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EFFECT OF ADDING HERB EXTRACTS AS ANTIMICROBIAL AND ANTIOXIDANT ON THE QUALITY CHARACTERISTICS OF SOME JUICES

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ABSTRACT

Antioxidant activity and inactivation pathogenic microorganism for extracts of sage, fennel, thyme, marjoram, chamomile and ginger as herb extracts were studied. Also, the effect adding of herb extracts on the antioxidant activity of mango, orange and carrot juices was also investigated. Results indicated that all herb extracts had different effect for the percentages of antioxidant activity ranged from 35.73 to 88.65%. Marjoram, thyme and sage had greatest antioxidant effect which was 88.65, 79.44 and 71.57%, respectively. Adding of thyme and marjoram extracts to juices recorded the highest effect of total antioxidant activity comparing with fennel and ginger extracts. The highest total phenolics content of thyme, marjoram and sage extracts was observed by adding to different juices while the fennel and ginger extracts recorded the lowest content. Results also showed that, antimicrobial activities in vitro the tested of herbs had affect for the potential activity of *Stapylococcus aureus, Salmonella typhi* and *Escherichia coli* and their activity potentials in inhibition zones as a result of presence of herb extracts. The results also showed that sage extract had greater potential antimicrobial agent against *Salmonella typhi* than other tested herb extracts. While, the highest inhibition zones against *Stapylococcus aureus* and *Salmonella typhi* was noticed in the presence of sage extract and the lowest inhibition was observed with ginger extract. On the other hand, the thyme and chamomile extracts had the highest inhibitions for *Escherichia coli* meanwhile; marjoram extract had the lowest effect for *Escherichia coli* inhibition.

INTRODUCTION

A large number of medicinal plants and their effective constituents have been shown as a beneficial therapeutic potential. Natural antioxidants may function as reducing agents, free radical scavengers, pro-oxidant metals complexes, and quenchers of singlet oxygen. They can be used in the food industry and there is evidence that they may exert antioxidant effects within the human body **(Ramadan *et al* 2003)**. Among Lamiaceae species, oregano (*Origanum vulgare* L.), thyme (*Thymus vulgaris* L.) and wild thyme (*Thymus serpyllum* L.) was widely studied for their antioxidant activity, due to the highest content of phenolic compounds **(Vichi *et al* 2001, Zandi & Ahmadi, 2000 and Takacsova *et al* 1995)**. On the other hand, the phenolic compounds of thyme have several medicinal benefits, as they strong antibacterial activity for common respiratory tract **(Inouye *et al* 2001)**. Antimicrobials are used in food for two main reasons: 1) to control natural spoilage processes (food preservation); and 2) to prevent/control growth of microorganisms, including pathogenic microorganisms to increase (food safety). **(Burt, 2004 and Gaysinsky & Weiss, 2007)**.

To achieve preservation of unstable nutrients for many processed juices was investigated alternative methods of thermal pasteurization, i.e un-

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pasteurized short shelf life juices with high retail value. This trend has continued within the European Union. However within the US recent regulations by the FDA have required processors to achieve the reduction in the numbers of the most resistant pathogens in their finished products. Pathogenic *E. coli* may survive in acid environments such as fruit juices for long periods **Patil *et al* (2009)**.

Antimicrobial activity for extracts of *Eupatorium lindleyanum* DC (EEL) was screened against eight selected food spoilage and food-borne pathogens. These extracts were used as a functional food ingredient and as a pathogenic microorganism inhibitor to preserve food products. They show effective antimicrobial effects with a remarkable dose–response relationship and broad antimicrobial spectrum on all tests of Gram-positive bacteria and Gram-negative species of bacteria. Acid environment or medium helps to enhance the antimicrobial activity. Also, the EEL was added in commercial orange juice at a concentration of 0.4 mg ml/1 had a similar antimicrobial effect potassium sorbate at the level of 0.2 mg ml/1 **(Ji *et al* 2008)**.

Food processors, food safety researchers and regulatory agencies have been increasingly concerned with the growing number of food-borne illness outbreaks caused by some pathogens and/or their enterotoxins. *Escherichia coli, Staphylococcus aureus, Salmonella spp., Yersinia spp. and Clostridium spp.* are responsible for many cases of intestinal disorders, causing vomiting and diarrhoea. However, some microorganisms are also associated with food spoilage, causing economic losses **(Demirci *et al* 2008 and Friedman *et al* 2002)**.

(Lampe, 1999) reported that foods containing antioxidant compounds, such as fruits and vegetables, could help for preventing of pathologies caused by oxidative stress.

The fennel *(Foeniculum vulgare)* seed is a potential source of natural antioxidant either water or ethanol seed extracts (FS) which was evaluated by various antioxidant assay, including total antioxidant, free radical scavenging. These antioxidant activities were compared to standard antioxidants. The water and ethanol extracts of FS seeds had a strong antioxidant activity depends on its used concentration. However, the antioxidant activity was increasing by adding large amounts of sample. **(Oktay *et al* 2003)**. On the other side, the antioxidant activity of the water extract of sage had the highest reducing power activity compared to tilia and black tea and no significant effect of these extracts on the antibacterial activity on the microorganisms under this investigation. **(Yildirim *et al* 2000)**.

Natural antimicrobials are receiving an increased of attention for several numbers of issues for controlling microorganism. Reducing the need for antibiotics, controlling microbial contamination in food, improving shelf-life extension technologies to eliminate undesirable pathogens and/or delay microbial spoilage, decreasing the development of antibiotic resistance by pathogenic microorganisms or strengthening immune cells in humans are other benefits **(Abou-taleb & Kawai, 2008 and Fisher & Phillips, 2008)**.

Even without a more comprehensive understanding of how natural antimicrobial substances work, there is a growing effort to develop new effective methods that rely primarily on their use to enhance food safety **(Ayala-Zavala *et al* 2008 and Brandi, *et al* 2006)**.

The aim of this investigation was carried out to study the effect of herb extracts for sage, fennel, thyme, marjoram, chamomile and ginger as antimicrobial and antioxidant. Also, the effect of adding these herb extracts to mango, orange and carrot juices on the antioxidant activity and sensory properties was also investigated.

MATERIALS AND METHODS

1. Materials

Leaves of sage (*Salvia officinalis* L.), thyme (*Thymus vulgaris* L.), marjoram (*Origanum majorana* L.) fennel (*Foeniculum vulgare* L.) seeds, chamomile (*Matricaria chamomilla* L.) florescence and ginger (*Zingiber officinale* L.) roots were obtained from Horticulture Research Institute, Agriculture Research Center, Giza, Egypt. These herbs were dried in the air protected against direct sunlight, grinding in blender mill, then extracted and stored at 5°C for further analysis.

2. Herbs material and extraction

The antioxidant activity of herb extracts were prepared according to **(Samec *et al* 2010)** with some modification by adding 5gm of dried herbs powder to 100 mL of distilled water was heated to 80°C to obtain the herb extracts where, the extraction proceeded for 5 min. in a closed plastic vial at room temperature, with continuous stirring. The obtained herb powders of each selected herb were filtered through cheese cloth. The antimicrobial

inhibition herbs extract was done by using 1gm of each herbs powder in 100ml ethanol (80%). Extraction was repeated for three times, and then the filtrates of all portions were collected in one vessel. The obtained ethanol extract was concentrated by using rotary evaporator, after that each extracts of herb was dissolved in the appropriate solvent for using as antimicrobial inhibitions.

3. Antioxidant activity for herbs adding to juices of mango, orange, and carrot

Fresh juices of mango, orange and carrot (without any additive) were prepared to test the effect of the previous extracted as natural antioxidant activity and /or antimicrobial preservative in the selected juices.

4. Antimicrobial susceptibility testing

Stapylococcus aureus (ATCC 20231), *Salmonella typhi* (ATCC 14028) and *Escherichia coli* (ATCC 69337) were used in vitro to assay the antimicrobial activities of selected extracted herbs (Extracts were prepared by using 100ml 80% ethanol and concentrated by rotary evaporator). The disk diffusion, turbidity adjusted to the McForland O.S turbidity stander. Sterile filter paper loaded with 12µl of extracted sit discs were applied to each petri dish, plates incubated at 37°C for 24h. The inhibition zones were measured in (mm) by **Bauer *et al* (1966).**

5. Total phenolic contents

The total phenolic contents of each extract were determined using the Folin–Ciocalteau reagent **(Zhou & Yu, 2006).** The reaction mixture contained 50µl of samples, 250µl of the Folin–Ciocalteau reagent, 0.75 ml of 20g/100ml sodium carbonate and 3ml of pure water. After 2h of reaction at ambient temperature, the absorbance at 765 nm was measured and used to calculate the phenolic contents using gallic acid as a standard. The total phenolic contents were then expressed as gallic acid equivalent (GAE), in mg/g dry sample. All experiments were performed in triplicate.

6. Assay of antioxidant activity by (DPPH)

The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity was determined by an assay modified by **Kwon *et al* (2006)** with some modification. To 3 mL of 60 µM DPPH was dissolved in 95% ethanol, 250 µL for each added sample extract, and the decrease in the absorbance was monitored after 30 min at 517 nm (A517 extract). The absorbance of a control (distilled water instead of sample extract) was also recorded after 30 min at the same wavelength (A517 control). Therefore, the percentage of inhibition was calculated as the following formula:

$$\%\ Inhibition = A517\ (control) - A517\ (extract)\ /A517\ (control) \times 100$$

7. Sensory evaluation

The sensory evaluation of prepared juices contained the herb extracts was carried out with three replicates by 10 trained panelists. The investigated sensory attributes were flavor (odor and taste), texture, colour and overall acceptability. All investigated attributes were scored of 10 degree.

8. Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA) using SPSS version 18 software (SPSS Inc., Chicago, USA). Where there was statistical significance (P = 0.05), the means were further separated using Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Total antioxidant activity in herbs extracts are shown in **Fig. (1)**.Results indicated that all extracts of herbs had different percentages of total antioxidant activity which ranged from 35.73 to 88.65%. Marjoram, thyme and sage showed the greatest percentages of antioxidant activity recorded 88.65, 79.44 and 71.57%, respectively correlation with the total of phenolics content. But the lowest percentages of total antioxidant activity observed with ginger and fennel which was 26.57 and 35.73%. Results also showed the extracted herbs under investigation had the higher effect as antioxidants than that for vegetables and fruit such as mango, orange and carrot. The variation in total antioxidant activity may relate to the content of total phenolic compound. **(Ranilla *et al* 2010)** found the antioxidant activity based on the DPPH radical inhibition assay for herb extracts had a significant correlation with total phenolics content.

Effects of adding herb extracts to some tested juices such as mango, orange and carrot on the change of total antioxidant activity are shown in **Fig. (2)**. Fresh mango juice had the highest percentage of total antioxidant activity (49.13%) followed by orange juice (37.44%) while carrot had the lowest one (14.22%). Significantly increased in

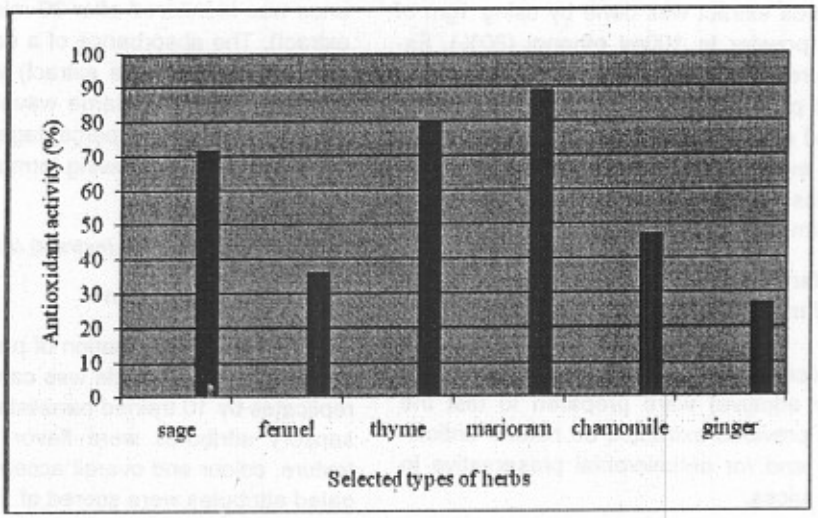

Fig.1. Antioxidant activity (%) of selected types of herb extracts

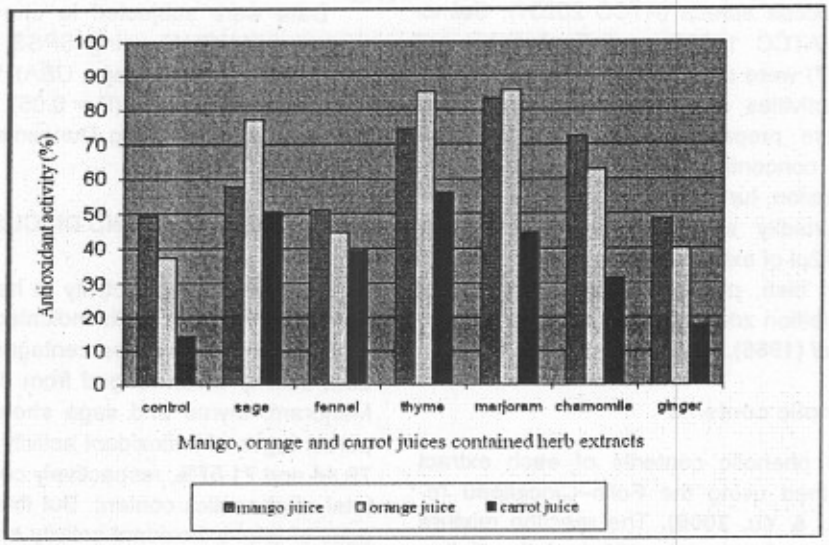

Fig. 2.The effect of adding herb extracts on total antioxidant activity (%) for tested juices

total antioxidant activity was observed of juices under investigation by adding different herb extracts. The highest total antioxidant activity showed with orange juices in the presence of both marjoram and thyme extracts where reached to 85.78 and 85.30%, respectively. Also, the total antioxidant activity of mango juice with adding marjoram extract was 83.23% and orange juice with sage extract was 77.07% followed by mango juice containing either thyme or chamomile extracts were 74.45 and 72.35%, respectively. Carrot juice with herb extracts had the lowest level of total antioxidant activity compared with that in mango or orange juices. The highest effect of extracted thyme as antioxidant activity showed with carrot juice 56.08% while the lowest effect showed with ginger extract (17.54%). Therefore, juices contained thyme and marjoram extracts had the highest total antioxidant activity than that contained fennel and ginger extracts. These results similar with obtained by **Djeridane *et al* (2006)** who found that the medicinal herbs exhibit clearly a higher antioxidant activity and contain significantly more phenolics than the common vegetables and fruits (nutritional plants).

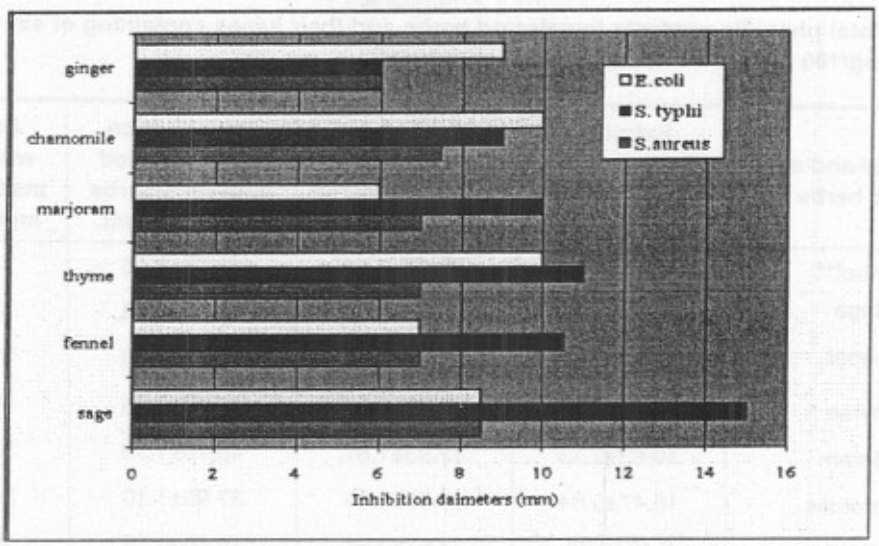

Fig. 3. Effect of herb extracts on inhibition diameters (mm) of *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli*

The total phenolic contents in some herb extracts expressed as gallic acid equivalent (GAE) per mg/gm on dry matter are shown in **Table (1)**. The mean values of total phenolics ranged from 9.08 to 30.92 mg/gm in ginger and thyme, respectively. Thyme and marjoram showed the highest total phenolic contents 30.92 and 30.83 mg/gm while fennel and ginger had the lowest content (9.08 and 9.46 mg/gm) respectively. Also, the total phenolics content of sage extract was 28.17mg/gm while chamomile extract was 16.47 mg/gm on dry matter. The extract of tested herbs were adding to mango, orange and carrot juices and the effect of these additives on the total phenolic contents was investigated and results are shown in **Table (1)**. The content of total phenolics was calculated as mg (GAE)/100mL juices. The total phenolic contents in fresh juices were 20.78, 22.64 and 14.43 in mango, orange and carrot juices, respectively. The additional of 10 mL (5%) herb extracts to 90 mL in fresh juices caused to increase of total phenolic contents. The highest total phenolic contents was showed in juices contained thyme, marjoram and sage extracts while the lowest incremental of total phenolics showed with juices contained fennel and ginger extracts. It is important to point out that, there was a positive correlation between antioxidant potential and phenolics content as estimated by **(Ksouri *et al* 2007)**.

The effect of investigated herb extracts as antimicrobial agents (at 12µl of extract sit disc) on inhibition zones of *Stapylococcus aureus* (ATCC 20231), *Salmonella typhi* (ATCC 14028) and *Escherichia coli* (ATCC 69337) in relation to their potentials activities are given in **Fig. (3)**. Results indicated that, sage extract had greater potential effect as antimicrobial agent against *Salmonella typhi* than other tested extracts of herbs. The highest inhibition zone, for *Salmonella typhi* recorded 15mm. While, sage extract with *Stapylococcus aureus* and *E.coli* recorded the inhibition zones of 8.5mm. Thyme and fennel extracts caused to inhibition zones of 11 and 10.5mm while using *Salmonella typhi* recorded 10mm inhibition diameter in the presence of marjoram extract. The extracted sage gave the highest inhibitions zones with *Stapylococcus aureus, Salmonella typhi* comparing with the extracted ginger (6mm). However, the highest inhibition zones for *E. coli* were noticed by using thyme and chamomile extracts (10mm) and the lowest inhibition diameter was obtained with marjoram extracted which was (6mm). Several studies attributed the inhibitor effect of plant extracts against pathogens bacteria to their phenolic composition **(Baydar *et al* 2004; Rodriguez Vaquero *et al* 2007)**. The inhibitor effect of these phenolics could be explained by adsorption of cell membranes, interaction with enzymes, substrate and metal ion deprivation **(Scalbert, 1991)**.

Sensory evaluation of mango, orange and carrot juices contained herb extracts are shown in **Table (2)**. Results indicated that no significant changes in colour as a result of adding herb extracts in comparing with control samples (fresh juices). Fresh mango juice and mango juice contained chamomile extract gave the highest

Table 1. Total phenolic contents in selected herbs and their juices containing of extracted herbs* mg/100 mL

Medicinal and aromatic herbs	Extracted medicinal herbs mg/gm (DM)	Mango juice with extracted medicinal herbs mgGAE/100mL	Orange juice with extracted medicinal herbs mgGAE/100mL	Carrot juice with extracted medicinal herbs mgGAE/100mL
Control**		20.78±2.04	22.64±0.98	14.43±1.01
Sage	28.17±1.15	41.25±0.89	42.09±1.58	38.26±1.44
Fennel	9.08±0.61	23.71±1.58	26.77±0.30	21.86±0.54
Thyme	30.92±0.98	43.76±2.22	44.17±2.55	40.51±1.72
Marjoram	30.83±2.13	42.93±1.51	45.75±1.39	37.25±1.38
Chamomile	16.47±0.84	33.59±2.45	37.96±1.10	25.51±0.55
Ginger	9.46±0.35	23.67±0.44	28.08±0.78	19.23±0.91

* Blend 90 mL juice to10 mL extracted herbs from 5gm/100ml in hot water at 80°C for 5min.on (DM)

** Fresh mango, orange and carrot juices

Table 2. Sensory evaluation of mango, orange and carrot juices containing extracted herbs

Medicinal and Aromatic herbs		Color	Taste	Odor	Texture	Overall acceptability
Control	Mango juice	8.50±1.32	8.00±1.00	7.33±1.11	8.60±1.00	8.00±1.00
Sage		8.17±0.76	7.07±0.32	6.83±0.35	8.43±0.49	8.13±0.71
Fennel		8.33±1.53	7.63±0.58	7.36±0.98	8.57±0.58	8.34±0.58
Thyme		8.31±0.29	7.78±1.53	8.15±1.00	8.76±1.52	8.00±1.60
Marjoram		8.00±1.00	7.47±1.13	7.30±1.53	8.35±1.13	7.33±1.53
Chamomile		8.50±1.50	7.00±1.73	8.00±1.44	8.19±1.73	8.00±1.73
Ginger		7.67±2.08	6.67±2.08	7.22±1.20	8.22±2.11	7.00±2.00
Control	Orange juice	8.00±1.41	8.20±1.30	7.36±1.64	8.50±0.50	7.80±0.84
Sage		7.10±1.00	7.30±0.83	6.77±0.44	8.41±0.55	8.00±0.79
Fennel		7.80±1.48	8.00±1.41	8.20±1.10	8.45±1.14	8.50±1.50
Thyme		8.20±1.09	8.40±1.14	8.34±1.48	8.80±1.10	8.90±0.89
Marjoram		8.40±1.14	8.00±1.58	8.08±1.22	8.22±1.10	8.10±1.14
Chamomile		7.40±0.89	7.60±1.12	7.62±0.55	8.60±0.89	8.20±0.83
Ginger		7.32±1.66	7.40±0.89	7.40±1.18	8.00±1.22	7.80±1.10
Control	Carrot juice	8.75±1.50	8.50±1.73	7.55±1.91	8.00±1.41	7.63±1.70
Sage		7.75±1.77	7.50±0.57	6.50±1.73	7.00±1.12	6.75±0.95
Fennel		7.92±0.54	7.74±0.50	7.28±0.96	7.33±0.50	6.75±0.50
Thyme		8.00±1.41	7.69±0.96	7.77±0.96	7.65±1.25	7.00±1.41
Marjoram		7.42±1.29	7.75±0.96	7.52±1.29	7.25±1.45	6.75±1.70
Chamomile		8.50±1.16	7.48±0.58	6.49±1.00	6.78±0.30	6.50±1.00
Ginger		7.68±0.50	7.25±0.50	6.71±0.96	6.57±1.50	7.00±1.15

Significantly different according to the Duncan test ($p<0.05$). Means ±SD

colour score in comparing with other juices contained another herb extracts. Also, orange juice contained marjoram extract had the highest score comparing with other tested orange juices. Also, no significant changes were noticed in taste juices contained different extracted of herbs. Therefore, adding fennel, thyme and marjoram extracts gave the best results while adding ginger extract gave the lowest score in all tested juices. Same findings are shown in odor and texture. Generally, adding herb extracts to investigated juices don't significantly effect on sensory evaluation but caused to improve juices properties especially for antioxidant or antimicrobial which may be increase the shelf life of these juices.

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حوليـــات العلـــوم الزراعية
جامعة عين شمس ، القاهرة
مجلد(٥٥)، عدد (٢)، ٢٦٣-٢٧١، ٢٠١٠

تأثير إضافة مستخلصات الأعشاب الطبية والعطرية كمثبطات ميكروبية ومضادات للأكسدة علي خصائص الجودة لبعض العصائر

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الموجـــز

تم دراسة تأثير إستخدام بعض مستخلصات الأعشاب الطبية والعطرية مثل المرمرية والشمر والزعتر والبردقوش والبابونج والزنجبيل كمثبطات للميكروبات المرضية ومضادات للأكسدة. مع الأخذ في الأعتبار دراسة تأثير اضافة هذه المستخلصات الي بعض العصائر مثل المانجو والبرتقال والجزر كمضادات للأكسدة حيث أظهرت النتائج ان مستخلصات هذه الأعشاب كانت ذات نشاط مضاد للأكسدة تراوح بين ٣٥,٧٣ و ٨٨,٦٥ % مع ملاحظة أن أعلي معدل للنشاط كمضادات للأكسدة كان نتيجة إضافة مستخلصات كل من البردقوش والزعتر والمرمرية التي سجلت ٨٨,٦٥ و ٧٩,٤٤ و٧١,٥٧ % علي التوالي. هذا وقد لوحظ ان العصائر التي اضيف اليها مستخلص الزعتر والبردقوش كانت ذات نشاط عالي كمضاد للأكسدة بينما كانت العصائر التي اضيف اليها مستخلص الشمر والزنجبيل كان تأثيرها كمضادات للأكسدة محدوداً. كذلك أوضحت النتائج ان أعلي محتوي من الفينولات الكلية كان في العصائر المضاف اليها مستخلصات الزعتر والبردقوش والمرمرية بينما كانت أقلها تلك التي اضيفت اليها مستخلصات الشمر والزنجبيل. أما بالنسبة لنشاط هذه المستخلصات كمثبطات للميكروبات المرضية مثل اشرشيا كولاي وستافيلوكوكس ايريس والسالمونيلا تايفي فلقد بينت الدراسة أن مستخلص المرمرية كان أكثر تأثيرا في تثبيط نشاط السالمونيلا تايفي مقارنة بمستخلصات الأعشاب الطبية والعطرية الأخري كذلك كان لمستخلص المرمرية أعلي معدل للتثبيط علي ميكروب ستافيلوكوكس ايريس بينما لوحظ أن مستخلص الزنجبيل كان ذات معدل منخفض في التثبيط. ومن ناحية اخري اظهرت النتائج ان أعلي معدل تثبيط لميكروب اشرشيا كولاي كان مع مستخلص الزعتر والبابونج بينما ادي إستخدام مستخلص البردقوش الي الحصول علي أقل معدل للثبيط.

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