Original Research Article

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Antimicrobial efficacy of leaf extracts of *Piper nigrum* against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*

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Abstract

This study investigated the antimicrobial activity of *Piper nigrum* leaves extracts (hot water, cold water and pepper soup) on *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*. The fresh leaves of climbing pepper were bought at Swali Market, Yenagoa, Bayelsa State of Nigeria. Hot and cold-water extracts and pepper soup made with the leaves were used for the sensitivity test. Filter paper disc method was used for the determination of zone of inhibition. Ampiclox (1%) was used as the positive control (AMP). The mean zone of inhibition for *S. aureus*, *E. coli* and *C. albicans* were 12.52 mm, 12.61 mm and 11.23 mm respectively (cold water leave viz: CWL), 13.16 mm, 13.57 mm and 11.49 mm respectively (hot water leave viz: HWL), 13.46 mm, 13.60 mm and 13.31 mm respectively (pepper soup extract leave viz: PEPL) and 12.07 mm, 12.31 mm and 12.03 mm respectively (1% concentration of the ampiclox used as positive control viz: AMP). Analysis of variance showed that there was a significant variation ($p < 0.05$) among the various isolates for each of the different extracts apart from PEPL. The phytochemical screening revealed the presence of tannins, flavonoids, cardiac glycosides and alkaloids in the leaves of *P. nigrum.*

Keywords: Antimicrobials, Microorganisms, Plant extracts, Leaves of *Piper nigrum*

1. Introduction

Due to the challenges of drug resistance, there have been an upsurge in search for effective alternative to sythenthic drugs. According to Pandey et al. [1], the development of effective antibacterial agents are needed due to the emergence of drug-resistant organisms because of the irrational and overuse of antibiotics, failure to complete a course of treatment, genetic versatility of microbes and horizontal transfer of resistant genes among bacterial species. In addition, the emerging and re-emerging diseases have also contributed to effective search of new antibiotics. Over the past few decades, the natural antioxidants of both nutritive and medicinal plants have been of significant interest to the pharmaceutical and food industries due to their roles in combating myriads of oxidative damages incurred by living cells and food products from free radicals' activities [2]. Recently, there have been increasing interests in the discovery of new antimicrobials, because of the increasing risk in the rate of infection with antibiotic-resistant microorganisms, and also due to the side and the residual effects of antibiotics. Plants have emerged as credible alternative [3,4] or a promising effect in therapeutics [5] due to their natural properties that can be easily absorbed.

The use of plant to treat human diseases can be traced to several centuries [6]. Till date, a large number of global population still depend on traditional medicine for treatment of a variety of diseases caused by microorganism especially in developing countries [3, 7, 8]. Authors have variously reported that over 80% of world population depend on traditional medicine practitioners for healthcare services [3, 9-12]. In developing countries, individuals, who patronize herbal medicine practitioners, are mostly in the rural areas [6].

Medicinal plants are plants whose roots, leaves, seeds, bark, or any other constituent possess therapeutic characteristics [3, 4, 6, 10]. Again, plants are source of food including carbohydrates, proteins, minerals, vitamins. As such, they are essential for the sustenance of life.

Plants are also used as spices in preparation of delicacies. According to Roy et al. [13], spices are natural food additives which are known to contribute immensely to the taste of food and are also known to possess tremendous medicinal properties. Several types of spices are used in developing countries like Nigeria. Some notable spices used include clove, nutmeg, garlic, ginger, various varieties of pepper such as *Piper nigrum*.

Piper nigrum, which belongs to Piperaceae family [5] is popularly known as black pepper and has culinary and medicial uses [13]. Medicinal properties of *Piper nigrum* have been comprehensively reviewed and documenetd by Ahmad et al. [14], Damanhouri et al. [15]. This could be due to several organic chemicals including essential oil, alkaloids, flavonoids, phenols, lignans, acids, amides and other aromatic compounds it contains [13].

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Piper nigrum is often referred to as ‘‘The King of Spices’’ [13, 15, 16] and have gained worldwide popularity in the spice industry [17]. Due to the bioactive and metabolites the plant contains, it has inordinate potential for the discovery of novel biologically active compounds [17]. Like other plants, *Piper nigrum* has antimicrobial potentials [1, 13- 24]. But, information about the comparison of the different extracts with pepper soup extract is scanty in literature. Hence, this study aimed at assessing the antimicrobial efficacy of leaf extracts of *piper nigrum* against enteric, superficial, etiologic microorganisms such as *Escherichia coli, Staphylococcus aureus and Candida albicans*.

2. Materials and methods

2.1. Source of plant samples and identification

The fresh leaves of climbing pepper were bought at Swali Market, Yenagoa, Bayelsa State of Nigeria at the early hours of the day (6:30 am - 7:00 am) in the month of June 2015. The samples were botanically identified using the morphological characteristics presented by Nyananyo [25].

2.2. Preparation of plant materials for extraction

The collected fresh plant materials were transported to the laboratory of the Department of Biological Sciences, Faculty of Sciences, Niger Delta University within 1 hr. The plant leaves were picked from the stems, cleaned and rinsed in deionized water. The leaves were allowed to air dry.

2.3. Extraction processes

One hundred grams (100 g) of the leaves was weighed and rinsed three times in deionized water and ground into a fine powder in a sterilized porcelain mortar. Two different solvents were used i.e. cold and hot water. Ten grams (10 g) of fine grounded *Piper nigrum* leaf powder was measured out using electronic balance (Series JT 302N) and then transferred into a conical flask containing 50 mL of sterile hot water at 96 ± 4 °C (which reduced to room temperature after about 2 hr). It was corked with a cotton wool and foil, shaken gently and allowed to stand in room temperature for 24 hr. The content was then transferred to a funnel bearing a sterile muslin cloth and further filtered using a Whatman No. 1 filter paper. This process was repeated for the cold water extract at room temperature of 29 ± 3 °C. All the extracts were stored in the refrigerator at 5 °C prior to use.

In the pepper soup extract, the initial quantity of water in cooking the pepper soup was 300 mL. Thirty grams of smoked fish (*Heterobranchus bidorsalis* which belong to the Clariidae family) was thoroughly washed and put inside a pot containing 300 mL of cold water. This was followed by 5 g of pepper, salt to taste, a cube of seasoning (Maggi), a small ball of onion and a teaspoon of grounded crayfish (*Macrobrachium felicinum* which belong to the Palaemonidae family) and then 10 g of fine grounded seed powder of *piper nigrum*. It was put on burning flame (gas cooker) and allowed to boil until the final quantity of water obtained was 20 mL. It was filtered using a muslin cloth on a funnel and further filtered using Whatman No.1 filter paper. The various extracts were also stored in the refrigerator at 5 °C prior to use. Figure 1 presents the pictorial nature of the pepper soup extract.

Figure 1. Pictorial nature of the Pepper soup extract of *Piper nigrum*

2.4. Experimental procedures

The various extracts i.e. hot water, cold water and pepper soup were used for the antimicrobial activity testing viz. cold water leaves (CWL) and hot water leaves (HWL).

2.5. Source and preparation of organisms

The fresh isolates of *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* used in this study were obtained from the stock culture from the Medical Microbiology and Parasitology Department, College of Health Sciences, Niger Delta University, Nigeria. The purity of the bacteria was checked by sub-culturing and the resultant growth was subjected to biochemical test using the scheme of Benson [26] and Cheesbrough [27]. Germ tube test was carried out for the *Candida albicans.*

2.6. Preparation of antibiotic disc and antibiotics medium

Antibiotic discs were prepared using a 6 mm diameter paper-perforating machine on Whatman No. 1 filter paper. Then, 0.1 mL of extract was dropped on a sterilized 7 mm Whatman No. 1 filter paper placed in sterilized petri dishes and was allowed to be absorbed in room temperature. After drying, they were safely packed in a petri-dish, wrapped in an aluminum foil and stored in the refrigerator.

The positive control antibiotic used was 500 mg Ampiclox (broad-spectrum antibiotic containing ampicillin 250 mg and cloxacillin 250 mg) and the media used was the multipurpose medium (nutrient agar) for *Staphylococcus aureus*, MacConkey agar for *Eschericia coli* and chocolate dextrose agar for *Candida albicans*. The contents of 500 mg

ampiclox capsule were dissolved in 500 mL of water giving out 1 mg/ml solution [28].

2.7. Antimicrobial screening of the extract

Filter paper disc method previously described by Benson [26], with modifications by Oguntoye et al. [29] and Kigigha et al. [30-35] was employed for the antimicrobial sensitivity testing. Nutrient agar was prepared according to the manufacturer's instruction. The liquefied agar was placed in a water bath at 50 °C to melt. The test organisms were labeled in the bottom of the petri dish. Then about 20 mL of the agar was poured on sterile petri dish and allowed to solidify. Sterile disc of 7 mm in diameter was dipped into the plant extract. The impregnated disc was placed in the solidified agar using forceps. The edge of the forceps was used to press down lightly the disc. A positive control was set up with 1% ampiclox. The plate was incubated at 37 °C for 48 hr and there after the resultant zone of inhibition was measured on bottom of the plate using meter rule.

2.8. Phytochemical screening of Piper nigrum leaves

Phytochemical analysis of the plant was carried out. Saponins, cardiac glycosides, alkaloids, flavonoids and tannins were determined using the scheme provided by previously reported procedures [2, 36-41].

2.9. Statistical analysis

SPSS software version 20 was used to carry out the statistical analysis. One-way analysis of variance was carried out at $\alpha = 0.05$, and Tukey Honestly Significance Difference (HSD) was used as the post hoc test to determine the source of the observed differences.

3. Results and discussion

The phytochemical analysis of leaves of *P. nigrum* is presented in Table 1. The active ingredients were tannins, flavonoids, cardiac glycoside (at high concentration), alkaloid (at moderate concentration) and saponin were absent in the leaves. The presents of these phytochemicals is in accordance with active ingredients found in plants as previously reported in literatures. Typically, plants constitute a diverse range of bioactive substances [3, 4]. The metabolites are typically classified into nitrogen compounds (alkaloids, non - protein amino acids, amines, alcamides, cyanogenic glycosides and glucosinolates) and non - nitrogen compounds (monoterpenes, diterpenes, triterpenes, tetraterpenes, sesquiterpenes, saponins, flavonoids, steroids and coumarins) [2, 42].

The occurrence of these phytochemicals alkaloids, flavonoids, tannins and cardiac glycosides suggested the usefulness of the leaves against microorganisms. Nahak et al. [20] suggested that the phytochemical properties of the plants are an indication that such a plant has medicinal and physiological activities against microbes. According to Shailesh [18], the potential therapeutic properties of plants vary at various degrees owing to the presence of several bioactive compounds in them. Furthermore, plants with certain phytochemicals suggest that they can be lethal to microbial cells [40]. Variation exists amongst different plants with regard to the concentration of the various bioactive ingredients. This could be due to compounding factors such as age and part of the plant [3, 12].

A variety of rich secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, phenols, steroids, glycosides and volatile oils are present in plants in general [43]. The presence of phytochemicals in plants is an indication that they have functions of biological activity [3]. For instance, alkaloids in plants have mechanisms by which they resist pests including microorganisms [3, 44]. Flavonoids, a hydroxylated phenolic compounds help plants to resist disease causing microorganisms [44] and they have antioxidant, anticarcinogenic, antimicrobial and antitumor properties [3, 46]. The presence of saponin in plants suggests that such a plant could be used as expectorant and cough suppressant [3, 46]. Plants containing tannins suggest that such plant can be used for the treatment of wounds, varicose ulcers, hemorrhoids, frostbite and burns [3, 6, 40, 46, 47].

Table 2 present zones of inhibition of various extracts of *P. nigrum* leaves. The mean zone of inhibition for *S. aureus, E. coli* and *C. albicans* were 12.52 mm, 12.61 mm and 11.23 mm respectively for cold water leave (CWL), 13.16 mm, 13.57 mm and 11.49 mm respectively for hot water leave (HWL), 13.46 mm, 13.60 mm and 13.31 mm respectively for pepper soup extract leave (PEPL) and 12.07 mm, 12.31 mm and 12.03 mm respectively for 1% concentration of the ampiclox used as control (AMP). In the AMP, CWL and HWL, there was significant differences ($p < 0.05$) among

Table 1. Phytochemical constituents of *P. nigrum* leaves

Tannins	Saponins	Flavonoids	Cardiac Glycoside	Alkaloids
++	-	++	++	+

++ = Highly present; + = Moderately present; - = Absent

Table 2. Zone of inhibition of various extracts of *P. nigrum* leaves

Microbes	**CWL (mm)**	**HWL (mm)**	**PEPL (mm)**	**AMP (mm)**
S. aureus	12.52 ± 0.05^{b}	13.16 ± 0.04^{b}	13.46 ± 0.04^{a}	12.07 ± 0.07^{ab}
E. coli	12.61 ± 0.03^{b}	13.57 ± 0.02^{c}	13.60 ± 0.10^{a}	12.31 ± 0.07^{b}
C. albicans	11.23 ± 0.05^{a}	11.49 ± 0.05^{a}	13.31 ± 0.11^{a}	12.03 ± 0.03^{a}

Data is expressed as mean ± standard error of mean (n=4); Different letters along the column indicate significant difference ($p < 0.05$) according to Tukey HSD statistics

the different isolates. Furthermore, there was no significant difference ($p > 0.05$) among the different isolates for PEPL. Based on the efficacy of extracts, they were in the order of CWL < HWL < PEPL.

The significant differences ($p < 0.05$) that existed among the various extract (CWL, HWL and PEPL) could be due to variation in their bioactive constituents as well as the biochemical/genetic makeup [3, 4, 6]. The hot-water extract has significantly higher efficacy than the cold water extract. This suggests that hot-water extract may have a higher extracted bioactive contents compared to cold-water extract. This is in agreement with the work of Kigigha et al. [6] that reported higher zone of inhibition for *E. coli*, *S. aureus* and *Bacillus* sp. exposed with hot-water extract of *Aframomum melegueta* (Alligator pepper) as compared to cold-water extract. The pepper soup extract has significantly higher efficacy than the cold and hot-water extracts apart from cold-water seed extract. This variation could be due to other active ingredients used for preparation of the pepper soup. Other spices used for the preparation of pepper soup may also contain phytochemical constituents with antimicrobial activity. Furthermore, the variation that exist among the microbes (*S. aureus*, *E. coli* and *C. albicans*) could be biochemical/genetic makeup of these organisms including their metabolism, nutrition and physiology [3, 4, 12].

4. Conclusion

P. nigrum is one of the common spices used for preparing delicacy especially in Nigeria. However, *P. nigrum* is harvested in the forest as a non-timber forest produce. Pepper soup is one of the common delicacies prepared with it especially for postpartum women to control postpartum hemorrhage and for treatment of individuals with symptoms of fever. This study assessed the phytochemical and antimicrobial activities of different leaves extracts (cold water, hot water and pepper soup) of *P. nigrum*. The study found that *P. nigrum* leaves contain phytochemicals such as tannins, flavonoids, cardiac glycosides and alkaloids. The *P. nigrum* leaf extracts exhibited significant antimicrobial effect against selected microorganisms. The superiority of the PEPL extracts among others type of extracts justifies the traditional uses of the PEPL by the indigenous people in the Niger Delta region of Nigeria.

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