Antibacterial Potential of Fruits of 'Bakayin' (*Melia azedarach* Linn.): A Unani Drug of Repute

***Sumbul Rehman and Abdul Latif**

Department of Ilmul Advia (Unani Pharmacology & Pharmaceutical Sciences), A.K. Tibbiya College, Aligarh Muslim University, Aligarh-202002

Abstract

Bakayin (*Melia azedarach* L.; Family Meliaceae) has been used in Traditional Systems of Medicine since long time to treat many diseases including infectious diseases. However, information to revalidate its use on scientific parameters is limited. Therefore, present study has been done to screen antibacterial activity of ethanolic extracts of fruits of Bakayin against pathological bacterial strains using disk diffusion method and its efficacy was compared with the standard drug Ciprofloxacin for gram positive and Gentamycin for gram negative bacterial strains. Qualitative analysis of the extracts was also done to confirm the presence of various phytoactive constituents. It was found that Bakayin contains alkaloids, glycosides, flavonoids, carbohydrates, tannins, starch, saponin, resins and terpenes. This study scientifically validates the antibacterial potential of Bakayin and reconfirms the claims of Unani physicians for its use in various infectious diseases as leprosy, scrofula, scabies, diarrhoea, dysentery, typhoid, suppurating wounds etc. The findings can usefully be applied by researchers elsewhere for comparison of its bioactive constituents, and this might be a helpful lead in the rational use of Bakayin.

Keywords: Infectious diseases, Bakayin (*Melia azedarach*), Antibacterial screening

Introduction

Unani system of medicine provides a large number of drugs of natural origin which can be used today to combat many dreadful diseases which are difficult to be treated with the western medicine along with the emerging health hazards nowadays. Ethno-botanical, medical and phytochemical literature available in our classical books has abundant remedies from natural resources as mentioned by eminent Unani physicians that need to be re-explored now for different health hazards. Unani medicine claims to possess a large number of effective and safe drugs to combat infectious diseases that are in use since centuries (Rehman and Latif, 2015).

Bacterial strains have developed resistance to almost all the antibiotics, further some antibiotics have serious undesirable effects (Rehman *et al*., 2011). This is an alarming situation and calls for serious consideration including drugs available in indigenous system of medicines. There appears no effort made to investigate the antimicrobial potential of 'bakayin' fruits through in-vitro testing. Present study is based on this rationale and presents results of phyto-chemical and antibacterial

*Author for correspondence; Email: dr.sumbulrehman@gmail.com

screening of this important Unani drug in an effort to revalidate its medical efficacy to combat infectious diseases.

Bakayin (*Melia azedarach* Linn.) is a medium sized deciduous tree; flowers liliac-purple; fruit drupe 4-seeded, yellowish with very hard endocarp. Wild in the sub-Himalayan tract at 700-1000m; cultivated and naturalized throughout India upto 1800 m; grows as a hedge plant also; known as Pride of India, Chinaberry, Persian liliac, Umbrella tree, Bead tree. Most of the parts of Bakayin have been used in various diseases as root bark, fruit or berry, seeds, flowers, leaves oils and gum. Root decoction or fluid extract used as anthelmintic while root bark is used as vermifuge, in intermittent fevers and dysentery, as a cathartic and emetic. In India, before quinine, its root bark was also used in malaria as infusion of bark used as febrifuge especially for periodic fevers; also, for thirst and nausea. Poultice of bark is used in leprosy and scrofulous ulcers (Pal and Bose, 2011; Chopra *et al.,* 1958; Panda *et* al., 2008; Sindhia and Bairwa, 2010; Nadkarni, 2002; Kiritikar and Basu, 1935).

Material and Methods

The study was undertaken in the department of Ilmul Advia, A.K. Tibbiya College, Aligarh, during 2012.

Plant Material

The berries of Bakayin were collected from the campus of Ajmal Khan Tibbiya College, Aligarh Muslim University, Aligarh, and was identified by the available literature.

Preparation of Berry Extracts

The test drug was dried at room temperature in a ventilated room, milled to fine powder and stored in a close air tight container in dark until use. Extraction was done according to the method described by Afaq *et al. (*1994) and Peach and Tracey (1955) with some minor modifications, keeping in mind that the thermo labile elements present in the drug are destroyed when exposed to a higher temperature beyond 55°C, so the heat wherever needed was kept as low as possible to prevent the loss of thermo-labile substances present in the drugs from destruction. The coarse drug material was extracted with 95% ethanol as a solvent at 50°C for 6 hours and dried under reduced pressure in the Lypholizer. Strict aseptic precautions were followed throughout the process. The stock solutions for extract were prepared from the dried extract so obtained in the Dimethyl Sulphoxide (DMSO) as a solvent for use. The respective stock solutions so prepared were refrigerated till further use.

Phytochemical Analysis

Phytochemical studies of the plant preparations are necessary for standardization, which helps in understanding the significance of phyto-constituents in terms of their observed activities. Phytochemistry also helps in standardizing the herbal preparations so as to get the optimal concentrations of known active constituents, and in preserving their activities. Qualitative analysis of the chemical constituents present in the drug sample. Standard tests and methodology has been followed to confirm the presence of chemical constituents (Bhattacharjee and Das, 2005 and Afaq *et al*., 1994) (Table-1).

Table 1: Qualitative Analysis of the Phytochemicals

S.No.	Chemical Constituents	Test Reagents	Inference
			Bakain (*M.azedarach* Linn.)
1.	Alkaloids	Dragendorff's reagent	+
		Wagner's reagent	+
		Mayer's reagent	+
2.	Carbohydrates	Molish Test	+
		Fehling Test	+
		Benedict Test	+
3.	Flavonoids	Mg Ribbon and dil. Hcl	_
4.	Glycosides	NaOH Test	+
5.	Tannins/Phenols	Ferric Chloride Test	_
		Liebermann's test	+
		Lead Acetate test	+
6.	Proteins	Xanthoproteic test	_
		Biuret test	+
7.	Starch	Iodine Test	_
8.	Saponins	Frothing with $NaHCO_3$	+
9.	Steroids/Terpenes	Salkowski Reaction	_
10.	Resins	Acetic anhydride test	+

Indications: ' - 'Absence and '+' Presence of constituents

Antibacterial Susceptibility Testing

Antimicrobial susceptibility testing was done by Kirby Bauer's disk diffusion method (Bauer *et al.,* 1996) as was performed on biofilm isolates by Kirby-Bauer on Muller Hinton Agar (Barry *et al.*, 1999).

Bacterial strains used for the study are listed in Table-2. The standard medium Mueller Hinton Agar, was poured to a depth of 4 mm in a 90 mm petridish (PW008, Himedia Labs Pvt. Ltd., Mumbai, India). The plate was inoculated by

Table 2: Microbial Strains Used

S.No.	Bacterial Strains	Type
1.	*Streptococcus mutans*	Gram Positive
2.	*Staphylococcus epidermidis*	"
3.	*Streptococcus pyrogenes*	"
4.	*Bacillus cereus*	"
5.	*Staphylococcus aureus*	"
6.	*Corynebacterium xerosis*	"
7.	*Escherichia coli*	Gram Negative
8.	*Proteus vulgaris*	"
9.	*Pseudomonas aeruginosa*	"
10.	*Klebseilla pneuomoniae*	"

Fig. 1: Fruit (Berry of Bakayin) *Melia azedirach* Linn.

streaking the entire surface in three planes with a sterile cotton swab (PW041, Himedia Labs Pvt. Ltd., Mumbai, India) dipped into standardized inoculums, spreaded evenly with the help of L-Spreader (PW1085, Himedia Labs Pvt. Ltd., Mumbai, India). The bacterial inoculum was prepared from an 18 hour broth culture of the microbe to be tested and was standardized with sterile physiologic saline to contain 10^6 cfu/ml. Standardized commercial paper disk containing amounts of the antimicrobial agents to be tested were placed on the surface of the agar. The plate was incubated in an inverted position at 37^0C for 18 hours. The diameter of zone of inhibition produced by the drug was measured with the help of Antibiotic zone scale (PW297, Himedia Labs Pvt. Ltd., Mumbai, India) from each disk (Kingsbury and Wagner, 1990).

Statistical Analysis

The statistical analysis was done using gpaid software, One way ANOVA and the post test named Bonferroni: Selected pairs of column with multiple comparison was performed with p-value <0.05.

Results and Discussion

Qualitative analysis of the phyto-chemicals of fruits of Bakayin was carried out for the determination of presence of alkaloids, amino acids, proteins, carbohydrates, flavonoids, glycosides, tannins, sterols, phenols, resins and volatile oil. As the therapeutic properties of the crude drugs are mainly due to physiologically active chemical constituents present in the drugs and the lower percentage of chemical constituents may cause lesser therapeutic values of the drugs and therefore, they are considered as low standard drugs. Moreover, the chemical constituents present in the drug have documented antimicrobial activities. Tannins have the property of precipitating proteins. By an analogous process they prevent the development of bacteria and since the proteins are necessary for their growth and development so, they not only stop their nutrition but also precipitate their own proteins (Said, 1996). The phytochemical analysis of the chemical constituents present in the test drug revealed that it contains alkaloids, phenol, resins, saponins, sugars and tannins. These were the encouraging results for our study as such types of chemical compounds are usually responsible for the therapeutic efficacy of that drug. As can be realized from the fact that alkaloids possess antimicrobial and anti-inflammatory activity, this effect has been confirmed by us in our in-vitro study of antimicrobial screening that the drugs were found to be sensitive to many microbes.

This study also confirms the presence of saponin by qualitative test, as they are generally considered as the soapy substances that are general cleansers, having

antiseptic properties (Hirat and Suga, 1983). Sterols either decrease the activity of *S.aureus, E.coli, P.vulgaris* and *Pseudomonas pyocyanea* or have no effect in case of *Klebseilla* and *S. dysentrica* (Anuradha and Goyal, 1995). Flavonoids possess anti-inflammatory, anti-spasmodic, antibacterial, antifungal and antitumor activity, they act as an anti-oxidant and are also thought to protect plants from UV radiations and micro-organisms (Cushnie and Lamb, 2005).These are few evidences in support of the therapeutic activity of the Unani drugs. Most of these findings were found to be helpful in showing its biological activity.

In an effort to validate the antibacterial efficacy of selected test drug 'Bakayin' all ethno- pharmacological knowledge was found to be in favour of our selection of the drug as per the guidelines by Cos *et al*. (2006) in which he described that how we can develop a stronger in vitro proof-of-concept in determining anti-infective potential of natural products. The literature survey revealed that an ample data is available regarding the authentication of Unani drugs to be used in infectious diseases, however, very little assay has been done on Unani drugs regarding their antimicrobial activity.

Present study is a step ahead in this regard to scientifically validate the antimicrobial activity of selected test drug viz. Bakayin using Kirby Bauer's disk diffusion and Agar well diffusion method against different Gram positive and Gram

Table-2 (a): Antibacterial activity of Bakayin against Gram positive bacterial strains

S. No.	Test strains	Zone of Inhibition (in mm) expressed as Mean ± S.E.M (S.D)[Probability of error]		
		Drug Extract (µg/ml)	Control (DMSO- 50µl)	Standard (Ciprofloxacin 30µg)
1.	*S.mutans* (ATCC 25175)	9.4±0.67(1.51)* (S)	6.6±0.24(0.54) (R)	21.2±0.37(0.83) (S)
2.	*S.epidermidis* (ATCC 155)	9.2±0.96(2.16)* (S)	6.4±0.24(0.54) (R)	21.6±0.24(0.54) (S)
3.	*S.pyrogenes* (ATCC 14289)	11.8±1.02(2.28)*** (S)	6.4±0.24(0.54) (R)	21.2±0.37(0.83) (S)
4.	*B.cereus* (ATCC 11778)	9.2±0.96(2.16)* (S)	6.6±0.24(0.54) (R)	21.4±0.24(0.54) (S)
5.	*S.aureus* (ATCC 29213)	22.8±1.02(2.28)* (S)	6.6±0.24(0.54) (R)	26.8±0.20(0.44) (S)
6.	*C.xerosis* (ATCC 373)	6.6±0.24(0.54) (R)	6.6±0.24(0.54) (R)	21.2±0.37(0.83) (S)

(S) – Sensitive (R) – Resistant

Table-2 (b): Antibacterial activity of Bakayin against Gram negative bacterial strains

S. No.	Test strains	Zone of Inhibition (in mm) expressed as Mean ± S.E.M (S.D)Probability of error		
		Drug Extract (µg/ml)	Control (DMSO- 50µl)	Standard (Gentamicin 30µg)
1.	*E.coli* (ATCC 25922)	11.2±0.37(0.83)*** (S)	6.4±0.24(0.54) (R)	14.8±0.20(0.44) (S)
2.	*P.vulgaris* (ATCC 6380)	9.4±0.67(1.51)* (S)	6.4±0.24(0.54) (R)	14.0±0.54(1.22) (S)
3.	*P.aeruginosa* (ATCC 25619)	9.6±0.24(0.54)* (S)	6.4±0.24(0.54) (R)	14.8±0.20(0.44) (S)
4.	*K.pneuomoniae* (ATCC 15380)	8.2±0.48(1.09)*** (S)	6.4±0.24(0.54) (R)	14.8±0.20(0.44) (S)

(S) – Sensitive (R) – Resistant

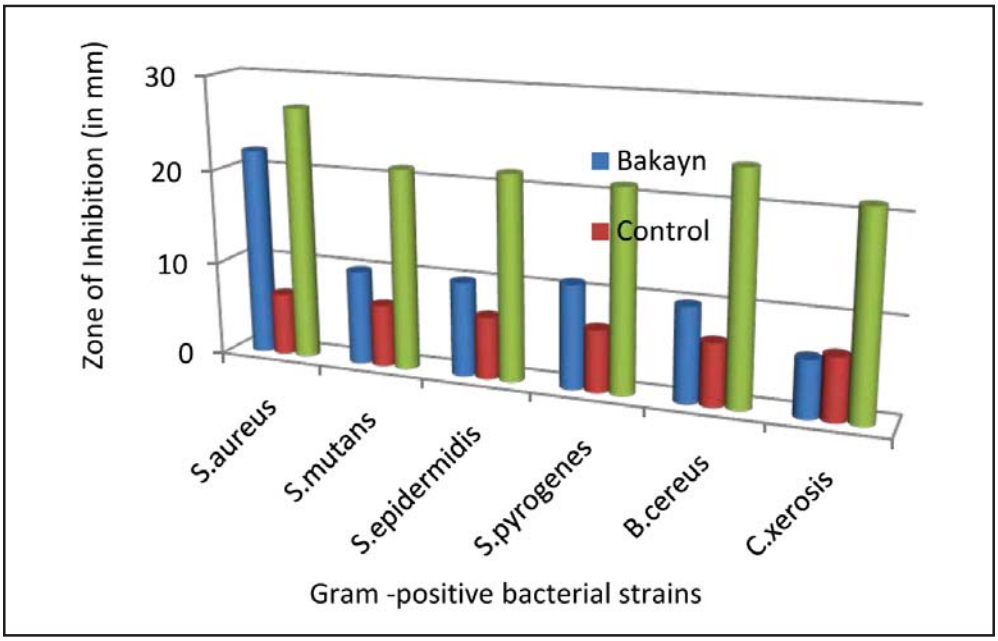

Fig. 2: Antibacterial activity of ethanolic extracts of the test drugs against Gram positive strains

Negative bacterial strains. A total volume of 40 µl of test drugs from concentrations viz. 40.0 µg/ml was used and compared with the standard drug Ciprofloxacin (30µg) for Gram positive and Gentamicin (30 µg) for Gram Negative bacteria and Plane control i.e. DMSO- (Dimethyl Sulphoxide) the solvent used (to exclude out any activity due to the solvent used to dissolve the plant extract).

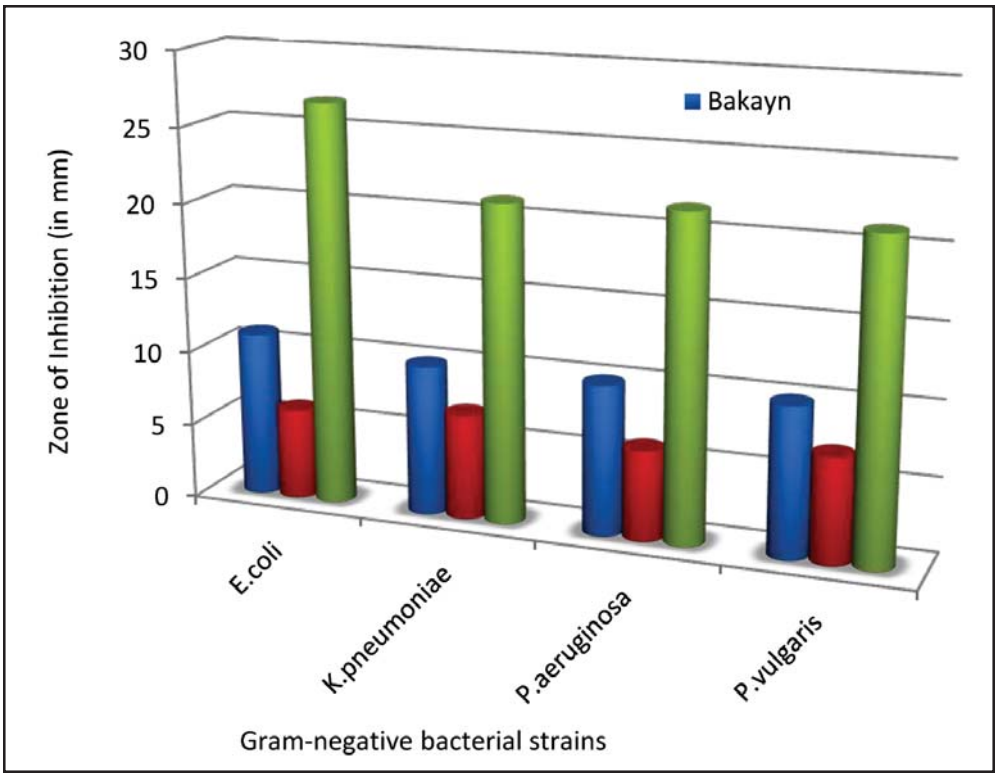

Fig. 3: Antibacterial activity of ethanolic extracts of the test drugs against gram negative strains

There was an increased inhibitory activity against most of the strains. Among Gram positive strains *S.aureus* (22.8±1.02) > *S.pyrogenes* (11.8±1.02) > *S.mutans* (9.4±0.67)> *B.cereus* (9.2±0.96) = *S.epidermidis* (9.2±0.96) while it was completely resistant to *C.xerosis* ATCC 373 at all concentration. For Gram negative strain used it showed moderate sensitivity to all strains and there was a significant inhibitory activity in the order of *E.coli* (11.2±0.37) > *P.aeruginosa* (9.6±0.24) = *P.vulgaris* (9.4±0.67) > *K.pneuomoniae* (8.2±0.48) > All showed a significant inhibition as compared to Gentamicin (ZOI- 14.0-14.8 mm) (table 2a & b) and (fig. 2 & 3).

Conclusion

The present study provides the first report to ameliorate the efficacy of 'Bakayin' in infectious diseases on scientific parameters. A large number of active phytoconstituents present in the drug have been found obviously to check the growth of bacteria and treat the disease. The study concludes Bakayin's fruit has a potent antibacterial activity against many pathogenic bacterial strains and thus could be used to derive antibacterial agents to fight against number of infectious diseases. Among the gram positive strains Bakayin's fruit was found to be sensitive to all the tested bacterial strains viz. S.*aureus, S.mutans,*

S.epidermidis, S.pyogenes, *B.cereus, E.coli, K.pneuomoniae, P.aeruginosa* and *P.vulgaris* while it showed a complete resistance to *C.xerosis*. Most significant sensitivity was shown towards *S.aureus* and *E.coli.*

Acknowledgement

Authors are thankful to DRS-I (SAP-II) UGC, Department of Ilmul Advia, Ajmal Khan Tibbiya College Hospital, A.M.U., Aligarh, for providing all the financial assistance for this study.

References

Afaq, S.H., Tajuddin., Siddiqui, M.M.H., 1994. Standardization of Herbal Drugs. AMU Publication Division, Aligarh Muslim University Press, Aligarh.

Anuradha, V. and Goyal, M.M., 1995. Phytochemical study on the leaves of *Alstonia scholaris* and their effects on pathogenic organisms. *Ancient Science of Life* 15 (1): 30-34.

Barry, L.A., Craig, A.W., Nadler, H., Reller, B.L., Sanders, C.C. and Swensor, J.M., 1999. Methods for determining bacteriacidal activity of antimicrobial agents. Approved Guidelines. Clinical and Laboratory Standard Institute (CLSI), September, Vol. 19 (18): M26-A:1-19.

Bauer, K., Sherris and Turck, 1996. Performance standards for antimicrobial disk susceptibility tests. CLSI (formerly NCCLS). *Am. J. Cl. Path.*45: 493.

Bhattacharjee, S.K., 2004. Handbook of aromatic plants. Pointer Publishers, Jaipur, p.283.

Bhattacharjee, S.K. and Das, L.C., 2005. Medicinal Herbs and Flowers. Aavishkar Publications, Jaipur.

Chopra, R.N., Chopra, J.C., Handa, K.L. and Kapur,L.D., 1958. Indigenous drugs of India, pp. 363-364.

Cos, P., Vlietinck, A.J., Berghe, D.V. and Maes, L., 2006. Anti-infective potential of natural products: How to develop a stronger in vitro 'proof of concept". *Journal of Ethnopharmacology* 106: 290-302.

Cushnie, T.P. and Lamb, A.J., 2005. Antimicrobial activity of flavonoids. *Int. J. Antimicrob. Agents* 26 (5): 343-56.

Hirat, T. and Suga, T., 1983. The efficiency of aloe plants, chemical constituents and biological activities. *Cosmetics and Toiletries* 98: 105-108.

Kirtikar, K.R. and Basu, B.D., 1935. Indian Medicinal Plants, Vol.1; Edn 2. Lalitmohan Basu Allahabad, India, pp. 785- 788.

Kingsbury, D.T. and Wagner, G.E., 1990. Microbiology, 2nd edition. Harvard Publishers, U.S.A, pp. 29-42.

Nadkarni, A. K., 2002. Indian Materia Medica. Bombay Prakashan Pvt. Ltd. Vol-I. pp. 784-785.

Pal, P and Bose, S., 2011. Phytopharmacological and Phytochemical Review of *Melia azadirachta*. *International Journal of Research in Pharmaceutical and Biomedical Sciences* 2 (3): 1374-1388.

Panda, S., Jafri, M., Kar, A. and Meheta, B.K., 2008. Thyroid inhibitory, antiperoxidative and hypoglycemic effects of stigmasterol isolated from *Melia azadirachta*. *Fitoterapia* 80:123–126

Peach, K. and Tracey, M.V., 1955. Modern methods of Plant analysis. Springer-Verlag, pp. 626-627.

Rehman, S., Latif, A., Ahmad, S. and Khan, A.U., 2011. In-vitro Antibacterial screening of *Swertia chirayita* Linn. against MRSA (Methicillin Resistant *Staphylococcus aureus*). *International Journal of Current Research and Review* 03 (6): 98-104.

Rehman, S. and Latif, A., 2015. Antibacterial Screening of Karanjwa Seeds (*Caesalpinia bonducella* Roxb.): An effective Unani Medicine for Infectious diseases. *Hippocratic Journal of Unani Medicine* 10 (2): 101-109.

Said, H.M., 1996. Medicinal herbs. Hamdard Foundation Pakistan, Pakistan, p. 144.

Sindhia, V.R. and Bairwa, R., 2010. Plant Review *Melia azadirachta*. *International Journal of Pharmaceutical and Clinical Research* 2(2): 90-94.