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Antioxidant and Antibacterial Influences of some Plant Leaves Cell sap

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ABSTRACT

Plant waste is of great importance from an environmental and economic perspective, and therefore they benefit from these secondary metabolites that these plant wastes contain, such as pomegranate leaves, moringa leaves, and olive leaves, which contain many important secondary metabolic products that play many important roles for the plant and also from terms of medical or nutritional roles. The total phenolic compounds in the cell juices of the studied plant leaves were estimated, and the results were as follows: Moringa leaf juice (MLE), olive leaf juice (OLE), and pomegranate leaf juice (PLE), respectively, (780, 698, 650 µg/g). Fractionation was carried out by HPLC to obtain the components of phenolic compounds from phenolic acids, phenols, or polyphenolic compounds "flavonoids". The evaluation of the activities of the studied plant juices for the antioxidant activity was carried out by two methods DPPH (2,2-diphenyl-1-picrylhydrazyl), and rancimat, which are three levels of concentrations of Moringa, olive, and pomegranate (200, 400 and 800 ppm) compared with BHT (Butylated hydroxytoluene) (200 ppm) and the results proved that for the three juices and the study examined the antioxidant activity because it contains phenolic compounds, which are attributed to this antioxidant activity. The antibacterial activity was also tested in some types of pathogenic bacteria, which are (*listeria spp –Bacillus subtitles –staphylococcus aureus*).

KEYWORDS: Cell sap extracts, Antioxidant activity, Antimicrobial activity.

1. INTRODUCTION

Plant product and waste are described as biomass or plant remember wooded area remnants (including lifeless timber, branches, leaves, and tree stumps) (Shihabudeen *et al.,* 2010) Plants derivative is now a prime subject matter each in growing and evolved international locations. A massive part of the waste is underused and can result in environmental troubles if now no longer satisfactorily handled (Jin *et al.,* 2018) Bioactive Compound: The period "bioactive" is accrued via way of words: bio- and -lively. In devil: bio- from the Greek (βίο-) "bios" [bio-, -bio], refers: existence. And –lively from the Latin "lively ", manner lively, complete of strength, with strength (Bernard and Dromard, 2011) or assumes a pastime (Alain, 1994).

(White *et al.,* 1995, Demirbas, 2011, Amasuomo and Baird, 2016 and Giudice *et al.,* 2021) plant wastes come in lots of numerous paperwork and its category may be said in quite a few paperwork. Some not unusual place traits used inside the category of waste encompass the bodily states, bodily houses, reusable potentials, biodegradable potentials, supply of manufacturing, and the diploma of environmental effect.

The plant substances and their remoted additives have proven a huge spectrum of *in-vitro* and *in-vivo* pharmacological results. The oil extract from leaves that are found as an agricultural waste derivative has many caps potential fitness blessings including antioxidant pastime, anti-HIV capabilities, anti-proliferative and apoptotic results, shielding impact effect human leukemia, and has lipid-few sports. Also, the usage of the olive leaf inside the sheep food regimen brought about a boom in omega-three fatty acids and conjugated linoleic acid (C18:2) quantities in Awassi sheep milk. in addition to higher the beef quality. Chemically, the leaves of olive comprise several bisphenols just like the different components of the olive tree. Oleuropein and its metabolites, together with tyrosol and hydroxytyrosol, are the maximum customary phenolic compounds acknowledged withinside the olive leaf. Oleuropein has antibacterial, antiviral, antitumor, blood antihypertensive, blood lipid-decreasing factor, anticancer, and cardioprotective houses handiest research was carried out on the leaves of the Egyptian olive tree. As a part of our look to evaluate the via way of merchandise of economically critical cultivated plant life for his or her price, olive leaves have been subjected to in-depth research chemically and biologically. The

fatty acid composition, overall phenolic, and flavonoid contents of various leaf extracts have been accomplished in addition to the dedication of microelements and macro elements, antimicrobial, and antioxidant sports (Mona *et al.,* 2019).

Basuny & Mohammed, (2020) The phenolic compounds of olive and pomegranate leaves juice has been extracted and determined. The all-out phenolic substance of the juice changed into measured and their potential to decrease the oxidation changed into attempted the usage of the rancimat approach and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) loose radical rummaging. The phenolic mixes of olive and pomegranate depart juice have been recognized via way of elite fluid chromatography (HPLC). The results confirmed that they confirmed an effective most cancers prevention agent impact in opposition to the DPPH radical and excessive oxidative dependability via way of rancimat of phenolic mixes of olive and pomegranate leaves juice.

Mohamed *et al.,* (2015) investigated the cap potential antimicrobial results of the depart- crude extracts of the moringa species, *M. peregrina* in the evaluation of the ones of *M. oleifera*. Most of the preceding research on antimicrobial, antifungal, and antioxidant results has been focused on (*M. oleifera*) due to its presence inside the negative regions in Africa and Asia in which maximum of the humans in rural locations look for fit to be eaten herbal meals assets. however additionally, *M. peregrina*, the tree that grows as a wild plant inside the Arabian barren region has gotten much less interest, and no targeted research have been traced on its chemical composition and organic pastime. Results indicated that *M. peregrina* has been discovered to have antimicrobial sports in opposition to 5 bacterial species however this pastime changed into much less than that found via way of *M. oleifera*.

Keskın *et al.,* (2012) Studied the antibacterial pastime in opposition to opportunistic infection changes discovered in olive leaf extract acquired from the Cine location in West Anatolia. These extracts ought to boom the shelf existence of foodstuffs. It can be a destiny goal for changing artificial antibacterial retailers. Results supplied right here can also additionally endorse that the olive leaves extract possesses an antibacterial impact and is a cap potential supply of antibacterial components for the meals and pharmaceutical enterprise. The aqueous extract of olive leaves was changed into examined for its antimicrobial pastime. This extract remarkably inhibited the boom of all examined Gram-advantageous and Gram-bad microorganisms besides *Bacillus cereus* CCM 99, *Enterobacter aerogenes* ATCC 13048, and *Enterobacter cloacae* ATCC 13047.

This study has been planned to estimate the content of total phenolic compounds & total flavonoids and identified using different chromatographic techniques, in addition, study it is effective as an antioxidant and antimicrobial of different extracts of peels prickly pear cactus originating from Giza, Egypt.

2. MATERIALS AND METHODS

2.1. Source of leaves

Samples of leaves of Moringa have been accrued in November 2020 from the farm of the school of agriculture, Beni–Suef University olive kalamata, manfaloty pomegranate leaves have been accrued in November 2020 from the farm of Agricultural Research Station in Sads, Beni–Suef, Egypt.

2.2. Physicochemical Analysis

Moisture Content, Ash Determination, Fiber Determination, Fat Determination, Protein Determination, and Carbohydrates Determination according to AOAC (2000).

2.3. Qualitative phytochemical Screening of pomegranate, olive, and Moringa crude juices:

Pomegranate leaves, Moringa leaves and olive leaves crude juices were screened for the presence of key families of phytochemicals (Carbohydrates, Detection of amino acids, Detection of sterols, Detection of phenols, Detection of flavonoids, Characterization of tannins, Detection of glycosides, Detection of saponins, Detection of alkaloids) according to the methods reported by Harbone, (1973).

2.4. Preparation of plant extract

Leave of Moringa, olive and pomegranate have been manually separated, wiped clean from dirt observed via way of seed elimination then routinely pressed via way of a Carver hydraulic laboratory press (Carver Model C S/N 37000- 156; Fred S. Carver NC, Menomonee Falls, WI, USA).

Figure 1. The hydraulic piston used in the era of the samples understudy.

2.5. Total phenolic Compounds

(TPCs) Following Folin's reagent colorimetric technique, the TPC of samples changed measured (Siriwoharn *et al.*, 2014).

2.6. Estimation of overall flavonoid content material (TFC)

Following the formerly defined technique by (Chang *et al.*, 2002).

2.7. Designation of induction length via way of Rancimat

The rancimat technique changed into used to assess oxidative balance due to the fact it's miles rapid and reliable according to Gutierrez, (1989).

2.8. Antioxidant Capacity Determination

The antioxidant evaluation of pomegranate leaves, Moringa leaves and olive leaves crude juices was assessed by DPPH (2,2-diphenyl-1-picrylhydrazyl) assay according to the procedure reported by Chen *et al.*, (2003).

2.9. Antimicrobial pastime assay

Bacterial lines: In the existing look 3 bacterial species *Bacillus subtilis* (*B. subtilis*), *Staphylococcus aureus* (*S. aureus*), and *Listeria spp.* have been examined. The microorganisms have been acquired from the Agricultural Microbiology department, Faculty of Agriculture, Beni-Suef University, Egypt.

Antibacterial pastime: The sensitivity of the studied microbes to the cell sap extract of samples changed into examined the usage of concentrations (50, a hundred, a hundred, and fifty mg/mL) *via* way Ohno *et al.*, (2003).

2.10. Determination of Antibacterial Activity

All the bacteria tested *(Bacillus subtilis (B. subtilis), Staphylococcus aureus (S. aureus), and Listeria spp*) were grown on Mueller Hinton Agar according to (*Tagg & Mcgiven, 1971; Bouderba et al.*, 2012).

2.11. Statistical analysis

The statistical analysis was performed using SPSS statistical software.

3. RESULTS AND DISCUSSION

Several *in vitro* and *in vivo* pharmacological results have proven a huge spectrum of the plant substances extract and their remoted additives of extract from leaves that's found as an agricultural waste derivative has many cap potential fitness blessings including antioxidant and antimicrobial activities.

Oleuropein and its metabolites, together with tyrosol and hydroxytyrosol, are the maximum customary phenolic compounds acknowledged inside the olive leaf. Oleuropein has antibacterial, antiviral, antitumor, blood antihypertensive, blood lipid-decreasing factor, anticancer, and cardioprotective houses handiest researches Mona *et al.*, (2019), Pereira *et al.*, (2007), Vasile *et al.*, (2019) and Walid *et al.*, (2012).

3.1. Gross chemical composition of pomegranate leaves, Moringa leaves, and olive leaves

The chemical composition analysis of pomegranate leaves, Moringa leaves and olive leaves are shown in (Table 1).

The finding of the present work revealed that Moringa leaves contain the heights moisture contents (97.20) while olive leaves contained the lowest level (96.50). Moreover, the height of ash content (0.90) and protein content (1.10), and oils content (0.41) in the olive leaves. The lowest level of oils in pomegranate leaves and Moringa leaves contained the heights total carbohydrates contents (1.55) while the lowest level of total carbohydrates contents in olive leaves (1.33).

Table 1. Gross chemical composition of pomegranate leaves, Moringa leaves, and olive leaves

Component %	P.L.	M.L.	O.L.
Moisture	97.11	97.20	96.50
Ash	0.10	0.10	0.90
Proteins	0.91	0.81	1.10
Oils	0.03	0.09	0.41
Total Carbohydrates	1.41	1.55	1.33

3.2. Phytochemical analysis

The results of the phytochemical scan analysis obtained proved that the cell sap extract of the three plants under study contained terpenes, flavonoids, saponins, steroids, cardiac glycosides, proteins, carbohydrates, phenolic compounds, and alkaloids (Table 2).

Table 2. Phytochemical analysis of moringa, olive, and pomegranate leaves juice.

	Alkaloids	Terpenes	Flavonoids	Saponins	Steroids	Cardiac glycosides	Proteins	Carbohydrates	Phenolic compounds
O.L.	+ +	+	+ +	+ +	+	+	+	+	+ +
M.L.	+ + +	+	+ + +	+ + +	+	+	+	+	+ + + +
P.L.	+ +	+	+ +	+ +	+	+	+	+	+ +

Through the results of the phytochemical analysis and from previous studies, phenolic compounds and flavonoids were identified as a primary targets for bioactive compounds and as defense compounds (Hansen *et al.*, 2007 & Jed and Fahey, 2005 & Pagliarulo *et al.*, 2016). Where the activity of the extracts understudy was tested as anti-microbial activity, antioxidant activity, and anti-insect.

3.3. Total Phenolic Compounds (TPCs)

The total phenolic compounds were estimated in the studied extracts, and the results were as follows Moringa Leaves Extract (MLE), Olive Leaves Extract (OLE), and Pomegranate Leaves Extract (PLE) respectively, (780, 698, and 650 µg/g) measured by Folin's reagent method (Table 3 and Fig. 2).

Table 3. The amount of total phenolic compounds measured by the Folin-Ciocalteu reagent

Samples	TPCs (µg/g)
MLE	780.00
OLE	698.00
PLE	650.00

* Value is mean ± S.D. (N=3) - MLE: Moringa Leaves Extract, OLE: Olive Leaves Extract and PLE: Pomegranate Leaves Extract

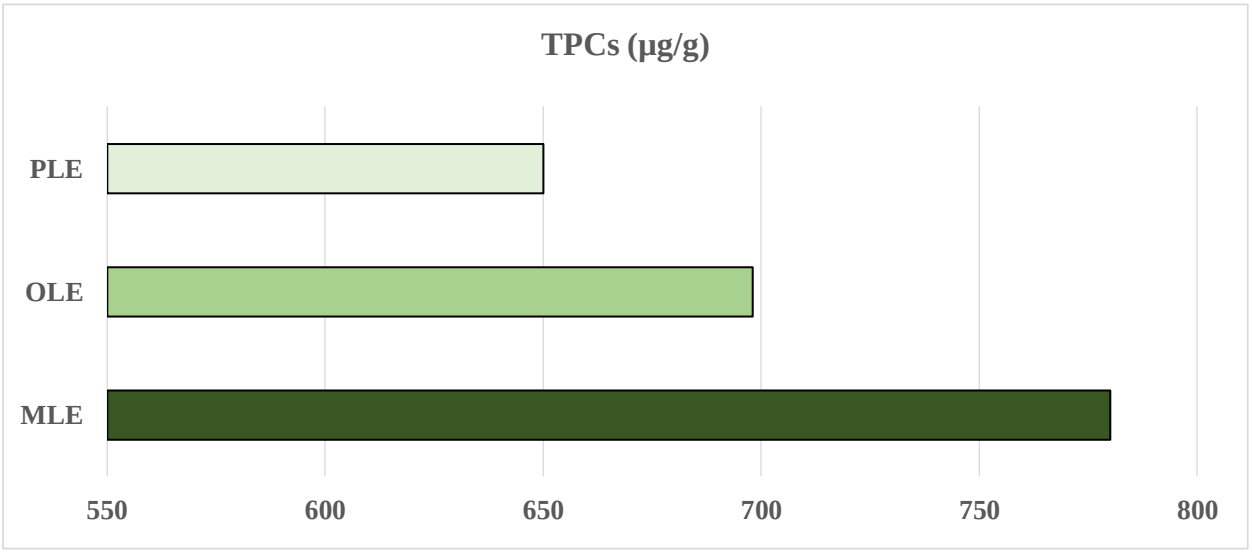

Figure 2. The amount of Total Phenolic Compounds measured by the Folin-Ciocalteu reagent.

* MLE: Moringa Leaves Extract, OLE: Olive Leaves Extract and PLE: Pomegranate Leaves Extract

3.4. Antioxidant Activity by Rancimat and DPPH methods:

Rancimat methods:

Table 4 and Fig. 3 show the effect of moringa, olive and pomegranate leaves the juice on the oxidative rancidity of sunflower oil. The results illustrate that all the various concentrations of olive and pomegranate leaves juice, exhibited antioxidant activity.

In the present study, simple model systems comprising sunflower oil with various concentrations of moringa, olive and pomegranate leaves juice were used to assess oxidation behavior. The antioxidant activities of moringa, olive, and pomegranate leaves juice extracted from leaves (moringa, olive, and pomegranate) were assessed by the rancimat method. This method assigned the induction period for the onset of oxidative rancidity in sunflower oil at 100ºC.

Table 4. Oxidative stability method by rancimat moringa, olive and pomegranate leaves juice, and BHT.

Samples	Induction period (hrs)
Control	7.30
BHT (200 ppm)	10.20
Moringa leaves (200 ppm)	9.50
Moringa leaves (400 ppm)	11.50
Moringa leaves (800 ppm)	16.00
Olive leaves (200 ppm)	9.00
Olive leaves (400 ppm)	11.00
Olive leaves (800 ppm)	15.00
Pomegranate leaves (200 ppm)	9.00
Pomegranate leaves (400 ppm)	10.80
Pomegranate leaves (800 ppm)	15.00

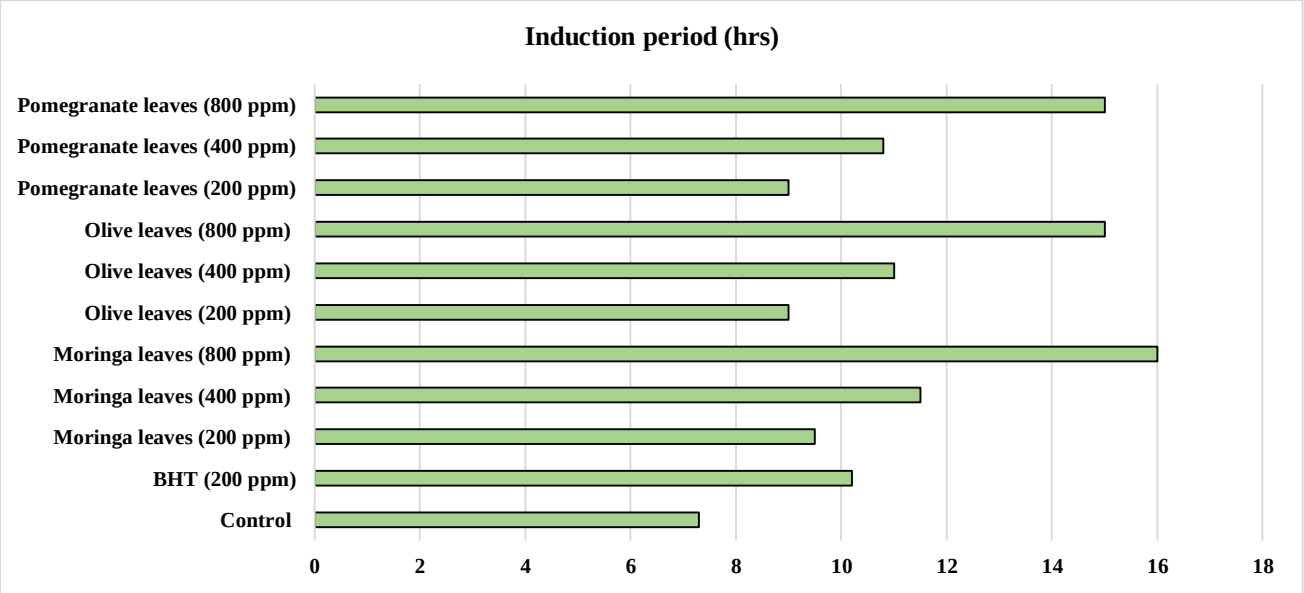

Figure 3. Oxidative stability method by Rancimat moringa, olive and pomegranate leaves juice and BHT

An experiment was performed with sunflower oil and BHT (200 ppm) to compare the antioxidant efficiency of the phenolic compounds from moringa, olive, and pomegranate leaves juice with the most commonly used synthetic antioxidant material. Statistical analysis showed that the moringa leaves juice had a significant antioxidant effect on sunflower oil stability. It is worth noting that moringa, olive, and pomegranate leaves juice at 400 ppm level superior to that of BHT 200 ppm in retarding sunflower oil oxidative rancidity.

DPPH methods:

Table 7 three levels of the concentrations of moringa, olive, and pomegranate leaves juice (200, 400, and 800 ppm) compared with BHT (200 ppm) were used with a very high scavenging capacity with an increased concentration of leaves juice. The reactions of BHT with DPPH were similar to moringa, olive and pomegranate leaves juice with DPPH; the scavenging capacities were similar at a concentration of 200 ppm.

The overall DPPH radical scavenging effect of the moringa, olive, and pomegranate leaves juice was to donate an electron, which could then react with free radicals to convert them to more stable products, terminating radical chain reactions (Jiao *et al.*, 2012) and (Ahmed *et al.*, 2020).

Table 7. Scavenging capacity of DPPH free radicals by moringa, olive, and pomegranate leaves juice and BHT.

Samples	Scavenging capacity (%)
Control	16.00
BHT (200 ppm)	27.20
Moringa leaves (200 ppm)	21.50
Moringa leaves (400 ppm)	26.30
Moringa leaves (800 ppm)	43.00
Olive leaves (200 ppm)	20.60
Olive leaves (400 ppm)	26.00
Olive leaves (800 ppm)	42.01
Pomegranate leaves (200 ppm)	20.00
Pomegranate leaves (400 ppm)	25.00
Pomegranate leaves (800 ppm)	41.00

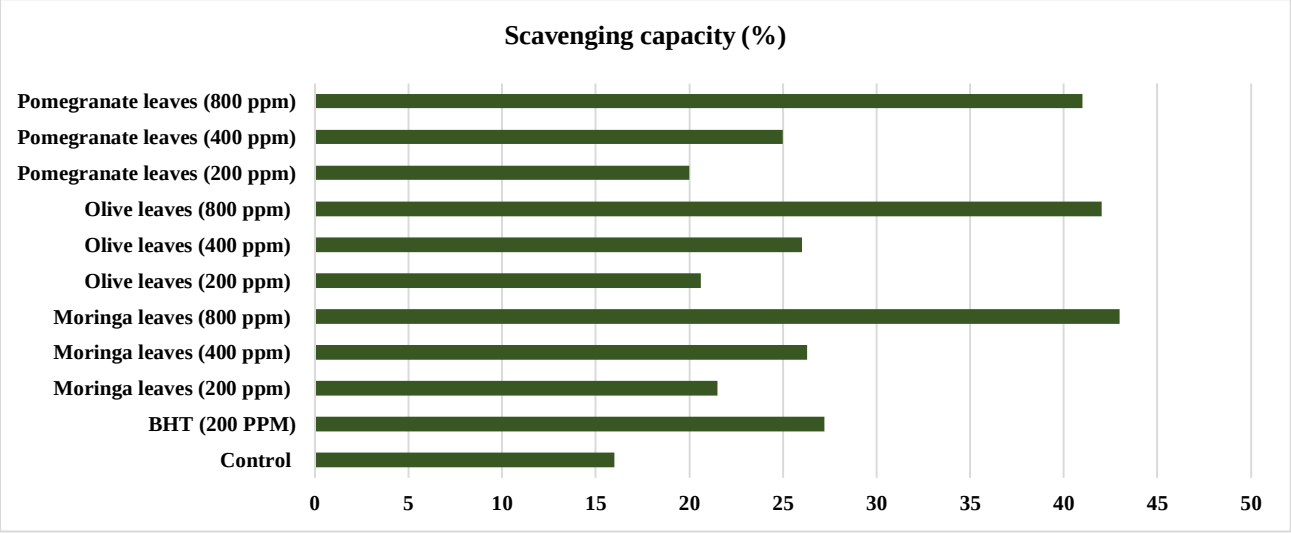

Figure 4. Scavenging capacity (%) by DPPH moringa, olive, and pomegranate leaves juice.

Antimicrobial Activity:

Fig. 5, 6, and 7 Experiments proved that olive leaf juice has an antibacterial action against three bacteria (*Bacillus subtitles, Staphylococcus aureus, listeria spp*)- and it was found that its effect is higher on *Bacillus subtitles* > *listeria spp* > *Staphylococcus aureus*.

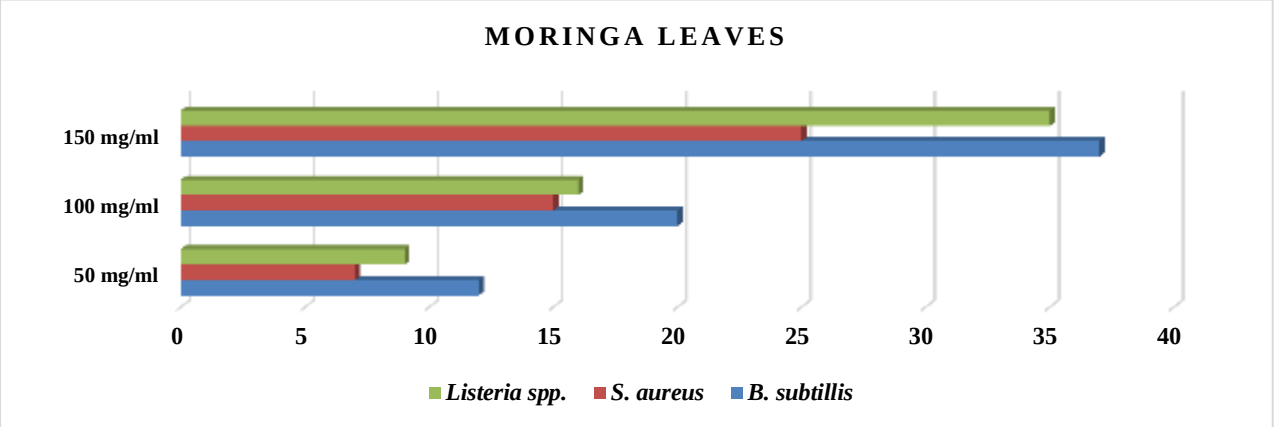

Figure 5. Antimicrobial Activity of moringa leaves juice

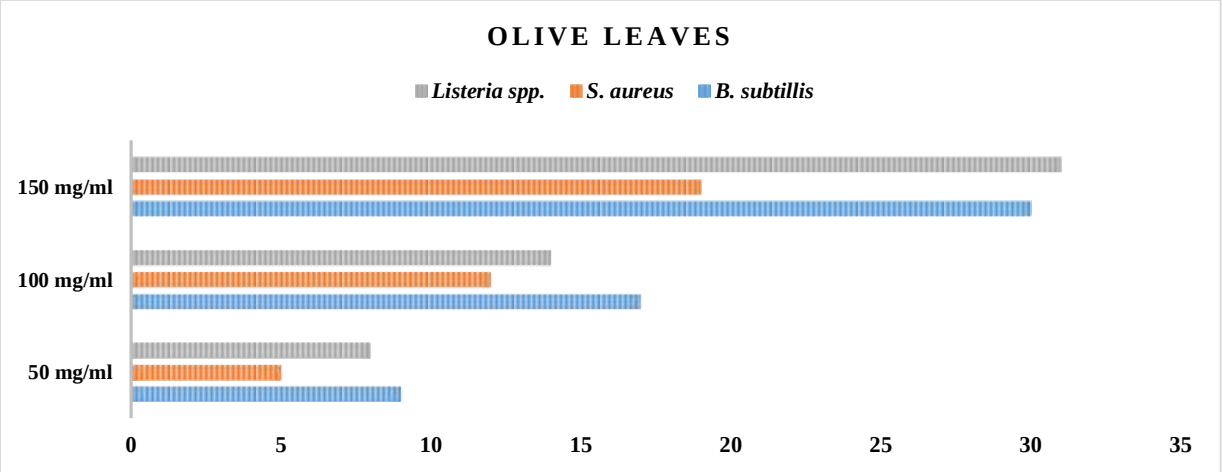

Figure 6. Antimicrobial Activity of olive leaves juice

Antimicrobial tests were performed to determine the effect of olive leaf extract, Moringa leaf extract, and pomegranate leaf extract against the growth of certain bacteria. Baycin *et al.* (2007) and Hassan *et al.* (2021) were exploring the antibacterial action against three bacteria (*Bacillus subtitles*, *Staphylococcus aureus*, *listeria spp*) determined by the disk diffusion method, where bacterial cultures were grown on agar overnight and after incubation.

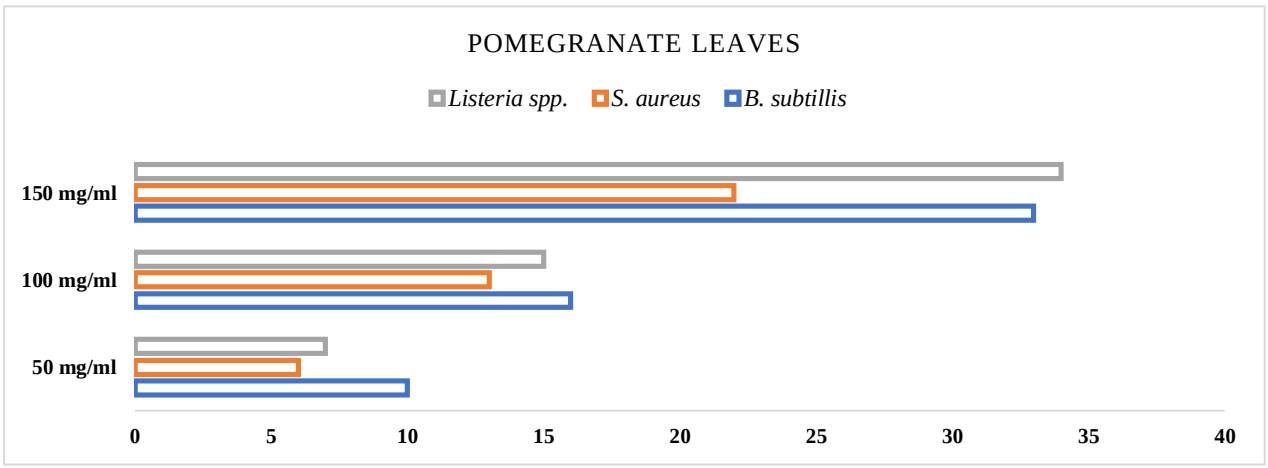

Figure 7. Antimicrobial Activity of pomegranate leaves juice

4. RECOMMENDATION

Through our study, we recommend the use of the cell sap of the leaves of the plants under study, as they are considered natural sources of bioactive chemical compounds, which have distinctive effects as antioxidant and antimicrobial activities because they contain phenolic compounds, which previous specialized studies have proven the beneficial effects of these compounds.

5. CONCLUSION

Using the cell sap of Moringa, olive, and pomegranate leaves by the hydraulic press to squeeze these leaves, gives impressive results in the prevention of very harmful free radicals in causing carcinogenic health effects and stopping the growth of pathogenic bacteria that lead to serious diseases for human health.

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الملخص العربي

التأثيرات المضادة للأكسدة ولنمو البكتريا للعصارة الخلوية لبعض الأوراق النباتية

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[٢] قسم الميكروبيولوجيا الزراعية – كلية الزراعة – جامعة بنى سويف

تمثل المخلفات النباتية أهمية كبيرة من المنظور بيئي واقتصادي، ومن ثم فإن الاستفادة من هذه المنتجات الثانوية والتي تحتويها تلك المخلفات النباتية، مثل أوراق الرمان وأوراق المورينجا وأوراق الزيتون والتي تحتوي على الكثير من نواتج التمثيل الغذائي الثانوي الهامة والتي تقوم بالكثير من الأدوار الهامة للنبات وأيضاً من حيث الأدوار الطبية أو التغذوية.

تم تقدير إجمالي المركبات الفينولية في العصائر الخلوية للأوراق النباتية محل الدراسة، وكانت النتائج كما يلي: عصير أوراق المورينجا (MLE)، عصير أوراق الزيتون (OLE) وعصير أوراق الرمان (PLE) على التوالي، (٧٨٠، ٦٩٨، ٦٥٠ ميكروجرام / جرام) وتم أجراء تفريد بواسطة جهاز HPLC للحصول على مكونات المركبات الفينولية من الأحماض الفينولية أو الفينولات أو المركبات الفينولية عديدة الفينول " الفلافينويدات". تم إجراء تقييم أنشطة العصائر النباتية محل الدراسة للنشاط المضاد للأكسدة بطريقتين (طريقتان DPPH وRancimat) في طريقة DPPH، وهما ثلاثة مستويات لتركيزات المورينجا والزيتون والرمان (٢٠٠ و٤٠٠ و٨٠٠ جزء في المليون) مقارنة مع BHT (٢٠٠ جزء في المليون) وأثبتت النتائج بأن للثلاث عصائر محل الدراسة نشاط مضاد للأكسدة لاحتوائها على المركبات الفينولية والتي يعزى لها تلك النشاط المضاد للأكسدة وتم ايضاً اختبار النشاط المضاد للبكتيريا في بعض أنواع البكتيريا الممرضة وهي (*Listeria spp – Bacillus subtitles – Staphylococcus aureus*).