



DETERMINATION OF THE PHYTOCHEMICAL CONSTITUENTS AND THE ANTIBACTERIAL ACTIVITY OF THE ROOT BARK EXTRACT OF *NEOCARYA MACROPHYLLA* (SABINE) PRANCE, AGAINST *KLEBSIELLA PNEUMONIAE*, AN EAR INFECTION-CAUSING PATHOGEN

*¹Datti, Y., ¹Musbahu, L., ¹Shuaibu, MM., ¹Abubakar, SA., ¹Salihu, I., ¹Isa, BK., ¹Usman, LA., ¹Nafisa, AM., ²Dikko MA. and ²Ahmed UU.

¹Department of Chemistry, Yusuf Maitama Sule University, Kano.

²Department of Science Laboratory Technology, Federal Polytechnic Nassarawa.

²Department of Science Laboratory Technology, Kano State Polytechnic.

*Correspondence Author email: vaudatti@gmail.com

ABSTRACT

Neocarya macrophylla root bark extract is a potential source of novel antimicrobial compounds, especially against *Klebsiella pneumoniae*, a bacterial pathogen. *N. macrophylla* is a medicinal plant commonly used in traditional medicine in Northern Nigeria to treat asthma, skin infections, treatment of wounds, pulmonary troubles, dysentery, inflammations and it is also used for the treatment of eye and ear infections. The susceptibility test results showed inhibition range of 15, 13, 11, 13 and 14mm for the NM, NM4, NM3, NM2 and NM1 respectively against test organism at 50mg/mL, and 09, 11, 09, 12 and 13mm for NM, NM4, NM3, NM2 and NM1 respectively at 25mg/mL, and finally -, 09, -, 09 and 09mm for NM, NM4, NM3, NM2 and NM1 respectively at 12.5mg/mL. This indicates NM, NM1, NM2 and NM4 to be the most active fractions against the organism at 50mg/mL in a respective manner and the least active fraction is NM3 at 25mg/mL, while fractions NM1, NM2 and NM4 showed no activity at 12.5mg/mL against the test organism. The test results indicated that root bark extract of *N. macrophylla* has antibacterial potency and could be used as an alternative antimicrobial therapy.

Keywords: *Neocarya macrophylla*, *Klebsiella pneumoniae*, Phytochemicals, Antibacteria.

INTRODUCTION

The rate at which life-threatening infections caused by pathogenic microorganisms is spreading throughout the world, and this is alarmingly becoming one of the major causes of morbidity and mortality especially in developing countries (Rahman *et al.* 2009). The prevalence of many strains of microorganism becoming highly resistant to many drugs is exponentially increasing the number of untreatable bacterial infections, and this necessitates the need for further researches with the view to finding new infection-fighting strategies (Shrutika *et al.* 2015). Recent studies have been highlighting the dangers and menace, as well as the socio-economic burdens of multi drug resistant bacteria in cosmopolitan cities (Shashikant *et al.* 2015). Therefore the need for research and development of new effective antimicrobial drugs cannot be overemphasized. Plant-based products represent an untapped source of new antimicrobials. The use of plant extract for medicinal treatment has become popular especially now when people are beginning to realize that the effective life span of antimicrobials is limited and over-prescription and misuse cause microbial resistance (Alam *et al.*, 2009). Medicinal plants produce a variety of compounds of known therapeutic properties (Harborne and Baxter, 1995). In recent years, antimicrobial properties of medicinal plants origin are being increasingly reported from different parts of the world (Grosvenor *et al.*, 1995; Ratnakar and Murthy, 1995; David, 1997; Saxena, 1997; Nimri *et al.*, 1999; Saxena and Sharma, 1999). One of such plants with high potentials to serve as an antimicrobial agent is *Neocarya macrophylla*.

N. macrophylla is one of the sources of under-explored oil seeds found in the wild and/or semi-cultivated in various part of the

World for its edible fruits (with a peculiar flavor sometimes likened to avocado) and peanut-like shaped kernels. The kernel is an excellent source of oil which is composed of oleic acid 40%, eleostearic acid 31%, linoleic acid 15%, palmitic acid 12% and stearic acid 2%. It also contains two phytosterols; parinicerium sterol A and B (Burkill, 1995) and some protein. It is traditionally used to treat numerous diseases which include: Asthma, skin infections, treatment of wounds, pulmonary troubles, dysentery, inflammations and it is also used for the treatment of eye and ear infections. (Halilu *et al.*, 2010).

MATERIALS AND METHODS

Collection of the Plant Materials

Root of *Neocarya macrophylla* was collected from Fitare, Kazaure Local Government, Jigawa State, North-Western, Nigeria. The plant was identified and authenticated (BS/176) by the Department of Biological Sciences, Yusuf Maitama Sule University, Kano, Nigeria.

Drying and Storage of the Sample

The root bark of *Neocarya macrophylla* was peeled off and then washed with running tap water and dried under shade for about 1 week. The dried sample was ground into fine powder with the aid of pestle and mortar. The root bark powder was stored in polythene bag until required for use.

Extraction Procedure

Extraction was carried out using maceration technique reported by Joshi *et al.* (2011) with slight modification. Hundred gram (100g) of the dried powdered sample was soaked in 300mL of

absolute ethanol in Winchester bottle, and was left to stay for seven days with constant shaking at regular intervals. The mixture was then filtered and concentrated using rotary evaporator to afford a reddish-brown residue (10g) subsequently referred to as the crude ethanol extract (NM). The percentage yield of the extract was calculated using the equation below:

$$\% \text{ yield of extract} = \frac{\text{weight of extract}}{\text{weight of sample}} \times 100$$

Fractionation of the Crude Extract

Portion of the crude ethanol extract was partitioned into different solvents in an increasing order of polarity (i.e *n*-hexane, chloroform, ethyl acetate and methanol) and labeled NM1, NM2, NM3 and NM4, respectively.

Phytochemical Analysis

All the fractions and the crude extract were phytochemically screened for the presence of secondary metabolites such as alkaloids, flavonoids, saponins, tannins, terpenoids and steroids in accordance with standard methods (Brain and Turner 1975; Harborne, 1975; Trease and Evans, 1989) with slight modification.

Detection of Alkaloids: Each fraction was dissolved in dilute Hydrochloric acid and filtered.

Mayer's Test: The filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide).

Detection of Flavonoids

Alkaline Reagent Test: The filtrates were treated with few drops of sodium hydroxide solution.

Detection of Saponins

Froth Test: The filtrates were diluted with distilled water to 5mL and this was shaken in a graduated cylinder for 5 minutes.

Detection of Phenols

Ferric Chloride Test: The filtrates were treated with 3-4 drops of ferric chloride solution.

Detection of Tannins

Lead acetate test: Few drops of 1% lead acetate were added to 2mL of each of the filtrates in a test tube.

Detection of Steroids

Chloroform (5mL) was added to 0.5mL of each of the filtrates in a test tube. Equal volume (5mL) of concentrated sulphuric acid was added by the sides of the test tube.

Antibacterial Screening

Bacterial Strain Used

Clinically isolated bacterium (*Klebsiella pneumoniae*) was obtained from Department of Medical Microbiology, Muhammad Abdullahi Wase Specialist Hospital, Nassarawa, Kano State, Nigeria. The isolate was cultured on nutrient agar slants using a sterile wire loop and incubated at 37°C for 24 hours and this served as the stock culture.

Determination of Antibacterial Activity (Disc Diffusion Method)

Nutrient agar plates were prepared and inoculated with test organisms by spreading the bacterial inoculum on the surface of the media using sterile swab. Discs (8mm diameter) were punched. Extracts of different concentrations (12.5, 25 and 50mg/mL) were impregnated on the discs. Ampicillin (50mg/mL) was used as a positive control. The plates were incubated at 37°C for 24 hours. The antibacterial activity was assessed by measuring the diameter of the zone of inhibition and recorded in mm.

RESULTS

The weights and the percentage yield of the extract is shown in Table 1, while Table 2 shows the appearance and colour of the extract and the fractions. The phytochemical constituents of the root bark extracts of the sample are presented in Table 3, and the susceptibility test of the microorganism against the plant extract.

Table 1: Weights and the Percentage Yield of the Extract

Part of Plant	Weight of Sample (g)	Weight of Extract (g)	Percentage Yield (%)
Root bark	100	10	10

Table 2: Appearance and Colour of the Extracts

Extracts	Appearance	Colour
Crude Ethanol Extract (NM)	Gummy	Dark brown
N-Hexane Fraction (NM1)	Gummy	Pale yellow
Chloroform Fraction (NM2)	Gummy	Brown
Ethyl Acetate Fraction (NM3)	Powdered	Reddish brown
Methanol Fraction (NM4)	Powdered	Reddish brown

Table 3: Phytochemical Constituents of the Root Bark of *Neocarya macrophylla*

Phytochemicals	NM	NM4	NM3	NM2	NM1
Alkaloids	+	+	+	+	-
Flavonoids	+	+	-	+	+
Saponins	+	+	+	-	-
Steroids	+	+	+	+	+
Phenols	+	+	+	+	+
Tannins	+	+	-	-	-

Key: NM = Crude extract, NM4 = methanol fraction, NM3 = ethyl acetate fraction, NM2 = chloroform fraction, NM1 = *n*-hexane fraction, + = present, - = absent

Table 4: Susceptibility Results of the Microorganism Against the Plant Extracts

Test Organism	Mean Zone of Inhibition (mm)														
	50mg/mL					25mg/mL				12.5mg/mL					
	NM	NM4	NM3	NM2	NM1	NM	NM4	NM3	NM2	NM1	NM	NM4	NM3	NM2	NM1
<i>K. pneumoniae</i>	15	13	11	13	14	09	11	09	12	13	-	09	-	09	09

Key: NM = Crude extract, NM4 = Methanol fraction, NM3 = Ethyl acetate fraction, NM2 = Chloroform fraction, NM1 = *n*-hexane fraction, - = No activity

Plate 1: Culture Plate Showing Bacterial Zones of Inhibition at Different Concentration of the Extracts

Key: A= 50mg/mL; B = 25mg/mL; C = 12.5mg/mL; D = Control

DISCUSSION

The beneficial medicinal effects of any plant material typically result from the secondary metabolites present in the plant. *Neocarya macrophylla* root bark extracts are potential sources of novel antimicrobial compounds, especially against bacterial pathogens. *N. macrophylla* is a medicinal plant commonly used in traditional medicine in Northern Nigeria to treat asthma, skin infections, treatment of wounds, pulmonary troubles, dysentery, inflammations and it is also used for the treatment of eye and ear infections. The antimicrobial activity of the plant extracts has been previously reviewed. According to Audu *et al.*, (2005), the ethyl acetate and hexane fractions of *N. macrophylla* showed antibacterial activity. *In vitro* studies in this work showed that the *N. macrophylla* root extracts inhibited bacterial growth. Phytochemical screening of the root bark of *N. macrophylla* in the present study revealed the presence of alkaloids, flavonoids, saponins, steroids, phenols and tannins in the crude ethanol extract while some phytochemicals are absent in the fractions as presented in Table 3. According to (Sharada *et al.*, 2008), Phenolic compounds like tannins and flavonoids have been reported to demonstrate antimicrobial activities.

Antibacterial activity of the fractions and the crude extract exhibited varying degree of antibacterial effect against the test organism in a concentration dependent manner and the solvents used for the extraction. The susceptibility test results showed inhibition range of 15, 13, 11, 13 and 14mm for the NM, NM4, NM3, NM2 and NM1 respectively against test organism at 50 mg/ml, then 09, 11, 09, 12 and 13mm for NM, NM4, NM3, NM2 and NM1 respectively at 25mg/mL, and finally -, 09, -, 09 and 09mm for the NM, NM4, NM3, NM2 and NM1 respectively

at 12.5mg/mL. This indicates NM, NM1, NM2 and NM4 to be the most active fractions against the test organism at 50mg/mL in a respective manner and the least active fractions are NM3 at 25mg/mL, and NM1, NM2 and NM4 12.5mg/mL. The test results indicated that root bark extract of *N. macrophylla* has antibacterial potency and could be used as an alternative antimicrobial therapy.

CONCLUSION

It can thus be concluded that the root bark extract of *N. macrophylla* is very active against *Klebsiella pneumoniae*. However, further research and investigation need to be directed towards extraction, isolation and elucidation of structure of the compound(s) that may be responsible for the reported bioactivity in the root bark extract of *Neocarya macrophylla*.

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