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ORIGINAL RESEARCH

Antimicrobial effect of different seed extracts of *Piper nigrum* against *Escherichia coli, Staphylococcus aureus* and *Candida albican*

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ABSTRACT
This study evaluated the antimicrobial effect of different seed extracts of *Piper nigrum* against *Escherichia coli, Staphylococcus aureus* and *Candida albican*. The three seed extracts (hot water, cold water and pepper soup) were made in replicates for the sensitivity study. Filter paper disk method was employed for the sensitivity. Results showed that the mean zone of inhibition results were 12.135mm, 13.293 mm, 13.775 mm and 13.497 mm for 1% concentration of the ampiclox used as control (AMP), cold water (CWS), hot water (HWS) and pepper soup (PEPS) extracts respectively. At P<0.05, the extracts were in the order AMP<CWS<HWS<PEPS. Also, the mean zone of inhibition was 12.604 mm, 13.548 mm and 13.374 mm for *C. albican*, *E.coli* and *S.aureus* respectively, which were in the order *C. albican* < *S.aureus* < *E.coli*. The phytochemicals analysis revealed that tannins and cardiac glycoside were highly present while saponin, flavonoids and alkaloid were moderately present. The occurrence of the bioactive constituents suggests the possible pharmacological characteristics of *P. nigrum.*

KEY WORDS: *Antimicrobials, Microbes, Peppersoup, Piper nigrum seed*

INTRODUCTION

The rise in the prevalence of multi-drug resistant microbes and the emergence and re-emergence of microbes of public health importance has led to search for alternative antibiotics. Deshwal (2013), Janovská *et al*. (2003), Deshwal and Vig (2011) opined that reduced susceptibility of antibiotics against infections caused by microbes have raised the scales of untreatable infections and have added urgency to the quest for new infection-fighting and safe strategies of combating diseases. On this perspective, Lokhande *et al*. (2007), Nahak and Sahu (2011) reported that many plant-derived molecules have shown a promising effect in therapeutics. The plants with pharmacological properties have severally been referred to as medicinal plants.

Typically, medicinal plants are plant in which any of the parts including seed, root, sap, leaves, fruit, flower etc have medicinal properties (Minocheherhomji and Vyas, 2014; Kigigha et al., 2015a). As such medicinal plants abounds especially in traditional systems. Ganesh *et al*. (2014) noted that large number of world population is increasingly using herbal medicines as dietary supplements to relieve and treat many different human disorders. In this perspective, Kigigha et al. (2016a), Tchouya and Nantia (2015), Minochecherhomi and Vyas (2014) opined that medicinal plants provide health coverage to 70 – 80% of world population especially in the developing nations. The applications depends mostly on the educational background of the people. In many developing countries, people residing in rural area patronize traditional medicine practitioners. The uses of medicinal plants as a source for relief from illness can be traced back to several millennia (Pandey *et al*., 2012).

Most spices and herbs are natural source of antioxidants which play important roles in chemoprevention of diseases and aging (Nahak and Sahu, 2011). Herbs and spices are also essential component of the human diet. They have been used for thousands of years to enhance the flavour, colour and aroma of food (Ganesh *et al*., 2014). Furthermore, pepper is one of the most valued spices used for cooking.

Several species of pepper exist and have been widely cultivated especially in the tropical region of the world. Some species of Pepper are found in the wild. One of the common genera found in the wild is Piper. The genus Piper which belongs to the family of *Piperacea* has over 1000 species globally (Suwanphakdee and Chantaranothai, 2008; Al-Tememy, 2013). Specifically, *Piper nigrum L*., a well known spice is often considered as ''The King of spices'' among various spices (Damanhouri and Ahmad, 2014; Roy and Vijayalaxmi, 2013; Wong and Ling, 2014). *Piper nigrum* is a flowering woody perennial climbing vine with root at the nodes (Deshwal *et al*., 2014; Damanhouri and Ahmad, 2014) *Piper nigrum* is found in tropical humid region of the world. It is native to the Malabar Coast of southwestern Ghats of India (Deshwal, 2013). *Piper nigrum* has several names depending in the region of the world including peppercorn, white pepper, green pepper, black pepper, madagascar pepper in english. In Nigeria, it is called different names depending on the locality. For instance, it is known as "Uziza" among Igbos tribes, Urire among the Urhobos.

Several of the common spices consumed as food adjuncts to impact flavour, aroma and colour to foods are also documented to exhibit several health beneficial physiological effects (Singh *et al*., 2015). The beneficial physiological effects of the *Piper nigrum* are often attributed to their active principles piperine (the biting principle of black pepper) (Singh *et al*., 2015; Damanhouri and Ahmad, 2014). Authors have reported that the presence of Piperine, pipene, piperamide and piperamine could account for their diverse pharmacological activities (Damanhouri and Ahmad, 2014; Ahmad *et al*., 2012; Parmar *et al*., 1997). Piperine is also an essential ingredient of black pepper and long pepper (Singh *et al*., 2015). Systemic pharmacological studies on piperine have shown that this compound stimulate several pharmacological activities; analgesic, anti-pyretic, anti-inflammatory, anti-convulsant and central nervous system-depressant activities (Singh *et al*., 2015), herbal cough syrups for its potent anti-tussive and bronchodilator properties Deshwal *et al*., 2014). It is also used as anti-inflammatory, anti-malarial, anti-leukemia (Deshwal *et al*., 2014), antihypertensive, antiplatelet, antioxidant, antitumor, anti-asthmatics, analgesic, anti-inflammatory, anti-diarrheal, antispasmodic, antidepressants, immunomodulatory, anticonvulsant, anti-thyroids, antibacterial, antifungal, hepato-protective, antipyretic, anti-apoptotic, anti-metastatic, antimutagenic, anti-spermatogenic, anti-colon toxin, anti-asthmatics, anti-anxiety, insecticidal and larvicidal activities etc (Damanhouri and Ahmad, 2014; Taqvi *et al*., 2008; Manoharan *et al*., 2009; Parganiha *et al*., 2011; Li *et al*., 2007; Matsuda *et al*., 2008; Acharya *et al*., 2012; Ahmad *et al*., 2012; Parganiha *et al*., 2011; Deshwal *et al*., 2014; Sujatha *et al*., 2003; Fan *et al*., 2011; Panda *et al*., 2013)

Basically, plants produce a diverse range of bioactive molecules, making them potential source medicine. Several plants have been reported to have antimicrobial activities. However, studies have been carried out on the antimicrobial activities of *Piper nigrum* (Pandey *et al*., 2012; Shailesh, 2015; Ahmad *et al*., 2012; Deshwal *et al*., 2014; Nahak and Sahu, 2011; Deshwal, 2013; Damanhouri and Ahmad, 2014; Singh *et al*., 2015; Abbasi *et al*., 2010; Roy and Vijayalaxmi, 2013; Gupta *et al*., 2013; Wong and Ling, 2014; Ganesh *et al*., 2014). But information on the comparison with the traditional uses of the *Piper nigrum* for pepper soup preparation and other extract types using solvents (hot and cold water) is rare in literature, hence the need for the study.

RESULTS AND DISCUSSION

The phytochemical analysis of the seed of *P. nigrum* is presented in Table 1. The active ingredients; tannins and cardiac glycoside were highly present, while saponin, flavonoids and alkaloid were moderately present. The findings of this study have some similarity with previous works on piper species. For instance, Al-Tememy (2013) reported that aqueous extract of Piper cubeba fruits contain tannin, flavonoids and glycoside and lacks alkaloids and saponin. Nahak and Sahu (2011) reported the presence of Alkaloid, Glycosides, Terpenoid, Steroid, Flavonoid, Tannins and other secondary metabolites in *Piper cubeba* and *Piper* *nigrum*. Shailesh (2015) reported that *P. nigrum* contain tannin, flavonoids and alkaloids and lacks saponin and cardiac glycoside. The occurrence of some phytochemical constituents plant suggest their pharmacological properties (Epidi et al., 2016a,b; Epidi, 2016).

Table 1: Phytochemical constituent in the seed of *P. nigrum*

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

++= Highly present; + = Moderately present; - = Absence

The effect of different seed extracts of *P.nigrum* on the zone of inhibition of some selected microbial isolates is presented in Figure 1. Mean zone of inhibition exhibited by the various extracts were 12.135mm, 13.293 mm, 13.775 mm and 13.497 mm for 1% concentration of the ampiclox used as control (AMP), cold water (CWS), hot water (HWS) and pepper soup (PEPS) extracts respectively. Basically there was significance difference (P<0.05) among the various group of extracts. Statistically, the extracts were in the order AMP<CWS<HWS<PEPS.

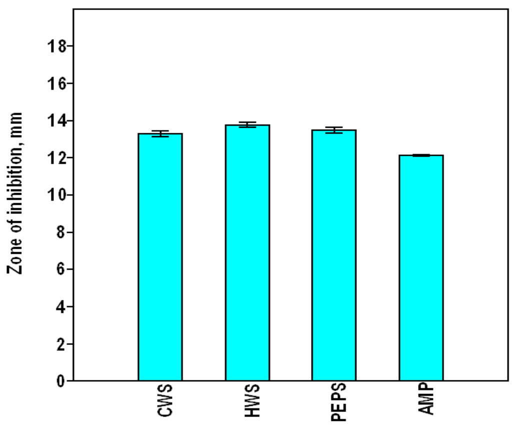

Figure 1: Effects of different seed extracts of *P.nigrum* on some selected microbial isolates.

Also, the effect of different seed extracts of *P.nigrum* on some selected microbial isolates is presented in Figure 2. Among the various organisms, the mean zone of inhibition was 12.604 mm, 13.548 mm and 13.374 mm for *C. albican*, *E.coli* and *S.aureus* respectively. There was significance difference (P<0.05) among the various microbial isolate tested. Statistically, the zone of inhibition exhibited by the various microbes were in the order *C. albican* < *S.aureus* < *E.coli*. Significance interaction exists between the microbial isolates and plant extracts.

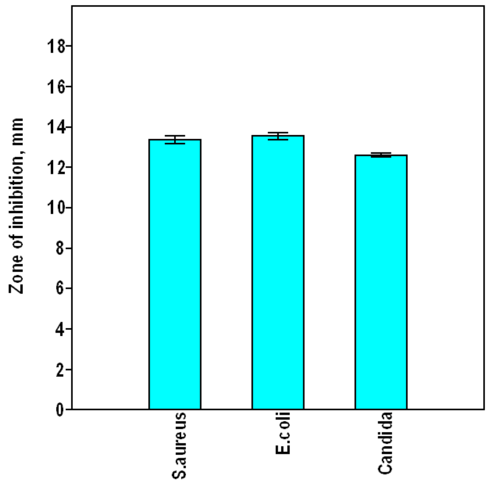

Figure 2: Zone of inhibition exhibited by some selected microbial isolates exposed to different extracts from seed of *P.nigrum*

The presence of insoluble active compound found in cold water extracts could be the contributing factor why there is low zone of inhibition among the various test organisms. While higher zone of inhibition exhibited by the pepper soup extract could be due to other constituents used for the pepper soup production. The findings of this study validate the traditional use of *P.nigurm* by indigenous people of Bayelsa state. Pepper soup is one of the common delicacies prepared with it especially for post-partum women as a stimulant to relieve constipation, aid lactation, prevent post-partum contraction and control passive uterine hemorrhage. The lower values of known antibiotics used as positive control could be due to the concentration applied (1%) in the cause of the experiment. This values obtained is close to the values previously for E.coli and S.aureus reported by Kigigha *et al*. (2015b, c), Kigigha *et al*. (2016b), Kigigha and Onyema (2015), Kigigha and Charlie (2012), Kigigha and Atuzie (2012).

E.coli which have superior or higher zone of inhibition could be due to biochemistry, nutrition, physiology and metabolism compared to other isolates (*S.aureus* and *C.albican*). This trend in zone of inhibition (for *E.coli*> *S.aureus*) on the antibacterial activity of *P.nigrum* against some bacteria isolates have been previously reported by Ganesh *et al*. (2014).

The zone of inhibition found in this study has some similarity with values previously reported plants parts including *Alstonia boonei* (Epidi *et al*., 2016b), *Vitex grandifolia* (Epidi *et al*., 2016a), *Aframomum melegueta* (Kigigha *et al*., 2015a), *Musanga cecropioides* (Kigigha *et al*., 2016a).

CONCLUSION

This study investigated the effect of different extracts (Pepper soup, hot water and cold water) of *P. nigrum* against *Escherichia coli, Staphylococcus aureus* and *Candida albican*. The study revealed that seed of *P. nigrum* contain some phytochemicals such as tannins, saponin, flavonoids, cardiac glycosides and alkaloids. The different extracts were statistically in the order: AMP<CWS<HWS<PEPS, while the microbes were in the order: *C. albican* < *S.aureus* < *E.coli* based on their effectiveness. The significant higher zone of inhibition by *E.coli* could be due to their biochemistry, metabolism and physiology. While the significant high efficacy of the PEPS extracts suggest the traditional utilization of the pepper for preparing pepper soup for post-partum women to control post-partum hemorrhage.

MATERIALS AND METHODS

Source of plant samples and identification

The dry seeds of *Piper nigrum* (black pepper) were purchased from Swali Market, Yenagoa, Bayelsa State of Nigeria and were transported to the laboratory of the Department of Biological Sciences, Faculty of Sciences, Niger Delta University, Wilberforce Island, Amassoma, Bayelsa State where the experimental set up was conducted.

Extraction processes

One hundred grams (100g) of Pepper corn was weighed and rinsed three times in deionized water and ground into a fine powder in a sterilized porcelain mortar. Ten grams (10g) of fine grounded *Piper nigrum* seed was measured out using electronic balance (Series JT 302N) and then transferred into a conical flask containing 50 ml of sterile hot water. It was corked with a cotton wool and foil, shaken gently and allowed to stand in room temperature for 24 hours. The content was then transferred to a funnel bearing a sterile muslin cloth and further filtered using a Whatman No 1 filter paper. The extracts were stored in a refrigerator at 5ºC prior to use. This procedure was applied for cold water extract except that the water used was not boiled.

In the pepper soup extract, the initial quantity of water in cooking the pepper soup was 300mls. 30 grams of smoked fish was thoroughly washed and put inside a pot containing 300mls of cold water. This was followed by 5 grams of pepper, salt to taste, a cube of seasoning (Maggi), a small ball of onion and a teaspoon of grounded crayfish and then 10 grams of fine grounded powder of *Piper nigrum*. It was put on burning flame (gas cooker) and allowed to boil until the final quantity of water obtained was 20mls. It was filtered using a muslin cloth on a funnel and further filtered using Whatman No1 filter paper. The various extracts were also stored in the refrigerator at 5^0C prior to use. The different extracts of the seed is presented in Figure 3

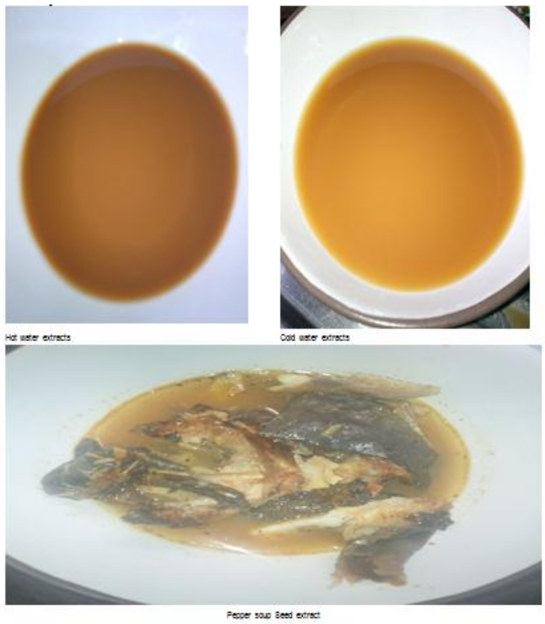

Figure 3: Pictorial nature of the seed extracts of *Piper nigrum* used in this study

The various extracts i.e. hot water, cold water and pepper soup was used for the antimicrobial activity for the different treatments viz: cold water seed (CWS), hot water seed (HWS) and peppersoup seed (PEPS) extracts.

Source and Preparation of organisms

The Fresh isolates of *Staphylococcus aureus*, *Escherichia coli* and *Candida albican* (microorganisms) used in this study were obtained from the stock culture in 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 (2002) and Cheesbrough (2004). Germ tube test was carried out for the *Candida albicans*.

Preparation of Antibiotic Disc and antibiotics medium

Antibiotic discs were prepared using a 6mm diameter paper perforating machine on Whatman No. 1 filter paper. 0.1ml of extract was dropped on a sterilized 7mm 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 to avoid contamination and then saved in the refrigerator

The antibiotic used was 500 mg Ampiclox (broad spectrum antibiotic) and the media were multipurpose (nutrient agar) for *Staphylococcus aureus,* macConkey agar for *Eschericia coli* and Chocolate dextrose agar for *Candida albican.* The 500 mg of ampiclox capsule was dissolved in 500 ml of water giving out 1g/ml (Prescott *et al*., 2008)

Antimicrobial screening of the extract

Filter paper disk method previously described by Benson (2002), with modification from Oguntoye *et al*. (2008), Kigigha and Onyema (2015), Kigigha and Atuzie (2012), Kigigha and Charlie (2012) and Kigigha *et al*. (2016b) was employed for the antimicrobial sensitivity testing. Nutrient agar was prepared according to the manufacturer's instruction. The liquefied agar was placed in water bath at 50ºC to cool. The test organisms were labeled in the bottom. Then about 20ml of the agar was poured on sterile Petri dish and allowed to solidify. Sterile disk of 7mm in diameter was dipped into the plant extract. The impregnated disk was placed in the solidified agar using forceps. The edge of the forceps was used to press down the disk lightly. A positive control was set up with 1% Ampiclox. The plate was incubated at 37ºC for 48 hours there after the resultant zone of inhibition was measured on the bottom of the plate using meter rule.

Phytochemical screening of the seed

Phytochemical analysis including Saponins, Cardiac glycosides, alkaloids, flavonoids and tannins were determined using the scheme provided by Sofowora (1993), Harborne (1973), Trease and Evans (1985), Okwu (2005), Doherty *et al*. (2010), Chiejina and Ukeh (2012), Epidi (2016).

Statistical analysis

Statistical analysis was carried out using SPSS software version 20. A two-way analysis of variance was carried out at P = 0.05, and Duncan statistics was used to separate the means. The charts with standard error bars chart was plotted at one sigma at 95% confidence interval using PAST statistical software (Hammer *et al*., 2001).

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