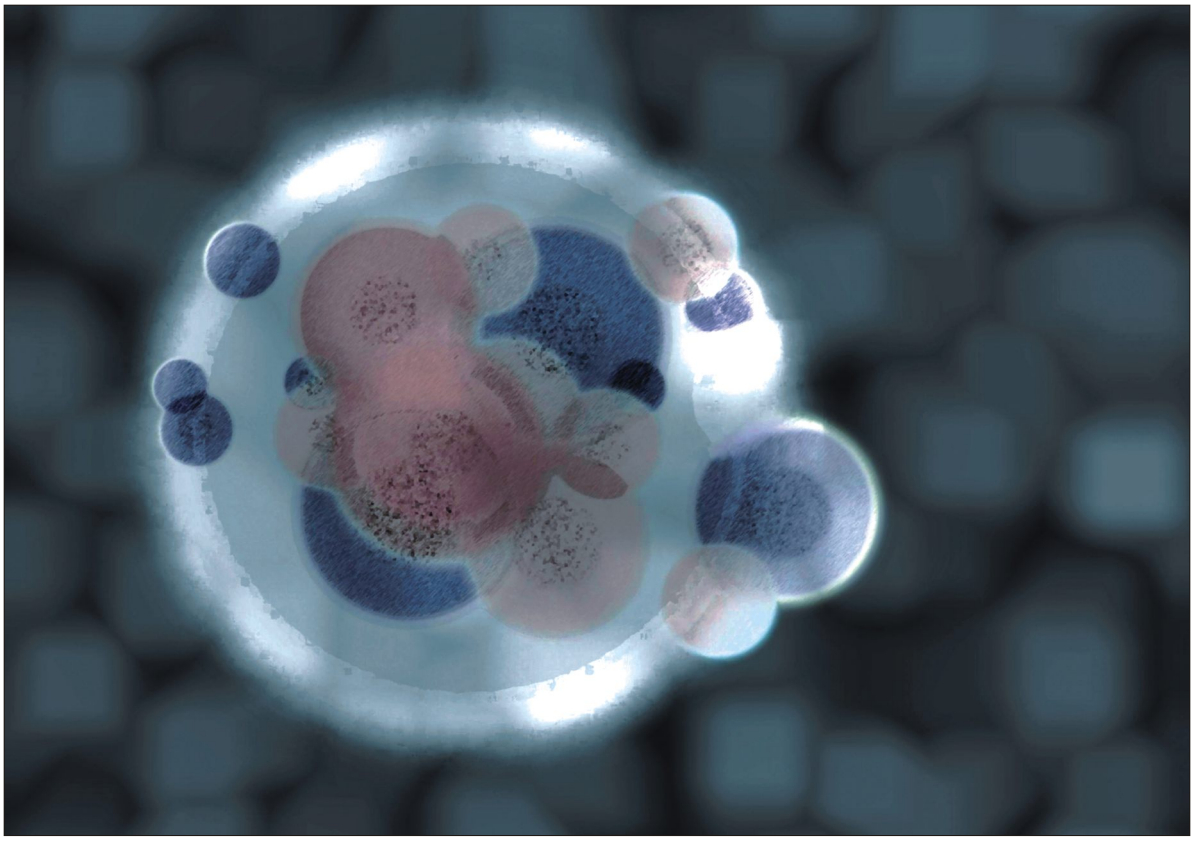

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Research Article
Prophylactic and Therapeutic Uses of Egyptian *Mentha spicata* L., *Mentha piperita* L. and *Ocimum basilicum* L. Stalks as Agro-industrial Byproducts

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Abstract

Background and Objective: Searching for new sources of important vital compounds has been and still concern the minds of scientific researchers. Agro-industrial wastes are great examples for such a purpose, it contains many bioactive compounds yet it mostly discarded causing environmental and health problems. This study sought to utilize the mint and basil stalks as agro-industrial wastes, these economically valuable byproducts have not yet been employed in previous studies. **Materials and Methods:** The aqueous extracts of both mint and basil stalks (wild and cultivated) were evaluated for their antioxidant activities and investigated as anti-inflammatory and antitumor agents. Total flavonoid, tannin and phenolic compounds were evaluated in the resulted extracts. Additionally, HPLC identification was implemented for effective compounds in all aqueous extracts. **Results:** The finding indicated that wild mint stalks extract possessed the highest levels of phenolic, flavonoid and tannin compounds. Wild mint also found to have a considerable amount of antioxidants and a vigorous *in-vitro* anti-inflammatory activity. Wild mint stalks extract also projected a significant activity for liver (HepG2) and breast (MCF-7) cell lines, even more potent than the standard drug as anti-proliferative agent. However, wild and cultivated basil stalk extracts showed a significant activity against (A549) human lung cell line. **Conclusion:** The results suggest that mint and basil stalks may be useful in the production of an economical and effective treatment for inflammation and cancer diseases, beside recycling a waste to reduce the health and environment hazards.

Key words: Mint stalks, basil stalks, antioxidants, anti-inflammatory, anticancer, HPLC

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Medicinal herbs have been always consumed due to their pharmacological effects. Without or with little side effects, they were preferable over chemicals alongside the healthy foods[1]. These plants are widely distributed worldwide, the *Lamiaceae* family is one of the aromatic plants that are extensively used for various objectives. Mint species like spearmint (*Mentha spicata* L.) and peppermint (*Mentha piperita* L.) beside Basil (*Ocimum basilicum* L.) are members of this family and consumed for their high content of antioxidants and aromatic compounds[2,3]. In fact, biochemical components and antioxidants activities of this family may differ due to geographical allocation beside the different parts of the same plant[4].

Investigation of the *Lamiaceae* family revealed that *Mentha* genus includes about 25-30 species which include many phenolic, flavonoid and terpenoid compounds. Using different extraction solvents many of these compounds have been recognized such as, acacetin, apigenin, luteolin and sideritoflavone[5,6]. Furthermore, basil is the major essential oil crop from more than 150 species of the genus *Ocimum*, which is commercially cultivated world wide for its ornamental value and abundance of natural components, such as phenolic acids, monoterpenes, sesquiterpenes, anthocyanins and phenylpropanoids[7,8]. Those above-mentioned phytochemicals have several health benefits, such as antitumor, anti-inflammatory, antioxidant, anti-hepatotoxic and antimicrobial activities[4].

As for chronic Inflammation, it is an important immune response, which ascribed to being a substantial baseline reaction that leads to various chronic diseases such as cancer, rheumatoid arthritis, atherosclerosis, diabetes and septic shock[9]. However, different mint and basil species found to have an anti-inflammatory influence in acute and chronic inflammation induced inhuman leukocytes cultures and *Wistar albino* rats[10,11].

Previous studies proved that polyphenols and terpenes are the main compounds responsible for the medicinal effect of this family[12]. Later, anticancer effect was observed for different mint species, where potent cytotoxic impact was reported on lung, breast, colon and liver cell lines[13,14]. On the other hand, different basil species also represents a cytotoxic effect to human cancer cells[15] while, Lawrence[16], illustrated the antitumor activity of basil extracts in mice.

Due to the high cost of treating chronic diseases and cancer, attention has been paid to the use of less expensive treatments, resulted from inexpensive, unconventional and natural sources such as agro-industrial wastes. Agro-industries are a fast-growing area, yet numerous amounts of generated wastes are generally discarded causing undesirable environmental and health impacts. However, appropriate biotechnological involvement could be effective to obtain valuable bioactive compounds from the produced wastes[17]. In Egypt, mint and basil stalks are considered as agro-industrial wastes generated during mint tea bags production and basil essential oil processing industries[18].

Most studies have focused on essential oils of *Lamiaceae* family extracted from leaves and flowers, while there are fewer reports regarding bioactive materials extracted from other parts of this family.

Therefore, the aim of the present study was to utilize the stalks as a waste generated from manufacturing of mint tea bags and basil essential oil processing to examine antioxidant, anti-inflammatory and anticancer activities of their aqueous extracts for *M. spicata* L. and *M. piperita* L. as well as *O. basilicum*.

MATERIALS AND METHODS

Chemicals and reagents: L-glutamine and fetal bovine serum (FBS) were obtained from Gibco Invitrogen Company (Scotland, UK). Dulbecco's modified Eagle's (DMEM) medium was provided from Cambrex (New Jersey, USA). DPPH$^{\bullet}$ (2, 2-diphenyl-1-picrylhydrazyl), ABTS$^{\bullet+}$ (2, 2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)), Ferrozine: (3- (2-pyridyl)- 5, 6- bis- (4-phenylsulfonic acid)-1, 2, 4-triazine, Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), BHT: Butyl Hydroxy toluene, Folin–Ciocalteu reagents, Quercetin, Gallic acid, Tannic acid, potassium ferricyanide. Penicillin, doxorubicin, streptomycin and Sulfo-Rhodamine-B stain (SRB) (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), Dimethyl sulfoxide (DMSO), were obtained from Sigma Chemical Company (St. Louis, MO, USA).

Normal cell line (human normal melanocyte, HFB4) as well as cancer cell lines, breast MCF-7, liver HepG2, colon HCT116 and lung A549 were purchased from the American Type Culture Collection (Rockville, MD, USA). Cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat inactivated fetal calf serum (GIBCO), penicillin (100 U mL^{-1}) and streptomycin (100 μg mL^{-1}) at 37°C in humidified atmosphere containing 5% CO2. The cell lines at a concentration of 0.50×10^{6} were grown in culture medium[19].

Plant materials: *Mentha spicata* (Spearmint) and *O. basilicum* (Basil) which belonged to family *Lamiaceae* were obtained from Matrouh governorate desert- Egypt, which considered as the wild types of mint and basil (W mint and W basil). However, *M. piperita* (peppermint) and *O. basilicum* (basil) were collected from local market, Cairo, Egypt as the cultivated types of this study (C mint and C basil).

Preparation and extraction of plants samples: Both mint and basil samples were air dried in a shade then the leaves and flowers were separated from the stalks. Samples were ground by electric miller (DING CANG-25000 r min^{-1}) and sieved to a particle size ranged from 10-200 mesh. The latest were then kept in freeze until extraction. Briefly, 100 g of the mint and basil stalks dried powder were soaked in 1 L of distilled water and shaking at room temperature for 48 h. The extract was filtered through Whatman No.1 filter paper. With the same volume of distilled water plant residue was re-extracted twice. The pooled filtrates were concentrated under vacuum at 40°C to dryness. Dried crude extract were re-dissolved in distilled water for the determination of phenolic, flavonoid, tannins, antioxidant, anti-inflammatory and antiproliferative activities.

Total phenolic content: The total phenolic (TPC) of mint and basil stalks extracts were spectrophotometrically determined by Folin Ciocalteu's reagent assay using gallic acid for standard curve preparation[20]. The absorbance was determined at 750 nm, by spectrophotometer (Unicum UV 300) against blank. A total phenolic content in the extracts was expressed as mg Gallic acid equivalents (GAE)/g dry weight (DW).

Total flavonoid content: Total flavonoid (TFC) of the extracted mint and basil stalks were spectrophotometrically determined by the aluminum chloride method using quercetin as a standard[21]. Absorbance was measured against blank at 510 nm by spectrophotometer. Total flavonoids in the sample were expressed as mg quercetin equivalents (QE)/g dry weight.

Total tannins content: Total tannins (TTC) of mint and basil stalks extracts were measured using the Folin-Ciocalteu's reagent according to Polshettiwar *et al.*[22]. Absorbance was determined against blank spectrophotometerically at 775 nm. Total tannins were expressed as mg tannic acid equivalent (TE)/g dry weight.

Antioxidant activity

DPPH• antioxidant assay: The activity of DPPH• free radical scavenging was measured spectrophotometrically[23]. The absorbance was measured at 515 nm against blank (methanol).

The capacity to scavenge the DPPH• radical was calculated using the following equation:

$$\text{DPPH}^{\bullet}\text{ scavenging effect (Inhibition \%)} = \frac{A_c - A_s}{A_c} \times 100$$

Where:

A_c = Absorbance of the control reaction
A_s = Absorbance in the presence of the plant extracts

Metal chelating activity: Metal chelating effects on ferrous ions was carried out calorimetrically[24]. The absorbance was recorded at 562 nm. Ferrous ion chelating activity (%) was determined by the following equation:

$$\text{Iron chelating activity (Inhibition \%)} = \frac{A_c - A_s}{A_c} \times 100$$

Where:

A_c = Is the absorbance of the control reaction
A_s = Is the absorbance of the plant samples

Reducing power: The reducing power was assayed spectrophotometrically according to Kuda *et al.*[25]. The absorbance was measured at 700 nm.

Determination of scavenging activities on $ABTS^{\bullet+}$ radicals: $ABTS^{\bullet+}$ assay was generated by oxidation of $ABTS^{\bullet+}$ with potassium persulphate according to Arnao *et al.*[26] as modified by Thaipong *et al.*[27]. Then the absorbance was read at 734 nm. Trolox was used as a standard.

The following equation used for calculation:

$$\text{Inhibition (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

Where:

A_0 = Is the $ABTS^{\bullet+}$ absorbance of the control reaction
A_1 = Is the $ABTS^{\bullet+}$ absorbance in the presence of the sample

Identification of phenolic compounds by HPLC: Phenolic compounds of mint and basil stalks extracts were identified

Table 1: Total phenolic, flavonoids and tannins of mint and basil stalks

Extracts	TPC (mg GAE g^{-1} DW)	TFC (mg QE g^{-1} DW)	TTC (mg TAE g^{-1} DW)
C Mint	9.49±0.05[b]	8.75±0.08[b]	5.66±0.04[b]
W Mint	57.10±1.33[d]	42.77±1.76[d]	18.60±0.43[d]
C Basil	7.16±0.06[a]	3.26±0.11[a]	3.30±0.04[a]
W Basil	22.45±0.19[c]	15.25±0.15[c]	12.79±0.18[c]
LSD at 0.05	1.26	1.67	0.44

*All values are the means of three replicates and values with the same letters are not significantly different, ±standard deviation, *C Mint: Cultivated mint, W Mint: Wild mint, C Basil: Cultivated basil, W Basil: Wild basil, *TPC: Total phenolic contents, TFC: Total flavonoids contents, TTC: Total tannins contents, *GAE: Gallic acid equivalents, QE: Quercetin equivalen, TAE: Tannic acid equivalen

according to Ben-Hammouda *et al.*[28]. Phenolic compounds were Identified using HPLC (Agilent 1100 series) coupled with UV-Vis detector (G1315B) and (G1322A) DEGASSER. Five µL of Sample was injected through auto-sampler (Agilent 1100 series), separations were carried out using ZORBAX-EclipseXDB-C18 column (4.6×250 mm, particle size 5 µm). A constant flow rate of 1 mL min^{-1} was used with two mobile phases: (A) 0.5% acetic acid in distilled water at pH 2.65 and solvent (B) 0.5% acetic acid in 99.5% acetonitrile. The elution gradient was linear with starting and ending over 50 min, at wavelength 280 nm using an UV detector. Phenolic compounds of mint and basil stalks extracts were identified by comparing their relative retention times with those of the standard mixture chromatogram. The concentration of an individual compound was calculated on the basis of peak area measurements and then converted to µg phenolic/g dry weight.

***In-vitro* anti-inflammatory activity of mint and basil stalks aqueous extracts:** Anti-inflammatory of mint and basil stalks aqueous extracts were tested according to Rahman *et al.*[29] using bovine albumin serum method. Different concentrations of plant extracts or standard drug diclofenac sodium (50, 100, 150 µg mL^{-1}) were mixed with bovine albumin serum.

The percentage inhibition of proteins can be calculated as:

$$\text{Percentage Inhibition} = \frac{\text{OD of test} - \text{OD of control}}{\text{OD of test}} \times 100$$

***In-vitro* antiproliferative activity assay:** Mint and basil stalks aqueous extracts were investigated for their antiproliferative activity using the Sulfo-Rhodamine-B stain (SRB) assay[30,31]. The samples were tested against doxorubicin (anticancer drug) as a reference drug. The survival curve for each cell line was obtained by drawing a relation between surviving fraction and drug concentration after the specified time. The IC_{50} (concentration for 50% inhibition of cell viability) was calculated and the results was summarized in Table 1. The results were compared to the antiproliferative effects of the reference drug[32,33].

Statistical analysis: Data were statistically analyzed using Costat statistical package data (ANOVA - complete randomize). The aqueous extracts of mint and basil stalks were analyzed in triplicates according to Snedecor and Cochran[34].

RESULTS AND DISCUSSION

Phenolic, flavonoids, tannins contents: Phenolic, flavonoids, tannins compounds have been shown to be responsible for the antioxidant activity of plant extracts[35] consequently, many associated applications in different fields such as pharmaceutical and food industries depending on the amounts of these particular compounds. Therefore, the amount of total phenols, flavonoids and tannins of mint and basil stalks aqueous extracts were investigated as gallic acid, quercetin and tannic acid equivalents (mg g^{-1} DW), respectively (Table 1).

The extracts of W mint followed by W basil stalks had the significantly highest total phenolics, flavonoids, tannins contents, while lowest content was attributed to C basil extract. In fact, the superiority of wild mint extract in total phenolics, flavonoids, tannins contents reflect its effect as a potentantioxidant agent. Total phenolic compounds were intensively investigated in many studies for different *M. species*. Comparing to current finding, the amounts of total phenolic compounds in wild mint stalk extract (*M. spicata*) were higher than that obtained previously for the aqueous and ethanol extracts of different *M.* species, which ranged from 0.7-13.3 mg g^{-1} DW[4,36,37]. A higher content of TPC was reported for aqueous, methanolic and ethanolic extracts of different *M. species*[12,38]. However, the results of the Cmint were comparable with the TPC results observed by Malik *et al.*[37] for the aqueous extract of mint leaves. As for, TF and TT contents, W mint was found to have the highest values compared to

that mentioned for different *M. species*[37-39]. The TPC of the C and W basil stalks were comparable with the results obtained for *O. basilicum* L. aerial parts extracted by water which ranged from 11.88-20.3 mg g^{-1} [40,41]. While, the TFC of the two studied basil types were lower than the previously obtained by Yesiloglu and Sit[42] and Kaurinovic *et al.*[41] for the water extracts of *O. basilicum* L. whole plant (26.42 and 42.5 mg g^{-1}, respectively).The high amounts of TPC, TFC and TTC existing in the stalk extracts comparing to previous studies, particularly in wild mint, reflect their possible potential applications economically in different fields as they are originated from an agro-industrial waste.

Antioxidant activity

DPPH· antioxidants assay: The color changing of DPPH· which is a stable free radical from violet to yellow was a result the process of hydrogen- or electron-donation, compounds that have the ability to prevent that donation are considered as antioxidants or radical scavengers[43]. IC_{50DPPH}. (Inhibitory concentration fifty percent) values of mint and basil stalks aqueous extracts showed in Fig. 1. Results illustrated that radical scavenging activity increased by increasing extracts concentration for all samples. IC_{50DPPH}. values ranked from 3.08 µg mL^{-1} for W mint to 64.15 µg mL^{-1} for Cbasil. Significant differences could be found for all extracts compared with reference substance (BHT).

Wild mint was observed to have more significant inhibitory effect against DPPH· than that represented by the standard BHT, while the W basil showed a moderate inhibitory effect followed by C mint, this maybe due to the accumulation of some active constituents such as phenolic compounds in the plant as a result of its natural development in the desert environment[44].

Fe^{2+}-Chelating activity: Foods are sometimes contaminated by transition iron element or other metals and during manufacturing processes. These ions have an important role as a catalyst in the oxidation process to form free radicals[45]. These processes can be reduced or removed by chelation of iron or metals. The ability of mints and basils aqueous extracts to chelate Fe^{2+} was determined and expressed as $IC_{50\,Fe^{2+}}$. The results were illustrated in Fig. 2.

The results revealed that the highly significant chelating activity was attributed to wild mint followed by wild basil. These results were confirmed by the strong significant correlations which were observed in iron chelating activity with tannins ($r^2 = 0.9345$), followed by flavonoids ($r^2 = 0.7626$)

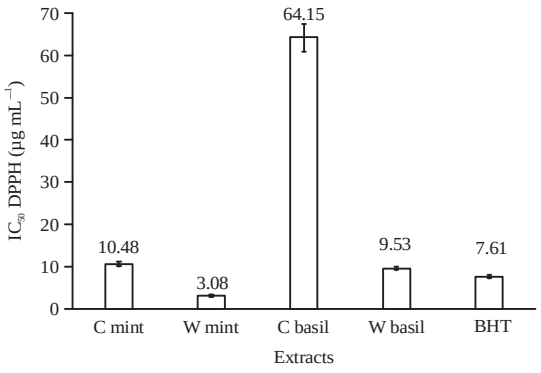

Fig. 1: IC_{50} of DPPH· radical scavenging of mint and basil stalks

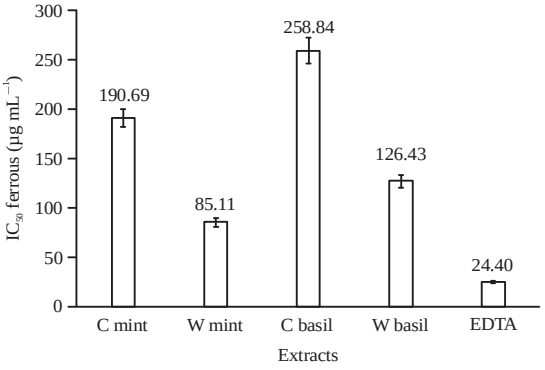

Fig. 2: IC_{50} of ferrous ion chelating of mint and basil stalks

and phenolic ($r^2 = 0.7460$). The estimation of iron chelating data at different concentrations indicated that wild type of both extracts would be useful against cellular damage[42].

Reducing power: The process of converting the ferric (Fe^{3+}) to ferrous (Fe^{2+}) is usually used as an indicator of electron donating activity, which is an important mechanism of phenolic antioxidant action[46]. Reducing power is expressed as (EC_{50} µg mL^{-1}: Effective concentration at which the absorbance is 0.5 at 700 nm). Data in Fig. 3 showed that the best activity was devoted to W mint stalks extract (20.74 µg mL^{-1}) followed by W basil (48.29 µg mL^{-1})compared with BHT(11.01 µg mL^{-1}). While, the lowest activities were observed in both cultivated mint and basil extracts. The ferric (Fe^{3+}) reducing power activity showed significant powerful correlation coefficient with the total phenolic content ($r^2 = 0.9064$), flavonoids ($r^2 = 0.9269$) and tannins ($r^2 = 0.9598$). Most of the antioxidant influence in medicinal plants was ascribed to the Oxidation-Reduction) redox)properties of phenolic compounds, which allow them to perform as hydrogen donors, reducing agents and scavenger for singlet oxygen[47].

ABTS· radical cation scavenging activity: The results of the ABTS•+ inhibitory activities for mint and basil stalks extracts as well as the positive control, Trolox are provided in Fig. 4. All the mint and basil extracts caused an inhibition in ABTS•+ activity. Whereas, the best activity obtained was corresponded to W mint extract, which maybe attributed to the presence of a considerable amount of bioactive compounds such as polyphenols. Also, the results of ABTS•+ scavenging activities revealed a vigorous significant correlation with phenolic ($r^2 = 0.9442$), flavonoids ($r^2 = 0.9566$) and tannins ($r^2 = 0.9654$). However, Stagos *et al.*[12] studied different mint species extracts (aqueous, ethanoland methanol) and they illustrated IC_{50} values for ABTS•+ ranged from 12-64 μg mL^{-1}.

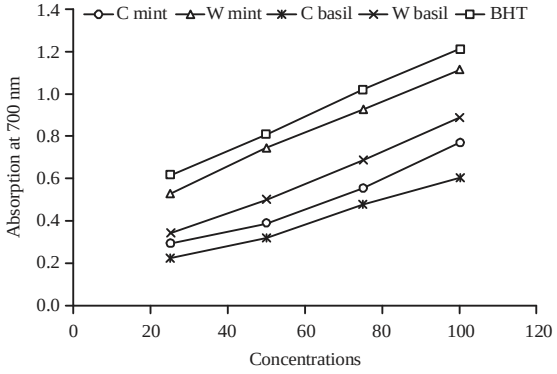

Fig. 3: Reducing power of mint and basil stalks aqueous extracts

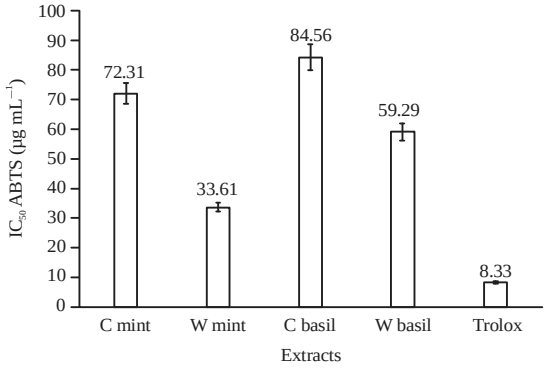

Fig. 4: IC_{50} of ABTS•+ radical cation scavenging of mint and basil stalks

HPLC profiling of phenolic compounds: Many studies showed that, the phenolic contents as measured by the Folin-Ciocalteus procedure, does not give a full concept of the quality and quantity of the phenolic compound in the plant extracts[48,49].

Data presented in Table 2 revealed that the aqueous extract of wild mint stalks had the highest amounts of most separated phenolic compounds. All extracts showed a high content of quercetin ranged from 8.15-13.25 mg/100 g DW. While, genistein was solely detected in C mint, andtannic acid was observed in both wild type of mint and basil.

HPLC profile also showed up that acacetin and catechin have been found only in both basil extracts. However, W mint possessed exclusively Luteolin and rutin. The extraordinary high amount of caffeic acid was attributed to W mint, which had about 10 folds than the nearest value of other extracts.

***In-vitro* anti-inflammatory activity of mint and basil stalks using bovine albumin serum:** A major factor responsible for inflammatory diseases is oxidative stress. In fact, many cancers arise from sites of inflammation, infection and chronic irritation[50]. The ability of the studied extracts to denature the protein in inflammation process was investigated. The anti-inflammatory effect of W mint and the mixture of W mint and basil (1:1) extracts were significantly higher than C mint at all tested concentrations (Table 3). These results were actually predicted, regarding to the high amounts of total phenolic, flavonoids and tannins compounds presented in the wild type of both plants.

Table 2: HPLC identification of phenolic compounds of mint and basil stalks

Phenolic compounds (mg/100 g DW)	C Mint	W Mint	C Basil	W Basil
Ellagic	2.02	4.03	3.23	7.47
Tannic acid	-	0.62	-	1.76
Gallic	0.58	0.63	-	-
Genistein	0.67	-	-	-
Quercetin	8.16	8.24	13.25	8.15
Coumarin	0.08	0.10	0.06	0.18
Catechin	-	-	3.34	1.70
Luteolin	-	1.12	-	-
Rutin	-	6.38	-	-
Acacetin	-	-	0.11	0.08
Caffeic acid	0.87	8.83	0.37	0.28

*C Mint: Cultivated mint, W Mint: Wild mint, C Basil: Cultivated basil, W Basil: Wild basil

Table 3: Rating of anti-inflammatory activity of mint and basil stalks

	Inhibition of denaturation at different conc. (%)		
Aqueous extracts	50 (μg mL^{-1})	100 (μg mL^{-1})	150 (μg mL^{-1})
C Mint	34.91±2.66^{a}	39.27±1.44^{a}	70.32±3.77^{a}
W Mint	71.03±4.17^{d}	84.31±2.92^{d}	96.79±4.27^{c}
C Basil	62.14±1.37^{b}	74.91±4.38^{b}	83.42±3.25^{b}
W Basil	64.63±0.49^{bc}	77.67±1.27^{bc}	87.43±0.44^{b}
W Mint : W Basil (1:1)	69.00±4.14^{cd}	82.43±2.84^{cd}	94.92±1.29^{c}
Standard Drug	74.05±0.37^{d}	81.74±0.64^{cd}	85.50±0.21^{b}
LSD at 0.05	4.85	4.64	4.90

All values are the means of three replicates and all values with the same letters are not significantly different,±standard deviation. Standard drug: diclofenac sodium. *C Mint: Cultivated mint, W Mint: Wild mint, C Basil: Cultivated basil, W Basil: Wild basil

Table 4: Antiproliferative activity of extract 1-5 against different cell lines as measured with SRB assay method

	IC_{50} (μg mL^{-1})				
Compounds	HepG2	MCF-7	A549	HCT116	HFB4
Doxorubicin	21.10±2.26	24.80±2.93	24.14±2.81	20.11±2.21	82.80±9.11
DMSO	NA	NA	NA	NA	86.90±9.27
C Mint	27.70±3.20	32.74±3.91	NA	NA	85.40±9.30
W Mint	21.00±2.90	24.30±2.76	NA	NA	NA
C Basil	31.20±3.80	NA	32.70±4.50	NA	81.60±8.85
W Basil	23.40±2.77	NA	27.20±2.90	NA	NA
W Mint:W Basil (1:1)	28.10±3.86	33.40±4.12	NA	NA	74.10±8.60

Data are expressed as Mean±standard deviation for at least three separate experiments. NA is no activity, *C Mint: Cultivated mint, W Mint: Wild mint, C Basil: Cultivated basil, W Basil: Wild basil

A significant reduction in the paw edema volume was noticed in adult male rat after 4 h following the *M. piperita* aqueous extract treatment[51]. Anti-inflammatory effect of aqueous and ethanolic extracts for *M. arvensis* whole plant was assayed on paw edema induced by histamine in mice. The results concluded that all *M. arvensis* extracts inhibited the acute allergic reactions[52]. Ethanol extract of fresh *O. basilicum* leaves have strongly reduced the inflammation of carrageenan-injection[53].

Antiproliferative activity of the extracts: This study is an attempt to examine the mint and basil (wild and cultivated) as well as a mixture of both wild extracts (1:1) for their antiproliferative activity against different human cancer cell lines and their toxicity on normal cells. The inhibition activities were expressed by median growth inhibitory concentration (IC_{50}). As shown in (Table 4), the proliferation inhibition activity of the extracts was evaluated against human liver HepG2, breast MCF-7, lung A549 and colon HCT116 cancer cell lines as well as their toxicity on normal cells using SRB assay, in comparison with doxorubicin as reference drug.

From the results it is evident that although W and C mint stalks aqueous extracts displayed potent growth inhibitory activity against liver HepG2 and breast MCF-7 cell lines, they had no activity against lung A549 or colon HCT116 cells. The C mint extract exerted antiproliferative activity with IC_{50} values of 27.70±3.20 and 32.74±3.91 μg mL^{-1} in HepG2 and MCF-7 cells respectively. While in case of W mint extract the IC_{50} values were 21.00±2.90 and 24.30±2.76 μg mL^{-1} in HepG2 and MCF-7 cells, respectively. It is clear that, while C mint extract had antiproliferative effect in HepG2 and MCF-7 cell lines closed to the doxorubicin, W mint extract was more potent effective than the reference drug as antitumor extract against both HepG2 and MCF-7 cells.

For W and C basil, the results revealed that both extracts showed potent proliferation inhibition effect against HepG2 and A549 cell lines while, there was no activity against MCF-7 and or HCT116 cell lines. The IC_{50} was 31.20±3.80 and 32.70±4.50 μg mL^{-1} in HepG2 and A549 cell lines, respectively for C basil extract as well as 23.40±2.77 and 27.20±2.90 μg mL^{-1} for W basil extract. It is clear that, while C basil stalks extract exerted moderate antiproliferative activity against HepG2 and A549 cells, W basil was similar potent to the doxorubicin.

In addition, although the mixture of both wiled types of mint and basil stalks aqueous extracts (1:1) had no effect on lung A549 or colon HCT116 cancer cell lines, it had moderate antiproliferative activity against HepG2 and MCF-7 cells with

IC_{50} value 28.10±3.86 and 33.40±4.12 μg mL^{-1}, respectively. The mixture of both wild types didn't potentiate the inhibitory action for each other. This may be attributed to the non-synergetic effect between the two extracts. Additionally, the results revealed that all extracts exerted no toxicity against normal cells.

Hajighasemi *et al.*[54] found that the aqueous extract derived from the leaves of *M. spicata* manifested a significant reduction in the proliferation of mouse fibrosarcoma Wehi-164 and human monocytic U937 cells. However, in a comparative study Abirami and Nirmala[55] investigated the ethanolic extracts of *M. piperita* and *O. basilicum* leaves against human laryngeal epidermoid carcinoma cells (Hep-2) and they obtained results lead them to conclude that *M. piperita* extract had a potent effect while *O. basilicum* gave a moderate one. Later, a comparison between *M. spicata* and *O. basilicum* methanol extract estimating the inhibition proliferation of human promyelocytic blood leukemia cells (HL60), illustrated that the *O. basilicum* extract had a superior effect with IC_{50} value of 45.7 μg mL^{-1} meanwhile, *M. spicate* extract showed a lower influence against HL60 cells with IC_{50} value of 98.1 μg mL^{-1} [56]. On contrary to current finding, Mohammadi *et al.*[57] revealed that the breast cancer cell (MCF-7) propagation was significantly inhibited by the methanolic extract of leaf and stem from *O. basilicum*.

CONCLUSION

The results confirmed the high availability and valuable bioactive compounds in some discarded agro-industrial wastes, yet many wastes are never been probably utilized in appropriate way. Taken together, these results suggest that the mint and basil stalks extracts showed interesting bio-activities that may be ascribed to the existing of antioxidant compounds namely phenolics, flavonoids and tannins. Thus, the aqueous extracts of mint and basil stalks may be useful in designing new treatment protocols for inflammation and cancer diseases, considering that further *in vivo* studies and characterization are required to investigate their active components and mechanism of action.

SIGNIFICANCE STATEMENT

This study discovered that mint and basil industrial wastes despite of being ignored and discarded for so long, they can participate in the therapeutic purposes of inflammation and cancer diseases in addition to their useful antioxidant content that may give them the potential use as protective agents. This will help the researchers to continue identification of the active ingredients and mechanism of action that are responsible for the cancer and inflammation treatment process.

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