Full Length Research Paper

Phytochemical screening and antibacterial activity of Parinari curatellifolia stem extract

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Microorganisms are increasingly developing resistance against commonly used antimicrobial agents, and the use of herbs in the treatment of diseases is becoming widely accepted. The aqueous and organic solvent extract of the stem of Parinari curatellifolia were screened for phytochemical and antibacterial activity by hole in plate bioassay procedure. The bacterial activity of aqueous extract of this plant was carried out against several bacteria Pseudomonas aeruginosa, Salmonella typhi, Klebsiella spp, Bacillus subtilis and Staphylococcus aureus. Phytochemical constituents present in the extract were found to include saponins, balsams, carbohydrate, alkaloids tannins, cardiac glycosides, flavonoids, digitalis glycosides, phenol, terpenes and steroids. Water (w) fraction at 2.8 g/100 ml showed significant (P < 0.05) inhibitory activity against all the species of microorganism tested in this study and most effective against S. aureus and Klebsiella spp. (P < 0.05). Methanol Extract (ME) fraction at the same concentration was effective (P < 0.05) on B. subtilis and P. aeruginosa. Diethyl and n-hexane extract did not show any antimicrobial activity (P < 0.05). The results shows that different solvent extracts of same plant may have different pharmacological properties.

Key words: Parinari curatellifolia stem extract, phytochemical constituents, antibacterial activity, zone of inhibition.

INTRODUCTION

The use of plants and their extracts for healing by native doctors, fetish priest and other specialists was the main method for treating various illnesses before the advent of modern medicine in rural areas; hence, the reliance on traditional medicine is high and is attributed to both economic and cultural factors (Aketch, 1992). In recent years, it has been indicated that medicinal plants and naturally occurring bioactive compounds from medicinal plants have been used as chemotherapeutic agent for various ailments without scientific validation (Fransworth, 1994). From an economic point of view the high cost of imported conventional drugs and/or inaccessibility to western health care facilities, implies that traditional mode of health care is only form of health care that is affordable and available to the rural people (Mungati, 1997) and as a result, traditional medicine usually exist side by side with western forms of health care (Sindiga, 1994). Herbal medicine whose bioactive components are unknown can be subjected to scientific investigations and adopted for treatment of infectious diseases (Hogreu, 1999). It has therefore become necessary to identify the phytochemical components of local medicinal plants usually employed by herbalist in the treatment of diseases especially with advocacy for the integration of traditional medicine in health care programmes in Nigeria (Aderotimi and Samuel, 2006). Parinari curatellifolia belongs to the rosaceace family and to the Rosales order. Rosaceae consist of about 100 genera and 2000 species of herbs and trees (Trease and Evans, 1989). P. curatellifolia plant is grown in Zuru and its environs in
The medicinal uses of the extract plant of this are numerous. The fruits extract of the plant are used as cardiac tonic, used for organic and functional heart diseases such as hypertension, dyspores as well as diuretic, the leaves are used as expectorant, sedative and is apply to heal inflammation and anemia.

Traditionally the bark is used for washing clothes, for vaginal douches, itchy scalp, dandruff and, cough (Qasem and Abublan, 1996). As a shrub, claimed to possess antimicrobial activities as a shrub are some common bacteria and fungi to determine the potency of the plant is much desired. It is in view of this that the study is undertaken to investigate the phytochemical constituents and the potential of *P. curatellifolia* aqueous and organic extracts for antimicrobial activity on some selected bacteria. In the present study, water, methanol, n-hexane and diethyl ether crude extracts were tested for antimicrobial activity on some selected bacteria.

### MATERIALS AND METHODS

**Plant material**

The stems of *P. curatellifolia* were collected from Zuru, in Southern part of Kebbi State Nigeria. The stems were taken down to the Usmanu Danfodiyo University, Sokoto, Nigeria. Department of Biological Sciences herbarium where they were identified. The stems were room dried and pulverized into powdered. The powder was subjected to aqueous and organic solvent extraction (Matawali et al., 2004).

**Preparation of extract and fractional procedure**

The stems of *P. curatellifolia* were fractionated by activity guided fraction using ethanol water (1.1) and different (hexane, petroleum ether and chloroform) organic solvents. The powdered extracts of stems (120 g) were extracted with ethanol water (1.1, 500 ml) (separately) at room temperature for 72 h, using soxhlet extractor (Springfield and Weitz, 2006). The extracts were filtered and evaporated to dryness using a rotary evaporator. The percentage yield residues were 12.56, 8.73, 5.26 and 7.06% (w/w), respectively.

**Phytochemical screening**

The phytochemical screening of *P. curatellifolia* stem extract has shown that the stem contains saponins, flavonoid, alkaloid, cardiac glycosides, tannins steroid, carbohydrate, balsams and terpenes in the water extract, diethyl ether extract and n-hexane extract. Which are very important constituent when looking for pharmacologically active phytochemicals in the plant (Table 3). This was carried out using standard procedure as described by Wall et al. (1954), Trease and Evans (1978) and El-Olemyl et al. (1994).

<table>
<thead>
<tr>
<th>Extracting solvent</th>
<th>Color and texture of crude extract</th>
<th>Percentage recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>Brownish only semi solid</td>
<td>15.07</td>
</tr>
<tr>
<td>Methanol</td>
<td>Dark-brown sticky semi-solid</td>
<td>10.47</td>
</tr>
<tr>
<td>n-hexane</td>
<td>Dark-green semi solid</td>
<td>6.31</td>
</tr>
<tr>
<td>Diethyl ether</td>
<td>Black shiny solid</td>
<td>8.47</td>
</tr>
</tbody>
</table>

### Microorganisms tested

The following bacterial cultures were used: *Pseudomonas aeruginosa*, *Salmonella typhi*, *Klebsiella spp.*, *Bacillus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus* were obtained from microbiology department, Usmanu Danfodiyo University Teaching Hospital (UDUTH) Sokoto and reidentified according to the method of cowas and steel (1992).

**Antibacterial testing**

Antibacterial testing was done by agar diffusion method as described by Hugo and Russell (1983) and Vlietinck et al. (1995). The microorganisms were cultured overnight at 37°C in nutrient agar suspension of the bacteria with an optical density of Mc Far lend 0.5 was made in isotonic sodium chloride solution. The suspension of the bacterium test growth in nutrient broth was pipetted in to Muller Hinton agar plates. The prepared culture plates were inoculated with different selected strains of bacteria using streak plate method wells were made on the agar surface with 6 mm cork borer. The concentrations of 10 to 20 mg/ml of the extract were poured in to well using sterile syringe. After incubation for 24 h at 37°C, the plates were observed for zone of inhibition and the diameter of these zones measured in millimeters including the well diameter. The readings were taken in three different fixed directions in all 3 replicates and average values were tabulated.

**Microbial activity**

The results of antimicrobial assay of the stem extracts of *P. curatellifolia* indicated that the plant exhibited antimicrobial activity against the tested microorganisms at concentration of 2.8 g/100 ml of four different stem extracts. The potential sensitivity of stem extracts were obtained against all the seven microorganisms tested and the zone of inhibition was recorded and presented in (Tables 1 and 2).

### DISCUSSION

In the present investigation, the active components of *P. curatellifolia* was studied and further the antimicrobial activity of the stems extract of water, methanol, it hexane and diethyl ether crude extracts were tested against some selected pathogenes, microorganisms such as *P. aeruginosa*, *S. typhi*, *klebsella* spp. *B. aureus*, *E. coli*, *B.*
The phytochemical screening of the stem water extract of *P. curatellifolia* showed the presence of saponins, flavonoids, balsams, carbohydrates, steroids, terpenes, alkaloids, tannins, and digitalis glycosides. While the methanol extract contains saponins, flavonoids, balsams, alkaloids, tannins, phenol, and digitalis glycosides, the diethyl ether extract contains flavonoids, balsams, alkaloids, tannins, phenol, and digitalis glycosides, and the n-hexane extract contains flavonoids, balsams, tannins, and digitalis glycosides. Alkaloid is a plant-derived compound that is toxic or physiologically active, contains nitrogen in a heterocyclic ring with complex structure. Alkaloids are formed as metabolic byproducts and have been reported to be responsible for the antibacterial activity (Doughari, 2006).

This is consistent with the reports of the De et al. (1999) of which 35 different Indian spices and herbs indicated that *Tamarindus indica* pulp among others had potent antimicrobial activities against the test organism's *B. subtilis, E. coli* and *Saccharomyces, cerevisiae*. Many phytochemicals are present in plants as glycosides (with a sugar moiety attached). Generally, glycosides are non-volatile and lack fragrance cleaning the glycosidic bond yields the glycon, which itself may be volatile and fragrant. Glycosides serve as defense mechanisms against predation by microorganisms, insects, and herbivorous (De et al., 1999). This may therefore explain the demonstration of antimicrobial activity by the stem extracts of *P. curatellifolia* (Doughari, 2006). It is probable that the antibacterial agents in the extract of *P. curatellifolia* act by inhibition of nucleic acid, protein, and membrane phospholipids biosynthesis (Franklin et al., 1989). The result of the study using water and methanol extracts of *P. curatellifolia* showed significant (P < 0.05) inhibitory activity on some isolates. Water extract showed inhibitory activities to all the isolates and was most effective on *S. aureus* among the gram positive bacteria and on *klebsiella spp* among the gram negative bacteria. The methanol extract of the same plant was not effective (P < 0.05) on *B. subtilis* among the gram positive bacteria and on *P. aeruginosa* among the gram negative bacteria. Extract of diethyl ether and n-hexane of the plant did not show any antimicrobial activity (Table 2). This, confirming the assertion that different solvent extracts of same plant have different pharmacological properties. Water is the commonly used solvent by traditional healers to extract pharmacologically active compounds because of its easy availability (Shale et al., 1999). From this study we can conclude that the stem extracts of *P. curatellifolia* showed significant antimicrobial activity. The stem extracts contains alkaloids flavonoids and glycosides, these chemical may be responsible for antimicrobial activity. These medicinal plants should be scientifically evaluated for the treatment of infectious diseases.

**Table 2.** Antibacterial activity of stem extracts of *P. curatellifolia*.

<table>
<thead>
<tr>
<th>S/No.</th>
<th>Name of the organism</th>
<th>Concentration of stem extracts added and Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Water extract (2.8 g/100 ml)</td>
</tr>
<tr>
<td>1</td>
<td><em>P. aeruginosa</em></td>
<td>25</td>
</tr>
<tr>
<td>2</td>
<td><em>S. typhi</em></td>
<td>27</td>
</tr>
<tr>
<td>3</td>
<td><em>klebsiella spp</em></td>
<td>28</td>
</tr>
<tr>
<td>4</td>
<td><em>B. aureus</em></td>
<td>25</td>
</tr>
<tr>
<td>5</td>
<td><em>E. coli</em></td>
<td>20</td>
</tr>
<tr>
<td>6</td>
<td><em>B. subtilis</em></td>
<td>29</td>
</tr>
<tr>
<td>7</td>
<td><em>S. aureus</em></td>
<td>36</td>
</tr>
</tbody>
</table>

**Table 3.** Phytochemical Screening of *P. curatellifolia* extract.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Sap</th>
<th>Bal</th>
<th>Ch</th>
<th>Alk</th>
<th>Ta</th>
<th>Cg</th>
<th>Fl</th>
<th>Dg</th>
<th>Phe</th>
<th>Ter</th>
<th>St</th>
</tr>
</thead>
<tbody>
<tr>
<td>W</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>ME</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>DE</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>nH</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

- = Absence, + = presence, W = Water, ME = Methanol, DE = Diethyl ether, nH = n-hexane, SAP = Saponins, Bal = Balsams, Ch = Carbohydrate, Alk = Alkaloid, Ta = Tannins, Cg = Cardiac glycosides, Fl = Flavonoids, Dg = Digitalis glycosides, Phe = Phenol, Ter = Terpenes, St = Steroids.
REFERENCES