In vitro anticandidal activity of the secondary metabolites extracted from Saussurea Costus (Falc.) lipschitz roots

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Abstract---The present study, was conducted to investigate the effect of the crude Alkaloids, Flavonoids, and Terpenoids compounds extract from roots of (Saussurea costus) against Candida species isolated from different clinical samples such as mouth and vagina in the province of Babil 2021 in Iraq. Anticandidal activity was achieved in vitro by using agar well diffusion method against Candida species by preparing three concentrations for each crude compound (25, 50, and 100) mg/ml and compared to positive control represented by Fluconazole 50mg/ml and negative control represented by 10% dimethyl sulfoxide. The aim of this study was to control of Candida species isolated different clinical samples such as mouth and vagina by using secondary metabolites extracted from seeds of Carthamus tinctorius L. The data collected from the study revealed that, the crude Alkaloids, Flavonoids, and Terpenoids compounds extract from seeds of (Saussurea costus) showed reduction at P≤ 0.05 in the growth of Candida species especially at 100mg/ml compared with negative control. Finally, it can be concluded that Saussurea costus is most effective in controlling Candida species, especially Terpenoid and Alkaloids compounds.

Keywords---anticandidal activity, saussurea costus, alkaloids, flavonoids, terpenoids.
Introduction

The use of herbal medicine to manage or cure diseases dates back to the Stone Age. There has been an advancement in pharmacological discoveries over the years that has resulted in the production of many synthetic drugs [1]. *Saussurea costus* (Falc.) Lipschitz, syn *Saussurea lappa* C.B. Clarke is belonging to *Asteraceae* family, is a Himalayan species that occurs at elevations from 2,700-4,000 m in Kashmir, Lahul Valley in Himachal Pradesh and Garhwal in Uttranchal [2, 3]. *Saussurea costus* (Falc.) Lipschitz, syn *Saussurea lappa* C.B. Clarke is a well known and important medicinal plant widely used in several indigenous systems of medicine for the treatment of various ailments, viz. asthma, inflammatory diseases, ulcer and stomach problems. Sesquiterpene lactones have been reported as the major phytoconstituents of this species. Different pharmacological experiments in a number of *in vitro* and *in vivo* models have convincingly demonstrated the ability of *Saussurea costus* to exhibit anti-inflammatory, anti-ulcer, anticancer and hepatoprotective activities, lending support to the rationale behind several of its traditional uses.

Costunolide, dehydrocostus lactone and cynaropicrin, isolated from this plant, have been identified to have potential to be developed as bioactive molecules. Due to the remarkable biological activity of *Saussurea costus* and its constituents it will be appropriate to develop them as a medicine [4]. Lappadilactone, lactone cyanaropicrin, dehydrocostus, germacrenes were isolated from fresh roots of plant [5]. *Candida* species are yeasts that can dangerously cause many types of fungal infections in human body/organs, whereas “Candidiasis” is the general term to categorize the infectious diseases from Candida contagion [6]. The antifungal resistance involves multifactorial complex phenomena that are not fully understood and haven’t been fully elucidated; resistance to antifungal drugs could develop as a result of mutations in the fungus’ DNA, which lead to alterations in the pathogen’s drug metabolism; the other potential mechanisms involving *Candida* spp. resistance especially biofilm forming cells include elevated efflux pump potentiality, excessive cells’ density within biofilm, consequences of biofilm matrix, nutrient and growth limitations, the persister cells occurrence, or increased sterols contents on cells’ membranes [7,8, 9]. However, the aim of this study was to control of *Candida* species isolated form different clinical samples by using secondary metabolites extracted from *Saussurea costus* roots.

Materials and Methods

Plant material

The roots of (*Saussurea costus*), had been purchased from local markets, identified and classified according to [10]. (Table: 1). Roots of this plant was cleaned, dried, and kept according to [11].
Table 1
Scientific, Local, English name, Family, and active parts

<table>
<thead>
<tr>
<th>Scientific name</th>
<th>Local name</th>
<th>English name</th>
<th>Family</th>
<th>Active part used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saussurea costus</td>
<td>Al-Kost Al-Hindi</td>
<td>Costus</td>
<td>Asteraceae</td>
<td>Roots</td>
</tr>
</tbody>
</table>

- **Extraction of the Crude Alkaloid Compounds:** Crude Alkaloid compounds were extracted according to [12].
- **Extraction of the Crude Flavonoid Compounds:** Crude Flavonoid compounds were extracted according to [13].
- **Extraction of the Crude Terpenoid Compounds:** Crude terpenoids compounds were extracted according to [14]. Stock solution of 100 mg/ml for Alkaloid, Flavonoid, and Terpenoid were prepared in 10% Dimethyl Sulfoxide (DMSO) then sterilized by Millipore filter (0.22µm) and stored at (-20C˚) until use [15].
- **Anticandidal Efficacy:** The anticandidal activity of the secondary metabolites compounds extracted from the root of *Saussurea costus* was tested against the isolates of *Candida* species by using agar-well diffusion method [16]. Wells were made by using cork porer (6mm) in diameter. Dimethyl sulfoxide 10% (DMSO) was used as a negative control and Fluconazole antibiotic as a positive control.
- **Candida Isolates:** All isolates used in this study was isolated from hospitals located at Hillah city, Iraq (Table: 2).

<table>
<thead>
<tr>
<th>NO</th>
<th>Fungal isolates</th>
<th>Type of specimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Candida Isolates</td>
<td>Mouth</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vagina</td>
</tr>
</tbody>
</table>

**Statistical analysis**

All data of treatments were dictated by three replicates. Data were subjected to an analysis of variance by using SPSS 16.0 program, a completely randomized design was used and least significant difference (L.S.D) was performed at P≤ 0.05.

**Results**

The results of antifungal activity of the crude Alkaloid compounds extracted from the roots of *Saussurea costus* against *Candida* species isolated from different clinical samples such as mouth and vagina are presented in (table 3). The antifungal activity of Alkaloid secondary metabolites with three concentrations (25, 50, and 100mg/ml) was screened by agar well diffusion methods. The results revealed that, the crude Alkaloid compounds extracted from the roots of *Saussurea costus* showed significant reduction at P≤ 0.05 in the growth of *Candida* species. Growth inhibition represented by zone of inhibition ranging from (20± 1.00mm in 25 mg/ml, 25± 1.00 mm in 50 mg/ ml, and 30± 1.00 mm in 100
mg/ml) (Figure: 1), compared with negative control representative by 10% DMSO
and positive control representative by Fluconazole 50mg/ml where inhibition zone
was (0.00 mm for negative control and 35± 1.00mm for positive control). On the
other hand, the crude Flavonoid compounds showed 8± 1.00mm of zone of
inhibition at (25 mg/ml) and 12± 1.00mm at (50 mg/ml), and 27± 1.00mm at (100
mg/ml) concentration (table 4), Thus, it differed significantly compared to the
control treatment (Figure: 2).

Table 3
Anti-candidiasis activity of the crude Alkaloid compounds extracted from
Saussurea costus roots

<table>
<thead>
<tr>
<th>Concentrations (mg/ml)</th>
<th>Alkaloids compounds Inhibition Zone/mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control</td>
<td>0± 0.00</td>
</tr>
<tr>
<td>25 mg/ml</td>
<td>20± 1.00</td>
</tr>
<tr>
<td>50 mg/ml</td>
<td>25± 1.00</td>
</tr>
<tr>
<td>100 mg/ml</td>
<td>30± 1.00</td>
</tr>
<tr>
<td>Positive Control</td>
<td>35± 1.00</td>
</tr>
<tr>
<td>L.S. D</td>
<td>1.62</td>
</tr>
</tbody>
</table>

*Mean± standard deviation

Table 4
Anti-candidiasis activity of the crude Flavonoid compounds extracted from
Saussurea costus roots

<table>
<thead>
<tr>
<th>Concentrations (mg/ml)</th>
<th>Flavonoid compounds Inhibition zone %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control</td>
<td>0± 0.00</td>
</tr>
<tr>
<td>25 mg/ml</td>
<td>8± 1.00</td>
</tr>
<tr>
<td>50 mg/ml</td>
<td>12± 1.00</td>
</tr>
<tr>
<td>100 mg/ml</td>
<td>27± 1.00</td>
</tr>
<tr>
<td>Positive Control</td>
<td>35± 1.00</td>
</tr>
<tr>
<td>L.S. D</td>
<td>1.60</td>
</tr>
</tbody>
</table>

*Mean± standard deviation

Table 5
Anti-candidiasis activity of the crude Terpenoid compounds extracted from
Saussurea costus roots

<table>
<thead>
<tr>
<th>Concentrations (mg/ml)</th>
<th>Terpenoid compounds Inhibition zone %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control</td>
<td>0± 0.00</td>
</tr>
<tr>
<td>25 mg/ml</td>
<td>22± 1.00</td>
</tr>
<tr>
<td>50 mg/ml</td>
<td>26± 1.00</td>
</tr>
<tr>
<td>100 mg/ml</td>
<td>31± 1.00</td>
</tr>
<tr>
<td>Positive Control</td>
<td>35± 1.00</td>
</tr>
<tr>
<td>L.S. D</td>
<td>1.61</td>
</tr>
</tbody>
</table>

*Mean± standard deviation
In the same context, the crude Terpenoid compounds showed significant activity at three concentrations (25, 50, and 100 mg/ml) compared with negative control against *Candida* species isolated from different clinical samples (Table 5). The highest zone of inhibition 31± 1.00mm was recorded at 100 mg/ml and 26± 1.00mm was recorded at 50 mg/ml (figure: 3). While the highest zone of inhibition in the crude Alkaloid compounds was reached up to 30± 1.00mm at 100 mg/ml concentration and the highest zone of inhibition in the crude Flavonoid compounds was reached up to 27± 1.00 at 100 mg/ml concentration. The terpenoid and alkaloid compounds were the most effective compared to flavonoid compounds and came close to the effect of the Fluconazole (35± 1.00mm), (figure: 4).

![Figure 3](image1.png) Anti-candidiasis activity of the crude Alkaloid compounds extracted from *Saussurea costus* roots at 100 mg/ml

![Figure 2](image2.png) Anti-candidiasis activity of the crude Flavonoid compounds extracted from *Saussurea costus* roots at 100 mg/ml

![Figure 4](image3.png) Anti-candidiasis activity of the crude Flavonoid compounds extracted from *Saussurea costus* roots at 50 and 100 mg/ml

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**Figure 4.** Inhibition zone of Alkaloid, Terpenoid, and Flavonoid at 100/ml against *Candida* species, LSD= 2.15
Discussion

The present study was proved that, the secondary metabolites include Alkaloids, Flavonoids, and Terpenoids extracted from the roots of (Saussurea costus) had antifungal activity against Candida species isolated from different clinical samples such as mouth and vagina. The plant kingdom provided and is still providing endless sources of medicinal plants of various uses for example, Secondary metabolites extracted from different active parts of numerous medicinal plants such as (Lactuca serriola leaves; Lepidium sativum leaves; Myrtus Communis leaves; Cassia senna leaves; Ricinus communis leaves; Cassia didymobotrya leaves; Melia azedarach leaves; Dianthus caryophyllus flowers bud; and Salvia hispanica seeds), possess ability of antibacterials for controlling several pathogenic microorganisms isolated from different clinical samples [17, 18, 19, 20, 21, 22, 23, 24, 25]. [26] Reported that, phytochemical compounds extracted from the unicellular primitive plant like Chlorella vulgaris possess ability of antibacterial counter to pathogenic bacteria. [27]

Used phytochemical compounds extracted from Hibiscus sabdarifa for controlling E. coli and Proteus sp. [28] Used phytochemical compounds extracted from of Ficus carica L. for controlling E. coli and Pseudomonas aeruginosa. [29] Used phytochemical compounds extracted from Boswellia carteri and Curcuma longa for controlling Fusarium sp. isolated from seeds of maize. [30, 31] Used terpenoids compounds extracted from C. tinctorius seeds and flavonoid compounds extracted from M. Communis leaves against Aspergillus species isolated from stored medicinal plant seeds. Secondary metabolites represented by Alkaloids and Flavonoids compounds extracted from M. Communis leaves respected a worthy source for controlling pathogenic microorganisms segregated from hemodialysis fluid specimens [32]. [33] Used Callistemon viminalis leaves extracts for controlling isolates of Urinary Tract Infections. S. costus has been screened as medicinally important plant, the various chemical compounds isolated from it possess medicinal properties [10].

Various compounds were isolated from the roots of S. costus and tested against the nine fungal strains i.e. Aspergillus flavus, Aspergillusniger, Aspergillus ochraceus, Aspergillus versicolor, Aspergillus flavus, Penicilium ochrochloron, Penicilium funiculosum, Trichoderma viride, Cladosporium cladosporioides and Alternaria. The compound showed antifungal effects which were moderate too high [34]. Ethanol and ethyl acetate extracts of S. costus had the highest levels of polyphenols followed by n-butanol, and then n-hexane extracts. The main phenolic compounds are Naringenin, Chlorogenic acid, Ferulic acid, Ellagic acid, Gallic acid and coffeic acid followed by taxifolin, catechin, syringic acid, methyl gallate, vanillin, kaempferol, cinnamic acid and rutin and this extracts had antibacterial and antifungal agents against broad range of microorganisms [35]. Saussurea lappa showed best antifungal activity against Aspergillus flavus followed by Trapa natans and Mangifera indica [36].

The methanol extract of roots of S. costus are rich in some bioactive phytochemical compounds such as alkaloids, phenols/polyphenols,flavonoids, terpenoids, tannins, coumarins, quinines, steroids, cardiac glycosides and resins and had antifungal activity against Aspergillus niger ATCC 6275 [37]. The crude
extracts of n-hexane and dichloromethane were effective against different strains and isolates of Candida [38]. On the other hands, the mode of the antifungal action of the Alkaloids is usually pleiotropic, where protein synthesis is inhibited, and the fungal DNA is intercalated or by boosting the development of fungi inhibitors [36]. Terpenoids reduced the mitochondrial content, thus modified the level of reactive oxygen species (ROS) and ATP generation.

It is also reported that triterpenoid possesses more potent antifungal activity as compared to the tetraterpenoid [37]. Terpenoids and flavonoids make their effects by disruption of microbial membranes [38]. Medicinal plant possessed antifungal effects by many mechanisms, they caused membrane disturbance resulting in the loss of membrane integrity, inhibited DNA transcription and reduced the cell populations, inhibited the activity of fungal antioxidant enzymes and inhibited fungal biofilm formation [39, 40]. Finally, anti-candidiasis activity of Carthamus tinctorius L. seeds might be belonging to secondary metabolites like Alkaloids, Flavonoids, and Terpenoids and their effect in proteins and DNA synthesis and disruption in membranes permeability or disturbance in metabolic activity.

Conclusion

Alkaloids, and Terpenoids extracted from the seeds of (Saussurea costus) have powerful antifungal activity against Candida species.

References


