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Enumerate the Role of *Piper Nigrum* Buffer Extract [PNBE] on its Therapeutical Applications

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ABSTRACT

Piper Nigrum Buffer Extract [PNBE] studies on its therapeutical application is the major scope of this research study. *Piper Nigrum Buffer* seeds were subjected to Soxhlet-extraction using Phosphate Buffer Saline (PBS) and final extract was termed as *Piper Nigrum* Buffer Extract [PNBE]. In phytochemical analysis of PNBE, it was found to be presence of carbohydrates, phenolic compounds, saponins and flavonoids in the extract. PNBE elutes only 4 peaks apart from the solvent peak in the HPLC analysis. But, in GCMS analysis PNBE elutes 7 peaks after the solvent peak. In addition, PNBE shows the presence of several minerals such as aluminium, boron, cadmium, iron, copper and etc., which is conformed in ICP-OES analysis. Furthermore, PNBE profound to be shown anti-microbial property as it generate zone of inhibition for *E.coli* and *S. aureus* cultured medias. Apart from all this above mentioned assays, fortunately we found that non-toxic property of PNBE when we perform non-toxic assay using packed RBC.

Key words: *Piper Nigrum* Buffer Extract [PNBE], GC-MS, RP-HPLC, ICP-OES, Antibacterial and Non- toxic property.

Introduction

Black pepper, scientifically known as Piper Nigrum, is among the most frequently utilized spices, earning the title "The King of Spices" due to its global trade significance [1]. It is also referred to by various names in different languages, including Kali Mirch in Urdu and Hindi, Pippali in Sanskrit, Milagu in Tamil, and peppercorn, white pepper, green pepper, and Black pepper in English [2]. Beyond its culinary uses, black pepper is valued for its medicinal properties, serving as a remedy, a preservative, and in the creation of perfumes [3]. The Piper genus encompasses over 1000 species, with the most renowned being Piper Nigrum, Piper longum, and Piper betli [4]. This versatile spice is utilized for a variety of purposes, including as a dietary supplement, medicine, preservative, and as a natural pest control agent [5]. It is a staple in numerous global cuisines, particularly in meat dishes, and is rich in the pungent alkaloid Piperine (1-peperoyl piperidine), which is associated with a range of pharmacological effects [6]. Research has shown that Piperine can stimulate digestive enzymes in the pancreas and intestine, increase bile acid secretion in the liver, and has a wide array of medicinal uses, including lowering blood pressure, reducing platelet aggregation, acting as an antioxidant, anticancer, antipyretic, analgesic, anti-inflammatory, anti-diarrheal, antispasmodic, liver-protective, antibacterial, antifungal, anti-thyroid, anti-apoptotic, anti-spermatogenic, insecticidal, and larvicidal activities [7]. Piperine has been shown to enhance the effectiveness of numerous drugs, vaccines, and nutrients by increasing their absorption through the oral route by inhibiting various metabolic enzymes [8].

Materials and Methods

All the chemicals used were of analytical grade. Microbial cultures were purchased from MTCC.

Preparation of PNBE

Piper Nigrum seeds were purchased from local market and it was subjected to Soxhlet extraction process using Phosphate Saline Buffer (PBS). The finally obtained extract was termed as *Piper Nigrum* Buffer Extract (PNBE) and it utilized for further assays.

Preliminary phytochemical screening of PNBE

PNBE was screened for terpenoids, phytosterol, tannin, phenolic, glycoside, saponins, flavonoids, carbohydrates, proteins, steroids and lipids [9].

Reverse Phase High Performance Liquid Chromatography analysis of PNBE

PNBE was subjected to RP-HPLC using C_{18} column (150mm×3mm, particle size 2.7µm) with VWD detector in Agilent 1260-infinity II. The column was pre-equilibrated with HPLC water and Acetonitrile and sample was eluted at the flow rate of 1ml/min in linear gradient mode [10].

Gas Chromatography-Mass Spectroscopy analysis of PNBE

PNBE was analyzed in GC-MSD, model number 5977B, Agilent Make on single quadrupole mass spectrometers in the Electron Impact Ionization Total Ion Chromatography (EITIC) mode with capillary column (30m lengthX0.25mm ID, 0.25µm film thickness, composed of 5% Phenyl methyl poly siloxane). Helium (99.999%) gas was used as carrier gas at the flow rate of 1ml/min and the injection volume of 2µl. Split ratio of 10:1, temperature program was set as follows, injector temperature 350°C; Auxiliary temperature 250°C, oven temperature initially 50°C (4min hold) with an increase in temperature of 10°C/min to 150°C (4min hold), thereafter 20°C/min to 200°C (4min hold), 25°C/min ramp to 250°C (4min hold), 30°C/min ramp to 280°C (4min hold). Total run time 35.5 min. Mass spectrum was taken at 70ev; a scan interval of 2.92s [11].

Direct hemolytic activity of PNBE

Direct hemolytic activity was determined by using washed human erythrocytes. Briefly, packed human erythrocytes and Phosphate Buffer Saline (PBS) (1:9v/v) were mixed; 1mL of this suspension was incubated independently with the various concentrations of PNBE (100µL & 200µL) for 1hr at 37°C. The reaction was terminated by adding 9mL of ice cold PBS and centrifuged at 1000g for 10min at 37°C [12]. The amount of hemoglobin released in the supernatant was measured at 540nm. Activity was expressed as percent of hemolysis against 100% lysis of cells due to the addition of water (positive control), whereas PBS served as negative control.

Antimicrobial assay of PNBE

The bacterial cultures (E. coli and S. aureus) were grown in Muller Hinton nutrient agar medium that contain peptone (1%), beef extract (1%) and NaCl (1%) at pH 6.8. Sterile nutrient agar petri plates were prepared and 0.1mL of the overnight grown bacterial culture was spread on the solidified agar plates evenly with the help of a glass spreader. Wells were made on the solidified agar using a cork borer. The test solution was made by dissolving 50mg of PNBE in 1.0mL of water to get 50mg/mL concentration followed by sonication for 2min. The 100µL of this test solution containing 5mg of PNBE added into the respective wells. The standard antibiotic drug Amoxycillin was kept as positive control and tested against both the pathogens. These plates were incubated at 37°C for 24hr. The diameter of 'zone of inhibition' at each well was measured and recorded [13]. The minimum inhibitory concentration (MIC) assay was carried out in triplicate and the average values were reported

Inductively Coupled Plasma Optical Emission spectroscopy analysis of PNBE

PNBE analyzed in Agilent Make ICP-OES instrument, model number 5110. To evaluate the content of minerals in the extract, the samples were aspirated at 12 RPM pump speed, 25 seconds sample uptake time, 30 seconds of rinse time, 5 seconds, read time, 1.2 KW RF power, 15 seconds stabilization time, Axial viewing mode, 8mm viewing height, 0.7 L/Min nebulizer flow, 12 L/Min plasma flow, 0.75 L/Min Aux flow [14].

Results and Discussion

Phytochemical analysis of PNBE

PNBE was found to be present of flavonoids, carbohydrates, phenolic compounds and saponins as per preliminary screening (Table 01). Moreover, PNBE withholds several minerals such as aluminium, cadmium, iron, boron, nickel, copper, zinc and etc., (Table 02).

SL NO	Phytochemical Analysis	Results
01	Terpenoid	Absent
02	Phytosterol	Absent
03	Tannin	Absent
04	Phenolic	Present
05	Glycoside	Absent
06	Saponin	Present
07	Flavonoid	Present
08	Carbohydrates	Present
09	Proteins	Absent
10	Alkaloid	Absent
11	Steroids	Absent

Table :01

SL.NO.	Name of The Metal	PNBE In ppm
01	Aluminium	3.04
02	Boron	0.32
03	Barium	1.07
04	Cadmium	0.00
05	Copper	1.32
06	Iron	6.57
07	Manganese	1.19
08	Molybdenum	0.02
09	Nickel	0.07
10	Lead	0.05
11	Zinc	0.50

Table.02

Reverse Phase-High Performance Liquid Chromatography analysis of PNBE

PNBE elutes only 4 peaks at different retention time in reverse phase HPLC attached to Variable Wavelength Detector. Sample was eluted at 216nm at room temperature (Fig.01).

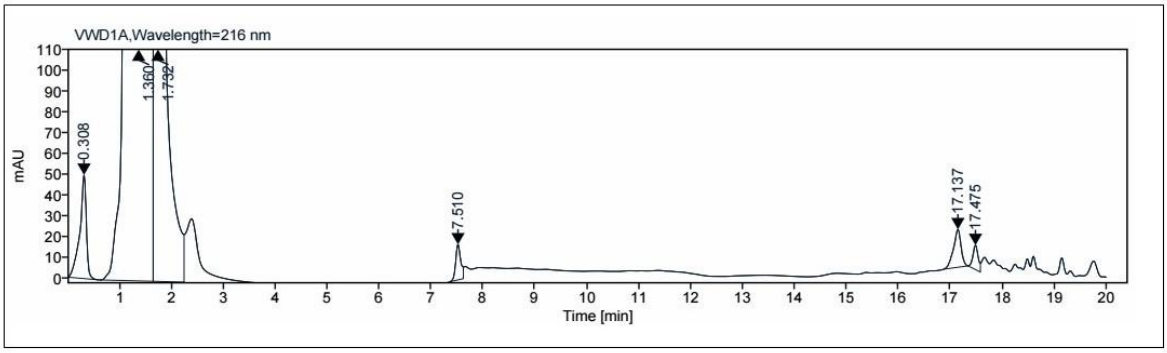

Fig.01: HPLC Chromatogram of PNBE

Gas Chromatography-Mass Spectroscopy analysis of PNBE

PNBE found to presence of 7 different set of compounds as it elutes 7 major peaks after the solvent peak in GC-MS analysis at the retention time of 11.5, 12.1, 14.4, 17.5, 23.4, 25.2, 26.0, 26.5, 38.5 and 39.3 respectively (Fig.02).

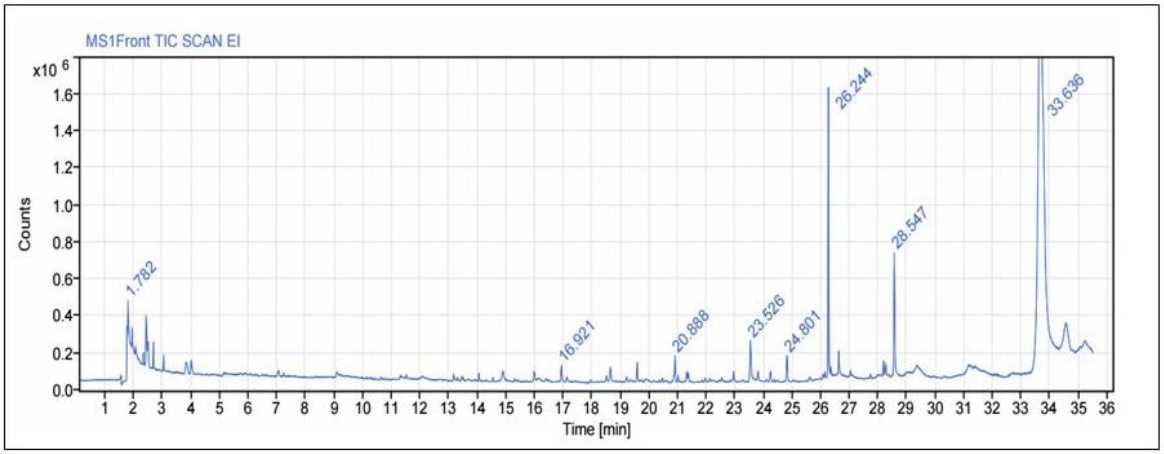

Fig.02: GC MS Chromatogram of PNBE

In addition, PNBE exhibits non-toxic property; as it was unable to degrade packed RBC in in-vitro study (Fig.03).

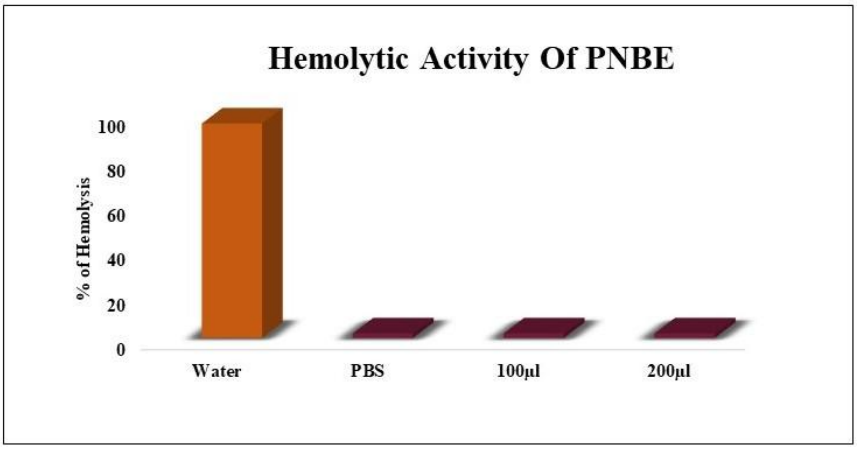

Fig.03: Hemolytic Activity Of PNBE

Antimicrobial activity of PNBE

PNBE exhibits anti-microbial property by inhibiting the growth of both gram negative bacteria (*E.coli)* and gram positive bacteria (*S.* aureus) (Fig.04). Identify the novel antimicrobial agent from herbal source with efficiency; play a pivotal role in the current research trend [15].

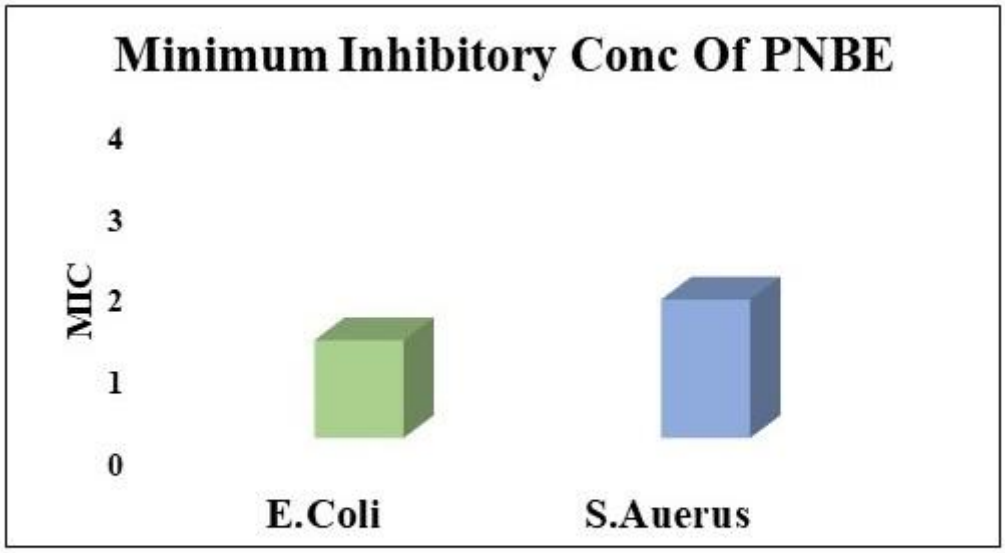

Fig.04: Antimicrobial Property Of PNBE

Conclusion

In conclusion, this study demonstrates the phytochemical analysis of PNBE and its anti-microbial property against gram positive and gram negative organisms.

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Declaration of Conflict of Interest

The authors declared no potential conflict of interest with respect to the authorship and publication.

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