Anticancer potential and antioxidant effect of clove (*Syzygium aromaticum*) hydro-alcoholic extract and his fractions

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ABSTRACT
The annual increase in female mortality caused by breast cancer, exacerbated by chemotherapy resistance, presents a serious concern. Our investigation aims to address specific aspects of chemotherapeutic resistance by capitalizing on the properties of medicinal plants. We prepared essential oil and hydroalcoholic extracts from Comorian cloves (*Syzygium aromaticum*). From the hydro-alcoholic extracts, we derived the following fractions: cold hexane and ethyl acetate fractions; as well as hot methanol, acetone, ethyl acetate, and hexane fractions. The hydroalcoholic extract, along with its fractions and essential oil, underwent cytotoxicity testing (MTT test) against the MCF7 tumor cell line. Remarkably, the essential oil, hot hexane fraction, methanol fraction, and cold ethyl acetate fraction exhibited a highly significant cytotoxic effect on MCF7 cells. Furthermore, the DPPH (2,2-diphenyl-1-picrylhydrazyl) test indicated a robust antioxidant activity in the hot hexane, hot acetone, and hexane and acetate fractions of cold ethyl acetate extracts. These findings emphasize the potential of Comorian cloves as a promising avenue to combat chemotherapeutic resistance and harness antioxidant benefits.

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1. Introduction:
The landscape of cancer diagnosis is constantly evolving, and within it, breast cancer has taken center stage as a significant global health concern [1]. In recent years, it has surpassed lung cancer to become the most commonly diagnosed cancer, with a staggering 2.3 million new cases reported in 2020, accounting for 11.7% of all new cancer cases worldwide. Among these cases, approximately 12% belong to the category of triple-negative breast cancer (TNBC), a particularly aggressive subtype that presents unique challenges in treatment and management [2-3].

Chemotherapy, while a cornerstone of cancer treatment, comes with a range of side effects that can significantly impact a patient's quality of life. The mechanism of chemotherapy involves targeting rapidly dividing cells, which includes cancer cells. However, it also affects normal, healthy cells that naturally divide rapidly, leading to adverse effects such as hair loss, nausea, fatigue, and compromised immune function. Furthermore, breast cancer cells can rapidly develop resistance to chemotherapy, rendering the treatment less effective over time [4-6].

In light of these challenges, researchers and medical professionals have been exploring alternative approaches to breast cancer treatment, and herbal medicine has emerged as a promising avenue. The use of herbal remedies dates back centuries and is deeply rooted in traditional medicine systems across the world. In recent years, there has been a growing interest in harnessing the potential of medicinal plants to complement conventional cancer therapies [7-10].

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One such medicinal plant that has garnered attention is *Syzygium aromaticum*, commonly known as cloves. Native to the Maluku Islands in eastern Indonesia, cloves have been used for their aromatic and medicinal properties for centuries. The plant yields dried flower buds (cloves), essential oil (EO), and oleoresin, with a high content of eugenol, a bioactive compound known for its antioxidant and anti-inflammatory properties [11]. Clove’s potential in cancer treatment lies in its diverse range of bioactive compounds that exhibit various therapeutic properties. Among its well-documented effects are analgesic and anti-inflammatory actions, making it a valuable natural remedy for pain relief and inflammation management. Additionally, cloves have demonstrated antimicrobial and antifungal activities, further highlighting their potential in disease prevention and management [12]. In the realm of breast cancer research, the focus has turned to investigating the cytotoxic and antioxidant effects of different fractions of clove extracts. The use of botanical extracts in cancer research is intriguing, as they offer a holistic approach that targets multiple pathways and mechanisms involved in cancer development and progression. By examining the impact of clove fractions on cell viability and oxidative stress, researchers aim to uncover potential therapeutic benefits that could enhance current breast cancer treatment strategies [13]. Early findings from studies assessing the cytotoxic and antioxidant effects of clove extracts are promising. Essential oils, hot hexane fractions, methanol fractions, and cold ethyl acetate fractions have demonstrated significant cytotoxic effects against MCF7 tumor cells, a well-established model for breast cancer research. Moreover, the antioxidant activity of specific clove fractions, as determined by the DPPH assay, has further emphasized the potential role of cloves in combating oxidative stress, a hallmark of cancer development.

As research continues to unfold, the role of clove extracts in breast cancer treatment becomes increasingly intriguing. While these findings present exciting possibilities, further exploration is needed to fully understand the mechanisms underlying clove’s effects on cancer cells and its potential to enhance existing treatment approaches. With its long history of use in traditional medicine and its growing recognition in modern research, cloves may hold the key to unlocking innovative strategies for addressing breast cancer and improving patient outcomes.

2. Materials and methods:

2.1. Chemicals and cell lines:
 Foetal calf serum and methyl tetrazolium (MTT) and 2,2-diphenyl-1-picrylhydrazyle, DPPH were purchased from Sigma Aldrish (St Quentin, France). The culture medium RPMI1640 with L-Glutamine (25mM HEPES) was purchased from Capricorn Scientific GmbH (Biotechnology company in Ebsdorfergrund, Germany).

The cell line MCF7 was from the stock cultures of the Laboratory of Biological Engineering, Faculty of Science & Technology, Sultan Moulay Slimane University, Morocco.

2.2. Extraction method:
In order to conduct this study, we purchased Comorian clove buds from the French company KLY GROUPE. As the cold hydro alcoholic extract, we crushed the cloves a little before putting 30g of this powder in maceration in an ethanolic solution (70% ethanol). After 72 hours, the macerate is filtered through cotton and filter paper and centrifuged at 1000 rpm for 15 min, and split twice with 100 ml of hexane and ethyl acetate. Using the Soxhlet, we prepared cartridges with 15g of cloves and subsequently used hexane, ethyl acetate, acetone, and methanol to prepare the hot fractions.

2.3. Cell culture:
MCF-7 cells were cultured in RPMI 1640 medium supplemented with 5% heat-inactivated fetal bovine serum and 1% antibiotic (penicillin G-streptomycin). After that, the cells were incubated at 37°C in a humid environment containing 5% CO₂.

2.4. Identification of cell viability by MTT Assay:
In 96-well microplates with 100 µl of complete medium, the human breast cancer cells MCF7 are distributed. Prior to treatment, the cells were allowed to adhere for one night. In 100 µl of RPMI 1640 medium, several quantities of extracts and their fractions in DMSO were added. DMSO alone was used to treat the control cells. The ultimate DMSO concentration was always less than 0.1% in every case, and we incubated the cells for 48 hours at 37°C in a humid environment containing 5% CO₂. The Colorimetric MTT (tetrazolium) assay is used according to the protocol of Mosman et al. (1983) with little modifications [14]. There were three separate sets of experiments that were duplicated. In order to determine the relative percentage of cell viability, the following formula was used:

\[
\text{% Viability} = 100 \times \left( \frac{A}{A_0} \right)
\]

In which 0. represents the absorbance of the negative control and A represents the absorbance of the test culture, respectively. IC50 values (concentrations of tested extracts that inhibit cell viability by 50%) were used to compare the cytotoxic effects of extracts and fractions against the cell line.
2.5. Antioxidant activity by DPPH Assay:
In order to assess antioxidant activity, the stable free radical DPPH (2,2-dioxygen) was used as a model. According to Abdoul-Latif et al. (2021) [15] with some modifications, extract solutions (50 mL) were mixed with 150 mL of freshly prepared DPPH solution. After shaking vigorously, the mixture was left to stand at room temperature and in the dark for 30 minutes. The reduction of the DPPH radical was measured at 517nm. This equation was used to calculate the DPPH scavenging activity:

\[
\text{%scavenging effect} = \left(\frac{A_{\text{DPPH}} - A_{\text{S}}}{A_{\text{DPPH}}}\right) \times 100
\]

Where \(A_{\text{S}}\) is the absorbance values of the sample and \(A_{\text{DPPH}}\) is the absorbance of the DPPH solution.

2.6. Statistical analysis:
The data are mean values ± standard deviation. A one-way analysis of variance (ANOVA) was applied to the results followed by the Tukey test using Graph Pad Prism 8 software. The differences were considered to be significant when \(p < 0.05\).

3. Results and discussion:
3.1. Identification of cell viability by MTT Assay:
Extensive research has been conducted to investigate the potential anticancer properties of clove, and multiple findings indicate that both clove itself and its chemical constituents possess attributes of a promising natural anticancer agent [16-18]. The antitumor efficacy of various extract fractions was assessed against MCF-7 tumor cells, and the summarized outcomes are presented in Table 1. The results indicate that the cytotoxic effects vary based on the nature of the fraction and the applied dosage against the tested cells. Particularly noteworthy are the essential oil derived from Comorian cloves and the hot hexane extract fraction, which exhibited the lowest IC50 values of 12.35±11.60µg/ml and 22±6.82µg/ml, respectively, against MCF7 cells. In a study by Lui et al. (2014) [19], the ethanol extract of clove buds demonstrated an IC50 of 455.0 ± 65.7µg/ml, while the ethyl acetate extract of clove buds showed an IC50 of 216.7 ±14.6 µg/ml against MCF7 cells. The ethyl acetate extract consistently displayed notable cytotoxicity levels against MCF7 and other cell lines compared to the fractions employed in our study. However, the ethyl acetate fraction used in our investigation revealed significant cytotoxicity in comparison to the ethyl acetate extract. Consistent with various studies, the essential oil of Comorian clove exhibited a highly significant IC50 value of 12.35±11.60, while the n-hexane and ethyl acetate extracts of clove buds demonstrated moderate cytotoxicity against the HUH7 cell line. Similarly, the methanol extract exhibited a pronounced effect, aligning with the outcomes observed in our study [20-21].

Table 1. IC50 of Comorian clove fractions against MCF7 cells.

<table>
<thead>
<tr>
<th>Fractions</th>
<th>IC50 (µg/ml)</th>
<th>IC50 paclitaxel (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hot hexane fraction</td>
<td>22.00±6.82</td>
<td>0.11±0.05</td>
</tr>
<tr>
<td>Hot Acetonic fraction</td>
<td>38.54±14.63</td>
<td></td>
</tr>
<tr>
<td>Hot Methanolic fraction</td>
<td>28.27±18.70</td>
<td></td>
</tr>
<tr>
<td>Cold hexane fraction</td>
<td>64.60±1.450</td>
<td></td>
</tr>
<tr>
<td>Hot ethyl acetate fraction</td>
<td>28.98±4.787</td>
<td></td>
</tr>
<tr>
<td>Cold ethyl acetate fraction</td>
<td>44.34±32.34</td>
<td></td>
</tr>
<tr>
<td>Essential oil</td>
<td>12.35±11.60</td>
<td></td>
</tr>
</tbody>
</table>

*: significant difference from essential oil at \(p<0.05\), \(b\): significant difference from paclitaxel at \(p<0.05\).

Figure 1. IC50 of tested fractions of Syzygium aromaticum and Paclitaxel against MCF7 cells.
3.2. Antioxidant activity by DPPH Assay:

DPPH, a free-radical compound, serves as a commonly utilized indicator for evaluating the free-radical scavenging potential of diverse molecules and extracts. Table 2 provides a comprehensive overview of the antioxidant prowess exhibited by fractions and essential oil derived from Comorian Syzygium aromaticum cloves. Particularly notable is the exceptional inhibitory activity demonstrated by clove oil, attaining an impressive 91.2% at a concentration of 0.5 µg/mL. Furthermore, our findings underscore the remarkable free-radical scavenging capability of the hot hexane fraction, surpassing that of other examined extracts and fractions [22].

Table 2. IC50 values of fractions obtained in DPPH assay.

<table>
<thead>
<tr>
<th>Anti-oxidant effect IC50 (µg/ml)</th>
<th>Hot hexane fraction</th>
<th>Hot Acetonic fraction</th>
<th>Hot Methanolic fraction</th>
<th>Cold hexane fraction</th>
<th>Hot ethyl acetate fraction</th>
<th>Cold ethyl acetate fraction</th>
<th>Essential oil</th>
<th>Ascorbic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.03±0.09</td>
<td>30.68±6.28</td>
<td>12.46±0.88</td>
<td>6.11±0.22</td>
<td>86.16±13.17</td>
<td>5.76±0.45</td>
<td>124.40±96.28</td>
<td>4.84±2.48</td>
</tr>
</tbody>
</table>

4. Conclusion:

In conclusion, the findings of this study underscore the significant potential of essential oil and various fractions (hot methanol, hexane, and ethyl acetate) derived from Comorian Syzygium aromaticum cloves in the field of cancer research. The observed high cytotoxic activity against MCF7 cells highlights their promising role as potential anti-cancer agents. Particularly noteworthy is the notable cytotoxic effect exhibited by the essential oil and the hot hexane fraction, both of which displayed low IC50 values, indicative of their strong potency in inhibiting tumor cell growth. Furthermore, the study revealed compelling evidence of the antioxidant prowess of these extracts and fractions, as demonstrated by their robust free-radical scavenging ability. The substantial inhibitory activity, particularly exhibited by the clove oil, underscores its potential as a potent natural antioxidant. The multifaceted attributes of Comorian clove extracts and fractions, including their significant cytotoxic and antioxidant effects, open new avenues for therapeutic interventions in breast cancer treatment. However, further exploration and comprehensive investigations are warranted to elucidate the underlying mechanisms of these bioactive components and their potential synergistic interactions. These promising findings provide a strong foundation for future studies aimed at harnessing the therapeutic potential of these natural compounds for breast cancer management and prevention.

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References:


