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

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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, 14 and 13 mm for the crude extract (NM), methanol extract (NM4), ethyl acetate extract (NM3), chloroform extract (NM2) and n-hexane extract (NM1) respectively against test organism at 50 mg/ml. The test results also showed inhibition range of 12, 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, 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 bark 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-preservation 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; 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%, elaeostearic acid 31%, stearic acid 2% and palmitic acid 12%. It also contains two phytosterols, namely parinenser 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:

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

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 for 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% hydrochloric 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 for 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:
Lead Acetate Test: A portion of the extract was treated with 3-4 drops of ferric chloride (FeCl₃) 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 °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, 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>
<thead>
<tr>
<th>Part of Plant</th>
<th>Weight of Sample (g)</th>
<th>Weight of Extract (g)</th>
<th>Percentage Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root bark</td>
<td>100</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

Table 2: Appearance and Colour of the Extracts

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Appearance</th>
<th>Colour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude Ethanol Extract (NM)</td>
<td>Gummy</td>
<td>Dark brown</td>
</tr>
<tr>
<td>N-Hexane Fraction (NM1)</td>
<td>Gummy</td>
<td>Pale yellow</td>
</tr>
<tr>
<td>Chloroform Fraction (NM2)</td>
<td>Gummy</td>
<td>Brown</td>
</tr>
<tr>
<td>Ethyl Acetate Fraction (NM3)</td>
<td>Powdered</td>
<td>Reddish-brown</td>
</tr>
<tr>
<td>Methanol Fraction (NM4)</td>
<td>Powdered</td>
<td>Reddish-brown</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>NM</th>
<th>NM1</th>
<th>NM2</th>
<th>NM3</th>
<th>NM4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phenols</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Test Organism</th>
<th>50 mg/ml</th>
<th>25 mg/ml</th>
<th>12.5 mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>NM</td>
<td>NM4</td>
<td>NM3</td>
</tr>
<tr>
<td>13</td>
<td>13</td>
<td>13</td>
<td>14</td>
</tr>
</tbody>
</table>

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


