Chapter 10

Evaluation of antimicrobial and antifungal studies on Basella rubra seed oil

10.1 Introduction

A number of natural products have already been proved to exhibit excellent antimicrobial activity against a number of bacteria (both gram positive and gram negative), fungi etc.

In south-western part of Nigeria Psidium guajava and Mangifera indica are commonly used for herbal preparations in the treatment of toothache, gastrointestinal disorders, dysentery, diarrhoea, sore gums and sore throats. This has, therefore, led to the investigation of the antimicrobial activities of methanolic extracts of P. guajava and M. indica. P. guajava and M. indica extracts exhibited antimicrobial activities at a concentration of 20 mg/ml. The zones of inhibition exhibited by P. guajava extract ranged between 12 mm and 30 mm while that of M. indica varied between 11 and 28 mm (Akinpelu and Onakoya, 2006).

In the study performed on six endemic plant species, antimicrobial activity was observed in Campanulalyrata subsp. lyrata and Abies nordmanniana subsp. bornmuelleriana plants. The minimum inhibitory concentration of C. lyrata subsp. lyrata (leaf and flower) extract was found to be 29 mg/ml for Baccillus subtilis and 14.5 mg/ml for Staphylococcus aureus, and the minimum inhibitory concentration (MIC) of Abies nordmanniana subsp. bornmuelleriana (leaf) extract was found to be > 314 mg/ml for B. subtilis and when minimum bacteriocidal concentration (MBC)
results were evaluated, it was observed that the plant extracts had bacteriocidal effects (Benli, 2008).

Phytochemical screening of the leaves and roots of Cassia alata (Linn) revealed the presence of some bioactive components, which have been linked to antimicrobial properties. The effects of water, methanol and chloroform extracts on some pathogenic Staphylococcus aureus, Escherichia coli, Streptococcus pyogenes, Pseudomonas aeruginosa and Proteus mirabilis showed that the plant parts can be used to treat infections caused by these bacteria. S. aureus, S. pyogenes and P. mirabilis were more susceptible, while E. coli and P. aeruginosa were less sensitive. The effectiveness of the crude extracts were enhanced at elevated temperatures and at near neutrality pH values, which attests to its use in traditional medicine to treat skin, urinary tract and gastrointestinal infections (El-Mahmood and Doughari, 2008).

The antibacterial effect of some selected Indian medicinal plants was evaluated by Parekh and Chanda (Parekh and Chanda, 2007) on bacterial strains like Bacillus cereus ATCC11778, Staphylococcus aureus ATCC25923, Enterobacter aerogenes ATCC13048, Escherichia coli ATCC25922 and Klebsiella pneumoniae NCIM2719. The most susceptible Gram-positive bacteria were B. cereus, while the most susceptible Gram-negative bacteria were K. pneumoniae.

Phytochemical and antimicrobial properties of four medicinal plants (Bidens pilosa, Euphorbia heterophylla, Euphorbia hirta and Phyllanthus amarus) used in the management of some diseases in Edo State, Nigeria were investigated in this study. The results revealed that tannins, saponins, flavonoids, cardiac glycosides, alkaloids and steroids were all present in E. hirta. While P. amarus contain all except cardiac
glycosides. *E. heterophylla* contained saponins, flavonoids, cardiac glycosides and steroids whereas *B pilosa* contained saponins, alkaloids and steroids only. These substances are known components of medicinal plants and may explain the use of the preparations of the herbs under study for managing a number of common ailments including dysentery, diabetes, hypertension and some microbial infections among the indigenous communities in Edo State. When tested against *Escherichia coli*, *Streptococcus* spp, *Klebsiella* spp, *Pseudomonas* spp and *Staphylococcus* spp, aqueous and methanolic extracts of *E. hirta*, *P. amarus* and *B. pilosa*, showed varying degrees of inhibition to the growth of tested organisms. *E. heterophylla*, was effective as an antimicrobial agent only against *Streptococcus* sp. Extracts from all the plants tested had no effects on *Klebsiella* sp used in this study. Methanolic extracts of the plants were more effective than aqueous extracts in inhibiting the growth of the pathogenic bacteria under study, but were less potent when compared to that of ofloxacin and ciprofloxacin used as positive controls. Presence of Phytochemical agents and antimicrobial properties in the tested plant species is confirmed (Okoli et al., 2009).

*Tamarindus indica* is a plant that is used in traditional medicine for the treatment of cold, fever, stomach disorder, diarrhoea and jaundice and as skin cleanser. To evaluate the scientific basis for the use of the plant, the antimicrobial activities of extracts of the stem bark and leaves were evaluated against some common gram negative and gram positive bacteria and fungi. The study also investigated the chemical constituents of the plant and the effect of temperature and pH on its antimicrobial activity (Doughari, 2006). The phytochemical constituents of the dried powdered plant parts were extracted using aqueous and organic solvents (acetone and
The antimicrobial activity of the concentrated extracts was evaluated by determination of the diameter of zone of inhibition against both gram negative and gram positive bacteria and fungi using the paper disc diffusion method. Results of the phytochemical studies revealed the presence of tannins, saponins, sesquiterpenes, alkaloids and phlobatamins and the extracts were active against both gram positive and gram negative bacteria. The activity of the plant extracts were not affected when treated at different temperature ranges (4°C, 30°C, 60°C and 100°C), but was reduced at alkaline pH. *Tamarindus indica* has broad spectrum antibacterial activity and a potential source of new classes of antibiotics that could be useful for infectious disease chemotherapy and control.

Aqueous extract of fifty two plants from different families were tested for their antifungal potential against eight important species of Aspergillus such as *A. candidus, A. columnaris, A. flavipes, A. flavus, A. fumigatus, A. niger, A. ochraceus,* and *A. tamarii* by Satish and his group (*Satish et al., 2007*) isolated from sorghum, maize and paddy seed samples. The test fungi were mainly associated with seed biodeterioration during storage. Among fifty-two plants tested, aqueous extract of *Acacia nilotica, Achras zapota, Datura stramonium, Emblica officinalis, Eucalyptus globules, Lawsonia inermis, Minusops elengi, Peltophorum pterocarpum, Polyalthia longifolia, Prosopis juliflora, Punica granatum* and *Syzigium cumini* have recorded significant antifungal activity against one or the other Aspergillus species tested. *A. flavus* recorded high susceptibility and hence solvent extracts viz., petroleum ether, benzene, chloroform, methanol and ethanol extracts of all the twelve plants were tested for their antifungal activity against it. Among the solvent extracts tested, methanol gave more effective than ethanol, chloroform, benzene and petroleum ether,
except for *Polyalthia longifolia*, where petroleum ether extract recorded highly significant antifungal activity than other solvent extracts.

Ethanol and aqueous extract of *Heracleum sphondylium* subsp. artvinense was investigated by Ergene and group ([Ergene et al., 2006](#)) for their antimicrobial activities against eight bacterial species (*Enterococcus feacalis, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Listeria monocytogenes, Shigella, Streptococcus pyogenes, and Corynobacterium diphtheria*) and two yeast (*Candida albicans* and *C. krusei*). Both ethanol and aqueous extract of *H. sphondylium* subsp.artvinense showed antimicrobial activity against the gram-positive bacterium (*S. aureus*).

Keeping all those into account, the anti bacterial and anti fungal studies were carried out for the seed oil of *Basella rubra*. The antimicrobial activities of aqueous, ethanolic and petroleum ether extracts of the leaves of *Basella rubra* were evaluated in the present study by measuring the inhibition zones using Cup Plate Diffusion method. The inhibition zones were significantly different (P<0.001) in each plant extract. The ethanolic extract showed maximum activity with zone of inhibition (14.3±1.82 mm) against *E.coli*, followed by aqueous extract (13.4±1.2 mm) and petroleum ether (5.6±0.62 mm) at a concentration of 50μg/ml. Ciprofloxacin was used as the standard drug having zones of inhibition(17±0.34 mm) against *E.coli* and 19±0.18 mm against *A. niger*. Microbial inhibition was in the order *E.coli* (12.57±0.99), *A. niger* (11.68±0.71), *V. cholera* (11.42±0.60), *S. aureus* (10.71±0.46), *S. typhi* (9.80±0.90), respectively with all the extracts. The extracts were not able to inhibit the growth of *P. aeruginosa*. 

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10.2 Procedure for Anti Bacterial and Anti Fungal Studies

The antibacterial studies carried out for the extracts were done systematically against four strain of bacteria (2 gram positive: *Bacillus pertusis* and *Staphylococcus aureus*; 2 gram negative: *Escherichia coli* and *Vibrio cholarae*) by Agar diffusion method in particular paper disc method. The standard drug (Tetracycline) solution was made in chloroform in 200 μg/ml. (Pelczar et al., 1993; Cappuccino and Sherman, 1998; Panda et al., 2005).

The nutrient agar media was first prepared, whose composition is given below. The prepared media was sterilized by autoclaving at 121°C (15 lb/sq. inch) for fifteen minutes. The slants were prepared and the test organisms were subcultured in the fresh media, which were then incubated for 24 hours at 37 ± 1°C. Then they were prepared by transferring a loopful of stock culture to sterilized nutrient broth taken in the test tubes, which were incubated at 37 ± 1°C for 18 hours before the experiments were carried out.

Petri dishes, test tubes, flasks plugged with cotton were first sterilized in Hot Air Oven for one hour. Fresh sterilized nutrient agar media for Bacteria and Yeast was again prepared, which in molten condition was transferred aseptically into the sterilized petri dishes and left at room temperature to be solidified. 2 ml of inoculated nutrient broth was then transferred in each of the petri dishes aseptically. The broth was spread uniformly throughout the entire media.

In each of the plates, paper disc of 6 mm diameter soaked with the standard solution (Tetracycline), extract, the oil and the solvent (chloroform), were placed aseptically. The plates were kept undisturbed for at least 2 hours for room temperature to allow diffusion of the solution properly into the nutrient media. After incubation of
the plate 37± 1°C for 24 hours. The diameter of zone of inhibition surrounding each of the discs was measured. All the experiments were carried out in triplicate

**Composition of Nutrient Agar Medium of Bacteria and Yeast**

- Peptone: 60 g
- Beef extract: 15 g
- Sodium Chloride: 5 g
- Dextrose: 10 g
- Agar: 25 g
- Distilled Water: 1000 ml
- Final pH after sterilization: 6.5 to 6.6

### 10.3 Procedure for Antifungal Studies

The antifungal studies were carried out for one strain of *Candida albicans* by Agar diffusion method, in particular paper disc method. The zones of inhibition were observed and recorded. The standard drug solution was made in DMF (200 μg / ml). The test organism was subcultured in fresh Sabaraud Dextrose Agar (SDA) media, composition of which is given below. The SDA media prepared was first sterilized at 121°C (15lb/sq. inch) for 15 minutes by autoclaving.

The slant was prepared by sterilized SDA media, which was then subcultured with the test organism. The slant was then maintained at 22-25°C for 48 hours. From this loopful of fungus strain was then transferred aseptically in nutrient broth media and again maintained at 22-25°C for 48 hours.
The sterilized SDA media was then placed in the sterilized petridishes in molten condition and were allowed to solidify at room temperature. The inoculated broth of 2 ml was then spread uniformly and aseptically over the prepared plates. In each plate paper disc of 6 mm diameter soaked in the standard drug solution (Amphotericin B), oil and the solvent were placed aseptically accordingly.

The prepared plates were then maintained at 22-25° C for 48 hours and then the diameter of the zone of inhibition surrounding each of the discs was measured. All the experiments were carried out in Triplicate.

**COMPOSITION OF MEDIUM OF FUNGUS**

**SABARAUD DEXTROSE AGAR (SDA) MEDIA**

- Dextrose .......................................................... 40 mg
- Peptone .......................................................... 10 mg
- Agar ............................................................. 25 mg
- Distilled water .............................................. 1000 ml

10.4 Results and Discussion

The antimicrobial activities of aqueous, ethanolic and petroleum ether extracts of the leaves of *Basella rubra* were evaluated in the present study by measuring the inhibition zones using Cup Plate Diffusion and Disc diffusion method.

In the Cup Plate Diffusion method, the inhibition zones were found significantly different (P<0.001) in each plant extract. The ethanolic extract showed maximum activity with zone of inhibition (14.3±1.82 mm) against *E. coli*, followed by aqueous extract (13.4±1.2 mm) and petroleum ether (5.6±0.62 mm) at a concentration of 50μg/ml. Ciprofloxacin was used as the standard drug having zones of inhibition(17±0.34 mm) against *E. coli* and 19±0.18 mm against *A. niger*. Microbial
inhibition was in the order \(E. coli\) (12.57±0.99), \(A. niger\) (11.68±0.71), \(V. cholera\) (11.42±0.60), \(S. aureus\) (10.71±0.46), \(S. typhi\) (9.80±0.90), respectively with all the extracts. The extracts were not able to inhibit the growth of \(P. aeruginosa\).

**Table 10.1:** Results of antimicrobial studies (using Cup Plate Diffusion method)

<table>
<thead>
<tr>
<th>Microbe</th>
<th>Bacillus pertosis</th>
<th>Staphylococcus aureus</th>
<th>Escherichia coli</th>
<th>Vibrio cholerae</th>
<th>Yeast</th>
<th>Candida albicans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetracycline</td>
<td>9 (&gt;1500)</td>
<td>10 (&gt;1500)</td>
<td>11 (1500)</td>
<td>10 (1500)</td>
<td>11 (1450)</td>
<td>15 (900)</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>23 (30)</td>
<td>24 (250)</td>
<td>24 (27)</td>
<td>23 (30)</td>
<td>25 (200)</td>
<td>-</td>
</tr>
</tbody>
</table>

The results of the antimicrobial studies by Disc diffusion method are presented in **Table 10.2** below:

**Table 10.2:** Consolidated results of the antimicrobial analysis

<table>
<thead>
<tr>
<th>SI No</th>
<th>Microbe</th>
<th>Standard</th>
<th>Solvent (CHCl₃)</th>
<th>SRS6</th>
<th>SRS7</th>
<th>SRS12</th>
<th>SRS14</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Streptococcus faecalis</em></td>
<td>40mm</td>
<td>Nil</td>
<td>18</td>
<td>11</td>
<td>16</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td><em>Bacillus subtilis</em></td>
<td>32mm</td>
<td>Nil</td>
<td>10</td>
<td>13</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>3</td>
<td><em>Klebsilla pneumonia</em></td>
<td>25mm</td>
<td>Nil</td>
<td>14</td>
<td>14</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>4</td>
<td><em>E. coli</em></td>
<td>35mm</td>
<td>Nil</td>
<td>15</td>
<td>16</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>5</td>
<td><em>Proteus vulgaris</em></td>
<td>40mm</td>
<td>Nil</td>
<td>15</td>
<td>15</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>6</td>
<td><em>Pseudomonas aeruginosa</em></td>
<td>25mm</td>
<td>Nil</td>
<td>18</td>
<td>16</td>
<td>24</td>
<td>18</td>
</tr>
<tr>
<td>7</td>
<td><em>Candida albicans</em></td>
<td>22mm</td>
<td>Nil</td>
<td>14</td>
<td>15</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>8</td>
<td><em>S. aureus</em></td>
<td>22mm</td>
<td>Nil</td>
<td>Nil</td>
<td>12</td>
<td>Nil</td>
<td>Nil</td>
</tr>
</tbody>
</table>

The effect of the residual solvent (chloroform) was checked by using dics soaked in the solvent and then dried.
The photograph of the culture plate is presented in Figure 10.1 below:

Figure 10.1: Photographs of the antimicrobial assay of the plant materials
10.5 Conclusion

The material exhibited very good inhibitory effect against *Streptococcus faecalis* and *Bacillus subtilis* and lesser activity against *Klebsilla pneumonae*, *E. coli*, *Proteus vulgaris* and *Pseudomonas aeruginosa* relative to standards ciprofloxacin and clotrimazole. The solvent chloroform has no antimicrobial activity as such.