Kigelia Africana (Bignoniaceae) stem bark. *Afr. J. Biotechnol.* 6 (14), 882-85.

Quale, J.M., Landman, D., Zaman, M.M., et al., 1996. In vitro activity of *Cinnamomum zeylanicum* against azole resistant and sensitive Candida species and a study of Cinnamon for oral candidiasis. *American Journal of Chinese Medicine* 24(2): 103-109.

Rahman, A., Tajuddin and Amin, K.M.Y., 2011. Identification and standardization of a Pharmacopoeial Unani formulation: estimation of marker compounds, *Unani Medicus: An International Journal* 01 (2): 58-62.

Rahman, A., Tajuddin, Amin, K.M.Y., Rehman, S., 2013. Antimicrobial assay of alcoholic and hydroalcoholic extract of Unani formulation by Agar well method. *Hippocratic Journal of Unani Medicine* 8 (1): 59-66.

Said, H.M., 1996. Medicinal Herbals. Hamdard Foundation, Karachi, Pakistan, pp. 27, 30.

Threlfall, E.J., Frost, J.A., Ward, L.R. and Rowe, B., 1996. Increasing spectrum of resistance in multi-resistant Salmonella typhimurium. *Lancet* 347: 1053-1054.

Tiwari, R., Dixit, R., and Dixit, S.N., 1994. Studies on fungi toxic properties of essential oil of *Cinnamomum zeylanicum Breyn., Indian perfumer* 38(3): 98-104.