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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 cynaropicrin, 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

Scientific name	Local name	English name	Family	Active part used
Saussurea costus	Al-Kost Al-Hindi	Costus	Asteraceae	Roots

- **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 2
Types of Candida Isolates and their sources

N0	Fungal isolates	Type of specimen
1	Candida Isolates	Mouth
		Vagina

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

Concentrations (mg/ml)	Alkaloids compounds
	Inhibition Zone/mm
Negative Control	0± 0.00
25 mg/ml	20± 1.00
50 mg/ml	25± 1.00
100 mg/ml	30± 1.00
Positive Control	35± 1.00
L.S. D	1.62
*Mean± standard deviation	

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

Concentrations (mg/ml)	Flavonoid compounds
	Inhibition zone %
Negative Control	0± 0.00
25 mg/ml	8± 1.00
50 mg/ml	12± 1.00
100 mg/ml	27± 1.00
Positive Control	35± 1.00
L.S. D	1.60
*Mean± standard deviation	

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

Concentrations (mg/ml)	Terpenoid compounds
	Inhibition zone %
Negative Control	0± 0.00
25 mg/ml	22± 1.00
50 mg/ml	26± 1.00
100 mg/ml	31± 1.00
Positive Control	35± 1.00
L.S. D	1.61
*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) .

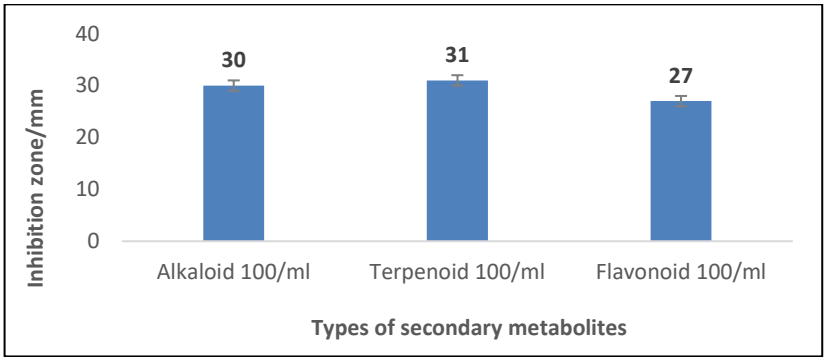

Figure 4. Inhibition zone of Alkaloid, Terpenoid, and Flavonoid at 100/ml against Candida species, LSD= 2.15

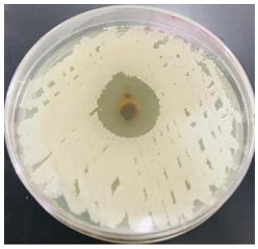

Figure 1: Anti-candidiasis activity of the crude Alkaloid compounds extracted from *Saussurea costus* roots at 100 mg/ml

Figure 2: Anti-candidiasis activity of the crude Flavonoid compounds extracted from *Saussurea costus* roots at 100 mg/ml

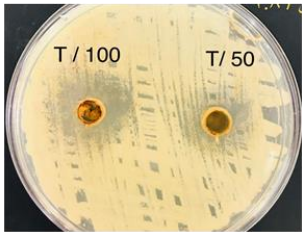

Figure 3: Anti-candidiasis activity of the crude Flavonoid compounds extracted from *Saussurea costus* roots at 50 ad 100 mg/ml

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, Trichodermaviride, 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

1. Abdallah EM, Qureshi KA, Ali AM, Elhassan GO. Evaluation of some biological properties of Saussurea costus crude root extract. Biosci. Biotechnol. Res. Commun. 2017 Oct 1;10(4):601-11.
2. Al-Jassani MJ. Tropaeolum Majus Leaves Extract as an Antifungal, Antiaflatoxigenic and Antiaflatoxin Agent. Journal of Global Pharma Technology. 2017; 12(09):328-333.
3. Al-Marzoqi AH, Al-Khafaji NM, Hussein HJ. In vitro Antibacterial Activity Assessment of the crude Phenolic, Alkaloid and Terpenoid compounds extracts of *Lepidium sativum* L. on Human Pathogenic Bacteria. International Journal of ChemTech Research, 2016; (4): 529-32.
4. Al-Marzoqi AH, Hussein HJ, Al-Khafaji NM. Antibacterial Activity of the Crude Phenolic, Alkaloid and Terpenoid Compounds Extracts of *Lactuca serriola* L. on Human Pathogenic Bacteria. Chemistry and Materials Research, 2015; 7(1): 8-10.
5. AL-Masoodi H, Hussein HJ, Al-Rubaye AF. Antifungal activity of the two medicinal plants (*Curcuma longa* L. and *Boswellia carteri* Birdwood) against Fusarium species isolated from maize seeds. International Journal of Pharmaceutical Research, 2020; 12(3): 408-14.
6. Arif T, Bhosale JT, Kumar N, Mandal TK, Bendre RS, Lavekar GS, Dabur R. Natural products–antifungal agents derived from plants. Journal of Asian natural products research. 2009; 11(7):621-38.
7. Boham BA and Kocipai-Abyazan R. Flavonoids and condensed tannins from leaves of Hawaiian Vaccinium vaticulatum and V. calycinium. Pacific Science, 1974 48:458-463.

8. Braun PC. The effects of tea polyphenols on Candida albicans: inhibition of biofilm formation and proteasome inactivation. Canadian Journal of Microbiology. 2009; 55 (9): 1033 – 1039.
9. Church NA, McKillip JL. Antibiotic resistance crisis: Challenges and imperatives. Biologia. 2021 May;76(5):1535-50.
10. Ciurea CN, Kosovski IB, Mare AD, Toma F, Pintea-Simon IA, Man A. Candida and candidiasis—opportunism versus pathogenicity: a review of the virulence traits. Microorganisms. 2020 Jun;8(6):857.
11. Deabes MM, Fatah AE, Sally I, Salem SH, Naguib KM. Antimicrobial activity of bioactive compounds extract from Saussurea costus against food spoilage microorganisms. Egyptian Journal of Chemistry. 2021 Jun 1;64(6):2833-43.
12. Haque E, Irfan S, Kamil M, Sheikh S, Hasan A, Ahmad A, Lakshmi V, Nazir A, Mir SS. Terpenoids with antifungal activity trigger mitochondrial dysfunction in Saccharomyces cerevisiae. Microbiology. 2016; 85(4):436-43.
13. Harborne J.B, Mabray T.Y, Marby H. Physiology and function of flavonoids. Academic Press, New York. 1975; 970.
14. Harborne JB. Phytochemical methods, London. Chapman and Hall, Ltd. 1973; 49-188.
15. Harborne, J.B. Phytochemical methods. Chapman and Hall, New York. 2nd ed. 1984 pp. 288.
16. Hasan Radhi Safa, Kamal SA, Mohammed Sahi N, Hussein HJ. Assessment of Antibacterial Efficacy of Callistemon viminalis (Sol. ex Gaertn.) G. Don against Some Isolates Obtained from Urinary Tract Infections. Archives of Razi Institute. 2022 Apr 1; 77(2): 891-7.
17. Hussain AY, Hussein HJ, Al-Rubaye AF. Antifungal Activity of the Secondary Metabolites Extracted from *Carthamus tinctorius* L. against *Aspergillus* Species Isolated from Stored Medicinal Plants Seeds in the Iraqi Markets. Clinical Schizophrenia and Related Psychoses, 2021; 15S: Doi:10.3371/CSRP.HAHH.081221.
18. Hussain AY, Hussein HJ, Al-Rubaye AF. Antifungal Efficacy of the crude Flavonoid, Terpenoid, and Alkaloid Extracted from Myrtus communis L. against Aspergillus species isolated from Stored Medicinal plants seeds in the Iraqi Markets. Journal of Biotechnology Research Center. 2021; 15 (2):73-80.
19. Hussein HJ, Al-Khafaji NM, Al-Mamoori AH, Al-Marzoqi AH. Evaluation of in vitro antibacterial properties of the crude Phenolic, Alkaloid and Terpenoid extracts of *Cassia senna* L. against Human gram-negative Pathogenic Bacteria. Plant archives, 2018; 18(1): 354-6.
20. Hussein HJ, Al-Khafaji NM, Al-Mamoori AH, Juaifer WA, Al-Marzoqi AH, Al-Zobiady RA. Antimicrobial Effect of the Crude Phenolic, Alkaloid and Terpenoid Compounds Extracts of *Myrtus Communis* L. against Human Gram-Negative Pathogenic Bacteria. Journal of Global Pharma Technology, 2017; (8); 130-3.
21. Hussein HJ, Al-Marzoqi AH. The Antibacterial efficacy of the secondary metabolites extracted from (*Melia azedarach* L.) leaves against pathogenic microorganisms isolated from burns and gingivitis infections. EurAsian Journal of Biosciences, 2020; 14(1): 561-5.
22. Hussein HJ, Kaizal AF, Al-Khafaji NM, Sadiq ZF, Shahad AS. Evaluation of antibacterial potential of the crude Phenolic, Alkaloid and Terpenoid extracts of *Ricinus communis* L. against gram-negative Pathogenic Bacteria. Journal of Global Pharma Technology, 2018; (05): 384-388.

23. Hussein HJ, Kamal SA, Sahi NM. Antibacterial Efficacy of The Seed Extract of *Saliva Hispanica* L. Against Pathogenic Bacteria Isolated from Diarrhea Cases. Biochemical and cellular archives, 2020; 20(supplement 2): 3491-94.
24. Hussein HJ, Naji SS, Al-Khafaji NM. Antibacterial properties of the *Chlorella vulgaris* isolated from polluted water in Iraq. Journal of Pharmaceutical Sciences and Research, 2018; 10(10): 2457-60.
25. Hussein HJ, Sahi NM, Saad AM, Altameme HJ. The Antibacterial Effect of bioactive compounds extracted from *Cassia didymobotrya* (Fresenius) Irwin & Barneby against Some Pathogenic Bacteria. Annals of Tropical Medicine and Public Health, 2019; 22(1): SPe 116.
26. Kamal AS, Hussein HJ, Tolaifeh ZA. (2019) Antibacterial potential of *Hibiscus sabdarifa* L. against some Enterobacteriaceae: in vitro. Biochemical and cellular archives, 2019; 19 (2): 4291-4294.
27. Kamal SA, Al-Kaim HW, Hussein HJ. Antibacterial activity of phytochemical compounds extracted from *Ficus carica* Linn. Leaves against human pathogenic bacteria. EurAsian Journal of BioSciences, 2020; 14(1):2293-8.
28. Kamalpreet LK, Singh A, Kaur J, Kaur N. A brief review of remedial uses of Saussurea lappa. Journal of Pharmacognosy and Phytochemistry. 2019;8(3):4423-30.
29. Kamil SS, Hussein HJ, Al-Marzoqi AH. Evolution of Antibacterial efficacy of *Dianthus caryophyllus* L. extracts against some hospitals pathogenic bacteria. International Journal of Pharmaceutical Research, 2020; 12(3): 1274-9.
30. Kaul MK. Medicinal plants of Kashmir and Ladakh: temperate and cold arid Himalaya. Indus publishing; 1997.
31. Mustafa, A. R., Ramadany, S., Sanusi, Y., Made, S., Stang, S., & Syarif, S. (2020). Learning media applications for toddler midwifery care about android-based fine motor development in improving midwifery students skills. International Journal of Health & Medical Sciences, 3(1), 130-135. https://doi.org/10.31295/ijhms.v3n1.290
32. Nadkarni KM. Indian materia medica, popular Book Depot. Bombay. 1954;7:946-8.
33. Nwauche KT, Monago CC, Anacletus FC. Antihyperglycemic activity of the aqueous extract of Costus afer stem alone and in combination with metformin. European journal of biotechnology and bioscience. 2014;1(5):19-25.
34. Okusa P.N, Stévigny C, Duez P. Medicinal Plants: A Tool to Overcome Antibiotic Resistance? In: Varela, A.; Ibañez, J. (eds). Medicinal plants: classification, biosynthesis and pharmacology. Nova Science Publishers, Incorporated, 2009 pp, 315.
35. Pandey MM, Rastogi S, Rawat AK. Saussurea costus: botanical, chemical and pharmacological review of an ayurvedic medicinal plant. Journal of ethnopharmacology. 2007 Apr 4;110(3):379-90.
36. Parekh J, Chanda S. In vitro antifungal activity of methanol extracts of some Indian medicinal plants against pathogenic yeast and moulds. African journal of Biotechnology. 2008;7(23).
37. Perez L, Pauli M, Bazequre P. Antibiotic assay by the agar well diffusion method. Journal of Actabiology. 1990; 15: 113-115.
38. Rao KS, Babu GV, Ramnareddy YV. Acylated flavone glycosides from the roots of Saussurea lappa and their antifungal activity. Molecules. 2007 Mar 7;12(3):328-44.

39. Salazar SB, Simões RS, Pedro NA, Pinheiro MJ, Carvalho MF, Mira NP. An overview on conventional and non-conventional therapeutic approaches for the treatment of candidiasis and underlying resistance mechanisms in clinical strains. Journal of Fungi. 2020 Feb 10;6(1):23.
40. Sharara DT, Al-Marzoqi AH, Hussein HJ. In Vitro Antibacterial efficacy of the Secondary Metabolites Extracted from Myrtus communis L. against some pathogenic bacteria isolated from Hemodialysis Fluid. Annals of the Romanian Society for Cell Biology, 2021; 25(6): 9267-9274.
41. Silva S, Rodrigues CF, Araújo D, Rodrigues ME, Henriques M. Candida species biofilms' antifungal resistance. Journal of Fungi. 2017 Feb 21;3(1):8.
42. Soliman MF, Shetaia YM, Tayel AA, Munshi AM, Alatawi FA, Alsieni MA, Al-Saman MA. Exploring the Antifungal Activity and Action of Saussurea costus Root Extracts against Candida albicans and Non-albicans Species. Antibiotics. 2022 Mar 1;11(3):327.
43. Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2021). Health and treatment of diabetes mellitus. *International Journal of Health Sciences*, *5*(1), i-v. https://doi.org/10.53730/ijhs.v5n1.2864
44. Taniguchi M, Kataoka T, Suzuki H, Uramoto M, Ando M, Arao K, Magae J, Nishimura T, Ōtake N, Nagai K. Costunolide and dehydrocostus lactone as inhibitors of killing function of cytotoxic T lymphocytes. Bioscience, biotechnology, and biochemistry. 1995 Jan 1;59(11):2064-7.
45. Wu T, He M, Zang X, Zhou Y, Qiu T, Pan S and Xu X. A structure–activity relationship study of flavonoids as inhibitors of E. coli by membrane interaction effect. Biochimica et Biophysica Acta (BBA) - Biomembranes 2013; 1828(11): 2751–2756.