3- Results and Discussion

3.1-Phytochemical screening

Seeds of *Acacia nilotica* and fruits of *Vangueria madagascariensis* were screened for different phytoconstituents. A tabulation of the results is presented in Table 3.1.

<table>
<thead>
<tr>
<th>Test</th>
<th><em>Vangueria madagascariensis</em></th>
<th><em>Acacia nilotica</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>

3.2. Characterization of isolated compounds

A successive silica gel column chromatography followed by further purification via thin layer chromatography allowed for the isolation of two components from *Vangueria madagascariensis* – compounds I and II. Identification of these compounds was based on extensive UV shifting reagents, IR, $^1$HNMR and mass spectroscopy data.
3.2.1--compound I

Compound I was isolated from fruits of *Vangueria madagascariensis* as yellow solid. The IR spectrum (Fig. 3.1) displayed absorption bands at $\nu$(KBr) 673 (C-H, Ar.), 1105 (C-O), 1550, 1645 (C=C, Ar) 1689 (C = O), 2854 (C-H) aliphatic, 3429 cm$^{-1}$ (OH).

![IR spectrum of compound I](image)

The presence of a C=O function excludes the presence of (i) anthocyanins and (ii) catechins.

The UV spectrum (Fig. 3.2) showed $\lambda_{\text{max}}$ (MeOH) 273 nm (Fig. 3.2) the presence of band II only indicates that the compound is probably a dihydroflavonol, dihydrochalcone, flavanone or an isoflavone. Among these classes only dihydroflavonols are characterized by a 3-OH function. The sodium methoxide spectrum indicated the presence of a 3-OH function (Fig. 3.3).
The shift reagent sodium methoxide is a strong base capable of ionizing all hydroxyl functions in the flavonoid nucleus, but it is diagnostic of 3- and 4'-OH groups. In both case it gives a bathochromic shift but with decrease in intensity in case 3-OH. Addition of the strong base NaOMe to a methanolic solution of compound I caused (Fig.3.3) a bathochromic shift (84nm) in band I with decrease in intensity. This indicates the presence of a hydroxyl group at C-3.
To investigate the hydroxylation pattern of this isolate, the UV shift reagents were employed. Soudim acetate is a weaker base than sodium methoxide and as such ionizes only the more acidic hydroxyl group in flavonoids i.e. 3-,7- and 4'- hydroxyl groups. Since ionization of 7- hydroxyl group mainly affects band II, sodium acetate is particularly useful diagnostic reagent for the specific detection of 7-hydroxy function. However, no bathochromic shift was observed in the sodium acetate spectrum (Fig.3.4). This indicates absence of a 7-OH function.

![Sodium acetate spectrum of compound I](image)

**Fig.3.4: Sodium acetate spectrum of compound I**

Aluminum chloride chelates with functional groups such as the 5-hydroxy-4-keto-,3-hydroxy-4-keto and ortho dihydroxyl systems and this is evidenced by bathochromic shifts of one or both bands in the spectrum. AlCl₃ complex between the C-4 keto function and either 3- or 5-hydroxyl group is stable in presence of HCl acid. However catechols yield acid-labile complexes.
The aluminium chloride spectrum did not reveal any bathochromic shift (Fig. 3.5) indicating absence of 3- and 5-OH function as well as catechol systems (Fig. 3.6).

![Aluminium chloride spectrum of compound I](image)

The $^1$HNMR spectrum (Fig. 3.6) showed signals for one methyl group at $\delta$1.23 (s, 3H) and three acetyl functions at $\delta$ 1.89 (s, 9H). The resonances at $\delta$6.10 (s, 1H) and $\delta$6.70 was assigned to H-6, H-8 respectively. The C-8 proton usually resonates at lower field relative to C-H due to the deshielding influence of neighbouring oxygen at position 1. The B-ring protons appeared as singlets at $\delta$ 6.98 and $\delta$7.31 ppm.
The ESI-MS(Fig.3.7) showed a molecular ion peak at m/z 381 [M⁺+H]. Other important fragmentns resulting from retro Diels-Alder fission(Schme 3.1) were shown at m/z 134, 246.Apparently these intact A and B rings respectively.
On the basis of the above cumulative, the following tentative structure was proposed for compound I.

![Compound I](image)

Scheme 3.1: Retro Diels-Alder fission of compound I

3.2.2. Compound II

The IR spectrum of compound II (Fig.3.7) displayed absorption bands at $\nu$(KBr): 671 (C-H, Ar), 1064 (C-O), 1456 (C=C, Ar), 1739 (C=O), 2923 (C-H) aliphatic, 3353 cm$^{-1}$ (OH).
The VU spectrum (Fig.3.11) showed $\lambda_{\text{max}}$(MeOH) 232,307 nm. Such absorption is characteristic of flavones.

The sodium methoxide spectrum(Fig.3.12) revealed a bathochromic shift without decrease in intensity and this is diagnostic of a 4`-OH function.

Also, the aluminum chloride spectrum(Fig.3.13) did not reveal a bathochromic shift indicating absence of 3- and 5-OH functions as well as catechol moieties.
The $^1$HNMR spectrum (Fig. 3.14) showed a signal for one methyl group at $\delta 1.23$, an acetyl function at $\delta 2.22$ and a methoxyl at $\delta 3.77$ppm. The resonances at $\delta 6.99$ and $\delta 7.71$ were assigned to the aromatic protons of A and B rings respectively.
The ESI-MS (Fig. 3.15) showed a peak at m/z 323 [M+H+] . Other important fragmentns resulting from retro Diels-Alder fission (Scheme 3.2) were shown at m/z 150, 175. Apparantly these correspond to intact A and B rings respectively.
On the basis of the above cumulative, the following tentative structure was proposed for compound II.

Scheme 3.2: Retro Diels-Alder fission of compound II
3.4-Antibacterial activity

In cup plate agar diffusion assay, the chloroform fraction of *Vangueria madagascariensis*, compound II and *Acacia nilotica* seed oil were evaluated for their antimicrobial activity. The aqueous extract of *Acacia nilotica* seeds was also evaluated. The chloroform fraction of *Vangueria madagascariensis* did not show antibacterial activity, but it showed significant inhibitory activity against the fungi: *Candida albicans* and *Aspergantillus niger*. Compounds II showed antifungal activity. However, it did not reveal antibacterial activity (Table 3.3). The activity is expressed as less active, if the zone of inhibition is 9-12 mm; active: 13-18mm; very active if the inhibition zone is greater than 18mm.

*Acacia nilotica* aqueous extract showed significant antibacterial activity and significant antifungal activity. *Acacia nilotica* oil also showed antibacterial and antifungal activity, but it was inactive against *Bacillus subtilis*. 
Table 3.3: Antimicrobial activity of studied species

<table>
<thead>
<tr>
<th>Sample</th>
<th>Conc mg/ml</th>
<th>E.c</th>
<th>P.s</th>
<th>S.a</th>
<th>B.s</th>
<th>C.a</th>
<th>A.s</th>
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<tbody>
<tr>
<td>Compound II</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td>Chloroform extract</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>17</td>
<td>20</td>
</tr>
<tr>
<td><em>Acacia nilotica</em> aq. extract</td>
<td>100</td>
<td>17</td>
<td>16</td>
<td>18</td>
<td>18</td>
<td>17</td>
<td>15</td>
</tr>
</tbody>
</table>

Table 3.4: Antibacterial activity of standard chemotherapeutic agents against standard bacteria: M.D.I.Z (mm)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Conc. mg/ml</th>
<th>B.s.</th>
<th>S.a.</th>
<th>E.c.</th>
<th>P.a.</th>
<th>S.t</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
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<tr>
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<td>12</td>
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</tbody>
</table>

Table 3.5: Antifungal activity of standard chemotherapeutic agents against standard fungi

<table>
<thead>
<tr>
<th>Drug</th>
<th>Conc. mg/ml</th>
<th>A.n</th>
<th>C.a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clotrimazole</td>
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<tr>
<td>7.5</td>
<td>16</td>
<td>29</td>
<td></td>
</tr>
</tbody>
</table>

- S.a: *Staphylococcus aureus*
- E.c: *Escherichia coli*
• P.a: *Pseudomonas aeruginosa*
• A.n: *Aspergillus niger*
• C.a: *Candida albicans*
• S.t: *Salmonella typhi*
• B.a: *Bacillus subtilis*
• M.D.I.Z: Mean diameter or growth inhibition zone (mm). Average or two replicates, inhibition zone >=15: sensitive, <15: resistant.