ANTIMICROBIAL EVALUATION OF LOZENGES FORMULATED WITH ETHANOLIC EXTRACT OF *Vernonia amygdalina*.

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ABSTRACT

The antimicrobial activity of ethanolic extract of *Vernonia amygdalina* was carried out on isolated cultures of *Staphylococcus aureus*, *Escherichia coli* and *Aspergillus fumigates* using standard procedures. *V. amygdalina* extract exhibited the highest activity on *S. aureus* and appreciable activity against cultures of *E. coli* and *A. fumigates*. The extract was then incorporated into a lozenge formulation and thereafter evaluated. Six batches of granulations for lozenges, weighing 500 mg each were produced. The granules’ flow rate, angle of repose, and compressibility indices were determined. The lozenges’ uniformity of weight, hardness, friability and disintegration time in distilled water were determined by established methods. Generally, there was poor flow but high compressibility for all the granulation batches. The hardness values for all the batches of the lozenges were not greater than 5 KgF and in the order: E > F > D > C > B > A. The friability values were in the order: E > A > C > B > F > D while disintegration times in distilled water followed the rank order C > D > F > E > B > A. Use of higher concentrations of binder would improve the quality parameters of the lozenge formulation which would encourage the application of the product in some oral infections.

**Keywords:** Antimicrobial activity; *Vernonia amygdalina*; lozenge formulation; ethanolic extract.

INTRODUCTION

Despite the tremendous breakthrough in modern medicine, infectious diseases are responsible for quite a large number of premature deaths in human beings.

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have been considered for the treatment of many of these infections throughout the world. \[^3\] It was on this background that the World Health Assembly adopted among its resolutions, the support of national trado-medicine program, drawing attention to herbal medicines as being of great importance to the health of both individuals and communities.\[^4\] World Health Organization estimated that between 70% and 95% of citizens in a majority of developing countries, especially those in Asia, Africa, Latin America and the Middle East, currently use herbal medicine for some aspects of Primary Health Care. \[^5\] The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotics.\[^2\]

_Vernonia amygdalina_ (family, Compositae) is a shrub that is widespread in East and West African countries.\[^6\] In Nigeria, it is commonly known as "bitter leaf" because the leaves and stem have a bitter taste when chewed. It is one of the many plants whose stems and roots are used as chewing sticks in Nigeria. Its leaves were used as a popular vegetable for soups particularly among the various ethnic groups in Nigeria. It is effective against gastrointestinal disorders\[^7\] including amoebic dysentery\[^8\] possibly due to its antimicrobial and antiparasitic activities\[^9,10\]. Formulation of these herbs or their extracts into appropriate dosage form would ensure that they are presented in acceptable pharmaceutically elegant dosage delivery systems which are convenient for use and are cost-effective. Lozenge formulation is one of such delivery systems.

Lozenges are solid unit drug delivery of one or more medicaments, usually in a flavored, sweetened base and that are intended to dissolve or disintegrate slowly in the mouth or pharynx.\[^11\] They can be prepared by molding (gelatin and/or fused sucrose and sorbitol base) or by compression of sugar based tablets. Molded lozenges are sometimes referred to as pastilles, whereas compressed lozenges may be referred to as troches.

This present study was to assess the antimicrobial activity of the ethanolic extract of _Vernonia amygdalina_ before and after incorporating it into a lozenge formulation and then assessing some quality parameters of the prepared lozenges.

**MATERIALS AND METHODS**

**Materials**

The materials used were 99% Ethanol (Novara House, Asbby Park, England), Avicel\(^{(R)}\) PH 101, Magnesium stearate (Sigma Aldrich, USA), Polyvinyl pyrrolidone, PVP (molecular weight 36,000).
weight 40,000; Aldrich Chemical Co. Ltd, Gillingham, Dorset UK), Lactose, Talc powder (BDH, England). All other chemicals and reagents used were of analytical grade.

**Microorganism**

The microorganisms used included the following: Isolated cultures of *Staphylococcus aureus* ATCC 6538, *Escherichia Coli* ATCC 25922 and *Aspergillus fumigates* ATCC 23955.

**Extraction of V. amygdalina leaves**

Fresh leaves of *V. amygdalina* plant were collected from Uyo, Akwa Ibom State, Nigeria and authenticated at the herbarium unit of the Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, Nigeria. The clean leaves were sorted, thoroughly washed and rinsed with cold water without squeezing and sun-dried for seven days. The dried leaves were pulverized and macerated in a transparent extraction tank using 70% ethanol for 72 hours at room temperature. There was intermittent stirring to allow for a good mix. The mixture was then filtered. using glass funnel filled with cotton wool. The filtrate collected in a beaker was subsequently concentrated to dryness in a water bath at 40°C to obtain the extract. The dried extract was weighed and stored in an air-tight container for further experiment.

**Phytochemical screening**

Phytochemical screening to detect the presence or otherwise of alkaloids, tannins, saponins, and flavonoids were carried out using simple standard methods described by Sofowora (2006).[[12]]

**Minimum inhibitory concentration (MIC) determination**

MIC was determined using Agar-well diffusion method described by Odoemena and Essien (1995).[[13]] Sterile petri dishes were aseptically inoculated with 0.1ml of suspension of the test organism in a petri dish and 20ml of the culture media (molten agar) poured into it. The mixture was swirled gently to mix and allowed to solidify (for *E.Coli* and *S. aureus*). Surface inoculation using a glass spreader was employed for *A. fumigates*.

Using the sterile flamed cork-borer, holes were bored on the seeded agar plates, discarding the removed agar rings into the disinfectant solution; the wells were aseptically filled with the different dilutions of the extract – 12.5, 25, 50 and 100 mg/mL of each extract. The plates were allowed to stay for 30 mins on the bench before incubation to allow for diffusion of the
micro-organisms. The plates were incubated at 37°C for 24 hours for bacteria but 25°C for 7-14 days for fungi. Observations were then made and diameter of the inhibition zone was measured.

**Preparation of granules**

The tableting technique employed is direct compression. Thus, granules were produced by simple bulk mixing. The following excipients were employed: *V. amygdalina* extract as active ingredient, Avicel\(^{(R)}\) PH-101 as disintegrant, polyvinylpyrrolidone (PVP) as binder, magnesium stearate as lubricant, talc as anti-adherent and glidant, and lactose as bulking agent. The formula for each batch, percentage composition and weight of ingredients for each batch is as shown in Table 1. The required quantities of the ingredients were weighed and mixed thoroughly for 5 minutes in figure 8 motion. Each resultant granule batch was exposed to heat (40°C) for 30 minutes to reduce the moisture content and then stored in an air-tight container for subsequent experiment.

**Table 1: Formula for each batch of granules**

<table>
<thead>
<tr>
<th>Batch</th>
<th>Extract</th>
<th>Avicel(^{(R)}) PH-101 %</th>
<th>Polyvinyl pyrrolidone (PVP) mg</th>
<th>Magnesium stearate (mg)</th>
<th>Talc (mg)</th>
<th>Lactose (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0</td>
<td>50</td>
<td>10</td>
<td>15</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>B</td>
<td>100</td>
<td>40</td>
<td>8</td>
<td>15</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>C</td>
<td>100</td>
<td>60</td>
<td>12</td>
<td>25</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>D</td>
<td>100</td>
<td>50</td>
<td>10</td>
<td>25</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>E</td>
<td>100</td>
<td>40</td>
<td>8</td>
<td>40</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>F</td>
<td>100</td>
<td>60</td>
<td>12</td>
<td>40</td>
<td>8</td>
<td>5</td>
</tr>
</tbody>
</table>

Unit weight of tablet = 500mg

**Determination of bulk and tapped densities**

A 30g quantity of the granule sample was placed in a 100 ml clean, dry measuring cylinder and the volume \( V_b \) occupied by the bulk powder was determined. Mechanical tapping of the cylinder on a padded horizontal surface was carried out until a constant volume \( V_t \) was obtained. The determinations were made in triplicate and the mean values were calculated. The bulk and tapped densities, \( D_b \) and \( D_t \), were calculated as the ratio of sample weight (30g) to bulk and tapped volumes, \( V_b \) and \( V_t \), respectively.

**Calculation of Hausner’s quotient (HQ)**

HQ of each batch sample was calculated as the ratio of its tapped density to the bulk density:

\[
HQ = \frac{D_t}{D_b} \quad (1)
\]
Calculation of Carr’s compressibility index (I)

Carr’s compressibility Index (I) was computed using the following equation:

\[ I(\%) = 100 \left( \frac{D_t - D_b}{D_t} \right) \]  \hspace{1cm} (2)

Determination of angle of repose

The static angle of repose was determined using the fixed base cone method.\textsuperscript{[14,15]} A 30 g quantity of the sample was transferred into an open-ended cylinder placed on a static base cone on a horizontal surface. The cylinder was gradually withdrawn vertically and the sample formed a cone-shaped heap. The height of the sample \((h)\) was determined using a cathetometer; the radius \((r)\) was gotten by dividing the fixed diameter by two. Angle of repose \((\Theta)\) for each sample was gotten using the equation:

\[ \Theta = \tan^{-1} \frac{h}{r} \]  \hspace{1cm} (3)

Determination of percentage fines

The percentage fines was determined for a 50g \((W_0)\) sample by screening using a 0.5mm aperture size sieve to separate the coarse from fine particles. The percentage fines was then calculated based on the quantity that passed through the sieve \((W_f)\):

\[ \% \text{ fines} = 100 \left( \frac{W_f}{W_0} \right) \]  \hspace{1cm} (4)

Compression

500mg of each batch of the granules was manually fed into the die cavity of the single punch tableting machine and then compressed at a load of 25 KgF.

Hardness test

The hardness test on the compacts was carried out using a Monsanto Tablet Hardness tester to measure the force required to break a tablet when the force generated by a coiled spring is applied diametrically. The force was measured in kilogram force (KgF). 10 tablets per batch were used for this determination. The mean hardness and the standard deviation values were then calculated.

Friability test

The friability test was conducted using Roche Friabitator, using 10 tablets for each batch with 100 revolutions (i.e. 25 revolutions per minute for 4 minutes). The tablets were dedusted, weighed together \((W_0)\) and friabilated. The friabilated tablets were re-weighed \((W_1)\). Friability was calculated as follows:
F = \{\text{Wo-\text{W}_1/\text{W}_0}\} \times 100\%.

**(Thickness)**

The thickness of each compact was measured using micrometer screw gauge and expressed in mm.

**Weight uniformity tests**

A quantity of 20 compacts was randomly selected from each batch. Then, the average weight, percentage deviation and standard deviation were determined for each batch.

**Disintegration time test**

The disintegration time of the compacts was determined in distilled water at 37 ± 0.5 °C using a B.P. Manesty disintegration test unit (Manesty Machines Ltd; Poole, UK). A compact each was placed on the wire mesh just above the surface of the distilled water in the test tube and the unit was switched on simultaneously with a stop clock. The time taken for the compacts to disintegrate and all particles to pass through the wire mesh was recorded as the disintegration time.

**RESULTS AND DISCUSSION**

**Phytochemicals in the extract**

The percentage yield of 9.8% was obtained. The phytochemical test results are shown in Table 2. The results reveal the presence of saponins, tannins, flavonoids, in appreciable quantities. Cardiac glycosides and Alkaloids are present in large quantity. These results correspond with the report by Nacoulna (1996).\textsuperscript{[16]} Hence the activity of the extract could be as a result of cardiac glycosides, tannins, saponins, or flavonoids. Tannins are known to be readily hydrolysed to yield phenolic acid which possesses anti-oxidant and antimicrobial properties. As a result of the latter property, tannins are used in the preparation of antiseptics.\textsuperscript{[17]}

<table>
<thead>
<tr>
<th>S/No</th>
<th>Test</th>
<th>Observation</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Alkaloid</td>
<td>+++</td>
<td>Present in Abundance</td>
</tr>
<tr>
<td>2.</td>
<td>Saponins (a) with water</td>
<td>++</td>
<td>Present in Abundance</td>
</tr>
<tr>
<td></td>
<td>(b) with sodium bicarbonate Tannins</td>
<td>++</td>
<td>Present in Abundance</td>
</tr>
</tbody>
</table>
The result of the antimicrobial screening is shown in Table 3. The extract effectively inhibited growth in the culture of *Staphylococcus aureus* at concentrations of 100mg/mL and 50mg/ml but no inhibition at 25mg/mL and 12.5 mg/mL. The extract exhibited no activity against the fungus *Aspergillus fumigatus* and *Escherichia coli*.

Table 3: Antimicrobial activity of *V. amygdalina* extract

<table>
<thead>
<tr>
<th>Concentration of extract (mg/ml)</th>
<th>Log concentration</th>
<th>S. aureus mean inhibition zone diameter (mm)</th>
<th>E. coli mean inhibition zone diameter (mm)</th>
<th>A. fumigates mean inhibitor zone diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>2.00</td>
<td>15</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>50</td>
<td>1.70</td>
<td>10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>25</td>
<td>1.40</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>12.5</td>
<td>1.09</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Compressibility and flowability properties of the powered mixes

The percentage fines obtained in this study was 16.92%. For good compacts to be formed, percentage fines in the prepared granules should not be more than 20%. Hence, lozenges produced from the granules are expected to exhibit optimum quality parameters.

The results obtained for the evaluation of flowability and compressibility of the formulated granules are presented in Table 4. The values generally indicated fair flow but high compressibility for all the batches. Batch A had the least Carr’s index and Hausner ratio of 25% and 1.29 respectively while batch F possessed the highest Carr’s index and Hausner’s ratio of 34% and 1.52 respectively. It is also observed that there is a significant decrease in the Carr’s index as the concentration of disintegrant (microcrystalline cellulose) increased to 12%. The reverse was the case at a low concentration of 8%. This might be due to increase in fine particles concentration obtained in the latter batch.
Table 4: Some flow and density parameters of the granules

<table>
<thead>
<tr>
<th>Batch</th>
<th>Bulk density g/cm$^3$</th>
<th>Tapped density g/cm$^3$</th>
<th>Hausner’s quotient</th>
<th>Carr’s index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.49</td>
<td>0.63</td>
<td>1.29</td>
<td>25</td>
</tr>
<tr>
<td>B</td>
<td>0.49</td>
<td>0.67</td>
<td>1.37</td>
<td>30</td>
</tr>
<tr>
<td>C</td>
<td>0.49</td>
<td>0.67</td>
<td>1.37</td>
<td>27</td>
</tr>
<tr>
<td>D</td>
<td>0.49</td>
<td>0.66</td>
<td>1.35</td>
<td>26</td>
</tr>
<tr>
<td>E</td>
<td>0.49</td>
<td>0.72</td>
<td>1.47</td>
<td>32</td>
</tr>
<tr>
<td>F</td>
<td>0.48</td>
<td>0.73</td>
<td>1.52</td>
<td>34</td>
</tr>
</tbody>
</table>

**Tablet properties**

Table 5 shows the values of the determined compacts’ quality parameters - average thickness, hardness, weight, friability and disintegration time. All the batches had the same average thickness of 2.5 mm showing uniformity of tablet. They also exhibited uniform tablet weight\(^{[18]}\) with good hardness (<5 KgF). The hardness results were in the order of E > F > D > C > B > A. Batches A and B had the lowest hardness perhaps as a result of low concentration of binder, while batches E and F with highest proportion of binder exhibited relatively high hardness values. The percentage friability values obtained for batches A, B, C, E and F were more than 1%. The friability were in the order of E > A > C > B > F > D>. Thus, Batch D had the lowest friability of 0.99% while E had the highest friability of 1.45. The disintegration time values are in the order of C > D > F > E > B > A>. Thus, Batch A had the lowest disintegration time of 10.46 mins while Batch C had the highest disintegration time of 41.53 minutes. Generally, a reduction in the quantity of lubricant in the formulation and increase in binder concentration would be required to obtain lozenges with desired optimum hardness, low friability and prolonged disintegration time.

Table 5: Some quality parameters of the compressed lozenges

<table>
<thead>
<tr>
<th>Batch</th>
<th>mean thickness (mm)</th>
<th>Mean Hardness kgF</th>
<th>Mean weight (g)</th>
<th>Friability (%)</th>
<th>Disintegration time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2.5</td>
<td>3.15</td>
<td>0.477</td>
<td>1.29</td>
<td>10.46</td>
</tr>
<tr>
<td>B</td>
<td>2.5</td>
<td>3.38</td>
<td>0.50</td>
<td>0.91</td>
<td>23:54</td>
</tr>
<tr>
<td>C</td>
<td>2.5</td>
<td>3.47</td>
<td>0.49</td>
<td>0.63</td>
<td>41:53</td>
</tr>
<tr>
<td>D</td>
<td>2.5</td>
<td>3.50</td>
<td>0.51</td>
<td>1.27</td>
<td>39.01</td>
</tr>
<tr>
<td>E</td>
<td>2.5</td>
<td>3.85</td>
<td>0.49</td>
<td>1.45</td>
<td>27.55</td>
</tr>
<tr>
<td>F</td>
<td>2.5</td>
<td>3.78</td>
<td>0.49</td>
<td>1.05</td>
<td>36.04</td>
</tr>
</tbody>
</table>

**CONCLUSIONS**

The ethanolic extract of sun-dried leaves of *Vernonia amygdalina* has significant activity against *Staphylococcus aureus* but no activity against *E.coli* and *Aspergillus fumigates*. With
some modifications in the formula employed in the lozenges preparation, such as use of high
concentrations of binder, the product would be a promising one especially in the treatment of
some oral infections involving S. aureus.

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