Assessing the Antioxidant Potential of Withania Somnifera Leaf Extract via DPPH Assay

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Abstract

The rise of antimicrobial resistance has spurred interest in exploring natural sources for new antibiotics and antifungals. In this study, we investigated the antimicrobial and antifungal potential of Withania somnifera extracts against common pathogens. Among various fractions (ethyl acetate, aqueous, hexane, and dichloromethane) obtained from Withania somnifera and tested using the agar well diffusion method, ethyl acetate extract proved to be the most effective one against Staphylococcus aureus, Escherichia coli by producing maximum zone of inhibition (10.5±1mm, 7.3±0.2mm, respectively). Similarly, strong antifungal activity was demonstrated against A. fumigatus, particularly by the ethyl acetate fraction (12.3±0.5mm). While these fractions showed promising activity, standard antibiotics generally displayed greater inhibition. Phytochemical analysis revealed the presence of bioactive compounds like tannins, phenols, saponins, and flavonoids, which likely contribute to the observed antimicrobial and antifungal effects. Anti-oxidant DPPH assay showed that the values of DDPH inhibition of Withiana somnifera samples collected from forest site samples were higher (81.63±0.56) than those of roadside collected samples (54.25±3.44). Additionally, environmental factors influenced the phytochemical composition of Withania somnifera extracts, with roadside plants exhibiting higher levels of phenolics and flavonoids (100.10±0.90 and 92.88±1.12, respectively) due to vehicular pollution exposure. These findings underscore the potential of Withania somnifera as a source of natural antimicrobial agents and highlight the importance of cultivation practices for preserving medicinal properties.

Keywords: Vehicular pollution; Total phenolics; Flavonoids; Antioxidant activity; Roadside plants

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INTRODUCTION

Withania somnifera belongs to the Solanaceae family, a group renowned for its diverse array of medicinal plants. This perennial shrub typically grows in the drier regions of the World, thriving in arid climates and sandy soils (Otmani et al., 2021). Characterized by small, green flowers and spherical orange-red berries, the plant possesses distinct morphological features that facilitate its identification in the wild (Chithiraikumar et al., 2017). *Withania somnifera* holds a venerable status in Ayurveda, the ancient Indian system of medicine, where it is classified as a rejuvenating herb (Nile et al., 2019). For centuries, Ayurvedic practitioners have prescribed *Withania somnifera* to enhance vitality, promote longevity, and alleviate various ailments.

The therapeutic potential of *Withania somnifera* can be attributed to its complex phytochemical composition, which encompasses a diverse array of biologically active compounds. Chief among these are alkaloids (such as withanine and somniferine), steroidal lactones (including withanolides and sitoindosides), and various other constituents like flavonoids, saponins, and amino acids (Alagesan & Venugopal, 2019; Alam et al., 2011; Fang et al., 2022; Ganguly et al., 2018; Yahia et al., 2020; Złotek et al., 2016) Numerous preclinical and clinical studies have validated its efficacy in ameliorating stress, enhancing cognitive function, improving sexual health, and bolstering immune function. Additionally, *Withania somnifera* exhibits neuroprotective, cardioprotective, and anti-cancer properties, making it a subject of intense scientific inquiry and pharmaceutical interest (Ezez & Tefera, 2021; Keneni et al., 2021).

In recent years, *Withania somnifera* has garnered increasing attention from researchers, healthcare practitioners, and consumers worldwide, owing to its potential therapeutic benefits and relatively low risk of adverse effects (Babbar et al., 2014; Dirar et al., 2019; Ezez & Tefera, 2021; Keneni et al., 2021; Khan et al., 2021). Its adaptogenic properties, in particular, have fueled its popularity as a natural remedy for stress management and overall well-being, amidst the growing prevalence of stress-related disorders in modern society (Mondal et al., 2021; Souza-Silva et al., 2015). *Withania somnifera* extracts and compounds are of interest to the pharmaceutical industry for their potential therapeutic applications. Research continues to explore their efficacy in treating various health conditions and developing novel pharmaceutical formulations. The current study was designed to determine the antioxidant and antimicrobial capacity of *Withania somnifera* leaf extract using the DPPH assay, and to investigate the concentration-dependent response of the extract in neutralizing DPPH radicals, providing insights into its dose-dependent antioxidant activity.

MATERIAL AND METHOD

Methodology

Fresh *Withania somnifera* leaves were obtained from a local village in Punjab and were botanically authenticated. All experiments were performed in triplicates or as per the experimental design. Positive and negative controls were included to ensure the validity of the assay.

Preparation of Withania somnifera Leaf Extract

To begin the extraction process, 1 gram of leaf was carefully weighed and then crushed using a mortar and pestle in 10 ml of 80% methanol. The resulting mixture was refrigerated at 0°C for 24 hours. Afterwards, the supernatant was separated and combined with an additional 80% methanol to achieve a final volume of 10

ml. This solution was then stored in a refrigerator for subsequent analyses.

Determination of Antioxidant Activity Using the DPPH Assay

Different concentrations of the *Withania somnifera* leaf extract were prepared in a solvent. To each test tube, a specific volume of the extract was added, followed by the addition of the DPPH solution. The reaction mixtures were incubated in the dark at an appropriate temperature for a fixed time. A standard curve was established using known concentrations of a reference antioxidant (e.g., ascorbic acid) under the same conditions. The scavenging activity of the extract was calculated as a percentage of DPPH radical inhibition and compared to the standard curve.

Antibacterial Activity Methodologies

Agar Diffusion (Kirby-Bauer) Assay

This method (Hudzicki, 2009) involves impregnating paper disks with the test substance and placing them on agar plates inoculated with bacterial cultures. The zones of inhibition around the disks indicate the effectiveness of the substance against the bacteria.

Broth Dilution Method (MIC and MBC)

Minimum Inhibitory Concentration (MIC) is determined by exposing serial dilutions of the test substance to bacterial cultures in liquid media. The lowest concentration that inhibits visible bacterial growth is considered the MIC. Minimum Bactericidal Concentration (MBC) is determined by sub-culturing from the MIC well onto agar plates to check for bacterial viability. Both these methods were used according to the method devised by Wiegand et al., (2008).

Time-Kill Assay

This method devised by Pankey and Ashcraft (2009) involves exposing bacterial cultures to the test substance at predetermined time intervals and assessing the reduction in bacterial count over time. It provides information on the rate and extent of bactericidal activity.

Antifungal Activity Methodologies

Agar Diffusion (Kirby-Bauer) Assay

Similar to the antibacterial assay, this method uses fungal cultures inoculated onto agar plates. Paper disks containing the test substance are placed on the agar, and zones of inhibition are measured to determine antifungal activity.

Broth Dilution Method (MIC and MFC)

Minimum Inhibitory Concentration (MIC) for fungi is determined by exposing serial dilutions of the test substance to fungal cultures in liquid media. The lowest concentration that inhibits fungal growth is recorded as the MIC. Minimum Fungicidal Concentration (MFC) is determined by sub-culturing from the MIC well onto agar plates to check for fungal viability.

Resazurin Microtiter Assay

This quantitative colourimetric assay developed by Rolón et al., (2006) uses resazurin, a dye that changes colour in response to the metabolic activity of fungi. The colour reduction indicates fungal growth inhibition.

Hyphal Elongation Assay

This method devised by Matsuoka et al., (1990) measures the effect of the test substance on fungal hyphal growth. Fungi were cultured in the presence of different concentrations of the substance, and the length of hyphal growth was measured to determine the antifungal effect.

Data Analysis

The data obtained from the DPPH assay were statistically analyzed. Graphical representations, such as dose-response curves, were generated to visualize the antioxidant activity of the extract. Calculations were made to determine the concentration at which the extract exhibited 50% inhibition of DPPH radicals (IC50) (Anbalagan et al., 2016; Ghosh et al., 2020; Saif et al., 2016; Thakur et al., 2018).

RESULTS

Antibacterial and antifungal activities of *Withania somnifera*

Bacteria and fungi were the driving force behind this investigation. Antimicrobial screening was conducted using the agar well diffusion method, by the national committee for clinical labs' standard protocol. The antibiotics that have been derived from plants are thought to be secure, and efficient, and typically have little or no adverse effects. To find new antibiotic medications, active phytochemicals with antimicrobial action against microorganisms are essential. This work is the first to examine the antibacterial and antifungal properties of the medicinal herb *Withania somnifera*. Table 1 summarizes the antibacterial properties of fractionated *Withania somnifera* extracts. These extracts affect the tested pathogens and show dose-dependent action.

Table 1: Antibacterial activity of *Withania somnifera* fractions.

Extract	Concentration (µl)	Staphylococcus aureus	Escherichia coli
Dichloromethane	30 µl	4.3 ± 0.2 mm	6.3 ± 0.5 mm
Water	30 µl	5.1 ± 0.2 mm	4.5 ± 0.5 mm
n-Hexane	30 µl	4.5 ± 0.5 mm	6.8 ± 0.2 mm
Ethyl acetate	30 µl	10.5 ± 1 mm	7.3 ± 0.2 mm
Ampicillin (+control)	10 mg	15.5 ± 0.5 mm	–
Gentamicin (+control)	10 mg	–	11.9 ± 0.4 mm
Ofloxacin (+control)	1 mg	20 ± 0.5 mm	11.5 ± 0.15 mm
DMSO (–control)	30 µl	1 ± 0 mm	1 ± 0 mm

The data are expressed as mean ± SD (standard deviation) in mm of zone of inhibition shown by each fraction. Compared using ANOVA, with significance level set at alpha of 0.05.

The unrefined ethyl acetate extract shows that at 30 µL concentration, the *Withania somnifera* ethyl acetate fraction demonstrated a maximal zone of inhibition of 10.5 ± 1 mm against *S. aureus* and 7.3 ± 0.2 mm against *E. coli*, as indicated in Table 1. In comparison to the ethyl acetate fraction, the hexane extract produced inhibitory zones against *S. aureus* and *E. coli* that were 6.3 ± 0.5 mm and 4.3 ± 0.2 mm, respectively (Figure 1). The inhibition zones of the dichloromethane fraction against S. aureus indicated decreased activity, in contrast to the more inhibiting conventional antibiotics ampicillin (11.9 ± 0.4 mm) and ofloxacin (11.5 ± 0.15 mm), while the most major inhibitor was gentamicin (15.5 ± 0.3 mm) (Figure 1).

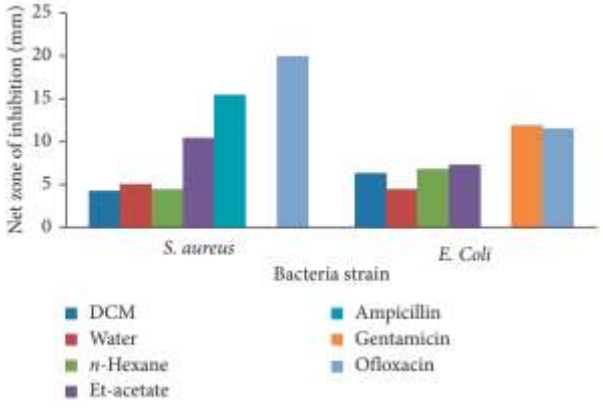

Figure. 1 Inhibition zones exhibited by *Withania somnifera* against different bacteria.

The antifungal assay results of *Withania somnifera* are summarized in Table 2. The crude extracts from *Withania somnifera* strongly inhibited *Aspergillus fumigatus* at the tested concentrations. Similarly, water, and dichloromethane extracts produced inhibition zones of 12.1 ± 0.2 mm, 10.6 ± 0.5 mm, and 8.3 ± 0.5 mm, respectively, at the same concentration. In comparison, the standard nystatin (test control) displayed a lower inhibition zone of 6.7 ± 0.5 mm at a 10 µL concentration compared to the extracted fractions of *Withania somnifera*. (Table 2, Figure 2).

Table 2: Antifungal activity of *Withania somnifera* fractions.

Extract	Concentration (µl)	*Aspergillus fumigatus*
Dichloromethane	10 µl	8.3 ± 0.5 mm
n-Hexane	10 µl	12.1 ± 0.2 mm
Water	10 µl	10.6 ± 0.5 mm
Ethyl acetate	10 µl	12.3 ± 0.5 mm
Oxytetracycline (test control)	10 µl	26 ± 0 mm
Nystatin (standard)	10 µl	6.7 ± 0.5 mm
DMSO (negative control)	10 µl	1 ± 0 mm

The data are expressed as mean ± SD (standard deviation) in mm of zone of inhibition shown by each fraction. Compared using ANOVA, with significance level set at alpha of 0.05.

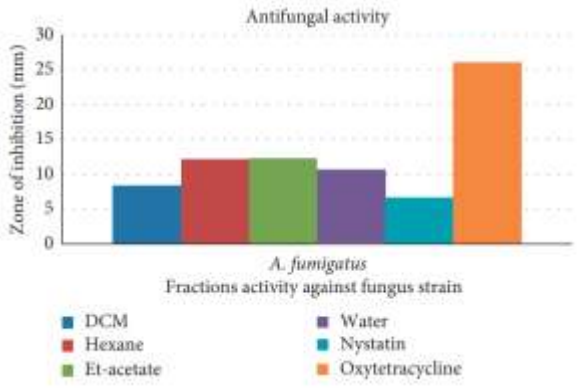

Figure 2: Inhibition zones demonstrated by each fraction of *Withania somnifera* against fungal strains.

DPPH inhibition potential of *Withiana somnifera* in methanol extracts

DPPH inhibition potential was evaluated in methanol extracts obtained of *Withiana somnifera* in a Bahawalpur city at two sites i.e. forest site and roadside. Results showed that methanol extract of roadside collected *Withiana somnifera* exhibited higher values of total phenolics and total flavonoids as compared to those collected from the forest side. Similarly, the values of DDPH inhibition from forest site samples were higher than those of roadside samples of *Withiana* (Table 3).

Table 3. DPPH inhibition potential, evaluated in methanol extracts obtained of *Withiana* somnifera in a Bahawalpur city.

Parameter	Methanol extracts	
	Forest site	Roadside
Total phenolics (µg tannic acid. g^{-1})	74.27 ± 0.76	100.10** ± 0.90
Total flavonoids (µg quercetin. g^{-1})	75.12 ± 0.89	92.88* ± 1.12
DPPH inhibition (%)	81.63 ± 0.56	54.25* ± 3.44

Values are means ± S.E. of five replicates. The significant differences between polluted and non-polluted sites were analyzed using the student's t-test. Level of significance: **ps0.01, *ps0.05

Likewise, the DPPH scavenging capacities (IC_{50}) assessed for methanol extracts were higher for roadside-collected *Withiana somnifera* relative to forest site samples. The trend was also noticed in high IC50 values of BHT and ascorbic acid (Table 4, Figure 3).

Table 4. The DPPH scavenging capacities were assessed for methanol extracts.

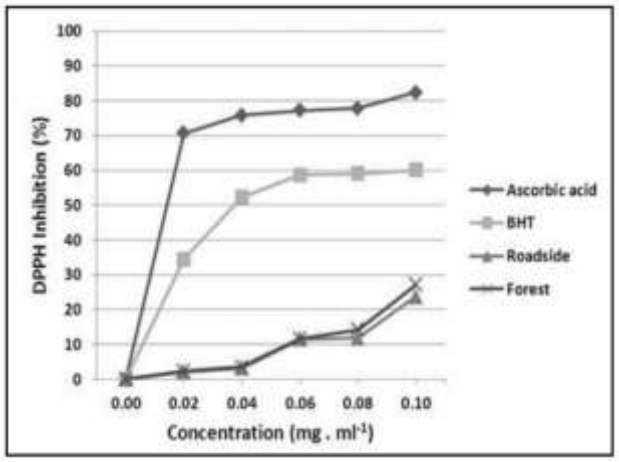

Figure 3. The free radical scavenging assay of samples collected from Bahawalpur, Punjab.

Discussion

The effectiveness of plant extracts against bacteria varies, with gram-positive bacteria being more susceptible than gram-negative bacteria. This disparity is attributed to differences in cell membrane structure and composition. Plant extracts have shown promising activity that reduces the uptake of antibiotics, leading to resistance. However, certain plant species contain bioactive compounds like terpenoids, steroids, saponins, tannins, and flavonoids, which exhibit antimicrobial properties. Withania somnifera, for instance, contains a range of bioactive compounds, contributing to its strong antimicrobial activity. Similarly, various Veronica species have been reported to exhibit notable antimicrobial activities, as documented by several researchers (Alagesan & Venugopal, 2019; Alam et al., 2011; Fang et al., 2022; Ganguly et al., 2018; Yahia et al., 2020; Złotek et al., 2016). In our study, the *Withania somnifera* ethyl acetate fraction demonstrated a maximal zone of inhibition of 10.5 ± 1 mm against *S. aureus* and 7.3 ± 0.2 mm against *E. coli*. On the contrary, the hexane extract produced

inhibitory zones against *S. aureus* and *E. coli* that were 6.3 ± 0.5 mm and 4.3 ± 0.2 mm, respectively. The inhibition zones of the dichloromethane fraction against *S. aureus* indicated decreased activity, in contrast to the more inhibiting conventional antibiotics ampicillin (11.9 ± 0.4 mm) and ofloxacin (11.5 ± 0.15 mm), while the most major inhibitor was gentamicin (15.5 ± 0.3 mm). Flavonoids, as a type of polyphenols, increase the effectiveness of antibiotics against microbes by forming complexes with fungal cell wall proteins and extracellular components. Terpenoids weaken the cell walls of fungal strains and promote the dissolution of membranes. Saponins interact with fungi to cause enzyme proteins to leak from cells, while steroids cause liposomes to leak from lipid bilayers (Dimitrić Marković et al., 2017; Treml & Šmejkal, 2016). This study comprehensively reported on the antifungal activity of *Withania somnifera* against *Aspergillus fumigatus*, highlighting its important therapeutic potential.

Higher concentrations of phenolics and flavonoids found in plant leaf extracts obtained from roadside areas could be attributed to elevated levels of free radicals induced by vehicular pollution. Typically, free radicals are inherently unstable and typically harmless, undergoing conversion into non-radical products. The reduced state of phenolic compounds functions as antioxidants; however, the oxidized form (phenoxy radical) can be cytotoxic, posing risks to living systems by potentially initiating free radical chain reactions within membranes and exhibiting tendencies to cross-link with diverse substances. It was found that the increase in phenolic content is correlated with the increase in the activity of enzymes involved in the metabolism of phenolic compounds under stressful conditions, and the increase in flavonoid content was mainly due to conjugation hydrolysis (Altemimi et al., 2017; Ali et al., 2020). Previous research has demonstrated that exposure of plants to ozone and heavy metals leads to a notable increase in the activity of antioxidants such as peroxidase, phenols, superoxide dismutase, and glutathione reductase. Mir et al. (2009) observed an elevation in total flavonoid and phenolic content in certain medicinal plants growing alongside roads. These results are in agreement with our findings of the DPPH inhibition potential of *Withiana somnifera* where samples in a Bahawalpur city were collected from two different sites i.e. forest site and roadside. Results showed that methanol extract of roadside collected *Withiana somnifera* exhibited higher values of total phenolics and total flavonoids as compared to those collected from the forest side. Similarly, the values of DDPH inhibition from forest site samples were higher than those of roadside samples of *Withiana somnifera*. Furthermore, the most noteworthy IC_{50} esteem was noticed for the plant extracts from dirtiest destinations, trailed by those from non-contaminated locales, BHT, and ascorbic corrosive. This study reports, interestingly, that methanol concentrates of *W. somnifera* starting from the side of the road areas in Punjab suggest that they may lose their medicinal properties from constant exposure to vehicle emissions.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

AUTHORS CONTRIBUTION

Nasr Ullah Khan and Naimat Ullah conceived the idea, designed the study and drafted the manuscript. Muhammad Waqas conducted the experiments and collected and analyzed the data.

REFERENCES

Alagesan V, Venugopal S. Green synthesis of selenium nanoparticle using leaves extract of *Withania somnifera* and

its biological applications and photocatalytic activities. Bionanoscience. 2019; 15:9:105-16.

Alam N, Hossain M, Khalil MI, Moniruzzaman M, Sulaiman SA, Gan SH. High catechin concentrations were detected in *Withania somnifera* (ashwagandha) by high-performance liquid chromatography analysis. BMC Complementary and Alternative Medicine. 2011; 11:1-8.

Ali AS, Bashir SH, Abdelshafeek KA. Isolation, identification of some chemical constituents and antimicrobial activity of different extracts from *Withania somnifera* growing at Albaha region, KSA. Biomedical and Pharmacology Journal. 2020; 13(2):635-45.

Altemimi A, Lakhssassi N, Baharlouei A, Watson DG, Lightfoot DA. Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. Plants. 2017; 22:6(4):42.

Anbalagan S, Sankareswaran M, Prabhavathi P, Manikandan A, Karthikeyan G. Green synthesis and characterization of Silver nanoparticles from Withania somnifera (L.) Dunal. Asian J Pharm Clin Res. 2016; 9(5):34-9.

Babbar N, Oberoi HS, Sandhu SK, Bhargav VK. Influence of different solvents in the extraction of phenolic compounds from vegetable residues and their evaluation as natural sources of antioxidants. Journal of food science and technology. 2014; 51:2568-75.

Chithiraikumar S, Gandhimathi S, Neelakantan MA. Structural characterization, surface characteristics and non-covalent interactions of a heterocyclic Schiff base: Evaluation of antioxidant potential by UV–visible spectroscopy and DFT. Journal of Molecular Structure. 2017; 1137:569-80.

Dirar AI, Alsaadi DH, Wada M, Mohamed MA, Watanabe T, Devkota HP. Effects of extraction solvents on total phenolic and flavonoid contents and biological activities of extracts from Sudanese medicinal plants. South African Journal of Botany. 2019; 120:261-7.

Ezez D, Tefera M. Effects of solvents on total phenolic content and antioxidant activity of ginger extracts. Journal of Chemistry. 2021; 2021(1):6635199.

Fang H, Yin X, He J, Xin S, Zhang H, Ye X, Yang Y, Tian J. Cooking methods affected the phytochemicals and antioxidant activities of potatoes from different varieties. Food Chemistry: X. 2022; 30:14:100339.

Ganguly B, Kumar N, Ahmad AH, Rastogi SK. Influence of phytochemical composition on in vitro antioxidant and reducing activities of Indian ginseng [Withania somnifera (L.) Dunal] root extracts. Journal of Ginseng Research. 2018; 42(4):463-9.

Ghosh MK, Sahu S, Gupta I, Ghorai TK. Green synthesis of copper nanoparticles from an extract of Jatropha curcas leaves: Characterization, optical properties, CT-DNA binding and photocatalytic activity. RSC advances. 2020; 10(37):22027-35.

Hudzicki J. Kirby-Bauer disk diffusion susceptibility test protocol. American society for microbiology. 2009;15(1):1-23.

Keneni YG, Bahiru LA, Marchetti JM. Effects of different extraction solvents on oil extracted from jatropha seeds and the potential of seed residues as a heat provider. BioEnergy Research. 2021; 14:1207-22.

Khan AU, Khan AU, Li B, Mahnashi MH, Alyami BA, Alqahtani YS, Alqarni AO, Khan ZU, Ullah S, Wasim M, Khan QU. Biosynthesis of silver-capped magnesium oxide nanocomposite using Olea cuspidata leaf extract and their photocatalytic, antioxidant and antibacterial activity. Photodiagnosis and Photodynamic Therapy. 2021; 33:102153.

Marković JM, Pejin B, Milenković D, Amić D, Begović N, Mojović M, Marković ZS. Antiradical activity of delphinidin, pelargonidin and malvin towards hydroxyl and nitric oxide radicals: The energy requirements calculations as a prediction of the possible antiradical mechanisms. Food chemistry. 2017; 218:440-6.

Matsuoka H, Ii Y, Takekawa Y, Teraoka T. Evaluation of antifungal volatile compounds based on the elongation rate of a single hypha. Applied and environmental microbiology. 1990 Dec;56(12):3779-84.

Mondal M, Hossen MS, Rahman MA, Saha S, Sarkar C, Bhoumik NC, Kundu SK. Antioxidant mediated protective effect of Bridelia tomentosa leaf extract against carbofuran induced oxidative hepatic toxicity. Toxicology Reports. 2021; 8:1369-80.

Nile SH, Nile A, Gansukh E, Baskar V, Kai G. Subcritical water extraction of withanosides and withanolides from ashwagandha (Withania somnifera L) and their biological activities. Food and Chemical Toxicology. 2019; 132:110659.

Otmani A, Amessis-Ouchemoukh N, Birinci C, Yahiaoui S, Kolayli S, Rodríguez-Flores MS, Escuredo O, Seijo MC, Ouchemoukh S. Phenolic compounds and antioxidant and antibacterial activities of Algerian honeys. Food Bioscience. 2021; 1;42:101070.

Pankey GA, Ashcraft DS. In vitro antibacterial activity of tigecycline against resistant Gram-negative bacilli and enterococci by time-kill assay. Diagnostic microbiology and infectious disease. 2009;64(3):300-4.

Qayoom Mir A, Yazdani T, Ahmad S, Yunus M. Total flavonoids and phenolics in Catharanthus roseus L. and Ocimum sanctum L. as biomarkers of urban auto pollution. Caspian Journal of Environmental Sciences. 2009; 1:7(1):9-16.

Rolón M, Vega C, Escario JA, Gómez-Barrio A. Development of resazurin microtiter assay for drug sensibility testing of Trypanosoma cruzi epimastigotes. Parasitology research. 2006; 99:103-7.

Saif S, Tahir A, Asim T, Chen Y. Plant mediated green synthesis of CuO nanoparticles: comparison of toxicity of engineered and plant-mediated CuO nanoparticles towards Daphnia magna. Nanomaterials. 2016; 9;6(11):205.

Souza-Silva ÉA, Gionfriddo E, Pawliszyn J. A critical review of the state of the art of solid-phase microextraction of complex matrices II. Food analysis. TrAC Trends in Analytical Chemistry. 2015; 71:236-48.

Thakur S, Sharma S, Thakur S, Rai R. Green synthesis of copper nanoparticles using Asparagus adscendens roxb. Root and leaf extract and their antimicrobial activities. Int. j. curr. Microbiol. appl. sci. 2018; 7(4):683-94.

Treml J, Šmejkal K. Flavonoids as potent scavengers of hydroxyl radicals. Comprehensive reviews in food science and food safety. 2016 Jul;15(4):720-38.

Wiegand I, Hilpert K, Hancock RE. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nature protocols. 2008;3(2):163-75.

Yahia, Y., Benabderrahim, M.A., Tlili, N., Hannachi, H., Ayadi, L. and Elfalleh, W., 2020. Comparison of three extraction protocols for the characterization of caper (Capparis spinosa L.) leaf extracts: Evaluation of phenolic acids and flavonoids by liquid chromatography-electrospray ionization–tandem mass spectrometry (LC–ESI–MS) and the antioxidant activity. Analytical Letters, 53(9), pp.1366-1377.

Złotek U, Mikulska S, Nagajek M, Świeca M. The effect of different solvents and number of extraction steps on the polyphenol content and antioxidant capacity of basil leaves (Ocimum basilicum L.) extracts. Saudi journal of biological sciences. 2016; 23(5):628-33