Antipseudomonal, Antioxidant, Anticoagulant, and Cytotoxic Activities of Novel Synthesized Heterocyclic Molecules

Article *in* Archives Of Pharmacy Practice · January 2024

DOI: 10.51847/DWeXd6w3Ja

CITATIONS
0

READS
42

5 authors, including:

Hind Abd Alati Alzletni
University of Benghazi
9 PUBLICATIONS **9** CITATIONS
SEE PROFILE

Stefania Marzocco
Università degli Studi di Salerno
174 PUBLICATIONS **4,795** CITATIONS
SEE PROFILE

Maha Mastouri
University of Monastir
164 PUBLICATIONS **2,473** CITATIONS
SEE PROFILE

Original Article

Antipseudomonal, Antioxidant, Anticoagulant, and Cytotoxic Activities of Novel Synthesized Heterocyclic Molecules

Randa Sakly[1], Hend A Elzletni[2], Stefania Marzocco[3], Maha Mastouri[1], Hayet Edziri[1]*

[1]Laboratory of Transmissible Diseases and Biologically Active Substances, Faculty of Pharmacy, University of Monastir 5000, Tunisia. [2]Department of Biology, Faculty of Education, University of Benghazi, Benghazi, Libya. [3] Department of Pharmacy, University of Salerno, Via Giovanni Paolo II 132, I-84084, Fisciano, Salerno, Italy.

Abstract

In this work, we evaluate the antipseudomonal, antioxidant, anticoagulant, and cytotoxic activities of new spirooxindolopyrrolizidine-linked 1,2,3-triazole were investigated for their antipseudomonal, antioxidant, anticoagulant and cytotoxic activities. The antipseudomonal activity of these novel molecules was tested by the microdilution method. DPPH, ABTS, and β-Carotene tests were used to estimate the antioxidant properties. Prothrombin time (PT) and activated partial thromboplastin time (aPTT) tests were measured to evaluate the anticoagulant potential and cytotoxic activity was assessed by MTT test. This study revealed that compounds P3 and P7 displayed excellent antipseudomonal activity against all tested *P. aeruginosa* Imp/R. with MIC values of 15.62µg/ml. the antioxidant activity demonstrated that all tested heterocyclic compounds exhibited important antioxidant activity by ABTS methods with IC_{50} ranging between, 25.5 and 35.84µg/ml. In addition, these new compounds were not toxic on the viability of macrophage cells J774A.1 at a concentration of 1 µM and the only non-toxic compound was P7.

Keywords: Molecules, Heterocyclic, Anticoagulant, Cytotoxic, Antipseudomonal, Activities

Introduction

Antibiotic resistance is a growing issue in public health. The spread of resistance to current antibiotics is a global concern [1]. While resistance to bacteria-fighting medication is a normal occurrence, different factors lead to this problem such as the social factor which raised transmission of infections, as well as inappropriate use of antibiotics. People may use antimicrobials for any infection, whether real or perceived, in varying dosages and for extended periods without medical guidance [2, 3]. Many antibiotic-resistant bacteria have no known treatments, and there is a continuous rise in the prevalence of resistance to commonly used antibiotics [4, 5]. In long-term care facilities, multiple drug-resistant organisms are frequently the cause of infections. Specifically, Pseudomonas aeruginosa accounts for 16% of pneumonia cases in hospitals [6, 7].

In the past decade, it has become apparent that bacteria are growing more resistant to antibiotics, making them less effective [8, 9]. This issue is compounded by the slow rate at which new drugs are introduced into the market [10, 11]. Additionally, many existing antifungal and antibacterial medications have undesirable side effects [12, 13]. Therefore, pharmaceutical companies are putting a lot of effort into developing new, more effective molecules [14, 15].

One particularly intriguing aspect is the potential of these molecules to combat *P. aeruginosa*, the main perpetrator of hospital-acquired infections. This bacterium has developed resistance to commonly used classes of antibiotics [16].

Heterocycles are vital biomolecules with a wealth of promising targets for organic synthesis due to their significant biological activities [17], Examples include spiro oxindole-pyrrolidines and triazoles, known for their antimicrobial, and antitumoral properties [18, 19], amongst others. These molecules have also shown potential in fighting inflammation

Address for correspondence: Hayet Edziri, Laboratory of Transmissible Diseases and Biologically Active Substances, Faculty of Pharmacy, University of Monastir 5000, Tunisia.
jaziri_hayet@yahoo.fr

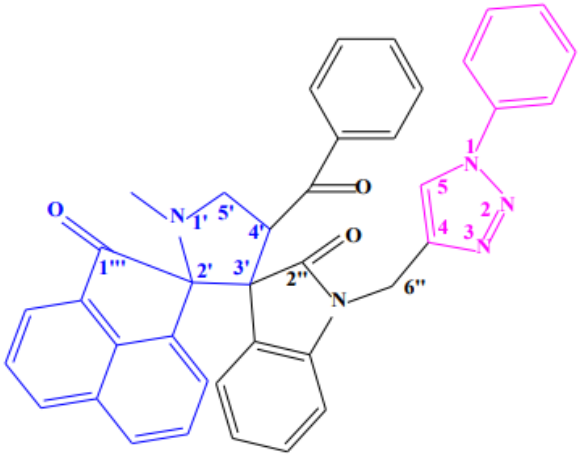
How to cite this article: Sakly R, Elzletni HA, Marzocco S, Mastouri M, Edziri H. Antipseudomonal, Antioxidant, Anticoagulant, and Cytotoxic Activities of Novel Synthesized Heterocyclic Molecules. Arch Pharm Pract. 2024;15(1):103-8. https://doi.org/10.51847/DWeXd6w3Ja

[20], and cancer [21, 22], as well as tuberculosis. Notably, similar properties can be found in existing drugs like fluconazole for fungal infections [18, 23], Metioprim for bacterial infections, and Flucytosine for fungal infections. With this in mind, our goal was to explore the capabilities of novel heterocyclic molecules, specifically regarding their activity against *P. aeruginosa*, as well as their antioxidant, anticoagulant, and cytotoxic properties.

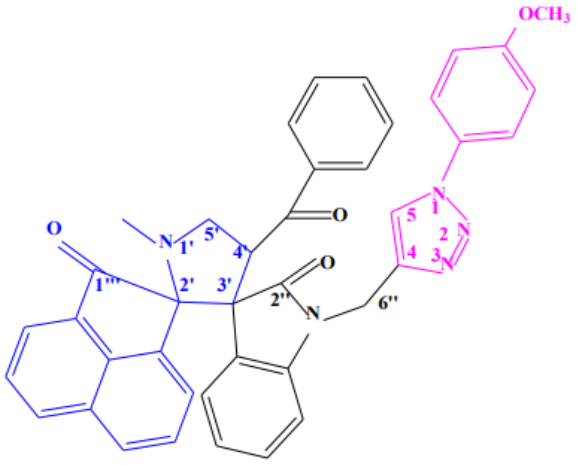
Materials and Methods

Compounds

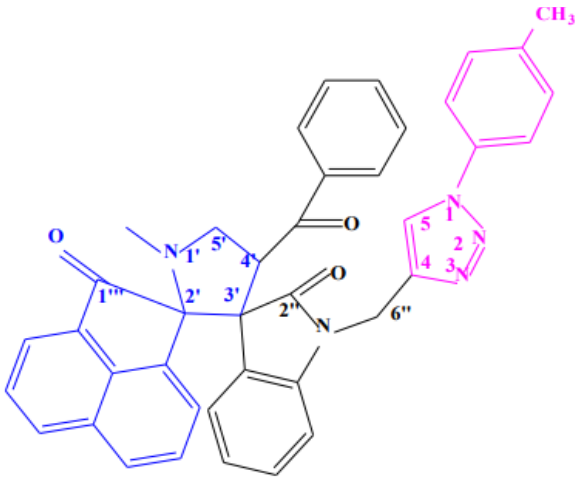
Seven novel spiro oxindole-pyrrolidine coupled with triazol **(Figure 1)** were synthesized by Sakly *et al*. (2018) [24] and we have continued to test their biological activities.



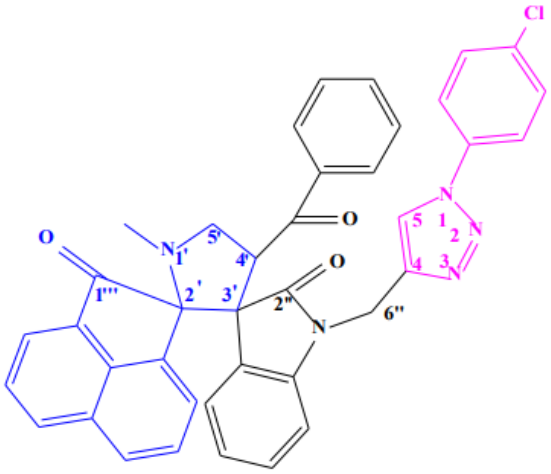
P1: 1-N-méthyl-spiro [2.3′] oxoacénaphthylen-2yl)-spiro[3.5″]-3″-N-((1-(4-phényl))- 1H-1,2,3-triazol-4-yl)méthyl)indolin-2-one-pyrrolidine



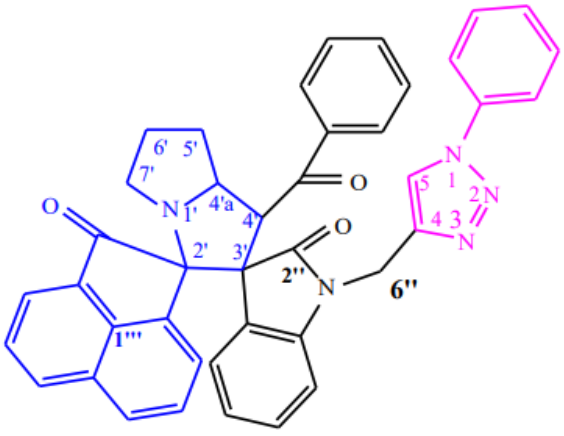
P2. 1-N-méthyl-spiro[2.3′]oxoacénaphthylen-2yl)-spiro [3.5″]-3″-N-((1-(4- méhoxyphényl))-1H-1, 2, 3-triazol-4-yl)méthyl)indolin-2-one-pyrrolidine



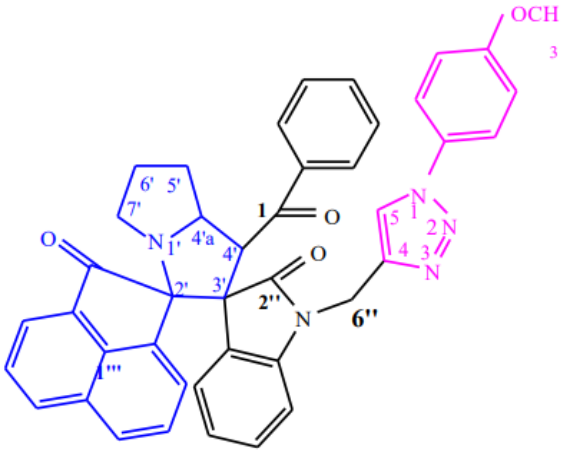
P3. 1-N-méthyl-spiro[2.3′]oxoacénaphthylen-2yl)-spiro[3.5″]-3″-N-((1-(4- méthylphényl))- 1H-1,2,3-triazol-4-yl)méthyl)indolin-2-one-pyrrolidine



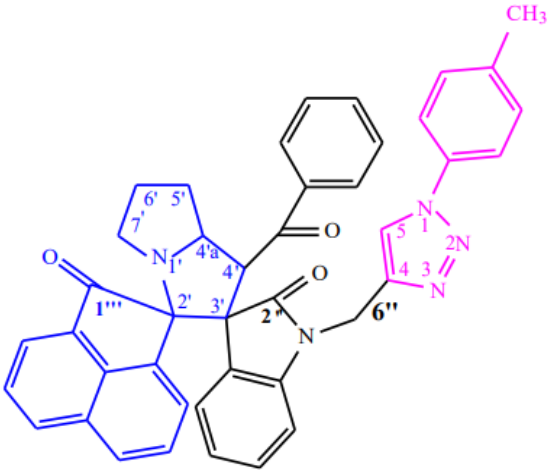
P4 1-N-méthyl-spiro[2.3′]oxoacénaphthylen-2yl)-spiro[3.5″]-3″-N-((1-(4- clhorophényl))- 1H-1,2,3-triazol-4-yl)méthyl)indolin-2-one-pyrrolidine



P5. 1-N-méthyl-spiro[2.3′]oxoacénaphthylen-2yl)-spiro[3.5″]-3″-N-((1-(4-phényl))-1H-1,2,3-triazol-4-yl)méthyl)indolin-2-one-pyrrolizidine



P6. 1-N-méthyl-spiro[2.3′]oxoacénaphthylen-2yl)-spiro[3.5″]-3″-N-((1-(4- méthoxyphényl))-1H-1,2,3-triazol-4-yl)méthyl)indolin-2-one-pyrrolizidine



P7. 1-N-méthyl-spiro[2.3′]oxoacénaphthylen-2yl)-spiro[3.5″]-3″-N-((1-(4- méthylphényl))- 1H-1,2,3-triazol-4-yl)méthyl)indolin-2-one-pyrrolizidine

Figure 1. Molecules structure

Antipseudomonal Activity

Origin of Pseudomonas Aeruginosa Strains

The isolates of *P. aeruginosa* (PA) were obtained from Pseudomonal infections and *P. aeruginosa* ATCC 27853 was provided from our laboratory in the faculty of pharmacy of Monastir.

Micro-Well Dilution Assay

To determine the Minimum inhibitory concentration (MIC) we used a microdilution method [25]. Briefly, tested molecules are first dissolved in dimethyl sulfoxide (DMSO, 10%). Then, they were diluted to an initial concentration of (250 µg/mL) and Cascade dilutions were carried out in 100 L of brain-heart broth which was placed in a 96-well plate.

20 L of bacterial suspension of the overnight culture was adjusted to a McFarland turbidity of 0.5 was added. finally, the plates were incubated for 24 h at 37°C. The MIC was defined as the lowest concentration that inhibits all growth visible.

Anticoagulant Activity

Prothrombin Time (PT) Test

According to Edziri *et al*. (2018), in the coagulometer cuvettes, 50 µl of citrated normal plasma pool containing 1 µl of buffer (control) or a sample at different concentrations are incubated for 2 minutes at 37°C. 100 µl of thromboplastin calcium tissue previously heated for 20 minutes at 37°C is then added and the clotting time is measured by the coagulometer. Heparin (1 IU/mL) was used as a positive control.

Activated Partial Thromboplastin Time (Aptt) Test

Concerning the aPTT test, based on Edziri *et al*. (2012) [26], 10µl of molecules are added to 100µl of platelet-poor plasma which is then incubated at 37C° for variable times. After incubation, 50 µl of a cephalin-Kaolin solution is added then the mixture is reincubated at 37C° for 3 minutes. Coagulation is then triggered by the addition of 50 µl of an aqueous solution of 0.025M CaCl2. The clotting time is then determined using an optical coagulometer based on the change in light transmission from the addition of calcium (starting the stopwatch) to the formation of the fibrin clot (stopping the stopwatch).

Antioxidant Activities

ABTS+• Cation Radical Scavenging Assay

The ABTS test was performed according to Edziri *et al*. (2018) [27]. The ABTS (2,2'-azinobis-3-ethylbenzothiazoline-6sulfonic acid) solution was diluted in ethanol and the OD was adjusted to 0.708 at 734 nm. Then 900 µL of the ABTS solution was mixed with 100 µL of different concentrations of molecules diluted in methanol. The IC50 represents the concentration that reduces 50% of the ABTS radical.

DPPH Assay

According to Ben Hsouna *et al*. (2017) [28], the DPPH solution was prepared by diluting 19.7 mg of DPPH in 50 ml of ethanol, then the solution was stored at 4°C. 100 µl of each dilution of the tested molecules previously diluted in ethanol were mixed with 100 µl of 0.1 mM DPPH in wells of 96-well plates. Then, the plate was incubated for 30 minutes in the dark and then the absorbance was read at 515 nm. BHT was used as a positive control. the percentage of DPPH radical inhibition was calculated according to the following formula:

$$\text{DPPH scavenging (\%)} = [1-(\text{As} - \text{Ab}/\text{Ac})] \times 100 \quad (1)$$

IC50 is the concentration that reduces 50% of the DPPH radical.

β-Carotene Bleaching Assay (BCB)

Briefly, the β-carotene bleaching method (BCB) was used according to the technique of Edziri *et al.* (2018) [27]. 950 µL of the β-carotene emulsion was mixed with 50 µL of the sample at 1.00 mg/mL. 250 µL of mixture was transferred to a 96-well plate. The wells were previously filled with 250 µL of water. The microplate was placed at 50 °C for 3 h and the absorbance was measured at 470 nm. The obtained results are calculated as a percentage of inhibition of β-carotene bleaching (% antioxidant activity).

Antiproliferative Activity of Tested Molecules in J774A.1 Cells

The cell viability test was evaluated by the MTT test (3-(4,5-dimethyl-2-thiazyl)-2,5-diphenyl-2H-tetrazolium bromide according to Havrylyuk *et al*. (2011). Indeed, the cells were cultured in 96-well plates (104 / well) for 4 h at 37 ° C in an atmosphere of 5% CO2. Different dilutions of the tested molecules (200 100–50 µg/ml) are incubated for 24 h. Then 50 µl of MTT was added and the cells were reincubated for 3 h. 100 µl of a solution containing 50% (v: v) of N, N-dimethylformamide, and 20% (w: v) SDS (pH of 4.5) was added, which solubilizes the lysed cells. Finally, the optical density (OD) of the plate was measured at 620 nm (Titertek Multiskan MCC/340). The percentage of viability was calculated as follows: % of dead cells = 100 × (treated OD/control OD).

Results and Discussion

Antipseudomonal Activity

The results of the anti-pseudomonal activity of the tested compounds are summarized in **Table 1**. As shown all tested compounds exhibited important antipseudomonal activity with MIC ranging between 125 and 15.62 µg/ml. The result showed that compounds P3 and P7 displayed excellent anti-pseudomonal activity against all tested *P. aeroginosa* with MIC values of 15.62 µg/ml, compared to the standard antibiotic ampicillin. On the other hand, P5 displayed important anti-bacterial activity against *P. aeroginosa* imipenem resistant with MIC of 31.25 µg/ml. we can conclude that compounds containing a nitro group at the triazol ring for example P3 and P7 contain a methyl group at the triazole ring. They were very active against *P. aeroginosa* imipenem resistant. the obtained results showed that the presence of certain substituents on the aryl ring of the triazole ring increases the antibacterial activity against *pseudomonas* strains. This is consistent with Kandsi *et al*. (2022) [2].

Table 1. Antipseudomonal activity of new molecules

Molecules	MIC(µg/ml) *P.aeroginosa*				
	ATCC 9027	*IMP/R 210*	*IMP/R 232*	*IMP/R 432*	*IMP/R 765*
P1	125	62.4	62.4	62.4	62.4
P 2	125	62.4	62.4	62.4	62.4
P3	250	15.62	15.62	15.62	15.62
P4	125	62.4	62.4	62.4	62.4
P5	250	31.25	31.25	31.25	31.25
P6	250	62.4	62.4	62.4	62.4
p7	125	15.62	15.62	15.62	15.62

Antioxidant Activity

The results of the antioxidant of the tested compound for DPPH, ABTS, and. β-Carotene Bleaching Assay were summarized in **Table 2**. According to the DPPH test P1, P2, P4, P5, and P6 showed the best antioxidant activity. They exhibited promising antiradical capacity when compared with the positive control BHT (IC50 = 18.64 µg/mL). Furthermore, P3 and P7 had almost the same IC50 as the positive control BHT. The ABTS assay demonstrated that all tested heterocyclic compounds exhibited important anti-oxidant activity with IC50 ranging between, 25.5 and 35.84µg/ml more active than reference standard drug BHT(IC50 = 50.31 µg/ml).

Table 2. Anticoagulant activity of leaves extract of *Ruscus hypophyllum*

Sample	Dose	PT (seconds)	aPTT (seconds)
Control	Saline	14.1 ± 1.87	34.1 ± 2
Heparin	1 IU/mL	42.18 ± 2.17	132.18 ± 1.48
P1	250	90.5 ± 2.9	120.94 ± 1.49
P2	250	82.8 ± 3.21	122.5 ± 2.21
P3	250	44.6 ± 4.15	109.4 ± 1.01
P4	250	50.9 ± 4.16	120.7 ± 3.16
P5	250	44.6 ± 3.15	114.6 ± 2.15
P6	250	40.9 ± 1.16	120.6 ± 3.16
P7	250	34.6 ± 2.15	114.6 ± 1.45

aPTT-Activated Partial Thromboplastin Time, PT- PT-
PT-Prothrombin Time

The β-carotene-linoleic bleach inhibition test affects membrane lipid oxidation. The oxidation of linoleic acid by heat causes the production of free radicals. These free radicals cause a whitening effect of beta-carotene, which will be blocked by the free radicals. To test the antiradical capacity of the tested molecules our results are summarized in **Table 3**. In this assay, we can conclude that compounds P3, P4, P5, P6, and P7 expressed the best antioxidant activity by comparing with the BHT inhibition percentage (80.5%). They exhibited good anti-oxidant capacity by comparing them with the positive controls (ascorbic acid, trolox, and BHT), suggesting that the presence of a hydroxyl group in their skeletons increases the antiradical capacity of the molecules this is consistent with Edziri *et al.* (2018) [27].

Table 3. Antioxidant activity of new molecules

Molecules	DPPH test (IC50) (µg/ml)	ABTS (IC50) µM	% β- Carotene linoleic acid
P1	14.8±1.54	26.26 ± 0.80	75.17 ± 0.20
P2	17.98 ± 0.80	35.84 ± 0.70	69.32 ± 0.40

P3	18.78 ± 1.00	25.5±2.12	85±1.32
P4	17.5±1.12	37.7 ± 0.63	87.7±1.23
P5	15.23±1.98	31.7±1.12	87.5 ± 2.20
P6	25±1.21	24.5±2.13	90.3±2.31
P7	18±1.23	20.22±2.3	87.4±2.32
BHT	18.64±0.02	50.31±0.01	80.5±2.6

DPPH radical scavenging assay, ABTS Anti-Oxidant Scavenging Assay, Total antioxidant
activity by the β–carotene–linoleic acid method.

Macrophage Viability

To explicate the effect of synthesized heterocyclic compounds on the viability of J774A.1 The latter were treated with these compounds (100–1 µM) for 24h. The obtained results showed that the macrophage viability was not altered by compound P7 in any concentration, but compounds P2 and P5 were not toxic only at a concentration of 1µM. P2 at a concentration upper than 10µM is toxique. So, we can conclude that the only non-toxic concentration of all tested compounds on J774A.1 cell was 1 µM. **Table 4**.

Table 4. Antiproliferative activity of new heterocycle

J774A.1 cells	MEAN±SEM of % of antiproliferative activity			
Dose	**P7**	**P2**	**P5**	**P6**
100 µM	16,00±1,15	26,00±0,06 #	24,70±1,86 #	31,00±4,36 ##
50 µM	13,67±3,76	27,00±1,00 #	25,70±1,20 #	32,67±1,67 ##
10 µM	5,00±5,00	22,00±2,60 #	9,33±0,88	20,67±2,60 #
1 µM	1,67±1,67	14,70±1,20	0,00±0,00	14,67±9,33

Conclusion

In summary, these newly synthesized heterocycles showed good antibacterial activity against *pseudomonas aeroginosa multiresistant drug bacteria* and important anti-oxidant activity by dpph, Abts, and betacarotene assays. In addition, these newly synthesized heterocycles possess good anticoagulant activity by pt and aptt assays but p6, p4, p2, and p1 had important anti-coagulant activity by aptt assays compared to positive control heparine. Furthermore, these new compounds were not toxic to the viability of macrophage cells j774a.1 at a concentration of 1 µm and the only non-toxic compound was p7. For this reason, spirooxindolopyrrolizidine-linked 1,2,3-triazole conjugates can be used as new anti-microbial drugs for the treatment of antibiotic resistance which represents a major public health problem, and may be used as a source of anticoagulants in the pharmaceutical industry.

ACKNOWLEDGMENTS: The authors are grateful to Pr Stefania Marzocco for his help in achieving the cytotoxic activity.
CONFLICT OF INTEREST: None
FINANCIAL SUPPORT: None
ETHICS STATEMENT: None

References

1. Okoye CO, Okeke ES, Ezeorba TP, Chukwudozie KI, Chiejina CO, Fomena Temgoua NS. Microbial and bio-based preservatives: recent advances in antimicrobial compounds. Mic Nat Food Add. 2023:53-74.
2. Kandsi F, Elbouzidi A, Lafdil FZ, Meskali N, Azghar A, Addi M, et al. Antibacterial and antioxidant activity of Dysphania ambrosioides (L.) mosyakin and clemants essential oils: Experimental and computational approaches. Antibiotics. 2022;11(4):482.
3. Bhardwaj M, Kapila R, Neha A, Jain R, Mittal P, Suri M. Awareness, perceived risk, and protective behavior towards Covid-19 among undergraduate students of Delhi and NCR, India. Int J Pharm Res Allied Sci. 2022;11(3):71-80.
4. Nicoară ND, Marian P, Petriș AO, Delcea C, Manole F. A review of the role of cognitive-behavioral therapy on anxiety disorders of children and adolescents. Pharmacophore. 2023;14(4):35-9.
5. Vartolomei L, Cotruș A, Stanciu C, Delcea C, Tozzi M, Lievore E, et al. Quality of life and psychological distress among patients with small renal masses. J Clin Med. 2022;11(14):3944.
6. Müller-Fabian A, Siserman C, Anițan ȘM, Delcea C. Juvenile delinquency in light of data recorded at the institute of forensic medicine. Rom J Leg Med. 2018;26(1):70-5.
7. Ceccato-Antonini SR, Shirahigue LD, Varano A, da Silva BN, Brianti CS, de Azevedo FA. Citrus essential oil: would it be feasible as antimicrobial in the bioethanol industry? Biotechnol Lett. 2023;45(1):1-2.
8. Delcea C, Enache A. Personality traits as predictor of crime. Rom J Leg Med. 2021;29(2):227-31.
9. Popa-Nedelcu R, Delcea C, Siserman C, Carmen Domnariu DC. The relationship between personality disorders and domestic violence in forensic context. Rom J Leg Med. 2020;28(2):166-71.
10. Elnaiem MAH, Alshammari AZB, Alanazi AOA, Alanazi IAR, Alshammari RHS, Alshamari NGS, et al. Acute appendicitis neglected to rupture: Review article. Int J Pharm Phytopharmacol Res. 2020;10(4):133-7.
11. Ahmadi Koulaei S, Hadjiakhoondi A, Delnavazi MR, Tofighi Z, Ajani Y, Kiashi F. Chemical composition and biological activity of Ferula aucheri essential oil. Res J Pharmacogn. 2020;7(2):21-31.
12. Sriset Y, Na Nakorn S, Chitsaitarn S, Dechsri K, Chatuphonprasert W, Jarukamjorn K. Bergenin exhibits a nephroprotective effect by improvement of the antioxidant system in xenobiotic-induced oxidative stress in ICR mice. Int J Pharm Phytopharmacol Res. 2020;10(3):13-21.
13. Solomennyi A, Drozdov D, Anna Drozdova OS, Davtian L, Shmatenko V. Medicines for local therapy of wounds in the Ukrainian pharmaceutical market. Int J Pharm Phytopharmacol Res. 2020;10(4):155-60.
14. Boghrati Z, Iranshahi M. Ferula species: A rich source of antimicrobial compounds. J Herb Med. 2019;16:100244.
15. Bruna F, Fernandez K, Urrejola F, Touma J, Navarro M, Sepulveda B, et al. Chemical composition, antioxidant, antimicrobial and antiproliferative activity of Laureliopsis philippiana essential oil of Chile, study in vitro and in silico. Arab J Chem. 2022;15(12):104271.
16. Meryem S, Mohamed D, Nour-eddine C, Faouzi E. Chemical composition, antibacterial and antioxidant properties of three Moroccan citrus peel essential oils. Sci Afr. 2023;20:e01592.
17. Wang J, Huang L, Cheng C, Li G, Xie J, Shen M, et al. Design, synthesis and biological evaluation of chalcone analogues with novel dual antioxidant mechanisms as potential anti-ischemic stroke agents. Acta Pharm Sin B. 2019;9(2):335-50.
18. Vásquez-Martínez YA, Osorio ME, San Martín DA, Carvajal MA, Vergara AP, Sanchez E, et al. Antimicrobial, anti-inflammatory and antioxidant activities of polyoxygenated chalcones. J Braz Chem Soc. 2019;30:286-304.
19. Alves-Silva JM, Zuzarte M, Girão H, Salgueiro L. The role of essential oils and their main compounds in the management of cardiovascular disease risk factors. Molecules. 2021;26(12):3506.
20. Cascaes MM, De Moraes ÂA, Cruz JN, Franco CD, E Silva RC, Nascimento LD, et al. Phytochemical profile, antioxidant potential and toxicity evaluation of the essential oils from Duguetia and Xylopia species (Annonaceae) from the Brazilian Amazon. Antioxidants. 2022;11(9):1709.

21. Maaroufi Z, Cojean S, Loiseau PM, Yahyaoui M, Agnely F, Abderraba M, et al. In vitro antileishmanial potentialities of essential oils from Citrus limon and Pistacia lentiscus harvested in Tunisia. Parasitol Res. 2021;120:1455-69.
22. Ekaette I, Saldaña MD. Ultrasound processing of rutin in food-grade solvents: Derivative compounds, antioxidant activities and optical rotation. Food Chem. 2021;344:128629.
23. Chortani S, Edziri H, Manachou M, Al-Ghamdi YO, Almalki SG, Alqurashi YE, et al. Novel 1, 3, 4-oxadiazole linked benzopyrimidinones conjugates: Synthesis, DFT study and antimicrobial evaluation. J Mol Struct. 2020;1217:128357.
24. Sakly R, Edziri H, Askri M, Knorr M, Strohmann C, Mastouri M. One-pot four-component domino strategy for the synthesis of novel spirooxindole–pyrrolidine/pyrrolizidine-linked 1, 2, 3-triazole conjugates via stereo-and regioselective [3+ 2] cycloaddition reactions: In vitro antibacterial and antifungal studies. Comptes Rendus Chimie. 2018;21(1):41-53.
25. Edziri H, Jaziri R, Haddad O, Anthonissen R, Aouni M, Mastouri M, et al. Phytochemical analysis, antioxidant, anticoagulant and in vitro toxicity and genotoxicity testing of methanolic and juice extracts of Beta vulgaris L. S Afr J Bot. 2019;126:170-5.
26. Edziri H, Mahjoub M, Mastouri M, Mighri Z, Aouni M. Antibacterial, antifungal and cytotoxic activities of two flavonoids from Retama raetam flowers. Molecules. 2012;17(6):7284-93.
27. Edziri H, Marzouk B, Mabrouk H, Garreb M, Dougui W, Mastouri M. Phytochemical screening, butyrylcholinesterase inhibitory activity and anti-inflammatory effect of some Tunisian medicinal plants. South Afr J Bot. 2018;114:84-8.
28. Ben Hsouna A, Ben Halima N, Smaoui S, Hamdi N. Citrus lemon essential oil: Chemical composition, antioxidant and antimicrobial activities with its preservative effect against Listeria monocytogenes inoculated in minced beef meat. Lipids Health Dis. 2017;16:146.