Antibacterial Screening of Karanjwa Seeds (*Caesalpinia bonducella* Roxb.): An Effective Unani Medicine for Infectious Diseases

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Abstract

Karanjwa (*Caesalpinia bonducella* Roxb.) has been reported in various Unani Classical books to be used in infectious diseases, but the study to confirm its efficacy scientifically is not done so far. Therefore, the present investigation was carried out to evaluate its antibacterial efficacy against various pathogenic bacterial strains by using Kirby Bauer's Disk Diffusion and Agar well method according to Clinical Laboratory Standard Institute (CLSI) Guidelines by W.H.O. Ethanolic and Aqueous extracts of the drug were used (40 µl/well) and the efficacy of the drug was compared with the standard drug i.e. Ciprofloxacin disk for Gram positive bacteria and Gentamicin for Gram negative strains and Control (Solvent used for dissolving the extracts i.e. Dimethyl Sulphoxide). Antibacterial effect was evaluated by measuring Zone of Inhibition (ZOI) (in mm). All the experiments were conducted in triplicates and in sterilized conditions. The results were analyzed statistically by using ANOVA. Phytochemical analysis of the drug confirmed the presence of alkaloids, flavonoids, glycosides, saponins, tannins and triterpenoids. Results indicate that *Caesalpinia bonducella* Roxb. has a significant activity against *Bacillus cereus*, *Coryne bacterium xerosis* and *Pseudomonas aeruginosa*, while moderate activity against *Proteus vulgaris* and *Staphylococcus aureus*. The study provides an *in-vitro* evidence of Karanjwa for having a very effective role against these pathogenic bacterial strains and can be considered as effective 'Drug Target' for further screening its effect in New Drug Development (NDD) in Research and Development (R & D) Unit in an effort to combat many infectious diseases.

Keywords: *Caesalpinia bonducella Roxb.*, CLSI Guidelines, Antibacterial effect, New Drug Development (NDL).

Introduction

Natural products have played a pivotal role in antibiotic drug discovery with most antibacterial drugs being derived from natural products (Buttler & Buss, 2006). The worldwide use of natural products including medicinal plants has become more and more important in primary health care especially in developing countries. With increased incidence of resistance to antibiotics, natural products from plants could be interesting alternatives. Some plant extracts and phytochemicals are known to have antimicrobial properties, and can be of great significance in therapeutic treatments. Scientific experiments on the antimicrobial properties of plant components were first documented in the late 19th century (Nair and Chanda, 2006).

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Caesalpinia bonducella Roxb. (Family : Caesalpinaceae) commonly known as 'Karanjwa' is used in Unani medicine since ancient times to treat various diseases. "Bonducella" the name of the species is derived from the Arabic word "Bonduce" meaning a "little ball" which indicates the globular shape of the seed *C. bonducella*. It is a climbing prickly shrub (Anonymous, 1987; Farooq, 2005) that grows in forests and near villages throughout the hotter parts of India. It has been used as an antipyretic, antimalarial, antimicrobial in traditional systems of medicine particularly in Unani Medicine (Ibne Sina, 1931; Hakim, 1343 H). Seeds of karanjwa are claimed to be styptic, purgative and anthelmintic and cures inflammations; useful in colic, malaria, hydrocele, skin diseases and leprosy (Anonymous, 1987; Chopra, 1958; Kiritikar and Basu, 1975). The powdered seeds mixed with equal part of pepper powder given to malaria patients were found to posses feeble antiperiodic properties (Farooq, 2005; Patil and Patil, 2007).

Seed of 'Karanjwa' and long pepper powders taken with honey gives good expectorant effect, The kernel of the seed is very useful and valuable in all cases of simple, continued and intermittent fevers (Ghani, 1921). Decoction of roasted kernels has been used in asthma (Chopra, 1958). Children unable to digest mother's milk are given the extract of the kernel or its powder along with ginger, salt and honey to get good stomachic effect (Farooq, 2005). Paste prepared from kernel gives relief from boils and other such swellings (Anonymous, 1950; Farooq, 2005). Chemically the seeds are found to contain a bitter substance bonducin, bonducellin phytosterinin, saponins (Rastogi and Mehrotra, 1993).

Present Work

The literature review undertaken on the test drug 'Karanjwa' reveals that the drug has been traditionally used by Unani physicians to combat many infectious diseases. Hence, the present study was designed to scientifically evaluate its antibacterial activity.

Material and Methods

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

Preparation of plant extracts

The test drug was dried at room temperature in a ventilated room, milled to a fine powder and stored in a closed 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 to maintain the low grade

temperature, 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.

Aqueous extract

The coarse powdered drug was 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 Lyophilizer (Macro Scientific works, New Delhi) under reduced pressure.

Ethanolic extract: The coarse powdered drug was extracted with 95% ethanol as a solvent at 500C for 6 hours as above and dried under reduced pressure in the Lyophilizer. The stock solutions for aqueous and ethanolic extract was prepared from the dried extract so obtained in the 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 phytoconstituents 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. The qualitative phytochemical analysis of the drugs was done according to the scheme proposed by Bhattacharjee & Das (1969) and Afaq *et al*. (1994) and are presented in table-1.

Test microorganisms

Bacterial strains were selected on the basis of their clinical importance in causing diseases in humans. These were obtained from different sources, clinical isolates of *Staphylococcus aureus* , *Streptococcus mutans*, *Acetobacter bovis*, *Staphylococcus epidermidis*, *Bacillus cereus* and *Corynebacterium xerosis* were collected locally from Jawaharlal Nehru Medical College & Hospital; Interdisciplinary Biotechnology Unit; Microbiology Unit, Gandhi Eye Institute, Aligarh Muslim University, Aligarh, while standard strains were obtained from Hi-media Labs Pvt. Ltd., Mumbai, India and Microbial Type Culture Collection, Chandigarh, Punjab, India. The strains so selected for the study are *Staphylococcus aureus* (ATCC 29213), *Streptococcus mutans* (ATCC 25175),

Streptococcus pyrogenes (MTCC 435), *Staphylococcus epidermidis* (MTCC 435), *Bacillus cereus* (MTCC 430) and *Corynebacterium xerosis* (ATCC 373). All strains were incubated at 37^0C for 24 hours followed by frequent sub culturing to fresh media and were used as 'test bacteria', cultures were checked to confirm the presence of sufficient number of bacterial cells on nutrient broth and maintained on nutrient agar slant.

Medium

The solid media namely Nutrient Agar No.2 (NA) (M 1269S-500G, Hi-media Labs Pvt. Ltd, Bombay, India) was used for preparing nutrient plates, while Nutrient Broth (NB) (M002500G, Hi-media Labs Pvt. Ltd, Bombay, India) was used for the liquid culture media.

Antimicrobial Susceptibility Testing

Antibacterial tests were performed as CLSI Guidelines (Anonymous, 2003; Barry, 1999). Aqueous and ethanolic extract (40µl) in the concentration of 5 mg/ml dissolved in DMSO was used for its antimicrobial activity using Agar well diffusion (Ananthanarayan and Paniker, 2009) on solid media. Brain Heart Infusion (BHI) Agar (SM 211 Himedia Labs, Mumbai, India) was used for *S.mutans* while Mueller Hinton Agar No.2 (M1084 Hi media Labs, India) & Nutrient Agar (Himedia Labs, Mumbai, India) for preparing plates for rest of the bacterial strains. The solid Agar was punched with 6mm diameter wells. The inoculums ($1.5´10^8$ cfu/ml) were spread on to their respective agar plates using sterile swabs (PW041 Himedia

Fig. 1: Karanjwa seeds (*Caeasalpinia bonducella)*

Labs, Mumbai, India) and then filled with 40 ml extract. All the plates were incubated at 37^0C for 24 hours. Ciprofloxacin disk (SD-142, Himedia labs, Mumbai, India) was used as standard drug for Gram positive while Gentamicin (SD170 Hi media Labs, Mumbai, India) for Gram negative bacteria. Wells containing respective solvent (DMSO) served as control.

Growth Inhibition was recorded by measuring the diameter of the Inhibitory Zones after the period of incubation. Triplicates were maintained and the experiment was repeated thrice and the Mean values along with Standard error (Mean +S.E) are presented in Table-2 & 3 and comparison can be readily evaluated by Fig. 2 & 3.

Results and Discussion

The world is heavily populated with bacteria, viruses and fungi. Infections are the major cause of human diseases. Bacterial world itself is heavily populated

Table 1: Qualitative Analysis of the Phytochemicals in 'Karanjwa'

S.No.	Chemical Constituents	Test Reagents	Inference
1.	Alkaloids	Dragendorff 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

Table 2: Antibacterial screening against Gram positive bacterial strains of Karanjwa (*Caesalpinia bonducella*)

S. No.	Strains	*Caesalpinia bonducella*		Standard
		Aqueous extract	Ethanolic extract	Cipro-floxacin (30µgm)
1.	*Staphylococcus aureus*	12±0.31*	12.4±0.40*	22*
2.	*Streptococcus mutans*	28.6±0.24*	11.33± 0.33*	21*
3.	*Streptococcus epidermidis*	_	_	23*
4.	*Staphylococcus pyrogenes*	_	_	22*
5.	*Corynebacterium xerosis*	26.6±0.50*	13.33±0.33*	21*
6.	*Bacillus cereus*	12.4±0.40*	12±0.31*	23*

*p-value>0.001

Table 3: Antibacterial screening against Gram negative bacterial strains of Karanjwa (*Caesalpinia bonducella*)

S. No.	Strains	*Caesalpinia bonducella*		Standard
		Aqueous extract	Ethanolic extract	Gentamicin (30µgm)
1.	*Escherichia coli*	11.33 ± 0.33*	17.2±0.37*	15*
2.	*Pseudomonas aeruginosa*	13.33 ± 0.33*	16±0.31*	14*
3.	*Proteus vulgaris*	_	_	14*
4.	*Klebsiella pneuomoniae*	11.33 ± 0.33*	19.2± 0.37*	15*

*p-value > 0.001

with too many species and produce fulminating infections like tetanus, gangrene, syphilis, gonorrhea, diphtheria, leprosy, tuberculosis, urinary tract infections, respiratory tract infections etc. (Mishal and Somani, 2000) in human beings and animals. The major problem that exists with the infectious diseases and the antibiotics used for them is the emergence of antibiotic resistance. This is a worldwide problem and now the time has come when one should think about its solution from alternative therapy. Evidence based medicine is the goal for western doctors nowadays and authorities request that for any drugs used in these medicines, they should solidify their evidence on the basis of scientific background to be presented, to make their use acceptable. So, natural drugs

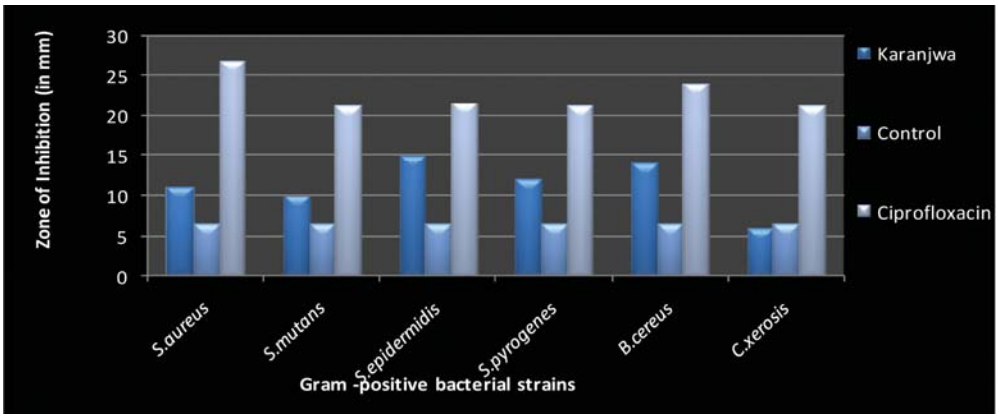

Figure 2: Antibacterial activity of Karanjwa extracts against Gram positive strains

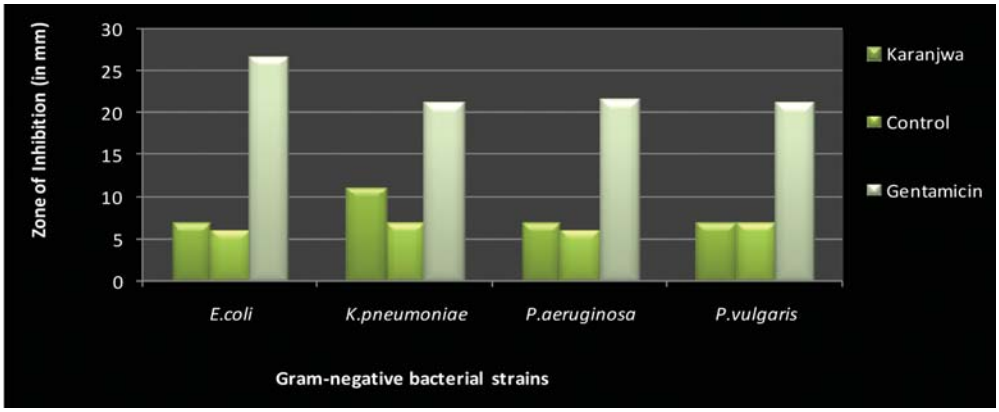

Figure 3: Antibacterial activity of Karanjwa extracts against Gram negative strains

fulfill this promise to a much extent as evident by the researches done so far. Certain antibiotics are there which have been derived from plant sources (Bhattacharjee and De, 2005). Keeping in mind, the side effects of antibiotics and emerging trends of development of resistance in microbes, present study assumes much significance in view of the fact that Karanjwa (*Caesalpinia bonducella* Roxb.) has been used in Unani medicine since ancient times for the treatment of infectious diseases, having great potential to kill or inhibit the growth of micro organisms.

Phytochemical analysis of *C. bonducella* has revealed the presence of alkaloids, flavonoids, glycosides, saponins, tannins and triterpenoids (Table-1). The results of antimicrobial activity of extracts showed a wide range of antibacterial activity against Gram positive and Gram negative bacteria. Results are expressed as ZOI (in mm) ± SEM (Standard error of mean). It was seen that aqueous extract of Karanjwa produces a significant results against *S.mutans, C.xerosis*, while the moderate activity was observe towards *S.aureus* and *B.cereus.* The ethanolic extract produces a significant ZOI against *E.coli*, *K.pneuomoniae*, *P.aeruginosa*,

moderate activity towards *S.aureus* and *B.cereus* and no activity was shown by either extract towards *S.pyrogenes, S.epidermidis* and *P.vulgaris*. Chemically the seeds are found to contain a bitter substance bonducin, bonducelli, phytosterinin, saponins (Rastogi and Mehrotra, 1993). These bitter component may be responsible for the antibacteria activity, however, further research work is needed to ensure its use clinically.

Conclusion

The study concludes that Karanjwa (*Caesalpinia bonducella* Roxb.) has a potent antibacterial activity against clinical and standards strains and thus could be used to derive antimicrobial agents to fight against the number of infectious diseases mainly against *S.mutans*, *B.cereus*, *C.xerosis, S.aureus, E.coli*, *K.pneuomoniae and P.aeruginosa.* This study not only provides the scientific evidence of antibacterial effect of 'Karanjwa' but also gives us a direction to search on, in detail, considering it as a Drug Target Molecule in New Drug Development series in an effort to discover new herbal antibiotics. The alarming and increasing scope of antibiotic resistance now further demands to search on for the newer drugs and establishing their antibacterial effect.

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