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RESEARCH ARTICLE

ANTIMICROBIAL INVESTIGATION OF *PIPER NIGRUM L.* AGAINST *SALMONELLA TYPHI*

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ABSTRACT

Aim of this study was to evaluate the antibacterial activity of different extracts of *Piper nigrum* L. against *Salmonella typhi*. Widal (serological) test positive patients were selected. *Salmonella* strains were isolated from blood of infected persons. 10 samples were analyzed for present study. Strains were cultured on blood agar medium and Bismuth Sulfite Agar medium. Pure isolated strains were characterized on the basis of Bergey's Manual of determinative bacteriology. Three extracts such as aqueous, ethanolic and chloroform extracts of *Piper nigrum* seeds were prepared. 4 different concentrations such as 15, 20, 25, 30 mg/ml of them were made. Antimicrobial activity was determined by Agar well diffusion method. Results showed that 30 mg/ml of aqueous, ethanol and chloroform extract of *Piper nigrum* gave 23.4±0.2, 21.8±0.4, 20.4±0.1 mm of inhibition zones, respectively. Aqueous extract was observed to be best among all extract of *Piper nigrum*.
Keywords: *Piper nigrum, Salmonella typhi*

INTRODUCTION

Medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi-synthesis[1]. Medicinal plants are a source of great medicinal value all over the world[2]. The increasing prevalence of multidrug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raises the spectra of untreatable bacterial infections and adds urgency to the search for new infection-fighting and safe strategies[3, 4].

Typhoid fever is caused by *Salmonella typhi* and is transmitted through the fecal-oral route by the consumption of contaminated water and food. Typhoid fever is an important health problem in many developing countries. The estimated global annual incidence of typhoid in 2000 was 21 million patients, with 217,000 deaths[5].

Black pepper (*Piper nigrum* L.) is members of botanical family Piperaceae. Black pepper is a perennial climbing vine grown for its berries extensively used as spice and in medicine. Black pepper is native to Malabar and Travancore coast of India. Other than India, it is mainly cultivated in Vietnam, Brazil, Indonesia, Malaysia, Sri Lanka, China, and Thailand. It is cultivated successfully between 200 North and 200 South of equator and 1500 MSL from sea level[6]. The fruits contain 1.0-2.5% volatile oil, 5-9% alkaloids, of which the major ones are piperine, chavicine, piperidine, and piperetine, and a resin[7]. Pharmacological and clinical studies have revealed that piperine has CNS depressant, antipyretic, analgesic, anti-inflammatory[8], hepatoprotective activity[9] and antioxidant[10]. Black pepper inhibits the growth of certain pathogens[11,12]. Previous finding reveled that Black pipper has good medicinal property and no side effect to human. So aim of this study was to evaluate the antimicrobial activity of *Piper nigrum* L. against *Salmonella.*

MATERIALS AND METHODS

Isolation of microorganism: Widal positive patients were selected for present study. *Salmonella* is isolated from the blood of infected persons. Pathogen was isolated on Blood agar, Bismuth Sulfite Agar medium. The plates were incubated at 37°C for 24-48 h.

Characterization of pathogen: Pure isolated strains were characterized according to Bergey's Manual of determinative bacteriology[13].

Plant materials: *Piper nigrum* L. seeds were used in present study. Seeds were purchased from certified herbal shop at Dehradun.

Preparation of the extracts: Three extract of medicinal plants was prepared according to Deshwal[12].

1. Aqueous extracts: 100g dried finely powdered seeds of *Piper nigrum* L. were infused in distilled water until completely exhausted. The extract was then filtered using Whatman No. 1 filter paper and the filtrate was evaporated and dried using rotary evaporator at 60°C. The final dried samples were stored at low temperature.

2. Ethanol extracts: Dried seeds were grounded and extracted in a percolator with 95% ethanol. About 10ml of ethanol per gram of sample was used. The ethanol extract was dried under a reduced pressure at 40°C. The dried extract was stored in sterile bottles for further use.

3. Chloroform extracts: Powdered sample (100g) of seeds were extracted with chloroform using a soxhlet extractor for continuously 10 h or until the used solvent turned pure and colorless. The solvent was removed by evaporation at 40°C to give a concentrated extract, which was then frozen and freeze-dried until further used.

Sterilization and preparation of different concentration of extract: The dried extracts were exposed to ultra violet light (UV) rays for 24h to sterilize[14]. Liquid extracts were sterilized using a membrane filter (0.45-micron sterile filter). Dry powder extracts were initially dissolved in 1ml of dimethyl sulfoxide (DMSO). Different dilution of extract was prepared. Norfloxacin antibiotic worked as control drug.

Antibacterial activity of *Piper nigrum* L.: Antibacterial activity was performed according to Deshwal and Vig[15]. The microorganism was activated by inoculating a loopful of the strain in muller hinton broth (30ml) and incubated on a rotary shaker. Then 0.2 ml of inoculum (inoculum size was 10^8 cells/ml as per McFarland standard) was inoculated into the molten Muller Hinton agar media and after proper homogenization it was poured into the sterilized Petri plate. For agar well diffusion method, a well was made in the seeded plates with the help of a sterilized cup-borer. 20µl test compound was introduced into the well and the plates were incubated at 37ºC for 24 h. Microbial growth was determined by measuring the diameter of zone of inhibition. For each bacterial strain, controls were maintained in which pure solvents were used instead of the extract.

RESULTS

10 typhoid patients were selected for present study. *Salmonella typhi* showed 2-3mm colony, grayish-white, circular, moist, convex colony on blood agar and typical *S. typhi* surface colonies are black, surrounded by black or brown-black zone with or without a metallic sheen on Bismuth Sulfite Agar medium.

Table 1: Biochemical characterization of *Salmonella typhi*

Biochemical test	*Salmonella typhi*	
	Reaction	Percentage (positive)
Motility	+	100
Yellow pigment	-	-
Red pigment	-	-
MacConkey growth	+	100
Simmon's citrate	-	-
Christensen's citrate	+	100
Urease	-	-
Gelatine hydrolysis	-	-
Growth in KCN medium	-	-
H_2S (PbAc paper)	+	100
H_2S from TSI	+	80
Gluconate	-	-
Malonate	-	-
ONPG	-	-
Phenylalanine	-	-
Arginine dihydrolase	+	100
Lysine decarboxylase	+	100
Ornithine decarboxylase	-	-
Oxidate	-	-
Selenite reduction	+	100
Casein reduction	-	-
DNAase	-	-
Carbohydrate (in peptone water)		
Gas from glucose	-	-
Acid from		
Arabinose	-	-
Cellobiose	-	-
Glycerol	+	80
Lactose	-	-
Maltose	+	100
Mannitol	+	100
Raffinose	-	-
Rhamnose	-	-
Sorbitol	+	100
Sucrose	-	-
Xylose	+	100
Starch	-	-
MR test 37°C for two days	+	100
MR test 22°C for five days	+	100
VP test 37°C for two days	-	-
VP test 22°C for five days	-	-
Indole	-	-
Catalase	+	100

Total number of samples 10 in 4 replicate

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All strains showed positive results in Motility, MacConkey growth, Christensen's citrate, H_2S (PbAc paper), H_2S from TSI (80%), Arginine dihydrolase, Lysine decarboxylase, Selenite reduction, Catalase, MR test (+). Strains showed variation in acid production form various sugars i.e. acid from Arabinose (-), Cellobiose (-), Glycerol (80), Lactose (-), Maltose (+), Mannitol (+), Raffinose (-), Rhamnose (-), Sorbitol (+), Sucrose (-), Xylose (+), Starch (-) and failed to produce gas from Glucose. Isolated strains did not show positive biochemicals test in VP (-), indole (-), Yellow pigment (-), Red pigment (-), Simmon's citrate (-), Urease (-), Gelatine hydrolysis (-), Growth in KCN medium (-), Gluconate (-), Malonate (-), ONPG (-), Phenylalanine (-), Ornithine decarboxylase (-), Oxidate (-), Casein reduction (-), DNAase (-) (table 1). Further, three extracts such as aqueous, ethanolic and chloroform extracts of *Piper nigrum* seeds were prepared. Inhibition zone was increased as increased the concentration of aqueous extract of *Piper nigram* but 15 mg/ml of ethanolic and chloroform extract showed less inhibition as compared to norfloxacin of same concentration. Maximum inhibition zone was observed in aqueous extract of *Piper nigrum* as compared to ethanol, chloroform, norfloxacin. Aqueous solution of medicinal plant (30 mg/ml) showed 25.8% more inhibition zone as compared to norfloxacin (30 mg/ml). Similar observation has been shown in ethanol (30 mg/ml), chloroform (30 mg/ml) by 17.2, 9.6% as compared to norfloxacin (30 mg/ml) (Table 2).

Table 2: *In vitro* antibacterial activity of different extracts of *Piper nigrum* L. on the growth of *Salmonella* by Well Diffusion test.

Concentration	Inhibition zone (mm)				
	Extract*				Control
	Aqueous	Ethanol	Chloroform		Norfloxacin
15 mg/ml	13.5±0.3	13.1±0.2	13.1±0.3		13.3±0.1
20 mg/ml	15.3±0.4	14.9±0.3	15.1±0.2		14.8±0.3
25 mg/ml	18.8±0.3	17.6±0.2	17.3±0.2		16.4±0.2
30 mg/ml	23.4±0.2	21.8±0.4	20.4±0.1		18.6±0.1

Values are mean of 10 replicates ± SD, * Inhibition zone = total inhibition zone – solvent inhibition zone

DISCUSSION

All these biochemical tests confirmed that isolated strains were *Salmonella typhi*. Similar observations were mentioned by Holt[13] and Cowan and Steel's manual for the identification of medical bacteria[16]. *Salmonella typhi* is pathogenic microorganism which causes typhoid. There are 16 million annual cases of typhoid fever, 1.3 billion cases of gastroenteritis and 3 million deaths worldwide due to *Salmonella*[17]. It is among the most commonly isolated foodborne pathogens associated with fresh fruits and vegetables[18]. All above literature suggest that *Salmonella typhi* is responsible for food borne disease. Our study showed that aqueous, ethanolic and chloroform extracts Extract of *Piper nigrum* L. showed antimicrobial activity against *Salmonella typhi*. Similarly, Mahida and Mohan[19] reported that extract of *Crytolepis buchanani* (Linn) Roem & schult, *Mangifera indica* Linn., *Manilkara hexandra* (Roxb.) Dubard and *Nyctanthes arbor-tristis* Linn exhibited significant antibacterial activity against *Staphylococcus* and *Salmonella* spp. Research studies have shown that several medicinal plants inhibit growth of bacterial pathogens[4,12,15,20]. Antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions[21]. The spices have a unique aroma and flavour which are derived from compounds known as phytochemicals or secondary metabolites[22]. Black pepper (*Piper nigrum* L.) is used to treat asthma, chronic indigestion, colon toxins, obesity, sinus, congestion, fever[23].

CONCLUSIONS:

Present study shows that *Piper nigrum* L. significantly inhibited the growth of *Salmonella typhi* and it's medicinal value improve its application. Use of antibiotic has side effect to human so it is necessary search for new antimicrobial substance. Medicinal plants are good alternative of chemical antibiotics.

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