

Full Length Research Paper

Phytochemical Profiling and Antibacterial Efficacy of *Cucurbita pepo* and *Brassica nigra* Seed Oils

R. K. Obi^{1*}, F. C. Nwanebu¹, U. U. Ndubuisi² and N. M. Orji³

¹Department of Microbiology, Federal University of Technology, Owerri, P. M. B. 1526, Owerri, Imo State, Nigeria.

²Department of Microbiology, University of Uyo, P.M.B. 1017, Uyo, Akwa Ibom State, Nigeria.

³Department of Biological Sciences, Anambra State University, Uli, P. O. Box 02, Uli, Anambra State, Nigeria.

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The oils of 2 seeds, *Brassica nigra* (black mustard) and *Curcubita pepo* (fluted pumpkin) obtained from St. Paul's catholic church, Owerri and Ihiagwa market respectively, were extracted using the Soxhlet extractor. The antibacterial activities of the extracted oils on 2 fresh hospital isolates, *Staphylococcus aureus* and *Escherischia coli* were determined using the Kirby Bauer disc diffusion method of sensitivity testing. Results obtained showed that the oil from *C. pepo* produced a higher zone of inhibition of 60% on *S. aureus* which is higher than that of the oil of *B. nigra* with a zone of inhibition of 40%. However, these 2 oil extracts showed similar inhibitory effects on *E. coli*. Results of phytochemical analyses of the extracts reveal that the oil from *C. pepo* contains tannins, flavonoids, saponins, cyanogenic glycosides and cardiac glycosides, while the oil from *B. nigra* consists of tannins, flavonoids and alkaloids. These seeds, apart from their roles as food additives and supplements, they may also be utilized as effective and cheap sources of antibacterial agents for the treatment of bacterial infections.

Key words: Oil, *Escherischia coli*, *Staphylococcus aureus*, antibacterial, *Curcubita pepo*, *Brassica nigra*.

INTRODUCTION

Bacterial infections contribute largely to general health problems of man and have been reported to be responsible for over 50% of deaths recorded in developing countries. This formidable threat posed by bacterial species appear not to have a solution in sight as some conventional antibacterial drugs have failed in their activity against the pathogens due to the development of drug resistance (Lamikanra, 1981; Cimanga et al., 1991). This increasing resistance to antibiotics has therefore resulted in the search for leads for new organic molecules from plants with antimicrobial properties (Cimanga et al., 1991).

The use of local plant extracts in the treatment of diseases is widespread in Nigeria today. In fact, there is a general belief that more than 60% of the population depend on traditional medicine for their health needs. Most traditional healers in the country employ a variety of plant extracts in concoctions as remedies for various ailment.

Claims of potency of the different plant extracts used in these therapies have generated increased interest in some herbs and seeds, leading to research and laboratory analysis of the extract from such plants (Ghani and Mbou, 1998).

In the search for drugs and new cures for human and animal ailments, medicinal plants are definitely the most popular targets. Consequently, all parts of the plant body and in fact, various plant products including their oils, juices and sap have been used in one form or the other in traditional and conventional medicine (Khan et al., 1988). This fact has been corroborated by Newell and Linda (1996) who reported that the antiseptic potentials of various products are found in their essential oils. Several other workers (Feldberg et al., 1988; Chowdhury et al., 1991; Weber et al., 1992) have also investigated the efficacy of the oil extracts of plants and their products and their findings have confirmed earlier reports made by previous workers. Consequently, oil extracts of seeds of 2 common, locally available medicinal plants, *Brassica nigra* commonly known for its spirituality in christianity and *Curcubita pepo*, much cherished because of the rich

*Corresponding author. E-mail: robertobi_2003@yahoo.co.

green vegetable it produces, were selected and investigated for the antibacterial potentials of their oil extracts.

B. nigra is a member of the *Brassicaceae* family that also includes *B. bois*, *B. hirta* and *B. juncea*. The seeds are globular, black and about 1mm in diameter. It has a pungent taste and rich nutty odor (www.ibiblio.org/lhtml/brassica). In addition to its importance as a food flavoring agent, the seeds of *B. nigra* also have important medicinal uses such as in treatment of rheumatism and joint pains, indurations of the liver and spleen, throat tumors and as a laxative (Gerald and Williams, 1989).

C. pepo is a member of the family of *Curcubitaceae* which also includes *C. maxima*, *C. mixta* and *C. moschata*. *C. pepo* (fluted pumpkin) is ovoid in shape with a curved green shell. Inside the shell is a flat, round, yellow and white seed enclosed in a husk. These seeds are chewable and sweet with a nutty flavor (www.botanical.org.pumpkin). Besides having important edible uses and another as an industrial food additive (www.botanical.org.pumpkin), *C. pepo* also has medicinal uses including antihelminthic as well as natural laxative. In addition it has been widely applied in the treatment of benign prostatic hyperplasia in men, obesity, skin problems and irritable bladder (enuresis) in children.

Hitherto, the main information about *B. nigra* (black mustard) around our part of the world is its connection with spirituality in christianity, while *C. pepo* has been known for its quality as an antihelminthic. However, information on the antibacterial qualities of the seeds of these plants is unavailable and justifies the need for this research.

MATERIALS AND METHODS

Preparation of plant samples

Healthy seeds of *B. nigra* (black mustard) and *C. pepo* (fluted pumpkin) were obtained from St. Paul's catholic church, Owerri, Nigeria and Ihiagwa market, Nigeria, respectively. A large quantity of the fluted pumpkin seed was decocted with a lancet and the naked seeds dried in the oven in the laboratory at 30°C for 2 h. Both the dried fluted pumpkin seeds and the mustard seeds were blended separately to a fine powder using Maulinex electric blender and preserved for extraction of the oil samples.

The oil extract of each of the plant samples was obtained using petroleum ether in continuous extraction in a Soxhlet reflux apparatus as described in earlier works (Reinhold, 1992; Ojiako and Nwanjo, 2006). On completion of extraction, the petroleum ether was completely evaporated through continuous heating of the oil and petroleum ether mixture as recommended by Reinhold (1992). The oil left after evaporation of the petroleum ether was the desired sample and each was then tested on the hospital isolates to determine their sensitivity.

Disc diffusion test

The level of sensitivity of the hospital isolates, *Staphylococcus aureus* and *Escherichia coli* was determined using the disc diffusion (Kirby Bauer) method as described by Nester et al. (2004). Circular paper discs measuring 7.0 mm were cut from Whatman No. 1 filter paper using a paper perforator and sterilized in an autoclave.

About 5 sterile discs were soaked in the oil and allowed to absorb the extract sufficiently. The extract-soaked discs were then used as the test "drugs".

Sterile nutrient agar plates were prepared and labeled in duplicates according to the test organisms. Each group of 2 plates was inoculated with each of the test organisms which were fully spread on the entire surface of the nutrient medium using the glass hockey stick spreader. Finally, the discs impregnated with the oil extracts were carefully placed into the culture plates and allowed to stand for a few minutes before being incubated for 18 h at 37°C. They were then examined for growth and signs of inhibition. A similar experiment was set-up but instead of the oil extracts, petroleum ether was used and this served as a control.

The zones of inhibition were determined by measuring the diameter of clearance across the disc, with a ruler. The measurements were aptly represented as +, ++, +++, which are equivalent to 0.2, 0.1 and 0.3 mm respectively.

Phytochemical analyses of herbal materials

Phytochemical analyses were carried out using the oil extracts as recommended by (Evans, 2002). The oil extracts were analyzed for possible presence/absence of tannins, flavonoids, saponins, cyanogenic glycosides, cardiac glycosides and alkaloids as in previous reports (Reinhold, 1992; Horbone, 1975).

RESULTS

Results of the disc diffusion test (Table 1) revealed that *S. aureus* was highly sensitive to the oil extract of *C. pepo* but only slightly sensitive to that of *B. nigra*. The results also showed that *E. coli* was sensitive to both oil extracts to equal degrees.

Percentage zone of inhibition as shown in Table 2 revealed that *B. nigra* and *C. pepo* oils produced equal zones of inhibition of 50% on *E. coli*. *C. pepo* oil produced a higher zone of inhibition of 60% and above, on *S. aureus*, while that of *B. nigra* produced activity of 40% on the same hospital isolate of *S. aureus*.

Results of phytochemical analyses (Table 3) revealed that the oil extract of both seeds contain tannins and flavonoids. While the oil extract of the seed of *B. nigra* alone contains alkaloids, that of *C. pepo* alone contains saponins, cyanogenic glycosides and cardiac glycosides.

DISCUSSION

Results of the disc diffusion tests (Table 1) showed that *S. aureus* was more sensitive to the oil extract of *C. pepo* (60%) than that for *B. nigra* which only slightly, (40%), inhibited the organism. The result of this test could mean that the oil extract of *C. pepo*, if properly processed, could be used to treat some stubborn *Staphylococcal* infections. A similar use of oil extract of both seeds could also be adopted in the treatment of infections due to *E. coli* as both extracts could inhibit growth (50%) of the organism efficiently. The fact that the extraction agent, petroleum ether, produced no visible sign of any activity against both hospital isolates could mean that petroleum ether made no contributions to the antimicrobial potency

Table 1. Disc diffusion test of the oil extracts on *S. aureus* and *E. coli*.

Sample	<i>S. aureus</i>	<i>E. coli</i>
<i>B. nigra</i> oil	+	++
<i>C. pepo</i> oil	+++	++
Petroleum ether	-	-

Key

+	=	30% - 44% inhibition
++	=	45% - 59% inhibition
+++	=	60% and above inhibition
-	=	0.00%/no inhibition

Table 2. Percentage (%) zones inhibition of oil extracts on *S. aureus* and *E. coli*.

Sample	<i>S. aureus</i> % zone of inhibition	<i>E. coli</i> % zone of inhibition
<i>B. nigra</i> oil	40	50
<i>C. pepo</i> oil	60	50

Table 3. Phytochemical analyses of oil extracts of seeds of *B. nigra* and *C. pepo*.

Sample	Tannins	Alkaloids	Cardiac glycosides	Flavonoids	Saponins	Cyanogenic glycosides
<i>B. nigra</i> oil	+	+	-	+	-	-
<i>C. pepo</i> oil	+	-	+	+	+	+

Key

+ = present
- = absent

of both oil extracts.

Phytochemical analyses as shown in Table 3 revealed that both oil extracts contain tannins and flavonoids which have been reported to be responsible for antimicrobial properties in some plant extracts and as a result, it could serve as antimicrobial agents for the treatment of microbial infections. In addition, the reported use of tannins to enhance the taste of foodstuff and beverages for human and animal consumption could underscore both oil extracts as potential flavoring agents in the food and beverage industries as well as in industries involved in the production of feed for herbivorous animals (Sofowora, 1982; Horbone, 1975). Besides tannins and flavonoids, the oil of *B. nigra* also contains alkaloids, a plant component, known for its antimicrobial potentials. The remaining three phytochemicals, saponins, cardiac glycosides and cyanogenic glycosides, were absent in the oil of *B. nigra* but present in that of *C. pepo*. Cardiac glycosides have been reported to have the medicinal value of helping to strengthen a failing heart (Horbone, 1975). In view of this, the oil of *C. pepo* could be administered to patients with a weak

heart to improve their condition. Cyanogenic glycosides, on hydrolysis, have been reported to release cyanic acid (Evans, 2002). Its continued presence in the extract could therefore cause the oil extract of *C. pepo* to be poisonous or toxic to use and this corroborates the report of Ojiako et al., (2007).

About one quarter of our present pharmaceuticals were originally derived from plant sources. Consequently there are numerous plant-derived agents that benefit health and eradicate or treat disease prompting the world health organization to estimate that about 80% of the earth's population uses some type or other of herbal remedy as their main form of medical treatment (<http://www.naturaldatabase.com/>). *B. nigra* and *C. pepo* oils could be added to this growing list of plant materials used for herbal remedies as our finding has shown.

Many traditional and attractive garden plants are indeed useful medicinal herbs, having antibacterial, antifungal, antiparasitic (<http://www.epocrates.com/>) and antiviral (Obi et al., 2006) properties. With our findings that the oils of *B. nigra* and *C. pepo*, 2 useful garden

plants are potential antibacterial agents, we have further established this fact.

B. nigra (black mustard), indeed, while servicing the need for spiritual growth among christians, could also be harnessed as a medicinal and food flavoring agent. Similarly, *C. pepo* which is commonly available in families within rural and urban settings in Nigeria has the most popular use attached to the seed is planting to generate rich green vegetables. This research has further established that these seeds possess antibacterial qualities. Their ready availability locally and at cheap, affordable prices, will surely enhance their application among other uses, as alternatives to antibiotics for effective treatment of bacterial infections.

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