A facile approach towards the synthesis of functionalized indenol derivatives identified as potent anti-oxidant and anti-bacterial agents

Sonia Taktouk¹, Jihène Ben Kraiem², Hedia Chaabane², Jacques Lebreton³ and Hassen Amri¹*

¹Department of Chemistry, University of Tunis El Manar, Faculty of Science, Campus, 2092 Tunis, Tunisia.
³Université de Nantes, CNRS, UMR 6230, Chimie Et Interdisciplinarité: Synthèse, Analyse, Modélisation, UFR Sciences et Techniques, 2, rue de la Houssinière, BP 92208, 44322 NANTES Cedex 3, France.

Abstract: An elegant one-pot synthesis of new indenols 2 was successfully carried out, starting from 1-hydroxy-1H-indene-2-carboxylic acid 1. Thus, esterification of acid 1 with aliphatic alcohols in toluene at reflux and a catalytic amount of para-toluene sulfonic acid afforded the corresponding alkyl esters 2 in moderate to good yields. This classical esterification process enables an efficient entry to a variety of new indenol-based molecular models 2, which could be adapted to a range of drug candidates. All the synthesized compounds 2a-e were subjected to the preliminary evaluation for their potential anti-oxidant and anti-bacterial activities. The assessment of radical scavenging capacity of the compounds 2a-e towards the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was measured and these compounds were found to scavenge DPPH free radical efficiently. Moreover, the in vitro antibacterial activity of derivatives 2a-e has been tested against a panel of pathogenic agents to show potent activity against all sensitive and resistant ones.

Keywords: 1-Hydroxy-1H-indene-2-carboxylic acid, Esterification, Indenol derivatives, Anti-bacterial and Anti-oxidant activities.

Introduction

For decades, the synthesis of molecules with an indenol core has been a topic of extensive interest for research as well as from an industrial standpoint¹. These compounds have a particular value due to their broad spectrum of biological activity² and their wide-ranging utility as synthetic tools in the design of various bioactive molecules³. Furthermore, some indenol-based natural products⁴ have shown analgesic and myorelaxation activity² and have been also used for the treatment of Alzheimer’s disease⁵. Despite the synthetic and pharmacological importance of indenols, few methods are known for the synthesis of these indene skeletons⁶. The typical method reported for the generation of indenols is the transition-metal-catalyzed carbocyclization⁷. In this context, the stoichiometric reaction of alkynes with ortho-manganated acetophenones⁸ gives rise to indenols. On the other hand, as

*corresponding author:
E-mail address: hassen.amri@fst.rnu.tn
DOI: http://dx.doi.org/10.13171/mjc.2.5.2014.07.01.22
previously described\textsuperscript{9}, the stoichiometric reaction of palladium (II) complexes of 2-acylaryl with alkynes affords indenol derivatives. Moreover, carbocyclization of \textit{ortho}-haloaromatic ketones or aldehydes with alkynes leads to substituted indenols\textsuperscript{10}. More recently\textsuperscript{11}, it has been reported that the regioselective rhodium-catalyzed carbocyclization reaction of \textit{ortho}-formylphenylboronic acid with alkynes is considered a promising way to produce substituted inden-1-ol derivatives. However, the above methods for synthesizing indenol derivatives require long reaction sequences, use expensive transition metals in strong acidic conditions\textsuperscript{12} and have less tolerance for sensitive organic functionalities. These limitations prompted us to develop a rarely reported protocol for the synthesis of various functionalized indenol derivatives, such as alkyl 1-hydroxy-1H-indene-2-carboxylates, using a simple low-cost methodology. In addition, interest in the research and study of biological activity showed that the synthesized cycloalkenols 2 showed potent antioxidant activity as DPPH radical scavenging as well as strong antibacterial activity against some pathogenic strains.

\textbf{Results and Discussion}

\textbf{Chemistry}

The starting material of our synthetic sequence depicted in Scheme 1 was the 1-hydroxy-1H-indene-2-carboxylic acid 1, obtained efficiently by a coupling reaction of a versatile reactant due to its special structure of two adjacent substituted formyl groups, such as \textit{ortho}-phthaldehyde, with malonic acid in basic medium in the presence of a catalytic amount of pyridine without organic solvent. This methodology afforded the functional acid 1 in 91\% yield, 24\% higher than the previous reported result\textsuperscript{13} (Scheme 1).

\begin{center}
\textbf{Scheme 1.} Synthesis of 1-hydroxy-1H-indene-2-carboxylic acid 1.
\end{center}

Motivated by this easy access to the acid 1, we then focused our investigations on the preparation of new functionalized indenols 2 using an esterification process as the key step. It is well known that the esterification reactions are among the oldest and most used reactions in organic chemistry\textsuperscript{14} and, even today, research in this field is still very active\textsuperscript{15}. They are considered the most expedient protocol for the one-pot conversion of carboxylic acids to their corresponding esters. In light of this, we sought to use a classic esterification process in the construction of the expected indene ring system. Our approach is based on an efficient coupling of primary and secondary alcohols to the acid 1.

The condensation of 1-hydroxy-1H-indene-2-carboxylic acid 1 with various alcohols in toluene at reflux and in the presence of a catalytic amount of \textit{para}-toluenesulfonic acid (PTSA) provided the corresponding esters 2 (Scheme 2 and Table 1).

All reactions worked well to give the desired bicyclic \(\beta\)-hydroxyesters 2 in moderate to good yields as shown in Table 1; these were characterized by spectroscopic methods such as \(\textsuperscript{1}H\) NMR, \(\textsuperscript{13}C\) NMR, IR and HRMS.
Scheme 2. Synthesis of cycloalkenols 2a-e

Table 1. Synthesis of new functionalized indenols 2a-e

<table>
<thead>
<tr>
<th>Entry</th>
<th>Substrate</th>
<th>Time</th>
<th>Product</th>
<th>Yield (%)^[a]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MeOH</td>
<td>72 h</td>
<td><img src="image1" alt="OH-CO2Me" /></td>
<td>65</td>
</tr>
<tr>
<td>2</td>
<td>EtOH</td>
<td>48 h</td>
<td><img src="image2" alt="OH-CO2Et" /></td>
<td>60</td>
</tr>
<tr>
<td>3</td>
<td>&quot;PrOH</td>
<td>52 h</td>
<td><img src="image3" alt="OH-CO2&quot;Pr" /></td>
<td>56</td>
</tr>
<tr>
<td>4</td>
<td>&quot;PrOH</td>
<td>60 h</td>
<td><img src="image4" alt="OH-CO2.&quot;Pr" /></td>
<td>62</td>
</tr>
<tr>
<td>5</td>
<td>&quot;BuOH</td>
<td>48 h</td>
<td><img src="image5" alt="OH-CO2.&quot;Bu" /></td>
<td>50</td>
</tr>
</tbody>
</table>

^[a]Yields obtained after chromatographic purification

To the best of our knowledge, the first synthesis of bicyclic functionalized cycloalkenols of type 2 was reported in 2005 in work related to the ring closing metathesis reactions of some Morita-Baylis-Hillman adducts^16. Consequently, we have tried to obtain compounds of type 2 via an esterification reaction of acid 1 through the use of some aliphatic alcohols in the presence of para-toluenesulfonic acid as catalyst in toluene at reflux. This coupling reaction afforded a new class of indene ring systems 2 bearing two different functional groups, which could be considered a new generation of Baylis-Hillman adducts^17.
Biological activities

Antioxidant studies

A freshly prepared DPPH solution exhibits a deep purple color with an absorption maximum at 515 nm. This purple color generally disappears when an antioxidant is present in the medium. Thus, antioxidant molecules can quench DPPH free radicals and convert them to colorless product\textsuperscript{18}. So, the RSA DPPH values of methanolic solutions of indenol derivatives 2 were examined and compared (Figure 1). Results are expressed as a percentage of the ratio of the decrease in absorbance at 515 nm. The antiradical activity was expressed as IC\textsubscript{50} (μg/ml), the concentration required to inhibit 50% of the DPPH, with lower IC\textsubscript{50} values corresponded to higher methanolic extract antioxidant activity. From analysis of the histogram (Figure 1), we can observe that the IC\textsubscript{50} values were found to vary from 373.67 μg/ml to 1294.34 μg/ml showing a wide range of variations in the reactivity of the indenols 2a-e. Compound 2c has the least IC\textsubscript{50} value of 373.67 μg/ml followed by compounds 2a, 2b, 2e; while compound 2d with the IC\textsubscript{50} = 1294.34 μg/ml showed the lowest DPPH scavenging activity. The wide variations in free radical scavenging activities may be due to the variations in the proton–electron transfer by the compounds due to the difference in their structures and stability.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{DPPH radical scavenging activities of the synthesized compounds 2a-e}
\end{figure}

Anti-bacterial studies

Access to highly functionalized building blocks 2 is of biological significance since these skeletons act as powerful agents against a panel of resistant pathogens\textsuperscript{19}. In fact, we found that all indenol derivatives 2a-e are capable of generating potent antibacterial activity against Gram-positive and Gram-negative bacteria such as \textit{Staphylococcus aureus} (ATCC 6538), \textit{Escherichia coli} (ATCC 8739), \textit{Salmonella typhimurium} (ATCC 14028), \textit{Streptococcus B}, \textit{Enterococcus faecium} (ATCC 19434), \textit{Pseudomonas aeruginosa} and \textit{Candida albicans} (ATCC 10231). The test results of the antibacterial activity of various indenol-esters 2a-e are summarized in Table 2.

As can be seen from the results collected in Table 2, all the compounds 2a-e showed an acceptable antibacterial activity against the Gram-negative and Gram-positive strains tested. Moreover, the zone of inhibition measured did not seem to be particularly affected by the length of the aliphatic skeleton group of the esters 2.
Table 2. Antibacterial activity of indenol-esters 2a-e against *S. aureus*, *E. coli*, *Streptococcus B*, *S. typhimurium*, *Pseudomonas aeruginosa*, *Enterococcus faecium*, *Candida albicans*.

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Standard (Ampicillin/Gentamicin)</th>
<th>2a</th>
<th>2b</th>
<th>2c</th>
<th>2d</th>
<th>2e</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em> ATCC 8739 Gram-</td>
<td>13 ± 1.4</td>
<td>8.5 ± 0.7</td>
<td>10.4 ± 0.7</td>
<td>10 ± 0.7</td>
<td>10.5 ± 0.7</td>
<td>9.5 ± 0.7</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em> Gram-</td>
<td>28.5 ± 2.1</td>
<td>8.25 ± 0.7</td>
<td>9</td>
<td>9 ± 2.8</td>
<td>9.5 ± 0.7</td>
<td></td>
</tr>
<tr>
<td><em>Salmonella typhimurium</em> ATCC</td>
<td>18 ± 2.8</td>
<td>8.5 ± 0.7</td>
<td>10</td>
<td>10.3 ± 0.7</td>
<td>10 ± 0.7</td>
<td>10.5 ± 0.7</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> ATCC</td>
<td>26.5 ± 2.1</td>
<td>-</td>
<td>-</td>
<td>11 ± 0.7</td>
<td>10.5 ± 17.5 ±</td>
<td></td>
</tr>
<tr>
<td><em>Enterococcus faecium</em> ATCC</td>
<td>39 ± 1.4</td>
<td>9.5 ± 0.7</td>
<td>10.5 ± 14.5 ±</td>
<td>17 ± 12 ±</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Streptococcus B</em> Gram+</td>
<td>34.5 ± 0.7</td>
<td>9.5 ± 14 ±</td>
<td>21 ± 19.5 ±</td>
<td>12 ±</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Candida albicans</em> ATCC 10231</td>
<td>18 - 13 11 11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The antibacterial activity of compounds 2a-e was determined in DMSO by the method of filter paper discs at a concentration of 10 mg/mL.

The results of the preliminary evaluation of the anti-bacterial activities of compounds 2a-e motivated us to determine the lower inhibitory concentration (MIC) of these esters 2. So, the MIC of different indenols 2 against the susceptible pathogens organisms is summarized in Table 3.

Table 3. Minimum inhibitory concentration (MIC, mg/ml) of indenols 2a-e against susceptible pathogen organisms

<table>
<thead>
<tr>
<th>Pathogens organisms</th>
<th>Standard (Ampicillin) (µg/ml)</th>
<th>2a</th>
<th>2b</th>
<th>2c</th>
<th>2d</th>
<th>2e</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em> ATCC 8739 Gram-</td>
<td>15.625</td>
<td>0.5</td>
<td>1.5</td>
<td>0.5</td>
<td>2</td>
<td>1.8</td>
</tr>
<tr>
<td><em>Salmonella typhimurium</em> ATCC 14028 Gram-</td>
<td>7.8125</td>
<td>0.5</td>
<td>1.8</td>
<td>1</td>
<td>1.5</td>
<td>1.8</td>
</tr>
<tr>
<td><em>Enterococcus faecium</em> ATCC 19434 Gram+</td>
<td>7.8125</td>
<td>0.5</td>
<td>1.5</td>
<td>0.5</td>
<td>0.4</td>
<td>1</td>
</tr>
<tr>
<td><em>Streptococcus B</em> Gram+</td>
<td>250</td>
<td>0.25</td>
<td>0.6</td>
<td>0.5</td>
<td>0.2</td>
<td>0.4</td>
</tr>
</tbody>
</table>

The results depicted in Table 3 revealed that indenols 2a-e showed moderate activities against the growth of some Gram-positive and Gram-negative bacteria (0.2 to 2 mg / mL), whereas the best activity against *Escherichia coli*, *Salmonella typhimurium* was referred to the compound 2a. The ester 2d was more effective against *Enterococcus faecium*, *Streptococcus B*. In this context, we intend to broaden the activity of these functionalized indenic esters 2a-e in other biological treatments.

Conclusion

In conclusion, we have developed a new and expeditious synthesis of functionalized indenol derivatives 2, which proved to be potent anti-bacterial and anti-oxidant agents, and could be useful as a template for future development of biologically active compounds. The extension of this method to the synthesis of other functionalized Michael acceptors, to study their electrophilic reactivity, synthetic utility, anti-bacterial and anti-oxidant activities, will be the subject of our future report.
Experimental Section

Chemistry

$^1$H-NMR and $^{13}$C-NMR spectra were recorded on a Bruker AMX 300 spectrometer working at 300 MHz and 75 MHz for the proton and $^{13}$C, respectively, with CDCl$_3$ (and sometimes DMSO or D$_2$O) as solvent and TMS as the internal standard. The chemical shifts ($\delta$) and coupling constants ($J$) are expressed in parts per million (ppm) and Hertz (Hz), respectively. All NMR spectra were acquired at room temperature. Assignments of proton ($^1$H-NMR) and carbon ($^{13}$C-NMR) signals were secured by DEPT experiments. IR spectra were recorded on an Equinox 55 spectrophotometer. High-Resolution Mass Spectrometry (HRMS) analyses were performed at the “Centre Commun de Spectrométrie de Masse” in Lyon (France), on a Micro-TOFII ThermoFischer Scientific for electro-spray ionization (ESI) measurements. Some products were also analysed with a Maldi-TOF-TOF technique on a Bruker Autoflex III Smartbeam in the laboratory. All reactions were monitored by TLC which was performed on Merck aluminum-backed plates pre-coated with silica (0.2 mm, 60 F$_{254}$). These were visualized either by quenching of ultraviolet fluorescence ($\lambda_{max} = 254$ nm) or by charring with KMnO$_4$ TLC dip. Flash chromatography (FC) was performed on silica gel (Merck Kieselgel 60 F$_{254}$, 230-400 mesh).

Procedure for the synthesis of 1-hydroxy-1H-indene-2-carboxylic acid 1

0.015 mole (2 g) of ortho-phthaldehyde was mixed with 0.015 mole (1.56 g) of malonic acid in an Erlenmeyer flask and warmed in a water-bath at 80°C until the aldehyde melted (about 1.5 h); then 0.15 mL of pyridine was added and the mixture was heated for 3 h at 80 °C. The resulting pasty mass was then stirred at room temperature overnight. Recrystallization, first from water and then from chloroform, gave 1-hydroxy-1H-indene-2-carboxylic acid 1. Yield: (91%) as a colorless needles; mp 163-164 °C. IR (neat): 3350, 1677 cm$^{-1}$. $^1$H NMR (300 MHz, D$_2$O) $\delta = 7.60$ (s, 1H, H ethylenic), 7.48-7.34 (m, 4H, H aromatic), 5.29 (s, 1H, C=OH). $^{13}$C NMR (75 MHz, D$_2$O) $\delta = 167.8$ (C=O), 146.2 (aromatic =C), 144.0 (=CH), 139.3 (aromatic =C), 138.8 (=C), 129.3 (aromatic =CH), 129.1 (aromatic =CH), 124.3 (aromatic =CH), 124.0 (aromatic =CH), 74.2 (CH). $^1$H NMR (300 MHz, DMSO) $\delta = 12.5$ (br, OH), 7.48 (s, 1H, H ethylenic), 7.43-7.29 (m, 4H, H aromatic), 5.5 (br, OH), 5.18 (s, 1H, CH).

General procedure for the esterification of 1-hydroxy-1H-indene-2-carboxylic acid 1 with alcohols

A mixture of 1-hydroxy-1H-indene-2-carboxylic acid 1 (1 g, 5 mmol) and the corresponding alcohol (15 mL) in toluene (20 mL) and a catalytic amount of para-toluene sulfonic acid (PTSA) was placed in a round-bottomed flask, and a Dean-Stark trap was attached. The reaction mixture was heated under reflux until evolution of water had ceased. After being cooled to room temperature, the solvent was removed under reduced pressure and the crude material was purified by column chromatography on silica gel (hexane/ethyl acetate, 6:4) to give the desired products 2a-e.
1-Hydroxy-1H-indene-2-carboxylic acid methyl ester (2a)
Yield: (65%) as a white solid; mp 99-100°C.
IR (neat) ν 3385, 1706 cm⁻¹. ¹H NMR (300 MHz, CDCl₃) δ= 7.61 (d, 1H, J= 6 Hz, H aromatic), 7.56 (s, 1H, H ethylenic), 7.43-7.36 (m, 3H, H aromatic), 5.43 (s, 1H, CH), 3.88 (s, 3H, OCH₃). ¹³C NMR (75 MHz, CDCl₃) δ= 165.1 (C=O), 145.8 (aromatic =C), 141.7 (=CH), 139.7 (aromatic =C), 139.2 (=C), 129.0 (aromatic =CH), 128.9 (aromatic =CH), 124.3 (aromatic =CH), 123.9 (aromatic =CH), 75.6 (CH), 51.8 (OCH₃). HRMS (ES) m/z calcd for C₁₃H₁₀O₃Na [M+Na⁺] 213.05186, found 213.05222.

1-Hydroxy-1H-indene-2-carboxylic acid ethyl ester (2b)
Yield: (60%) as a white solid; mp 59-60°C.
IR (neat) ν 3358, 1709 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ= 7.57 (d, 1H, J= 6 Hz, H aromatic), 7.52 (s, 1H, H ethylenic), 7.40-7.30 (m, 3H, H aromatic), 5.41 (s, 1H, CH), 4.30 (q, 2H, J= 7 Hz, OCH₂), 3.48 (br, OH), 1.35 (t, 3H, J= 7 Hz, CH₃). ¹³C NMR (75 MHz, CDCl₃) δ= 164.7 (C=O), 145.9 (aromatic =C), 141.3 (=CH), 139.7 (aromatic =C), 139.4 (=C), 128.8 (aromatic =CH), 124.3 (aromatic =CH), 123.7 (aromatic =CH), 75.5 (CH), 60.7 (OCH₂), 14.3 (CH₃). HRMS (ES) m/z calcd for C₁₃H₁₂O₃Na [M+Na⁺] 227.06787, found 227.06766.

1-Hydroxy-1H-indene-2-carboxylic acid propyl ester (2c)
Yield: (56%) as a white solid; mp 57-58°C.
IR (neat) ν 3355, 1707 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ= 7.58 (d, 1H, J= 6 Hz, H aromatic), 7.53 (s, 1H, H ethylenic), 7.40-7.31 (m, 3H, H aromatic), 5.41 (s, 1H, CH), 4.42 (t, 2H, J= 7 Hz, OCH₂), 3.35 (br, OH), 1.74 (m, 2H, CH₂), 1.01 (t, 3H, J= 7 Hz, CH₃). ¹³C NMR (75 MHz, CDCl₃) δ= 164.8 (C=O), 145.9 (aromatic =C), 141.2 (=CH), 139.7 (aromatic =C), 139.5 (=C), 128.8 (aromatic =CH), 124.3 (aromatic =CH), 123.7 (aromatic =CH), 75.6 (CH), 66.2 (OCH₂), 22.1 (CH₂), 10.4 (CH₃). HRMS (ES) m/z calcd for C₁₃H₁₄O₃Na [M+Na⁺] 241.0835, found 241.0830.

1-Hydroxy-1H-indene-2-carboxylic acid isopropyl ester (2d)
Yield: (62%) as a yellow oil.
¹H NMR (300 MHz, CDCl₃) δ= 7.58 (d, 1H, J= 6 Hz, H aromatic), 7.49 (s, 1H, H ethylenic), 7.36-7.30 (m, 3H, H aromatic), 5.39 (s, 1H, CH), 5.18 (m, 1H, CH), 3.71 (br, OH), 1.33 (d, 3H, J= 6 Hz, CH₃), 1.31 (d, 3H, J= 6.3 Hz, CH₃). ¹³C NMR (75 MHz, CDCl₃) δ= 164.3 (C=O), 146.0 (aromatic =C), 141.0 (=CH), 139.9 (aromatic =C), 139.7 (=C), 128.7 (aromatic =CH), 124.3 (aromatic =CH), 123.6 (aromatic =CH), 75.5 (CH), 68.2 (OCH₂), 21.9 (2xCH₃).

1-Hydroxy-1H-indene-2-carboxylic acid butyl ester (2e)
Yield: (50%) as a white solid; mp 53-54°C.
IR (neat) ν 3367, 1704 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ= 7.60 (d, 1H, J= 6 Hz, H aromatic), 7.54 (s, 1H, H ethylenic), 7.41-7.35 (m, 3H, H aromatic), 5.42 (s, 1H, CH), 4.27 (t, 2H, J= 6 Hz, OCH₂), 3.25 (br, OH), 1.70 (m, 2H, CH₂), 1.47 (m, 2H, CH₂), 0.89 (t, 3H, J= 6 Hz, CH₃). ¹³C NMR (75 MHz, CDCl₃) δ= 164.8 (C=O), 145.9 (aromatic =C), 141.2 (=CH), 139.7 (aromatic =C), 139.4 (=C), 128.9 (aromatic =CH), 124.3 (aromatic =CH), 123.8 (aromatic =CH), 75.6 (CH), 64.6 (OCH₂), 30.7 (CH₂), 19.2 (CH₂), 13.7 (CH₃). HRMS (ES) m/z calcd for C₁₄H₁₆O₃Na [M+Na⁺] 255.0992, found 255.0993.

Antioxidant activity studies: DPPH radical scavenging activity
The capacity of compounds to scavenge the free radical DPPH was monitored according to the method of Hatano et al.²⁰ Different concentrations of methanolic compounds solutions (1 mL) were mixed with methanolic solution containing DPPH radicals (10⁻⁴ mol.L⁻¹). The mixtures were incubated for 4h in the dark at room temperature (until stable absorption values
were obtained. Scavenging capacity was measured spectrophotometrically (UV-visible 515 nm optizen 2120 UV) by monitoring the decrease in absorbance 515 nm. The antiradical activity was expressed as IC50 (μg/ml), the concentration required to inhibit 50% of the DPPH. The DPPH radical-scavenging activity was calculated as follows:

\[ \%RSA = \% inhibition = \left( \frac{A_{DPPH} - A_S}{A_{DPPH}} \right) \times 100, \]

where \( A_S \) is the absorbance of the solution when the compound has been added at a particular level and \( A_{DPPH} \) is the absorbance of the DPPH solution. Trxol was used as standard.

**Anti-bacterial testing**

All the strains chosen in this test were supplied by the Institute for Research and Physico-Chemical Analysis (INRAP) Pôle Technologique Sidi Thabet, Tunisia. The *in vitro* antimicrobial activity of the prepared indenols 2a-e against *S. aureus*, *E. coli*, *Streptococcus B*, *S. typhimurium*, *Pseudomonas aeruginosa*, *Enterococcus faecium*, *Candida albicans* was determined as recommended by the NCCLS. So, the antibacterial test of synthesized indenols 2a-e was carried out using the agar well diffusion method. Ampicillin and Gentamicin are used as the standard drug. The bacteria from stock culture were lightly inoculated into the Mueller Hinton broth (MHB) and allowed to grow overnight at 37 °C in an ambient air incubator. The culture was diluted with a new MHB to achieve an absorbance value of 2.0 × 10^6 colony-forming units (CFU/mL). Sterile cotton swab was dipped into the broth culture and inoculated on the Mueller Hinton agar. Sterile paper disks with 5 mm diameter were placed on the agar at equal distance. The recommended concentration of the test sample (10 mg/mL in DMSO) was introduced individually to each of the disks. The agar plates were incubated immediately at 37 °C for 20 h. For each plate, DMSO mixture and reference antibacterial drug such as Ampicillin and Gentamicin served as negative and positive controls, respectively. The activity was determined by measuring the diameter of zones showing complete inhibition (mm). Growth inhibition was calculated with reference to the positive control. For the MIC (defined as the lowest concentration of inhibitor at which microbial growth was not apparent disregarding a single colony) it was determinate by the agar dilution method using Mueller-Hinton agar; the bacterial inocula contained approximately 10^6 colony-forming units and the bacterial growth was assessed by visual inspection after 24h incubation at 37°C.

**Acknowledgements**

The authors thank the Tunisian Ministry of Higher Education and Scientific Research for financial support. We are also thankful to the Institute for Research and Physico Chemical Analysis (INRAP) Pôle Technologique Sidi Thabet for anti-oxidant studies and biological testing.

**References**