Phytochemical Analysis and Antibacterial Screening of Gul-e-Tesu [Butea monosperma (Lam.) Taub]

***Sumbul Rehman and Abdul Latif**

Department of Ilmul Advia, A.K. Tibbiya College, Aligarh Muslim University, Aligarh-202002

Abstract

Gul-e-Tesu (flowers of *Butea monosperma*; Family Fabaceae) has been reported to be an effective drug in Unani classical literature for treating various diseases including infectious diseases. However, it appears that no work has been done on scientific validation of its use in infectious diseases. Therefore, in the present study in-vitro screening of Gul-e-Tesu has been done for antibacterial activity against bacterial strains using Kirby Bauer's Disk Diffusion method. The efficacy was compared with the standard drug used as Positive Control and the solvent used to dissolve the test drug- Dimethyl Sulphoxide (DMSO) as Negative Control. Physico-chemical analysis was also done to confirm the presence of various phytoactive constituents present in the test drug. It was revealed that Gul-e-Tesu contains alkaloids, glycosides, flavonoids, carbohydrates, tannins, starch, saponin, resins and terpenes. The study demonstrates the in-vitro antimicrobial effect of Unani drug Gul-e-Tesu.

Keywords: Phytochemical standardization, Infectious diseases, Gul-e-Tesu (*Butea monosperma*), Antibacterial screening.

Introduction

Infectious diseases are a leading cause of death accounting for a quarter to a third of estimated 54 million deaths worldwide in 1998 (Gannon, 2000). This demonstrates that the number of multiple drug resistance microbial strains or those with a reduced susceptibility to antibiotics are increasing and this could be attributed to indiscriminate use of broad spectrum antibiotics. It has been reported that 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 time-tested drugs available in indigenous systems of medicine. A review of literature indicates that 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). However, there appears limited efforts made to validate medical efficacy of Unani drugs based on modern scientific tools and parameters.

Gul-e-tesu (*Butea monosperma*) is a medium-sized deciduous tree, commonly known as 'Flame of forest', Palash, Mutthuga, Bijasneha, Khakara, Chichara, Bastard teak, Bengal kino (Kritikar and Basu, 1935). Bark, flowers, leaves, gum and even seeds are used to prepare herbal remedies. Medicinally the plant exhibits astringent, antidiarrhoeal, antidysenteric, insecticide, febrifuge,

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

aphrodisiac, purgative and anthelmintic properties (Sehrawat, 2006; Sindhia 2010; Pal and Bose, 2011; Panda *et al*., 2008 and Nadkarni, 2002). The flowers of *B. monosperma* are used in Unani medicine as anti-stress formulation, 'Jigrine' as rejuvinator (Soman *et al*., 2004). The drug Gul-e-Tesu has also been traditionally used in many infectious diseases as influenza, coryza, scabies, skin infectious diseases, wounds (Anonymous, 1988; Nadkarni, 2002; Bhattacharjee and Das, 2005; Chopra, 1958). Therefore, present study was aimed to scientifically validate the medical efficacy of this well-known Unani drug Gul-e-Tesu to combat infectious diseases and evaluate its anti-microbial potential. The study was undertaken between 2012 and 2013 at the department of Ilmul Advia, A.K. Tibbiya College, Aligarh Muslim University, Aligarh.

Material and Methods

Plant Material: The herb was procured from the local market of Baradari in Aligarh city and was properly identified by the available literature (Fig.1)

Table 1: Qualitative Analysis of the Phytochemicals present in Gul-e-tesu

S.No.	Chemical Constituents	Test Reagents	Gul-e-Tesu
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

Figure 1: Gul-e-Tesu (*Butea monosperma*)

(i) Preparation of Plant 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 drugs are destroyed when exposed to a higher temperature beyond 55^0C, so the heat wherever was needed was kept as low as possible to prevent the loss of thermo-labile substances present in the drugs from destruction. Strict aseptic precautions were followed throughout the process.

(ii) Aqueous extract:The coarse powdered drugs were extracted using soxhlet apparatus, by reflux method with double distilled water (DDW) as a solvent at 50^0C for 6 hours or until the extracting return in the siphon was colorless. The extract obtained was subjected to dryness in the Lypholizer (Macro Scientific Works, New Delhi) under reduced pressure.

(iii) Ethanolic extract:The coarse drug material was extracted with 95% ethanol as a solvent at 50^0C for 6 hours and dried under reduced pressure in the Lypholizer.

The stock solutions for aqueous and ethanolic extract was 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 were carried out to confirm the presence of chemical constituents in the drug sample (Bhattacharjee and Das, 2005; Afaq *et al.*, 1994).

Antibacterial Susceptibility Testing

Antimicrobial susceptibility testing was done by Kirby Bauer's disk diffusion method (1996).

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 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 (Kingsbury and Wagner, 1990).

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

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.	*Corynebacteriumxerosis*	"
7.	*Escherichia coli*	Gram Negative
8.	*Proteus vulgaris*	"
9.	*Pseudomonas aeruginosa*	"
10.	*Klebseilla pneuomoniae*	"

Table 2 (a): Antibacterial activity Gul-e-Tesu 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*	16.0±0.54(1.22)*** (S)	6.6±0.24(0.54) (R)	21.2±0.37(0.83) (S)
2.	*S.epidermidis*	18.0±0.89(2.00)* (S)	6.4±0.24(0.54) (R)	21.6±0.24(0.54) (S)
3.	*S.pyrogenes*	7.8±0.37(0.83)*** (S)	6.4±0.24(0.54) (R)	21.2±0.37(0.83) (S)
4.	*B.cereus*	18.8±0.58(1.30)* (S)	6.6±0.24(0.54) (R)	21.4±0.24(0.54) (S)
5.	*S.aureus*	16.2±1.20(2.68)*** (S)	6.6±0.24(0.54) (R)	26.8±0.20(0.44) (S)
6.	*C.xerosis*	6.6±0.24(0.54) (R)	6.6±0.24(0.54) (R)	21.2±0.37(0.83) (S)

Table 2 (b): Antibacterial activity of Gul-e-Tesu 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*	9.2±0.96(2.16)* (S)	6.4±0.24(0.54) (R)	14.8±0.20(0.44) (S)
2.	*P.vulgaris*	9.4±0.67(1.51)* (S)	6.4±0.24(0.54) (R)	14.0±0.54(1.22) (S)
3.	*P.aeruginosa*	13.8±1.31(2.95)* (S)	6.4±0.24(0.54) (R)	14.8±0.20(0.44) (S)
4.	*K.pneuomoniae*	16.6±0.24(0.54)*** (S)	6.4±0.24(0.54) (R)	14.8±0.20(0.44) (S)

(S) – Sensitive (R) – Resistant

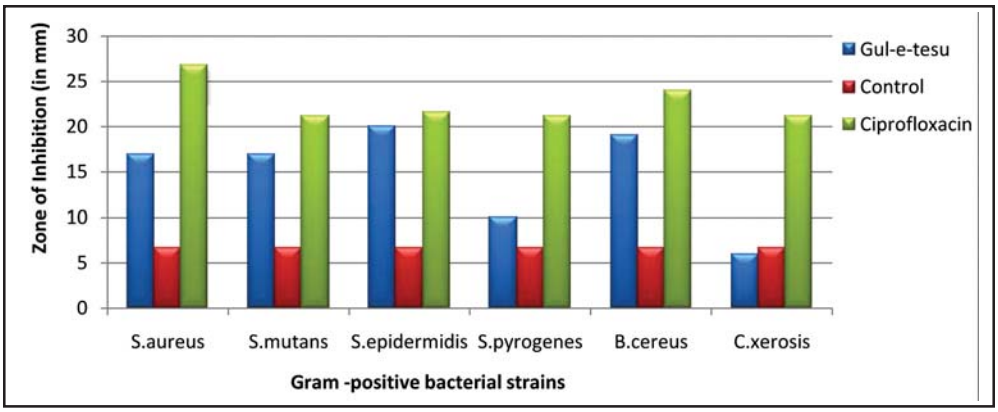

Figure 2(a): Antibacterial activity of Gul-e-Tesu against Gram positive bacterial strains

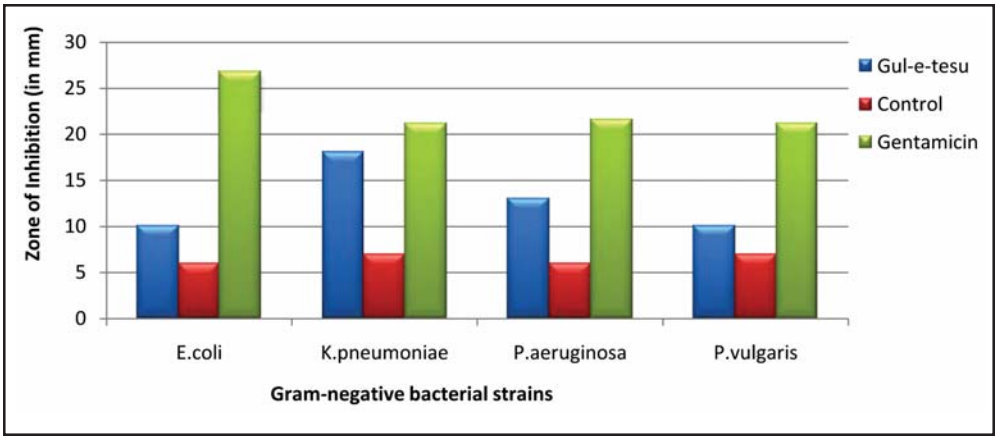

Figure 2(b): Antibacterial activity of Gul-e-Tesu against Gram negative bacterial strains

and Gentamicin (30 µg) for Gram Negative bacteria and Plane control i.e. DMSO (Dimethyl Sulphoxide).

Statistical Analysis: 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

The phytochemical analysis of the chemical constituents present in the drugs revealed that it contains alkaloids, phenol, resins, saponins, sugars and tannins. These chemical compounds may be responsible for the therapeutic efficiency 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.

There was an increased inhibitory activity against most of the strains. Among Gram positive strains *B.cereus* (18.8±0.58)>*S.epidermidis* (18.0±0.89)>*S.aureus* (16.2±1.20) >*S.mutans* (16.0±0.54)>*S.pyrogenes* (7.8±0.37) while it was completely resistant to *C.xerosis* ATCC 373 at all concentration. For Gram negative strain used it showed sensitivity to all strains and there was equal inhibitory activity in the order of *K.pneuomoniae* (16.6±0.24) >*P.aeruginosa* (13.8±1.31)>*P.vulgaris* (9.4±0.67)>*E.coli* (9.2±0.96). All showed a significant inhibition as compared to Gentamicin (ZOI- 14.0-14.8 mm).

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 along with other biological activities have also been reported to possess significant anti-bacterial activity. Presence of flavonoid in the test drug and the subsequent anti-microbial activity confirms the findings reported by other authors in respect of flavonoids (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 all ethno-pharmacological knowledge was found to be in favor of our selection of the drug as per the guidelines (Cos *et al*., 2006). The study demonstrated that the test drug possesses significant anti-microbial activity against a number of gram +ve and gram –ve bacteria. Thus, the study revealed the use of Gule-e-Tesu by Unani physicians in the management of various infective diseases.

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