ChemSearch Journal 10(2): 41 – 45, December, 2019
Publication of Chemical Society of Nigeria, Kano Chapter

Received:13/09/2019 Accepted:24/10/2019
http://www.ajol.info/index.php/csj

Phytochemical Screening and Antibacterial activity of the Root Bark Extracts of *Neocarya macrophylla*

[1]Bayero, A. S., [1*]Datti Y., [1]Shuaibu, M. M., [1]Nafisatu, A. M., [1]Asma'u, A. A., [2]Dikko, M. A., [2]Zakari, A. H. and [2]Yusuf, M.

[1]Department of Chemistry, Yusuf Maitama Sule University, Kano.
[2]Department of Science Laboratory Technology, Federal Polytechnic Nassarawa
***Correspondence Email:** yaudatti@gmail.com

ABSTRACT

Neocarya macrophylla is a medicinal plant commonly used in traditional medicine in Northern Nigeria to treat asthma, skin infections, treatment of wounds, dysentery, pulmonary troubles and a number of inflammations, as well as treatment of eye and ear infections. In this work, the root back extracts of *Neocarya macrophylla* were screened for phytochemical constituents as well as the antibacterial activity against *Escherichia coli*, an ear infection-causing pathogen. The results revealed that some of the common phytoconstituents are present in most of the extracts. The susceptibility test results gives inhibition range of 13, 13, 13, 14 and 13 mm for the crude extract (NM), methanol extract (NM4), ethyl acetate extract (NM3), chloroform extract (NM2) and *n*-hexame extract (NM1) respectively against test organism at 50 mg/ml. The test results also showed inhibition range of 12, 11, 11, 12 and 12 mm for NM, NM4, NM3, NM2 and NM1 respectively at 25 mg/ml. Lastly, the results showed inhibition range of 11, 10, 09, 10 and 11 mm for NM, NM4, NM3, NM2 and NM1 respectively at 12.5 mg/ml. This indicates that NM2 is the most active fraction against the organism at 50 mg/ml, and the least active fraction was NM3 at 12.5 mg/ml. The test results also show that the root back extract NM2 has the potentials of providing the active components that could be developed into new antibacterial agents.

Keywords: Antibacterial activity, *Escherichia coli*, *Neocarya macrophylla*, Phytochemicals

INTRODUCTION

The rate at which life-threatening infections caused by pathogenic microorganisms is spreading is increasing throughout the world. This trend 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 today 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 multidrug-resistant bacteria in cosmopolitan cities (Shashikant *et al.* 2015). Therefore the need for research and development of new effective antimicrobial drugs cannot be overemphasized.

The use of plant extracts 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). Many traditionally used medicinal plants produce a variety of different compounds of known therapeutic properties. Specifically, medicinal plants with antimicrobial properties have been 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 the high potentiality of serving as an antimicrobial agent is *Neocarya macrophylla*.

Neocarya macrophylla is a genus of plant in family *Chrysobalanaceae*, and it is native to western and central Africa from Senegal to the Nigeria, and also in South Sudan. It is a small, bushy, evergreen tree growing up to 10 metres tall. It has a gnarled bole and a rounded, bushy crown with densely tomentose branchlets. The kernel of this seed is an excellent source of oil which is reported to compose of linoleic acid 15%, oleic acid 40%, eleostearic acid 31%, stearic acid 2% and palmitic acid 12%. It also contains two phytosterols, namely parincerium sterol A and B (Burkill, 1995) and some proteins. *N. macrophylla* is traditionally used to treat numerous diseases which include, asthma, skin infections, pulmonary troubles, dysentery, treatment of wounds, inflammations and ear infections (Halilu *et al.*, 1995).

The aim of this research is to determine the phytochemical constituents as well as the antibacterial potency of the root bark extracts of

Neocarya macrophylla against *Escherichia coli*, an ear infection-causing pathogen.

MATERIALS AND METHODS

Collection and Authentication of Plant Materials

Neocarya macrophylla plant was collected from Fitare village, Kazaure Local Government, Jigawa State, North-Western, Nigeria. The plant was identified and authenticated at the Department of Biological Sciences, Yusuf Maitama Sule University, Kano, Nigeria.

Extraction Procedure

The root bark of *Neocarya macrophylla* was peeled off and then washed with running tap water and dried under shade for approximately 1 week. The dried sample was then ground into a fine powder with the aid of pestle and mortar. To obtain the crude extract maceration technique was adopted as reported by Joshi *et al.* 2011 with a slight modification. Thus 100 g of the dried powdered sample was soaked in 300 ml 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 a rotary evaporator to afford a reddish-brown residue (10 g) subsequently referred to as the crude ethanolic extract (NM). The percentage yield of the extract was calculated using the equation below:

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

Fractionation of the Crude Extract

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

Phytochemical Analysis

The crude extract as well as the fractions were phytochemically screened fort the presence of secondary metabolites such as alkaloids, flavonoids, saponins, tannins, terpenoids, and steroids in accordance with standard methods (Tripathi and Mishra 2015) with slight modification.

Test for Alkaloids:

Each fraction was dissolved in dilute 1% hydrocloric acid and filtered.

Mayer's Test: A portion of the sample (1 ml) was treated with a few drops of Mayer's reagent (potassium iodide). Formation of white or pale yellow precipitate indicates the presence of alkaloids.

Test for Flavonoids:

Alkaline Reagent Test: A fraction of the sample was treated with a few drops of 1% sodium hydroxide. Formation of yellow colour indicates the presence of flavonoids.

Test of Saponin:

Froth Test: A portion of the extract was diluted with distilled water to 5 ml, and this was shaken in a graduated cylinder for 5 minutes. Formation of honeycomb froth confirms the presence of saponin.

Test for Tannins:

Lead Acetate Test: A portion of the filterate (2 ml) was treated with 3-4 drops of 1% lead acetate in a test tube. Formation of a blue-black colour indicates the presence of tannins.

Test for phenols:

A portion of extract was treated with 3-4 drops of ferric chloride ($FeCl_3$) solution. Formation of a blue-green colour confirms the presence of phenols.

Test for Steroids:

Salkowski Tests: Chloroform (5 ml) was added to 0.5ml of each of the filtrates in a test tube. An equal volume of concentrated sulphuric acid (5 ml) was added by the sides of the test tube. Formation of a red colour on standing indicates the presence of steroids.

ANTIBACTERIAL SCREENING

Bacterial Strain Used

Clinically isolated bacterium (*Escherichia coli*) was obtained from the 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 ^{O}C for 24 hours, and this served as the stock culture (Sanders, 2012).

Determination of Antibacterial Activity (Disc Diffusion Method)

The nutrient agar plates were prepared and inoculated with the test organisms by spreading the bacterial inoculum on the surface of the media using sterile swab. Discs (8mm in diameter) were punched and soaked into the DMSO solutions of the extracts of different concentrations (12.5 mg/ml, 25 mg/ml and 50 mg/ml). To serve as a positive control, 50 mg/ml of ampicillin disc was used. 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 millimeter.

RESULTS AND DISCUSSION

The physical states of the extracts are given in Table 1. While the phytochemical constituents detected and the zones of inhibition of the bacterial growth are presented in Table 2 and 3 respectively. Phytochemical screening of the root bark of *Neocarya macrophylla* in the present study revealed the presence of alkaloids, flavonoids, saponins, steroids, phenols and tannins in the crude ethanol extract while some chemicals are absent from the fractions as shown in Table 3. According to Sharada *et al*., (2008), phenolic compounds like tannins and flavonoids have been reported to show 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. The solvent type used for the extraction also played a major role. The susceptibility test results showed inhibition range of 13, 13, 13, 14 and 13 mm for the NM, NM4, NM3, NM2, and NM1 fractions respectively against test organism at 50 mg/ml. Also 12, 11, 11, 12 and 12 mm mean zone of inhibition was observed for NM, NM4, NM3, NM2 and NM1 fractions respectively at 25 mg/ml. Finally 11, 10, 09, 10 and 11 mm mean zone of inhibition was observed for NM, NM4, NM3, NM2 and NM1 fractions respectively at 12.5 mg/ml. The results indicate that NM2 fraction is the most active fraction against the test organism at 50 mg/ml and the least active fraction was NM3 fraction at 12.5 mg/ml.

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	NM1	NM2	NM3	NM4
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)														
	50 mg/ml					25 mg/ml					12.5 mg/ml				
	NM	NM4	NM3	NM2	NM1	NM	NM4	NM3	NM2	NM1	NM	NM4	NM3	NM2	NM1
E. coli	13	13	13	14	13	12	11	11	12	12	11	10	09	10	11

Key: NM = Crude extract, NM4 = Methanol fraction, NM3 = Ethyl acetate fraction, NM2 = Chloroform fraction, NM1 = *n*-hexane fraction

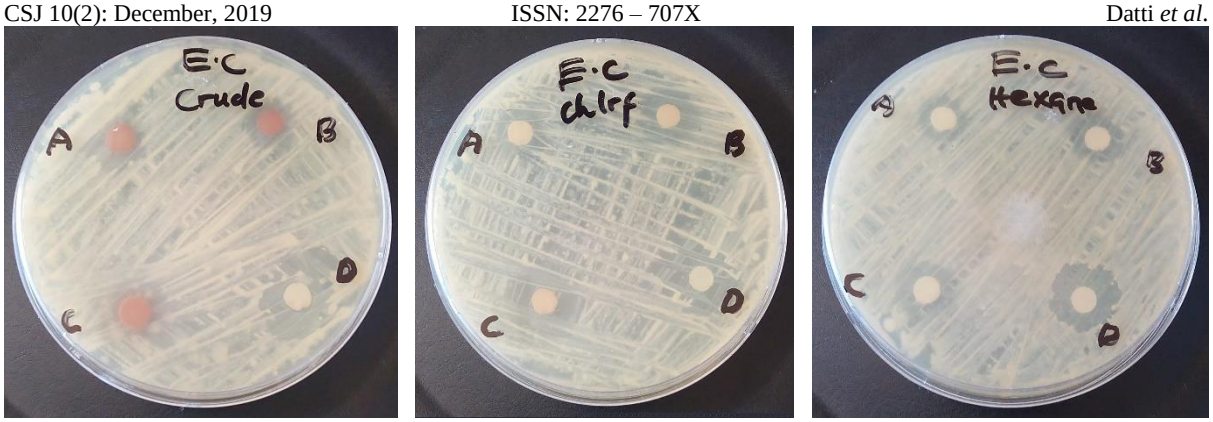

Figure 1: Culture Plate Showing Bacterial Zones of Inhibition at Different Concentration of the Extracts
Key: A= 50 mg/ml; B = 25 mg/ml; C = 12.5 mg/ml; D = Control

CONCLUSION

It can thus be concluded that the root bark of *N. macrophylla* is very active against *Escherichia coli*. However, further research and investigation need to be directed towards extraction, isolation, and elucidation of the structure of the phytochemical compounds that are responsible for the reported bioactivity in the root bark of *Neocarya macrophylla*.

REFERENCES

Alam, M.T., Karim, M.M., and Khan, S.N. (2009). Antibacterial Activity of Different Organic Extracts of *Achyranthes aspera* and *Cassia alata*. *Journal of Science Res.* 1:393-398.

Audu, O.T., Oyewale, O, and Amupitan J.O. (2005). The Biological Activities of Secondary Metabolites of *Parinari macrophylla*-Sabine. *Chemclass Journal*. 2:19-21.

Burkill, H.M. (1995). The Useful Plants of West Tropical Africa. 2nd Edition. Volume 3, Families J–L. Royal Botanic Gardens, Kew, Richmond, United Kingdom. P857.

David, M. (1997). Antimicrobial Activity of Garlic. *Antimicrob. Agents and Chem.* 41:2286.

Grosvenor, P.W., Supriono, A. and Gray, D.O. (1995). Medicinal Plants from Riau Province, Sumatra, Indonesia. Part 2, Antibacterial, and antifungal activity. *Journal of Ethnopharm* 45:97–111.

Halilu ME, Abah JO, Almustapha NL and Achor M. (2010) Phytochemical Screening and Mineral Element Analysis of the Root Bark of *Parinari macrophylla sabine* (chrysobalanaceae) and its Effect on Microorganisms. *Continental Journal of Biological Sciences* 3:46–50.

Nimri, L.F., Meqdam, M.M., and Alkofahi, A. (1999). Antibacterial Activity of Jordanian Medicinal Plants. *Pharm. Biol*.37 (3):196–201.

Rahman, M. M., Sheikh, M. M. I., Sharmin, S.A., Islam, M. S., Rahman, M. A., Rahman, M. M., and Alam, M. (2009). Antibacterial Activity of Leaf Juice and Extracts of *Moringa oleifera* Lam. against Some Human Pathogenic Bacteria. *CMU Journal of Natural Science* 8(2):219.

Ratnakar, P., and Murthy, P.S. (1995). Purification and Mechanisms of Action of Antitubercular Principle from Garlic (*Allium sati*6*um*) against Isoniazid Susceptible and Resistant *Mycobacterium tuberculae* H37RV. *Ind. J. Clinic. Biochem* 10:14–18.

Sanders, E.R. (2012): Aseptic Laboratory Techniques: Plating Methods. *Journal of Visualized Experiments* 63:3064

Saxena, V.K., and Sharma, R.N. (1999). Antimicrobial Activity of Essential Oil of *Lantana aculeata*. *Fitoterapia* 70(1):59–60.

Saxena, K. (1997). Antimicrobial Screening of Selected Medicinal Plants from Ind. *J. Ethnopharm* 58(2):75–83.

Sharada L.D., Khadabadi S.S., Lalita B. and Ghorpada K. (2008). In vitro Antimicrobial and Antioxidant Studies on *Enicostemma axillare* (Lam) Raynal leaves. *Natural Product Radiance*, 7(5):409- 412.

Shrutika, W., Sanap, S., Mukadam, T., Vaidya S., and Chowdhary A. (2015). Prevalence of Candidiasis in Children in Mumbai. *LIFE: International Journal of Health and Life-Sciences* (Special Issue):25-36.

Shashikant, V., M. Shreyasi, K. Mohan and K. Geeta (2015). "To Study the Incidence of Multi Drug-Resistant Tuberculosis in Mumbai." *LIFE: International Journal of Health and Life-Sciences* (Special Issues):122-138.

Tripathi, I.P. and Mishra, C. (2015): Phytochemical Screening of Some Medicinal Plants of Chitrakoot Region. *Indian Journal of Applied Research* 5(12):56-60.