Antibacterial, Antifungal, Antioxidant Activities Assessment Of Some Newly Registered Plant Extracts Collected From Izmir City

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ABSTRACT

In recent times, especially during the antibiotic resistance era, it is crucial to find active and sustainable alternatives that work against microbes. In this study, seven plants *Minuartia nifensis* McNeill, *Sideritis sipylea* Boiss, *Asperula daphneola* O.Schwarz, *Teucrium montanum* L, *Stachys cretica* L. subsp, *Smyrnaea* rech. Fil and *Euphorbia erythrodon* Boiss/Heldr growing in Turkey were collected and newly registered. Alcoholic extracts were prepared to investigate their antimicrobial and antioxidant activity. These plants are widespread in the mountains of Izmir City (Turkey). Three assessments were performed in this project: agar well diffusion method, a standard antimicrobial (antibacterial and anti-fungal) activity test, followed by MIC test, which determines the lowest concentration and lowest quantity that affects microbes after adding resazurin indicator, and finally, antioxidant activity by ELISA microplate reader. The findings showed activity against *S. aureus* and *E. coli*. Antifungal activity against *C. albicans* and *C. glabrata* was the highest activity shown by most extracts. Antioxidant activity was also confirmed by quantification of radical scavenging activity via DPPH assay.

KEYWORDS: *Smyrnaea* Rech. Fil, Antimicrobial Potential, Antibiotic Resistance, DPPH, Izmir Mountains.

ABBREVIATIONS: MIC: Minimum Inhibitory Concentration; AOA: Anti-Oxidant Activity; DPPH: 2,2-Diphenyl-1-Picryl Hydrazyl.

1. INTRODUCTION

Recently, the major challenge in the recent antimicrobial resistance crisis is the need for effective, affordable and sustainable remedies to treat microbial infections, especially bacterial and fungal infections, due to expanding of antibiotic resistance in the past three decades [1,2]. Random and inappropriate use of antibiotics is considered one of the most common causes of antibiotic resistance development in bacteria and fungi [3]. So, the development of healthy and safer alternative treatments is urgently required to overcome this problem [1,4] as well as to minimize environmental and health hazards derived from synthetic chemical products [5], and, finally, to add the benefits of an environmentally safe and economically viable product [6].

Natural products of plant extracts are considered good resources for producing drug agents that could act as alternatives to synthetic antimicrobials [7] and represent a novel therapeutic substance for combating serious diseases [8].

Over many years, plants have been used in classic medicine; among anti-microbial agents in conventional clinical use, there are efforts to incorporate plant-derived compounds in remedies despite the traditional use of plant extracts [9].

Only a few of the 70,000 plant species benefited from using the different applications; about 90% of plant species are located in Europe. In Turkey, 75% of aromatic and medicinal plants are collected from the forest. There is a limited area for cultured plant species [10].

Generally, İzmir city has a geographical structure, rich water sources and suitable climate conditions, making it a suitable environment for many plant species with its splendid potential that can be seen in a few regions holding a variety of medical and aromatic plants.

Although many plant species have been studied and used as a sustainable remedy, many medicinal plants in Izmir city have not been studied as antibacterial and antifungal. These plants are naturally available and awaiting discovery. Many studies investigated its antiparasitic activities [11]. For this reason, we aimed to study the antibacterial, antifungal and antioxidant activity of some plants. To our knowledge, the current study considers the first work for these plants in Izmir city.

2. METHOD(S)

2.1. COLLECTION OF PLANT MATERIALS

All plants of interest were collected from two spots in Izmir city. The first group was collected from Nif Mountain, summit and slopes near Kemalpaşa district, and the second group was collected from Ödemiş, Bozdağ Mountain, near Sky Center, slopes of Izmir, Turkey, during their flowering season.

Collection, classification, and identification of these plants were performed by Dr. Hasan Yildirim and also deposited in the Herbarium of Ege University, belonging to the Faculty of Science, under special accession numbers mentioned in Table 1.

Table 1. Plant names and their specifications.

Plant name	Family	Location	Collector number	Herbarium code and number	Used Parts
Minuartia nifensis McNeill	Caryophyllaceae	İZMİR: Kemalpaşa, Nif Mountain, summit and slopes near the summit, 1290-1400 m	H.Yıldırım 8090	EGE 43209	leaves
Sideritis sipylea Boiss.	Lamiaceae	İZMİR: Kemalpaşa, Nif Mountain, summit and slopes near summit, 1290-1400 m	H.Yıldırım 8084	EGE 43210	leaves
Asperula daphneola O.Schwarz	Rubiaceae	İZMİR: Kemalpaşa, Nif Mountain, summit and slopes near summit, 1290-1400 m	H.Yıldırım 8093	EGE 43211	leaves
Teucrium montanum L.	Lamiaceae	İZMİR: Kemalpaşa, Nif Mountain, summit and slopes near summit, 1290-1400 m	H.Yıldırım 8085	EGE 43212	leaves
Stachys cretica L. subsp. *smyrnaea* Rech. fil.	Lamiaceae	İZMİR: Ödemiş, Bozdağ Mountain, near Sky Center, slopes, 1550 m	H.Yıldırım 7915a	EGE 43213	Roses+ leaves
Euphorbia erythrodon Boiss. and Heldr.	Euphorbia	İZMİR: Kemalpaşa, Nif Mountain, summit and slopes near summit, 1290-1400 m	H.Yıldırım 8092	EGE 43214	leaves
Smyrnium rotundifolium Mill.	Apiaceae	İZMİR: Karaburun, Akdağ Mountain, slopes, 750 m	H.Yıldırım 7764	EGE 43215	Roses

2.2. PREPARATIONS AND EXTRACTION PROCESSES

The plants were obtained from Ege University, Department of Biology. Spoiled plant parts were firstly removed. Main parts were carefully cleaned and washed many times with distilled water until complete removal of soil and was then press-dried for 7-10 days under room temperature (20°C ± 1) to avoid any loss of active compounds. Aerial parts were separated and powdered using a grinder, then weighted before extraction. All ground plants were macerated in dark bottles containing 80% ethanol (Sigma-Aldrich, Germany) 1:5 w/v ratio for 2-3 days with slow shaking [5]. Then filtration was done through Whatman-filter paper (No. 1) and then concentrated to constant dryness [12]. The yield was about 30-35 % (w/w), and the extract was stored in a refrigerator at +4°C for further use.

2.3. TEST MICRO-ORGANISMS

The antibacterial activity of these extracts was evaluated in vitro against the four bacterial species *Listerịa monocytogens, Staphylococcus aureus, Escherichịa coli* and *Pseudomonas aerugịnosa*, while the antifungal activity was evaluated against two pathogenic yeast *Candida albịcans* and *Candida glabrata*.

2.4. ANTIMICROBIAL ACTIVITY METHOD

The antibacterial and antifungal activity testing was performed by agar-diffusion as described minutely by Abdallah *et al*. [13] and Al-Quhli *et al*. [14]; the crude extracts were tested on targeted bacterịa and yeast, and the concentration of microorganism's inoculum was 10^7 cells mL^{-1}, 80% of ethanol was used as positive control.

Plates were incubated at 37°C for bacterial strains; yeasts were incubated at 25°C until detectable bacterịal growth in the control plates. The antimicrobial activity was expressed as the growth inhibition zones that may be formed around the extracts-impregnated paper discs.

2.5. MIC

Minimum-inhibitory-concentration or "MIC", which is well-known as the lowest quantity of any antimicrobial that kills or inhibịt the microorganism, the test was performed with some modifications [15-18].

Muelle-Hinton broth was prepared and sterilized, 100 µL aseptically were poured in each well of the 96-microtiter plate (MTP), the double concentration of interested material was added to the first well of each 96 MTP column then diluted subsequently to the end well of each treated column, the same operation was applied on other columns that specified for different material and different microorganism. Columns 11 and 12 of MTP were left for negative and positive control, respectively.

About 5 µL of standard bacteria or fungi (0.5 Macfarland) that were prepared previously was added to each well in a single column; plates were incubated at 37°C for 18-24 hrs; after the incubation period, the presence or absence of microorganism growth was tested by adding 30 µL of growth indicator resazurin which turns to pink color when growth is still available (no activity) [19]. The result of MIC was considered for the last well and showed no growth (blue color of resazurin).

2.6. ANTIOXIDANT ACTIVITY

The AOA of prepared extracts was performed by the DPPH standard method [20-22]; DPPH acts as a capture hook of any free H. or unwanted Free radicals in the DPPH molecule (Violet color) reduced to DPPH.H (colorless) owing to the reaction with an antioxidant [23].

The AOA was tested in two methods: Qualitatively by screening whether extracts possess AO activity or not by observing the turning of the DPPH mixture color. A quantitative test was performed by microtiter plate method followed by reading the absorbance via microtiter plate reader (Biotek ELX800, USA) at 517 nm. The final results were expressed as radical scavenging activity percentage (RSA%) according to the below equation; for all experiments, low absorbance reflected high antioxidant activity.

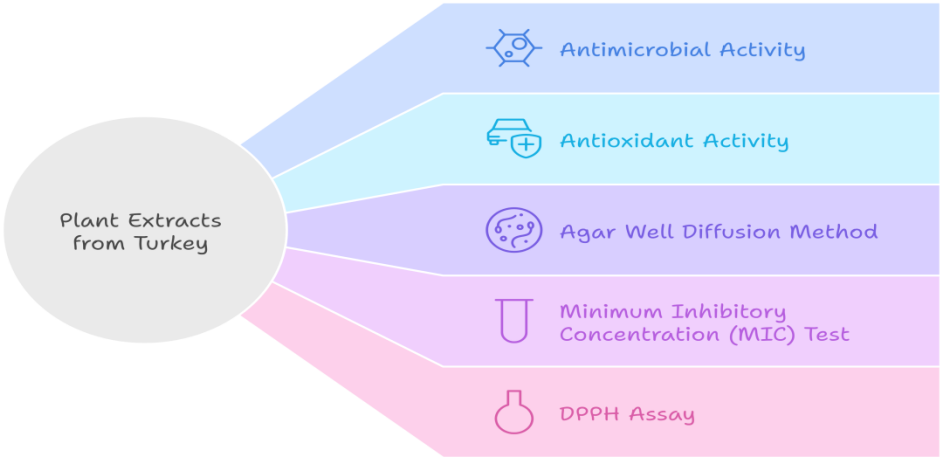
$$\mathrm{RSA}(\%) = \left[\frac{(\mathrm{Ac} - \mathrm{As})}{\mathrm{Ac}}\right] \times 100 \text{ or } \mathrm{RSA}(\%) = \left[1 - \frac{\mathrm{Ac}}{\mathrm{As}}\right] \times 100$$

Ac (Absorbance of control), As (Absorbance of the sample)

For all tests, serial dilution of extracts prepared and mixed with DPPH indicator constant quantity 15:10 separately, incubated in a dark environment at temperature (25-30ºC) for 30 minutes; for all tests, the solvent was DMSO or methanol, the reference chemical was ascorbic acid (Vitamin C) which is a good antioxidant reagent so it's considered as standard for this test, the control was DPPH + DMSO.

2.7. STATISTICAL DATA ANALYSIS

Data analysis and statistical data were expressed as 'mean' and 'standard deviation' with the aid of the Office software package 2016. The differences were evaluated using a one-way analysis of differences (ANOVA) with $p < 0.05$.



3. RESULTS AND DISCUSSION

3.1. ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY

In this project, seven plant extracts from plant species were tested by disk diffusion method against four common pathogenic bacterial strains (*S. aureus, P. aeruginosa, E. coli, L. monocytogenes*) and two yeast strains (*C. albicans, C. glabrata*). These microbes are antibiotic-resistant and cause many diseases.

The main results of plant extracts against tested micro-organisms are summarized in Table 2. According to the investigations, the most effective plant was *Smyrnium rotundifolium* Mill extract, which was potentially effective in suppressing microbial growth of both *S. aureus* and *E. coli* but failed to show any effects against *P. aeruginosa* and *L. monocytogenes.*

Asperula daphneola O. Schwarz, *Teucrium montanum* L., *Stachys cretica* L. subsp. *Smyrnaea* Rech. fil., *Euphorbia erythrodon* Boiss. and Heldr and *Smyrnium rotundifolium* Mill. showed potential activity against *C. albicans* and *C. glabrata* yeasts with different ranges of effects.

While *P. aeruginosa* and *L. monocytogenes* revealed 100% resistance against all extracts, both *Minuartia nifensis* McNeill and *Sideritis sipylea* Boiss did not show any effect against bacteria and yeasts.

Table 2. Antimicrobial screening test of plant extract.

Organism	Extracts/inhibition zone (mm)±1						
	Minuartia nifensis McNeill	*Sideritis sipylea* Boiss.	*Asperula daphneola* O. Schwarz	*Teucrium montanum* L.	*Stachys cretica* L. subsp. *smyrnaea* Rech. Fil.	*Euphorbia erythrodon* Boiss. and Heldr.	*Smyrnium rotundifolium* Mill.
S. aureus	-	-	-	-	-	-	18
P. aeruginosa	-	-	-	-	-	-	-
E. coli	-	-	-	-	-	-	18
L. monocytogens	-	-	-	-	-	-	-
C. glabrata	-	-	17.5	21	21.2	18.2	15.4
C. albicans	-	-	14	19	16.5	15.5	16

Due to continuous development of antibiotics and antibacterial resistance emerging in different micro-organisms, it has become important to find new compounds (natural or synthetic) to defend against virulence of micro-organisms, nowadays the researchers interested in preparing newly discovered compounds that act against antibiotic-resistant micro-organisms. For example, the micro-organisms in this study reveal high resistance to antibiotics. Overall, the potential of the ethanolic plant extracts of *Asperula daphneola* O. Schwarz, *Teucrium montanum* L., *Stachys cretica* L. subsp. *Smyrnaea*, *Euphorbia erythrodon* Boiss. and Heldr., and *Smyrnium rotundifolium* Mill. are promising for further investigation against both *C. albicans* and *C. glabrata* pathogenic yeast and *S. aureus* bacteria.

Antimicrobial activity in *T. montanum* bioactive compounds was reported from different projects. Sailović [24] found that using different solvents exhibited activity against a wide range of micro-organisms; in contrast to our finding, Sailović [24] found that different extracts can act as antifungal more than antibacterial due to the chemical composition of plant extracts that reacted with different solvents like water and acetone as well as the plant that was collected from Ozren mountain located in northern Bosnia and Herzegovina. All these differences can affect the chemical composition as a result of its activity.

The phenolic profile and antimicrobial, as well as antioxidant potential of *Stachys* extracts, were evaluated by Benedec [25], who found that *Stachys* was rich in 15 different polyphenolic compounds, including protocatehuic, rosmarinic, vanillic, gallic, gentisic, *p*-coumaric caftaric, chlorogenic, syringic and ferulic acids as well as 5 flavonoid compounds like apigenin, rutin, isoquercitrin, quercitrin and luteolin. LC-MS detected all these bioactive ingredients.

Benedec found that extracts revealed a strong antimicrobial activity against gram +ve *S. aureus* bacteria, similar to the current investigation; another similarity was extracted revealed limited activity against gram -ve bacteria.

Regarding other plant extracts of *Asperula daphneola* O. Schwarz, *Euphorbia erythrodon* Boiss. and Heldr, *Smyrnium rotundifolium* Mill, and *Minuartia nifensis* McNeill, we did not find an investigation on their bioactivity except *Sideritis* was investigated by Çarıkçı *et al*. [26] and Loğoğlu *et al*. [27] with different perspectives.

3.2. AOA

Antioxidants have drawn the wide interest of the medical, industrial and scientific communities due to their benefits for the prevention and treatment of various degenerative diseases, especially during the AI era. The antioxidant capacity in this work has been tested in vitro. Therefore, the current study's target is to evaluate the antioxidant activity of natural compounds.

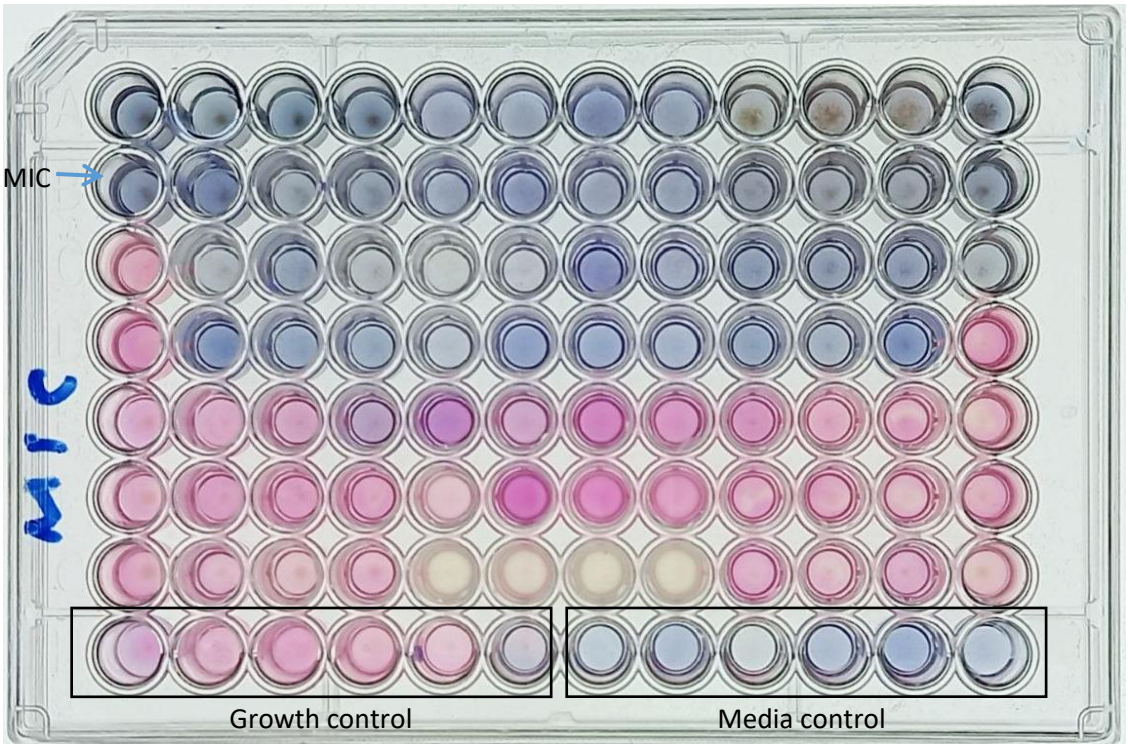


Figure 1. MIC assay by 96-well microtiter plate.

The investigations of qualitative antioxidant activity revealed that all extracts possess antioxidant activity by noticing color changes during 30 minutes of reaction between DPPH and plant extracts in micro-Eppendorf tube; the standard color of the DPPH indicator is purple, the antioxidant of material indicated when the purple color turn to different as in Figure 2.

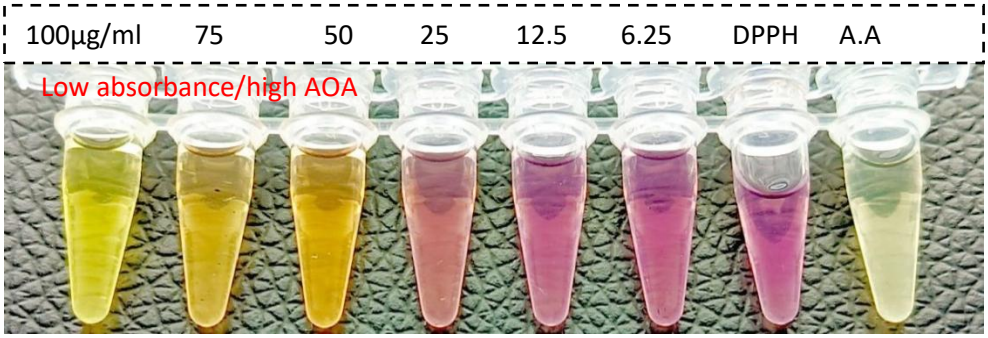


Figure 2. The qualitative assay of antioxidants in a micro-Eppendorf tube to test whether the extract possesses AOA or not, as seen above, the more reduction of DPPH and the less purple color.

Quantitative assessment or RSA% of DPPH for *Smyrnium rotundifolium* Mill, *Euphorbia erythrodon* Boiss. and Heldr, *Stachys cretica* L. subsp. *smyrnaea* Rech. Fil and *Teucrium montanum* L. revealed a high range of antioxidant activity due to high polyphenol content that acts as a strong antioxidant bioactive compound; the RSA ranged from 41% to 60% compared to the strongest AOA of ascorbic acid that revealed 75% RSA against DPPH indicator.

After concentrating on investigations, we observed that the extracts of *Teucrium montanum* had the highest antiradical RSA action due to high Total Phenolic Contents as well as caffeic acid and flavonoids. These results are compatible with the study findings of Sailović [24] as well as Benedec [25].

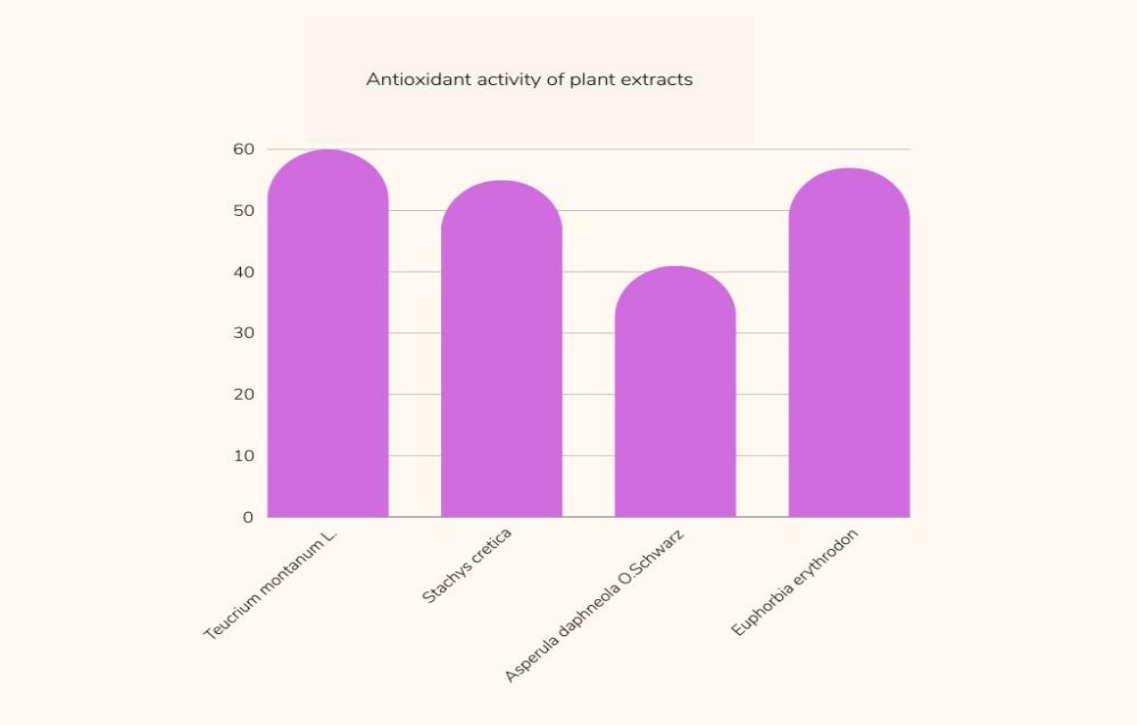


Figure 3. The RSA percentage of different plant extracts.

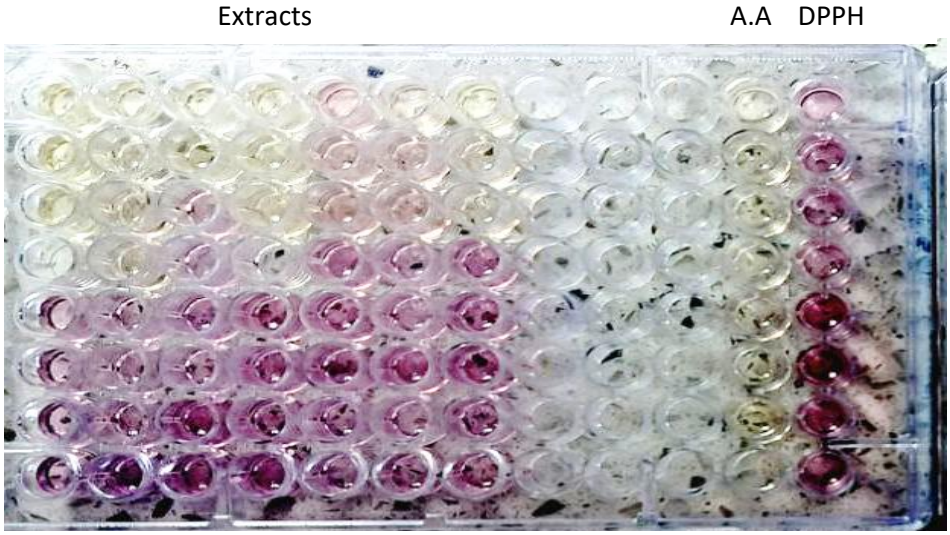


Figure 4. Antioxidant activity test of extracts on MTP with comparison to DPPH control and reference material A.A serial concentrations were made vertically on MTP.

4. CONCLUSION

The overall investigation results of the current study reveal the great bioactive potential of these plant extracts that may be applied in different industrial applications like cosmetics, food, and the drug industry. An important point of the project is that the extracts are considered eco-friendly and sustainable as alternatives to synthetic chemicals that may be hazardous to the environment.

AUTHORS' CONTRIBUTION

Both authors contributed equally to this study.

CONFLICT OF INTEREST

None.

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