Antimicrobial Assay of Alcoholic and Hydroalcoholic Extract of a Unani Formulation by Agar Well Method

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Abstract

The worldwide use of natural products including medicinal plants has become more important in primary health care for various pharmacological effects including antimicrobial activity. Further, it is being appreciated that with increased incidence of resistance to antibiotics, natural products especially from medicinal plants could be interesting alternatives. In this regard a study was conducted to investigate antibacterial activity of a Unani formulation containing (i) Sonth (Zanjbeel) (*Zingiber officinale*) (ii) Suranjan (*Colchicum luteum)* and (iii) Elwa (*Aloe vera*). The alcoholic and hydro-alcoholic extracts dissolved in DMSO (Dimethyl Sulphoxaside) were used to determine antibacterial activity by Agar Well Method. Zone of Inhibition (in mm) was taken as the parameter of measurement against a number of bacterial strains viz. *Staphylococcus aureus, Streptococcus mutans, Bacillus cereus, Corynebacterium xerosis, Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa, Klebseilla pneuomoniae*. The efficacy of Unani formulation against *Bacillus cereus* and *Pseudomonas aeruginosa*, was found even better than Ciprofloxacin and Amikacin, respectively. The study demonstrated that Unani formulation possesses significant antibacterial activity and can be used in infectious diseases caused by a number of Gram +ve and Gram -ve microorganisms.

Keywords: Unani Medicine, *Zingiber officinale*, *Colchicum luteum*, *Aloe vera*, Antimicrobial, Agar Well Method.

Introduction

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). Many works have been done 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). Unani medicine also offers a number of single and compound preparations that are used successfully in the management of various infectious diseases. Although a number of single drugs have been investigated scientifically but the compound preparations have largely not been studied for antimicrobial and other pharmacological activities.

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In this regard a study has been conducted to find antibacterial activity of a pharmacopoeal Unani preparation containing (1) Ginger (*Zingiber officinale* Linn.— Dried Rhizome- 3.5 g) (2) Colchicum (*Colchicum luteum* Baker— Dried Corm- 3.5 g) and (3) Aloe (*Aloe vera* Linn.—Dried Exudate- 7.0 g) (Khan, 1870). This combination has been described to be useful in Wajaul Mafasil (Arthritis) and other joints ailments, and the physicians of Unani medicine are prescribing it for the management of joint diseases since long time. Further, an experimental study has shown significant analgesic, anti-inflammatory and anti-arthritic effect possessed by this compound formulation (Rahman *et al.,* 2010, 2011). But certain other studies conducted on the ingredients of this formulation have demonstrated that they possess significant antibacterial activity against a number of Gram +ve and Gram -ve bacteria suggesting that this combination may also be used as an antibacterial agent.

Z. officinale (Zanjabeel) has been reported to inhibit the growth of both Gram-positive and Gram-negative bacteria significantly (Mascolo *et al.,* 1989, Samy, 2005) along with possessing anti-inflammatory, antiemetic, antioxidant, antiulcer, anticarcinogenic properties (Ali *et al.,* 2008; Evans, 2009; Rhode *et al.,* 2007; Minaiyan *et al.,* 2006). *C. luteum* is mainly used as anti-inflammatory and analgesic agent in arthritic conditions (Ghani, 2005; Konda and Rao, 2010) but its crude methanolic extract and subsequent fractions have been shown to possess antimicrobial activity against *Lipoxygenase* and *Bacillus subtilis* (Ahmad *et al.,* 2006). *A. vera* (Sibr or Elwa) is one of the earliest known purgatives used in Unani system of medicine but recently it has been shown to promote wound healing due to the presence of antibacterial, antifungal and antiviral properties (Agarry *et al.,* 2005). Mpala et al. (2010) have also reported that *A.vera* has significant antimicrobial activity. There are several Unani pharmacopoeial preparations having anti-microbial property include these herbs such as Ushban, Sadri, Sharbat-e-Adrak, Qurs-e- Sual, (Anonymous, 2011) Jauhar-e-Kibreet Qawi, (Anonymous, 2007), Kushta Marjan Sada (Anonymous, 2008) etc.

In view of the above, the formulation was hypothesized to be effective in infectious diseases and the present study was designed to evaluate its efficacy against a number of bacterial strains viz. *Staphylococcus aureus, Streptococcus mutans, Bacillus cereus, Corynebacterium xerosis, Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa* and *Klebseilla pneuomoniae.*

Methodology

Collection of plant material

The raw materials were purchased from the local market of Aligarh. The sample was authenticated in Pharmacognosy section of Department of Ilmul Advia, Faculty of Unani Medicine, AMU, Aligarh by Professor S.H. Afaq and a voucher specimen was deposited in the Dept. of Ilmul Advia.

Preparation of extracts

All the ingredients of test formulation were powdered coarsely in an electric grinder. The powder of each drug was extracted separately in absolute alcohol (alcoholic) and in 50% alcohol (hydro-alcoholic) with the help of Soxhlet's apparatus for 6 hours. The extracts were filtered and dried by evaporation under reduced pressure in a lyophilizer (Macro scientific works, Delhi) and the lyophilized extracts were dissolved in DMSO (Dimethyl sulfoxide) to the desired concentration (20 mg/ml) before the experimentation.

Microorganisms used in the study

The clinical bacterial strains used in the study were *Staphylococcus aureus, Streptococcus mutans, Bacillus cereus* and *Corynebacterium xerosis* from Gram positive and *Escherichia coli, Klebsiella pneuomoniae, Pseudomonas aeruginosa* and *Proteus vulgaris* from Gram negative bacteria. These strains were procured from the Department of Microbiology, Jawaharlal Nehru Medical College & Hospital and Interdisciplinary Biotechnology Unit, Aligarh Muslim University, Aligarh.

S. mutans were grown in Brain Heart Infusion (BHI) broth (LQ210 Himedia Labs, Mumbai, India) and the remaining strains were grown in Nutrient broth (M002 Himedia Labs, Mumbai, India) and incubated at 37^0C for 24 hours followed by frequent sub-culturing to fresh media. Bacterial cultures were checked to confirm the presence of sufficient number of bacterial cells on nutrient broth and maintained on nutrient agar slant.

Antimicrobial susceptibility testing

Antibacterial tests were performed as per CLSI (Clinical and Laboratory Standard Institute) guidelines. The extracts were tested for their antimicrobial activity using agar well diffusion 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 Himedia Labs, India) and Nutrient agar for preparing plates for rest of the bacterial strains. The solid agar was punched with 6 mm diameter wells. The inoculums were spread on to agar plates using sterile swabs and then filled with 40 ml of the prepared extract. The concentration of the extract employed was 0.02 g/ml /well. All the plates were incubated at 37 ^{0}C for 24 hours. Ciprofloxacin disks (SD142 Himedia Labs, Mumbai, India) were used as standard drug for Gram positive, while Amikacin disks (SD035 Himedia Labs, Mumbai, India) were used for Gram negative bacteria. Wells containing respective solvent served as control. Growth inhibition was recorded by measuring the diameter of the inhibitory zones after the period of incubation of 24 hours.

Statistical analysis

The results have been expressed as Mean ± SE. The findings were analyzed to determine significance of difference by one-way ANOVA test followed by pair-wise comparison of various groups by Tukey-Karmar test with 95% confidence limit. The analysis was carried out by using the software analyseit.com.

Results

Antibacterial activity against Gram positive bacterial strains

Both the extracts viz. alcoholic and hydroalcoholic of the formulation exhibited varying degree of inhibitory effect against all tested pathogenic strains which have been shown in Table–1. The antibacterial activity exhibited by these extracts was found to be significant and greater than DMSO ($p<0.01$) against all the tested bacterial strains.

Against *S. mutans*, the alc. extract showed significantly greater effect than hydroalcoholic extract ($p<0.01$), while against *B. cereus* both the extract showed an effect that was significantly better than that induced by the standard drug Ciprofloxacin ($p<0.01$).

Antibacterial activity against Gram negative bacterial strains

Both the extracts of the formulation demonstrated inhibitory effect against all tested Gram negative pathogenic strains (Table-2). The alcoholic and hydroalcoholic extracts exhibited significantly greater effect than that produced by DMSO ($p<0.01$) against all pathogenic organism especially against *P. aeruginosa* and *P. vulgaris.* Hydroalcoholic extract demonstrated better effect than the alc. extract ($p<0.01$) against *E. coli.*

Table 1: Antibacterial activity against Gram positive bacterial strains

S. No.	Microbial strains	Zone of inhibition (ZOI) in mm (Mean ± S.E.M)			
		Hydro-alcoholic extract (50% alc.)	Alcoholic extract	DMSO (40 µl)	Ciprofloxacin (30 µgm/ disk)
1.	Staphylococcus aureus	7.4±0.21	10.4±0.09 (ac*)	7.0±0.1	26.8±0.22 (abc*)
2.	Streptococcus mutans	10.4±0.09 (c*)	12.2±0.22 (ac*)	7.4±0.12	21.2±0.14 (abc*)
3.	Bacillus cereus	27.2±0.22 (cd*)	27.2±0.14 (cd*)	7.4±0.22	21.6±0.20 (c*)
4.	Corynebacterium xerosis	13.8±0.17 (c*)	13.8±0.15 (c*)	7.2±0.10	21.2±0.21 (abc*)

*p<0.01
a = against 50% alc. extract
b = against alc. extract
c = against DMSO (Dimethyl Sulphoxaside)
d = against Ciprofloxacin (Cf)

Table 2: Antibacterial activity against Gram negative bacterial strains

S. No.	Microbial strains	Zone of inhibition (ZOI) in mm (Mean ± S.E.M)			
		Hydro-alcoholic extract (50% alc.)	Alcoholic extract	DMSO (40 µl)	Amikacin (30 µgm/ disk)
1.	Escherichia coli	12.4±0.14 (bc*)	10.0±0.19 (c*)	7.0±0..16	28.0±0.19 (abc*)
2.	Pseudomonas aeruginosa	26.8±0.15 (c*)	27.2±0.21 (c*)	7.4±0.14	33.2±0.14 (abc*)
3.	Proteus vulgaris	13.4±0.14 (c*)	14.6±0.13 (c*)	7.4±0.14	29.4±0.13 (abc*)
4.	Klebsiella pneuomoniae	12.6±0.17 (c*)	11.8±0.13 (c*)	7.2±0.13	18.6±0.17 (abc*)

*p<0.01
a = against 50 % alc. extract
b = against alc. extract
c = against DMSO
d = against Amikacin (Ak)

Discussion

In the present study antimicrobial activity of the alcoholic and hydro-alcoholic extracts of Unani formulation was quantitatively assessed using agar well method on the basis of Zone of Inhibition (ZOI) which was expressed in mm.

The study demonstrated that the alcoholic extract possessed more pronounced antimicrobial activity as compared to hydro-alcoholic extract. The results were found comparable with that of the standard drugs and the effect induced by the extracts against *B. cereus* (Gram positive) was even better than Ciprofloxacin and that against *Pseudomonas aeruginosa* it was found better than Amikacin. They also possessed moderate activity against certain Gram positive bacteria such as *C. xerosis and S. mutans* and few Gram negative bacteria such as *E. coli, P. vulgaris* and *K. pneuomoniae.* The results of the present investigation suggested that the formulation containing *C. luteum, Z. officinale* and *A. vera* has a salient antimicrobial effect against *B. cereus* which is resistant to a number of allopathic drugs. 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.

In previous studies the constituents of this formulation were studied alone or in combination with other natural products for their antimicrobial activity. The crude extract and subsequent fractions of *C. luteum* showed mild to moderate activity in an antibacterial bioassay with maximum antibacterial activity of 58% against *Bacillus subtilis* (Bashir *et al.,* 2006). The acetone extracts of *Aloe vera* leaves showed antibacterial activities against the selected human clinical pathogens *Staphylococcus aureus, Streptococcus pyogens, Pseudomonas aeruginosa and Escherichia coli* (Arunkumar and Muthuselvam, 2009). A number of studies have confirmed that the alcoholic extract of the *Zingiber officinale* and its flavonoids have antibacterial activities. Study conducted by Demin and Yingying (2010) revealed that the ethanolic extracts of ginger and crude flavonoids exhibited antimicrobial activity against *Escherichia coli, Staphylococcus aureus, Shigella fiexneri, Proteus vulgaris* and *Pseudomonas aeruginos*[1].

Our findings thus conformed that the ingredients of the compound preparation which are reported to possess antibacterial activity also retain the effect in combination form and even demonstrated better response than the allopathic antibacterial agents in respect of certain strains. This is probably the earliest report on this pharmacopoeal drug demonstrating antibacterial activity. This combination now can also be used as an antimicrobial agent against diverse types of microorganism. Further, the present study has revealed the importance of natural products to control antibiotic resistant bacteria, which are a major threat to human health.

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