Antimicrobial Activity of Four Medicinal Plants Used by Sudanese Traditional Medicine

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Abstract—This study described chemical and biological screening of crude ethanolic extract from four species, Ozoroa insiginis Del., Oliv; (Anacardiaceae), Ximenia americana L.; (Olacaceae), Boscia salicifolia Oliv; (Capparidaceae), and Terminalia brownii Fresen; (Combretaceae). The four species were selected for this study according to ethnobotanical literature and their significant as traditional medicine practiced worldwide in Alangasana area, in West of Sudan. The four species revealed high availability of tannins, saponins, steroids, flavonoids, and terpinoids. Alkaloids were present only in O. insiginis. The antimicrobial activity of the ethanolic extracts from the barks of the four species was performed to determine quantitatively the presence or absence of inhibition zones, and measurement of zone diameter. O.insiginis extract showed good activity against Pseudomonas aeruginosa. Whereas X. americana extract exhibited significant activity against Staphylococcus aeruginos and low activity against Pseudomonas aeruginos. Consequently T. brownii showed significant activity against Staphylococcus aureus, and good activity against Pseudomonas aeruginos. B. salicifolia exhibited no activity against all types of tested bacteria.

Index Terms—Phytochemical, Antibacterial, Ximenia americana, Ozoroa insiginis, Terminalia brownii.

I. INTRODUCTION

For many centuries, plants have been main source for drug development. Human use of plants as medicinal agents predates recorded history [1]. Ethnomedical plant-use data in many forms has been heavily utilized in the development of formularies and pharmacopoeias, providing a major focus in global health care, as well as contributing substantially to the drug development process [2]. Medicinal plants contain physiologically active principles that over the years have been exploited in traditional medicine for the treatment of various ailments [3] as they contain anti-microbial properties [4]. These medicinal herbs constitute indispensable components of the traditional medicine practiced worldwide due to the low cost, easy access and ancestral experience [5].

In Sudan, people have been tapping their herbal remedies for medication for time immemorial. For this purpose they use a vast variety of plants ranging from the rain forest vegetation in the south, to the desert vegetation of the north, and from the semi-Mediterranean climatic zone of the red sea, to the rich savanna of the west [6]. Sudan folklore-medicine represents a unique blend of indigenous cultures with Egyptian, Indian, Arabian, East and West African cultures. This in view of a number of factors, such as draught, desertification, expansion of agricultural schemes and the introduction of health services to primitive areas, which initiated astonishingly rapid changes, leading to the least use of native medicines, which would eventually disappear [7].

Four Sudanese plants were selected for the present study namely (B. salicifolia, O. insiginis, T. brownii, and X. americanaL;) respectively (Figs: 1, 2, 3, and 4). The field of the study was Alangasana Mountains in Eastern South of Sudan. Selection of the plant samples based on the limited use of these plants in early research study and their traditional uses in treatment of many diseases.

The aim of this study was to carry out preclinical evaluation of some popular medicinal plant species, i.e., biological and phytochemical screening, with particular emphasis on those that seem to have very little or no scientific information in the areas intended for the investigation. It is also hoped that the study will facilitate the selection, for further investigation, of plants with relatively high level of potency and/or with wide range of biological activities.

It is believed that plants which are rich in a wide variety of secondary metabolites, belonging to chemical classes such as tannins, terpenoids, alkaloids, polyphenols are generally superior in their anti-microbial activities [8]. This suggests that the strength of biological activities of a natural product is dependent on the diversity and quantity of such constituents. Therefore, simultaneous determination of the compounds that is possibly responsible for any biological activity would, inter
alia, facilitate decision-making process as in the selection of the plants for in-depth future investigation. In view of this, we have also undertaken chemical screening of all the plants that were subjected to the biological screening.

Fig 1: Boscia salicifolia Oliv.

Fig 2: Ozoroa insignis Del.

Fig 3: Terminalia brownii Fresen.

Fig 4: Ximenia americana L

II. MATERIALS AND METHODS
Bark of four plants (B. salicifolia, O. insignis, T. brownie Fresen, and X. americana L;) were collected from Alangasana area, were identified and authenticated by Professor Syadat, Department of Botany, Khartoum University. The specimens were deposited at the same department. The bark was carefully peeled, chopped into pieces and shade dried. These plant samples were extracted with aqueous ethanol (80 %). (0.5Kg of the bark of each sample was macerated in aqueous ethanol for three days) .The extracts were filtered, concentrated, dried, and weighed (Table 1). The extracts obtained were used for further tests.
**Phytochemical screening**

Chemical tests were carried out on the aqueous ethanolic extracts and on the powdered specimens using standard procedures to identify chemical constituents as described by Harbone [9] (Table 2).

**Screening for anti-bacterial activity**

A total of six bacterial cultures (Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhii and Shigella flexenari) were used in this study. The bacterial strains were grown in Muller-Hinton agar plates at 37°C and maintained on nutrient agar slants.

**Well-in agar method**

Anti-bacterial activity of plant extracts was tested by a modified well-in agar method [10]. Two ml of the standardized bacterial stock suspension were thoroughly mixed with 250 ml of sterile melted nutrient agar and maintained at 40°C. 20 ml aliquots of the inoculated media were poured into a sterile Petri dish (9 cm in diameter). The agar was left to set, and in each of these plates, four holes (6 mm in diameter) were made using a sterile cork borer and agar discs were removed. Alternate holes (wells) were filled with 0.1 ml (100 µl) of 3 mg/ml sample of each of the extracts (dissolved in DMSO), and allowed to diffuse at room temperature for two hours. The plates were then incubated, in the upright position, at 37°C for 18 hours. Three replicates were carried out for each extract against each of the test organisms. Simultaneously; controls involving the addition of the respective solvents instead of the extracts were carried out. The results were recorded by measuring the diameter of inhibition zone at the end of 24–48 h (Table 3). The experiments were performed in the Natural product laboratory, of Husein Ebrahim Jamal (HEJ) research institute of chemistry, University of Karachi, Pakistan. The drug used as standard (Imipenum) was dissolved in DMSO (5 µg/ml). It was obtained from the same institute.

**Table 1**

Medicinal plants profile and their extracts yield

<table>
<thead>
<tr>
<th>No.</th>
<th>Botanical name</th>
<th>Family</th>
<th>Vern name</th>
<th>Plant part</th>
<th>Traditional use</th>
<th>Yield % (w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Boscia Salicifolia</td>
<td>Capparidaceae</td>
<td>Tella</td>
<td>bark</td>
<td>The maceration of the bark are used against cough and malaria</td>
<td>13.00</td>
</tr>
<tr>
<td>2</td>
<td>Ozoroa insignis</td>
<td>Anacardiaceae</td>
<td>Tugul</td>
<td>bark</td>
<td>The maceration of the powdered root used to treat pharangitis</td>
<td>20.00</td>
</tr>
<tr>
<td>3</td>
<td>Terminalia brownii</td>
<td>Combretaceae</td>
<td>Arza</td>
<td>bark</td>
<td>The maceration of the bark is used for cough.</td>
<td>15.00</td>
</tr>
<tr>
<td>4</td>
<td>Ximenia Americana</td>
<td>Olacaceae</td>
<td>Beuok</td>
<td>bark</td>
<td>The maceration of the roots is used as antiseptic after child birth whereas that of the leaves are used against measles.</td>
<td>12.00</td>
</tr>
</tbody>
</table>
III. RESULTS AND DISCUSSION

The four plants exhibited high availability of tannins, saponins, terpinoids, and steroids. Alkaloids were present only in B. Salicilfolia and O. insignis whereas the other two samples (T. brownii, and X. americana) showed negative result (Table 1). Also B. Salicilfolia exhibited negative test for the flavonoids. Whereas the other three samples (T. brownii, X. americana, and O. insignis) showed positive test for this class of natural product. The result of this chemical screening revealed that, these plants are rich in secondary metabolites and this support their therapeutic effect and traditional use.

As it was clear in Table 3. B. salicilfolia exhibited no activity against all types of tested bacteria. Whereas the other plant samples showed good to significant activity against certain types of tested bacteria. O. insignis exhibited good activity against P. aeruginosa. On the other hand T. brownii showed significant activity against S. aureus, and good activity against P. aeruginosa. At the same time X. americana showed significant activity against S. aureus, and low activity against P. aeruginosa.

The species, O. insignis, T. brownii, and X. americana, have proved to be rich in biologically active compounds (Table 3); yet further separation of extracts and high purification could cropout more active components.

X. americana in this study exhibited higher activity against P. aeruginosa (14) and S. aureus (20) compared with the results obtained by Francisco, et al. P. aeruginosa (14) and S. aureus (18), [14], and that determined by Jemes, D. B, et al. P. aeruginosa (11.5), [16]. T. brownii in present investigation showed significant activity against P. aeruginosa (17) and S. aureus (18), relative to low activity obtained by Mbwambo, Z. H, et al. P. aeruginosa (8.7) and S. aureus (11.3), [13].

Mathabe, M. C, et al. explained that, the extract of O. insignis, was active against S. aureus (25.3), Which was more active than the extract of this study (15), [12].

Generally the extracts of the stem bark of T. brownii, X. americana, O. insignis in this study have indicated varied levels of antibacterial activity. The traditional use of these extracts to treat diarrhea, cut wounds, gonorrhea, and other infections have been supported by laboratory results from this study, suggesting a need to isolate and evaluate active constituents responsible for the exhibited biological activities.

This study justifies the claimed uses of X. americana, O. insignis, and T. brownii, in the traditional system of managing various infections diseases caused by microorganisms.

### Table 2

Results of preliminary Phytochemical screening of plant extracts

<table>
<thead>
<tr>
<th>Screened plants</th>
<th>Alkaloids</th>
<th>Tannins</th>
<th>Saponins</th>
<th>Flavonoids</th>
<th>Terpinoids</th>
<th>Steroids</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. salicilfolia</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>_</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>O. insignis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>T. brownii</td>
<td>_</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>X. americana</td>
<td>_</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = positive result (present) - = negative result (absent)

### Table 3

Results of in vitro antibacterial Bioassay of selected plant, ethanolic extract (the average values of inhibition zone diameter in mm)

<table>
<thead>
<tr>
<th>Screened plants</th>
<th>E. coli</th>
<th>B. subtilis</th>
<th>S. flexenari</th>
<th>S. aureus</th>
<th>P. aeruginosa</th>
<th>S. typhi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zone of inhibition of std. Drug(mm)</td>
<td>35</td>
<td>36</td>
<td>36</td>
<td>43</td>
<td>32</td>
<td>40</td>
</tr>
<tr>
<td>B. salicilfolia</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>O. insignis</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>15</td>
<td>_</td>
</tr>
<tr>
<td>T. brownii</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>18</td>
<td>17</td>
<td>_</td>
</tr>
<tr>
<td>X. americana</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>20</td>
<td>14</td>
<td>_</td>
</tr>
</tbody>
</table>

Key: Concentration of sample 3 mg/ml of DMSO, size of well 6 mm (diameter) std. Std. drug Imipenum 10µg/disc. (5 µg/ml).

15 and above = active; less than 15 = not active. ( - ) = no distinct zone of inhibition
IV. CONCLUSIONS

The antibacterial activity of the plants under the study T. brownii, X. americana, O. insiginis, and B. salicifolia, exhibited: significant, good, and low activity, the inhibition zone diameters were [(18, 17), (20, 14), and 15] against S.aures and P. aeruginosa respectively. The phytochemical screening of the ethanolic extracts revealed the presence of tannins, saponins, terpinoids, steroids, and flavonoids in all species. The antibacterial and phytochemical screening insured the importance of these plants and the intensive use by the healers as traditional medicines and pointed a good guiding for further research studies in these plants.

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REFERENCES


