Standardization and Evaluation of Antibacterial Activity of Some Unani Drugs

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

[1]Department of Ilmul Advia;

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

Abstract

Microorganisms have developed resistance due to indiscriminate use of many antibiotics which are also associated with severe adverse effects. To overcome these problems plants and their products provides a better substitute for treating infectious diseases. Afsanteen (*Artemisia absenthium*), flowers of Banafshah (*Viola odorata*) and Bisheri booti (*Aerva lanata*) are used in Unani System of Medicine since long time either as single drug or in compound formulation. All the three drugs were screened for antibacterial activity against different strains of gram positive and gram negative bacteria such as *Bacillus sp. Staphylococcus aureus, Streptococcus pyogenes Escherichia coli, Citrobacter sp., Klebsiella sp. Salmonella typhimurium* and *Proteus sp. etc.* The Aqueous and alcoholic extracts of these crude drugs were used to determine antibacterial activity by disc-agar diffusion technique at four concentration levels (100, 50, 25 and 12.5µg/disc) and compared with standard drug ciprofloxacin at concentration of 5 µg/disc. Zone of Inhibition was taken as the parameter for the assessment of antibacterial activity. Of all the drugs tested, the alcoholic extract of *Viola odorata* was found to be the most active against *Salmonella typhimurium* at the doses of 50 and 100µg/disc. Furthermore, before testing these drugs for antimicrobial activity, their samples were also checked for authenticity based on certain physico-chemical parameters.

Keywords: *Artemisia absenthium, Viola odorata, Aerva lanata,* Antibacterial, Physico-chemical

Introduction

It is well known that infectious diseases account for a high proportion of the health problems in the developing countries, hence it becomes necessary to search for quick, effective and safe remedies for these health problems and this gave a stimulus to the search for new anti-microbial substances. World Health Organization noted that majority of the world's population depends on traditional medicine for primary healthcare. Plants which are widely used as medicines constitute a major source of natural organic compounds (Prabuseenivasan *et al.,* 2006). During the recent past there has been a growing interest in traditional and alternative systems of medicine in industrialized 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. Nature has been a source of medicinal agents for thousands of years and a good number of modern drugs have been isolated from natural sources, many of these isolations were based on the use of the agents in traditional medicine (Owolabi *et al*., 2007). In India

[2*]Author for correspondence; Email: rahman.mdi@gmail.com

medicinal plants are a source of great economic value. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of medicinal plants grow in different parts of the country (Parekh *et al.,* 2005).

Microorganisms have developed resistance due to indiscriminate use of many antibiotics, thus creating an immense clinical problem in the treatment of infectious diseases (Davis 1994). In addition, antibiotics are also sometimes associated with adverse effects on patients, which include hypersensitivity, depletion of beneficial gut mucosal flora, immune-suppression and allergic reactions (Ahmad *et al.,* 1998). Thus there is an urgent and constant need to develop new and effective therapeutic agents for the treatment of infectious diseases (Khan and Jain, 2003; Monroe and Polk, 2000; Bhavnani and Ballow, 2000).

Several screening studies have been carried out in different parts of the world which aim at knowing the different phytochemical constituents of medicinal plants possessing antimicrobial activity so as to use them for the treatment of microbial infections as a possible alternative to chemically synthetic drugs, to which many infectious microorganisms have become resistant (Akinpelu and Onakoya, 2006). There are several reports on the antimicrobial activity of different herbal extracts in different regions of the world (Chung *et al*., 2004; Nair and Chanda, 2004; De Boer *et al.,* 2005; Nair *et al.,* 2005). Because of the side effects and the resistance that pathogenic microorganisms build against antibiotics, recently much attention has been paid to extracts and biologically active compounds isolated from plant species used in herbal medicine (Essawi and Srour, 2000). Plant-based antimicrobials represent a vast untapped source of medicines and further exploration of plant antimicrobials needs to occur. Antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials (Iwu *et al.,* 1999).Unani medicine also offers a number of single and compound preparations that are used successfully in the management of various infectious diseases (Rahman *et al.,* 2013).

Therefore, present study was undertaken to evaluate the anti-microbial potential of aqueous and alcoholic extract of Afsanteen *(Artemisia absinthium*) - whole herb, Banafshah *(Viola odorata*) - flowers and Bisehri Booti *(Aerva lanata*) - whole herb. 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 as anti- infective and blood purifier, or are in clinical practices of Unani physicians for treatment of various infectious diseases.

Since the efficacy, safety and global acceptance of medicinal plants depends upon its authenticity and quality, therefore, standardization of these drugs is mandatory to make them less controversial and more efficacious to use (Rahman *et al.,* 2013). Hence, these drugs were also tested for their originality based on certain physico-chemical parameters before evaluating for antimicrobial activity.

Materials and Methods

The study was carried out at the Department of Ilmul Advia, A.K. Tibbiya College, A.M.U., during 2004.

Collection of plants:

The plant materials were procured from different sources. Afsanteen and Banafshah were purchased from the local market of Srinagar (Jammu and Kashmir), Bisehri booti was obtained from the herbarium of Department of Ilmul Advia, Faculty of Unani Medicine, 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, 1984, 1992, 2007; Khory and Katrak, 1985). The samples have been submitted to the Department of Ilmul Advia for future reference. All the drugs were dried in shade and then powdered.

Physico-chemical parameters

It included successive 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 (Anonymous, 1968; Jenkins *et al.,*1967; Anonymous, 1987), chemical treatment and fluorescence analysis (Anonymous, 1992). Qualitative estimation of chemical constituents present in the powder form of test drugs was carried out according to the scheme proposed by Bhattacharjee and Das (1969) depicted in figure 1.

Anti-bacterial studies

Extracts preparation

Alcoholic extracts of the coarsely powdered drugs were prepared by the method of Ibrahim and Osman (1995) with some modifications. Coarse powdered drugs were extracted using a Soxhlet's apparatus with 95% ethanol as solvent, at 55°C for 06 hours or until the extracting return in the Siphon was colorless. The resultant extract was then concentrated in a water bath, at 40°C. The, stock solutions were prepared in 95% ethanol. Strict aseptic precautions were followed throughout the process and the heat wherever needed was kept as low as possible to prevent the thermolabile substances present in the drugs from destruction. Same procedure, as mentioned above, was carried out to prepare aqueous extracts using distilled water as solvent at 60°C and concentrated at 50°C.

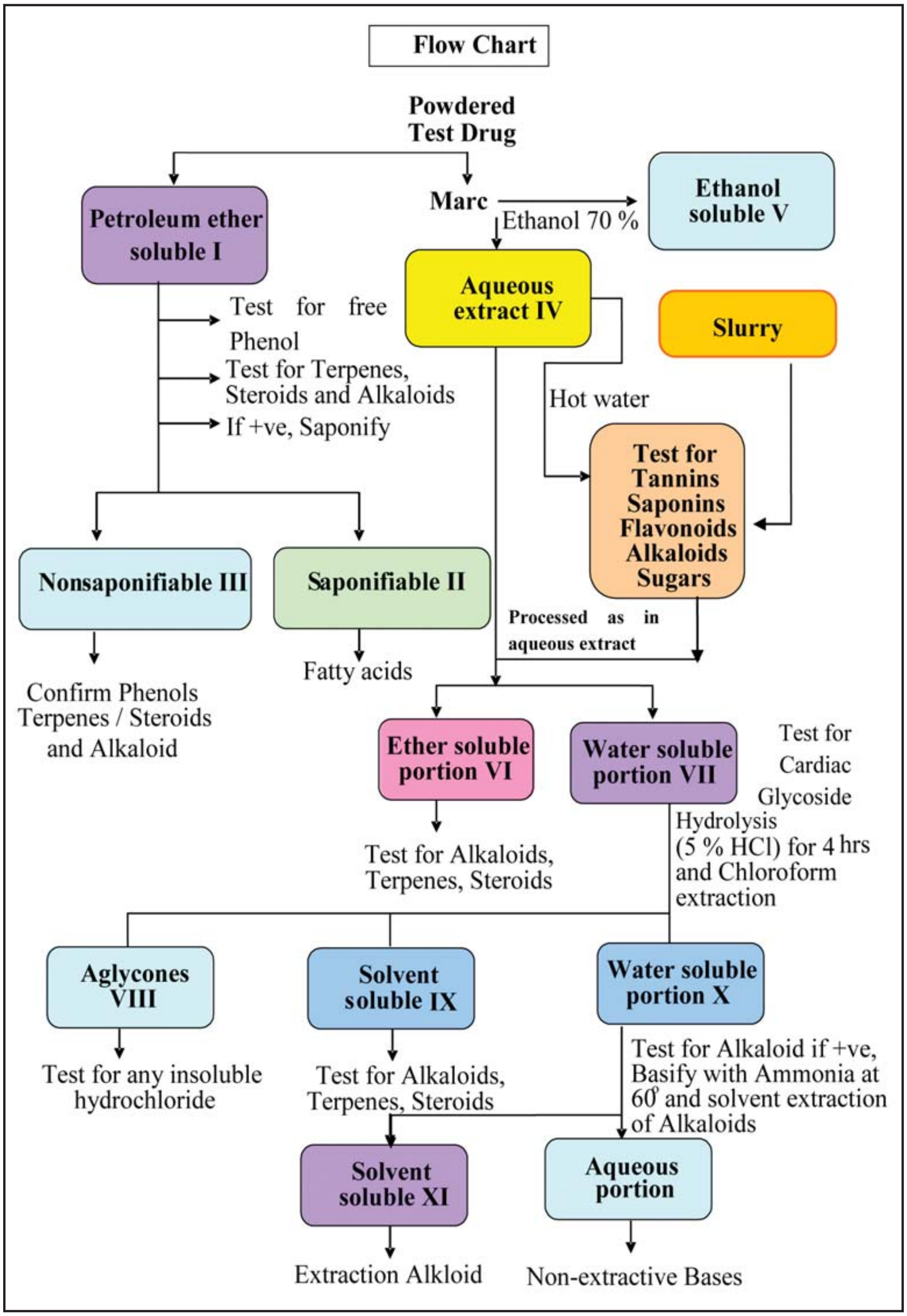

Figure 1: Scheme for qualitative analysis of chemical constituents present in the test drugs

Bacterial strains used in the study

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

Antibacterial susceptibility testing

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.

Anti-bacterial activity was determined using the disc-agar, diffusion technique according to the method described by Finegold and Martin, 1982. Overnight cultures of the bacterial isolates were prepared in Mueller-Hinton Broth (M 173, HiMedia, India) standardized to match 0.5 McFarland Barium sulphate tubes and, used to inoculate Mueller-Hinton agar plates. Discs impregnated with different amounts of the extracts were placed in the surface of these plates and incubated at 37^0C overnight. Diameters of zones of inhibition were recorded.

For fastidious organisms such as *Streptococci,* the agar supplemented with 05% sterile, defibrinated blood was used. The cultures of *Streptococci* were incubated

Table 1: Standard and clinical bacterial strains used for the detection of antibacterial activities of the drug

S.No.	Gram Negative bacterial strains	S.No.	Gram Positive bacterial strains
1.	*Citrobacter sp.*	1.	*Bacillus sp.*
2.	*Escherichia coli*	2.	*Staphylococcus aureus*
3.	*Klebsiella sp*	3.	*Staphylococcus aureus ATCC25923*
4.	*Proteus sp.*	4.	*Streptococcus pyogenes*
5.	*Pseudomonas aerugenosa*		
6.	*Pseudomonas aerugenosa ATCC 27583*		
7.	*Salmonella typhimurium*		
8.	*Shigella sp.*		

in an atmosphere enriched with 10% CO_2, The whole experiment was performed in triplicate.

The concentration levels used to determine antibacterial activity of test drug was 50, 25 and 12.5µg/disc for Afsanteen while concentration levels for Gul-e-Banfshah and Bisehri Booti were 100, 50, 25 and 12.5µg/disc. Commercial diagnostic sensitivity disc (Hi Media) of Ciprofloxacin was used for comparison at concentration of 5µg/disc. Furthermore, results were analyzed statistically by Student's 't' test and P value d" 0.05 were considered statistically significant.

Results

Physico-chemical parameters

The analytical values of different physicochemical parameters such as solubility, moisture content, loss of weight on drying, ash values and pH values of the test drugs were determined and are depicted in table 2. Successive 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 3.

Table 2: Physico-chemical values of the test drugs

Physico-chemical parameters	Afsanteen (Mean±SE)	Gul-e-Banfshah (Mean±SE)	Bisehri Booti (Mean±SE)
Water soluble contents (1% solution)	10.10±0.05	13.23±0.10	48.07±0.05
Water soluble contents (10% solution)	6.34±0.04	7.55±0.04	8.35±0.02
Alcohol soluble contents (1% solution)	8.08±0.04	6.13±0.05	3.53±0.04
Alcohol soluble contents (10% solution)	0.06±0.02	3.57±0.04	1.35±0.02
Moisture content (%)	8.83±0.14	11.17±0.14	12.33±0.14
Loss on drying at 105^0C (%)	12.07±0.32	12.63±0.19	13.50±0.09
Total Ash (%)	4.70±0.06	11.21±0.09	8.78±0.03
Acid Insoluble Ash (%)	2.63±0.05	6.27±0.12	2.48±0.19
Water Soluble Ash (%)	4.32±0.04	1.73±0.06	1.01±0.01
pH (1% solution)	1.60±0.03	1.50±0.00	1.70±0.03
pH (10% solution)	1.70±0.00	1.80±0.03	2.50±0.00

Table 3: Successive extractive values of the test drugs

Solvent	Extractive values in % (Mean±SE)		
	Afsanteen (Mean±SE)	Gul-e-Banfshah (Mean±SE)	Bisehri Booti (Mean±SE)
Petroleum Ether	2.95±0.02	1.43±0.01	4.90±0.15
Diethyl Ether	2.64±0.00	0.83±0.01	1.05±0.02
Chloroform	1.79±0.02	0.77±0.01	0.83±0.03
Benzene	0.21±0.00	0.11±0.00	0.23±0.00
Alcohol	22.36±0.16	9.19±0.01	11.12±0.06
Distilled Water	12.47±0.16	11.41±0.01	9.97±0.03

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 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.

Chemical treatment and fluorescence analysis

The powdered test drugs were treated with different chemicals and the changes in color were observed in day light and shown in table 5. For fluorescence

Table 4: Qualitative analysis of chemical constituents

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

analysis powder of all the three drugs were treated with different reagents and change in color was observed in Ultra-violet (UV) radiations alongwith day light, results are depicted in table 6.

Table 5: Treatment of test drugs with chemicals

Chemical Treatment	Afsanteen Change in color	Gul-e-Banfshah Change in color	Bisehri Booti Change in color
Concentrated HNO_3	Golden Brown	Dark Brown	Yellow
Concentrated HCL	Green	Brownish Green	Green
Concentrated H_2SO_4	Black	Black	Black
2% Iodine Solution	Yellow	Brown	Green
Acetic Acid	Brown	Brown	Green
10% NaOH Solution	Brown	Brown	Green
Acetic Acid + H_2SO_4	Brownish Yellow	Brown	Green
10% NaOH + few drops of $CuSO_4$ sol.	Black	Black	Green
10% NaOH + few drops of 5% lead acetate	Golden Brown	Golden Brown	Green
Acetic Acid + few drops of 5% ferric chloride + Concentrated H_2SO_4	Yellowish Brown	Yellowish Brown	Yellowish Green
5% ferric chloride	Fluorescent Green	Fluorescent Green	Fluorescent Green

Table 6: Fluorescence analysis of test drugs

Reagents	Afsanteen Change in color		Gul-e-Banfshah Change in color		Bisehri Booti Change in color	
	Day light	UV Light	Day light	UV Light	Day light	UV Light
1N HCL	Dull Yellow	Green	Light Brown	Brown	Dull Green	Green
Concentrated HNO_3	Golden Brown	Green	Dark Brown	Brown	Yellow	Green
Concentrated H_2SO_4	Black	Green	Black	Blackish Green	Black	Green
Water	Straw	Dull Green	Brown	Brownish Green	Parrot Green	Green
Acetic Acid	Straw	Green	Brown	Black	Green	Green
5% Ferric Chloride	Straw	Dark Green	Fluorescent Green	Green	Fluorescent Green	Green
10% NaOH	Straw	Dark Green	Brown	Dark Green	Green	Parrot Green
2N HCL	Light Brown	Green	Brown	Black	Dull Green	Green
1N H_2SO_4	Straw	Green	Brown	Black	Dull Green	Brown
4N H_2SO_4	Straw	Green	Brown	Black	Yellowish Green	Green

Anti-bacterial studies

Aqueous and alcoholic extracts of Afsanteen, Gul-e-Banfshah and Bisehri Booti were screened against Gram positive and Gram negative bacterial strains. Results are depicted in table 7 (a,b,c).

Table 7 (a): Antibacterial activity of Afsanteen *(Artemisia absinthium)*

Microbial Strains	**Test Drug Concentration (µg/disc)**	**Zone of inhibition (ZOI) in mm (Mean ± S.E.)**		
		Alcoholic extract	**Aqueous Extract**	**Ciprofloxacin (5µg/disc)**
Bacillus sp.	50 25 12.50	No Effect No Effect Not Tested	No Effect No Effect Not Tested	17.10±0.01
Staphylococcus aureus	50 25 12.50	No Effect No Effect Not Tested	No Effect No Effect Not Tested	25.16±0.02
Streptococcus pyogenes	50 25 12.50	No Effect No Effect Not Tested	No Effect No Effect Not Tested	10.13±0.01
Escherichia coli	50 25 12.50	No Effect No Effect Not Tested	No Effect No Effect Not Tested	19.10±0.00
Klebsiella sp	50 25 12.50	No Effect No Effect Not Tested	No Effect No Effect Not Tested	25.03±0.08
Pseudomonas aerugenosa	50 25 12.50	No Effect No Effect Not Tested	No Effect No Effect Not Tested	23.16±0.17
Proteus sp.	50 25 12.50	No Effect No Effect Not Tested	No Effect No Effect Not Tested	13.16±0.02
Salmonella typhimurium	50 25 12.50	No Effect No Effect Not Tested	No Effect No Effect Not Tested	10.13±0.02

Table 7 (b): Antibacterial activity of Banafshah *(Viola odorata)*

Microbial Strains	Test Drug Concentration (µg/disc)	Zone of inhibition (ZOI) in mm (Mean ± S.E.)		
		Alcoholic extract	Aqueous Extract	Ciprofloxacin (5µg/disc)
Staphylococcus aureus ATCC25923	100 50 25 12.50	No Effect No Effect Not Tested Not Tested	No Effect No Effect Not Tested Not Tested	25.16±0.02
Streptococcus pyogenes	100 50 25 12.50	No Effect No Effect Not Tested Not Tested	No Effect No Effect Not Tested Not Tested	10.13±0.02
Escherichia coli	100 50 25 12.50	No Effect No Effect Not Tested Not Tested	No Effect No Effect Not Tested Not Tested	19.10±0.00
Shigella sp.	100 50 25 12.50	No Effect No Effect Not Tested Not Tested	No Effect No Effect Not Tested Not Tested	16.10±0.01
Pseudomonas aerugenosa ATCC 27583	100 50 25 12.50	No Effect No Effect Not Tested Not Tested	No Effect No Effect Not Tested Not Tested	23.16±0.17
Salmonella typhimurium	100 50 25 12.50	11.40±0.15(P<0.01) 08.23±0.02(P<0.01) Not Tested Not Tested	No Effect No Effect Not Tested Not Tested	10.13±0.02
Citrobacter sp.	100 50 25 12.50	No Effect No Effect Not Tested Not Tested	No Effect No Effect Not Tested Not Tested	22.00±0.00

Discussion and Conclusion

Standardization of the test drugs

An impressive number of modern drugs have been isolated from natural sources; many of these isolations were based on the uses of the agents in traditional medicine (Rahman *et al.,* 2011). This plant-based, traditional medicine system

Table 7 (c): Antibacterial activity of Bisehri Booti *(Aerva lanata)*

Microbial Strains	Test Drug Concentration (µg/disc)	Zone of inhibition (ZOI) in mm (Mean ± S.E.)		
		Alcoholic extract	Aqueous Extract	Ciprofloxacin (5µg/disc)
Staphylococcus aureus ATCC25923	100 50 25 12.50	No Effect No Effect Not Tested Not Tested	No Effect No Effect Not Tested Not Tested	25.16±0.02
E. Coli	100 50 25 12.50	No Effect No Effect Not Tested Not Tested	No Effect No Effect Not Tested Not Tested	19.10±0.00
Shigella sp.	100 50 25 12.50	No Effect No Effect Not Tested Not Tested	No Effect No Effect Not Tested Not Tested	16.10±0.01
Pseudomonas aerugenosa ATCC 27583	100 50 25 12.50	No Effect No Effect Not Tested Not Tested	No Effect No Effect Not Tested Not Tested	23.16±0.17
Salmonella typhimurium	100 50 25 12.50	No Effect No Effect Not Tested Not Tested	No Effect No Effect Not Tested Not Tested	10.13±0.02
Citrobacter sp.	100 50 25 12.50	No Effect No Effect Not Tested Not Tested	No Effect No Effect Not Tested Not Tested	22.00±0.00

continues to play an essential role in health care, with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care (Doughari *et al.,* 2008). Therefore, with the ever-increasing use of herbal medicines worldwide and the rapid extension of the global market for these products, the safety and quality of medicinal plant materials and finished products have become a major concern for health authorities and pharmaceutical industries. From the time of collection of drug to its storage and up to the production of medicine, chances of deterioration in quality are quite frequent, resulting in the decline of the efficacy of drug. To overcome these problems of Unani drugs it is almost inevitable to standardize the drugs for their rational therapeutic use. A disease cannot be managed comprehensively until the delivery of genuine samples of drug is ensured (Jahan *et al.,* 2008; Alam and Khan, 2010).

These physico-chemical parameters are considered as tools of checking quality, identity, purity and strength of the Unani drugs. The solubility and extractive values sometimes are used as an index of purity for formulations. The amount of the extract that the drug yields in a solvent is often an approximate measure of the amount of certain constituents that the drug contains. Therefore, for establishing the standards of any drug these extractive values and solubility play an important role, as the adulterated or exhausted drug material will give different values rather than the extractive percentage of the genuine one (Jenkins *et al.,*1967). The Moisture content of the test drugs determines the release of their active ingredients as well as their chemical, physical, microbial, shelf-life properties and adulterations (Jacob *et al*., 2006). Loss in weight on drying at 105^0C also indicates towards the loss of volatile substances along with water. pH value of the drug determines whether the drug absorb in stomach or intestine because the drugs in the opposite pH are unionized and absorbed rapidly from stomach or intestine. The pH of all the three test drugs were found to be acidic therefore, test drugs gets ionized in stomach and absorbs somewhere in intestines. Adulteration of herbal drugs with unwanted materials like earthy matters resulting in higher ash percentage. Therefore, Estimation of Ash values are important criterion for judging impurities along with identity and quality of the drugs (Jenkins *et al.,*1967).

The fluorescence analysis under UV –light and treatment of test drugs with chemicals are very characteristic and helps in the identification of the drugs (Chase and Pratt, 1949) from the adulterated ones. Many compounds show distinct colours under UV-light after treatment e.g. aconitine (light blue), berberine (yellow) and emetine (orange). Hence, the fluorescence analysis provides valuable information regarding the genuineness of drugs. The efficacy of plant drugs is mainly because of their active chemical constituents therefore, it is necessary to determine active compounds in the test drugs at least qualitatively. Alkaloids, tannins, sterols, phenols, flavonoids, oils etc possesses antibacterial property. If all or some of these are present in drugs then drug may have antimicrobial efficacy (Said 1996; Katzung, 2001; Anuradha and Goyal, 1995; Evans, 2003).

Antimicrobial studies

Many diseases are being spread in the world by microorganisms and the discovery of microorganisms as the causative agent of many infectious diseases of man naturally created an interest in substances toxic to these organisms and this gave a stimulus to the search for new antimicrobial substances. The antibiotics in modern therapeutic system have tremendous effect in controlling the infectious diseases (Finland, M. 1978). However, the advent of escape mechanism (cf.drug resistance) adopted by most of the pathogens certainly needs a suitable replacement of the presently available antibiotics (Conly *et al.,* 1992; Threlfall *et al.,* 1996).

As plant drugs which constitute the major chunk of Unani therapeutics are considered important because they are physiologically innocuous and safe and also because they may be useful against resistant microorganisms (Rahman *et al.,* 2013). According to Unani literature in infectious diseases the blood has become impure and the treatment is attempted with a class of drugs described as blood purifiers. In all the conditions like bacteraemia, septicaemia, viraemia, toxaemia, pyrexia, pyaemia, uraemia, etc., the blood contains such substances that should not be normally present in it or an abnormally high concentration of normal constituents. There may be bacteria, bacterial toxins, viruses, allergens, pus, metabolic products, bile pigments, etc. They are listed as impurities that have rendered the blood impure (Vohola, 1985).The antibiotics and other chemotherapeutic agents purify the blood by destroying the infective organisms.

In the present study antimicrobial activity of the alcoholic and aqueous extracts of Unani drugs was quantitatively assessed using agar well method on the basis of Zone of Inhibition (ZOI) which was expressed in mm. Extracts of Afsanteen and Bisehri Booti at given concentration level doesn't show any significant activity against any of the bacterial species tested. While, the alcoholic extract of Gul-e-Banfshah showed significant antibacterial activity against *Salmonella typhimurium* at the concentration level of 50 and100 µg/disc.

As bacterial strains were the clinical isolates and may develop resistance. So Afsanteen didn't show any effect at the particular test doses against any of the test species. This also correlates partially with the studies carried out by Anesini and Perez, 1993 and Perez and Anesini, 1994 in which Afsanteen extracts doesn't show any effect against *Salmonella typhimurium* and penicillin G resistant *Staphylococcus aureus, Escherichia coli* and *Aspergillus niger.* Gul-e-Banafshah doesn't depict effect against any of the other test strains except *Salmonella typhimurium*. This result is totally in contrast to the study carried out by Afaq *et al.,* 2004 where they have shown the aqueous extract of the drug effective against *Escherichia coli* K-12, *Staphylococcus aureus, Bacillus subtilis* ATCC 6051, but no effect was seen against *Salmonella typhimurium* ATCC 23564. The difference in the results may be due to a number of reasons as antibacterial activity of different medicinal plants shows variability due to different chemicals found in them as well as the quality and quantity produced during different seasons, at different stages and in different species of plants. Variation in results may also be related to extraction methodology (Prasad *et al.,* 1999). The difference may also be due to the differences in the bacterial strains tested. No effect was shown by Bisehri booti at any dose against any of the bacterial strain. Although it is usually recommended in urinary tract infections may be because of other actions like anti-inflammatory and diuretic.

There are many documented, scientifically proved and globally accepted Unani drugs having striking and significant antimicrobial effects. But the present study has partially revealed the activity of test drugs to control antibiotic resistant bacteria, which are a major threat to human health. However the alcoholic extracts of *Gul-e-Banafshah* could be a possible source to obtain new and effective herbal medicines to treat infections specially caused by multi-drug resistant strains of *Salmonella typhimurium.* Further, it is necessary to determine the toxicity of the test drugs, their side effects and pharmaco-kinetic properties.

References

Afaq, S.H., Shehbaz, A., Masood, A.K., Owais, M. and Nadeem, A., 2004. Antibiotic screening of certain Unani medicinal plants. *Indian Drugs* 41(4): 236-239.

Ahmad, I., Mehmood, Z., Mohammad, F., Screening of some Indian medicinal plants for their antimicrobial properties. *J Ethnopharmacol.* 62: 183-193, 1998.

Akinpelu, D.A. and Onakoya, T.M., 2006. Antimicrobial activities of medicinal plants used in folklore remedies in south-western. *African Journal of Biotechnology* 5 (11), 1078-1081.

Alam, S. and Khan, N.A., 2010. A Physicochemical Study of Qurs-e-Rewand. *Unani Medicus* 1(1): 92-101.

Anesini, C., and Perez, C., 1993. Screening of plants used in Argentine folk medicine for antimicrobial activity, *Journal of Ethnopharmacology* 39: 119-128.

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

Anonymous, 1984. Medicinal Plants of Gwalior Forest Division, Madhya Pradesh, CCRUM, New Delhi, p. 6.

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. New Delhi: Ministry of Health and Family Welfare, Deptt. of AYUSH, Govt. of India; Part-I, (1 and 3), pp. 3-4, 12-16.

Anonymous. 1987. Physiochemical Standards of Unani Formulations. New Delhi: Central Council for Research in Unani Medicine (CCRUM), Part-1 & 2, 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

Bhavnani, S.M. and Ballow, C.H., 2000. New agents for Gram-positive bacteria. *Curr Opin Microbiol* 3: 528-534

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.

Chung, P.Y., Chung, L.Y., Ngeow, Y.F. et al., 2004. Antimicrobial activities of Malaysian plant species. *Pharm Biol* 42: 292-300.

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.

Davies, J., 1994. Inactivation of the antibiotics and the dissemination of resistance genes. *Science* 264: 375-382.

De Boer, H.J., Kool, A., Broberg, A., et al., 2005. Antifungal and antibacterial activity of some herbal remedies from Tanzania. *J Ethnopharmacol.* 96: 461-469.

Doughari, J.H., El-Mahmood, A.M., 2008. Antimicrobial activity of leaf extracts of *Senna obtusifolia* (L). *Indian Journal of Pharmacology* 2 (1):7-13.

Essawi, T., Srour, M., 2000. Screening of some Palestinian medicinal plants for antibacterial activity. *J Ethnopharmacol* 70: 343-349.

Evans, W.C., 2003. Trease and Evan's pharmacognosy, WB Saunders Company Ltd. London, UK 14: pp. 23-38, 251,257.

Finegold, S.M., and Martin, H.J., 1982. Diagnostic Microbiology, C.V. Mosby Company, London (Ed.) 6, 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.

Iwu, M.W., Duncan, A.R., Okunji, C.O., 1999. New antimicrobials of plant origin In: Janick, J. (Ed), Perspectives on New Crops and New Uses. ASHS Press, Alexandria, VA, p. 457-462.

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.

Jahan, N., Afaq, S.H. and Khan, N.A., et al., 2008. Physico-Chemical studies of the Gum Acacia. *Natural Product Radiance* 7(4): 335-337.

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 8: p. 850.

Khan, K.H. and Jain, S.K., 2003. Medicinal plants a retrospective. *Hamdard Medicus* XLVI (3): 23-33.

Khory, R.N. and Katrak, N.N., 1985. Materia medica of India and their therapeutics, Neeraj publishing house, Delhi, pp. 54-55, 351-352 and 505.

Monroe, S. and Polk, R., 2000. Antimicrobial use and bacterial resistance. *Curr Opin Microbiol* 3: 496-501.

Nair, R. and Chanda, S.V., 2004. Antibacterial activity of some medicinal plants of Saurashtra region. *J. Tissue Res* 4: 117-120.

Nair, R., Kalariya, T., Chanda, S., 2005. Antibacterial activity of some selected Indian medicinal flora. *Turk J Biol* 29: 41-47.

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.

Perez, C. and Anesini, C., 1994. In vitro antibacterial activity of Argentine folk medicinal plants against *Salmonella typhi. Journal of Ethnopharmacology* 44: 41-46.

Prabuseenivasan, S., Jayakumar, M., and Ignacimuthu, S., 2006. In vitro antibacterial activity of some plant essential oils. *BMC Complementary and Alternative Medicine* 6(39): 1-8.

Prasad, G., Pandey, S. and Gupta, K.K., 1999. Antibacterial activity of certain indigenous drugs and drug plants against certain bacterial species. *Hamdard Medicine* XLII (3): 53-57.

Rahman, A., Tajuddin, Amin, K.M.Y., 2013. Physico-chemical assay alongwith HPLC profile of a Pharmacopeial preparation containing colchicum, ginger and aloe. *International Journal of Pharmaceutical Research* 5 (4): 51-55.

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.

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.

Said, H.M., 1996. Medicinal herbal, Hamdard foundation, Karachi, Pakistan, 1: 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.

Vohola, S.B., 1985. Skin diseases: Two Unani concepts, Proceedings of the symposium on Dermatology and Unani System of Medicine. Hamdard National Foundation, New Delhi, pp. 8-13.