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Antioxidant Activity, Anti-Inflammatory Activities, Anti-Cancer and Chemical Composition of Spring Onion (Allium *Fistolisum*) Extracts.

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ABSTRACT

Spring Onion (Allium *fistolisum*) is a promising source of bioactive moieties such as quercetin and flavonoids that exhibited various biological activities as anticancer and antioxidant. Samples of spring onion were analyzed using HPLC. Minerals profiling, antioxidant potential, and quantification of polyphenols were measured as indicators of antibacterial, anticancer, and anti-inflammatory activities. The quercetin and gallic acid contents in leaves and bulb portion were reported at 23.42±2.34 & 16.96±1.69 and 15.06±1.56 & 99.57±9.55, respectively. Likewise, chlorogenic and p-coumaric acid were quantified in leaves at 10.37±1.03, where as it was 12.33±1.23 in bulb portion. Results revealed that ethanol extract of the bulb portion exhibited the highest percentage inhibition of 10.45± 1.04% against anti-inflammatory activity as compared to methanol extract, 8.53 ± 0.85%.Similarly, bulb ethanolic and methanolic extracts of spring onion showed anticancer inhibition activities as 10.03 ± 1.00 & 31.00 ± 1.77%.In brief, spring onion has good anti-inflammatory, anti-microbial and anticancer potential and it is recommended to use these extracts on the pharmaceutical level.
Keywords: HPLC; Anti-inflammatory; Anti-microbial; Antioxidant; Anticancer; Spring onion; Bulbs; Leaves

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INTRODUCTION

Natural antioxidants are gaining popularity owing to their safe status and effective enessin the physiological system. There is a growing interest among the consumers against synthetic additives, thereby diverting their trend towards natural counterparts [1]. The diverse phenolic compounds of plant origin exhibit differential anti-oxidative activity against reactive oxygen species by scavenging hydroxyl & peroxy radicals and singlet oxygen quenching thereby inhibit lipid per-oxidation [2].

Spring Onion *(Allium fistolisum)* is a promising source of bioactive moieties such as quercetin and flavonoids that exhibited various biological activities such as anticancer, antioxidant, antimicrobial [3] antiplatelet, antidiabetic, anti-inflammatory, and antiasthmatic effects, antithrombotic, antihyperlipidemic, and antihypertensive [4:6]. These biological activities are performed due to the presence of high content of sulfur compounds and flavonoids [7]. There are several polyphenols such as 3,5,7-trihydroxyflavone (kaempferol), N-trans-p-coumaroyltyramine, N-trans-feruloyl-3m-methoxytyramine, and N-cis-feruloyl-3m-methoxytyramine found on the spectroscopic data of MS and NMR. Compound N-trans-feruloyl-3m-methoxytyramine and N-cis-feruloyl-3m-methoxytyramine quantified in methanolic extracts of spring onion through column chromatography [8]. These compounds exhibit antibacterial activity against *Escherichia coli,Bacillus subtilis, Staphylococcusblepharitis* and *Streptococcus pyogenes*, respectively [9:12]. Spring onion containing sulphur compounds exhibits anticancer effects against Wehi164, Jurkat and K562, and normal cell line (HUVEC) cancer cell lines using Trypan blue and LDH assays [13].

Bioactive compounds of spring onion suppress the inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), and inhibit the development of different cellular markers, which are responsible for tumor apoptosis, proliferation, the development of new blood vessels (angiogenesis) and tumor invasion [14]. These compounds also lower the risk of gastrointestinal tract cancer through repressing Helicobacter pylori and other bacterial action, and lowering the endogenous arrangement of compounds cancer-causing N-nitroso [15]. Spring onion performs the following mechanism such as modulation of enzyme, hindrance of mutagenesis, and cell flagging pathways, free-radical cleaning, programmed cell death, and different consequences for cell growth and tumor development [16:18]. They also show defending role on bosom tumor cells by expanding the movement of reductase proteins known to deactivate cytotoxic cancer-causing compounds [19].

So, the aim of this research work is to investigate the chemical composition, total phenolic content (TPC) and the polyphenols quantity of Spring Onion by using high performance liquid chromatography (HPLC). Also, to evaluate the bioactivity of spring onion extracts as antioxidant, anti-inflammatory, antimicrobial, and anti-cancer activities for the purpose to be used functional food &drug industries.

MATERIALS AND METHODS

This work was done in the Laboratory of Food Microbiology and Biotechnology, Food Analysis Laboratory, Institute of Food Science and Nutrition (IFSN), Bahauddin Zakariya University, Multan. Samples of spring onion have been analyzed for the proximate composition, minerals profiling, antioxidant potential, and quantification of polyphenols through HPLC, antibacterial, anticancer, and anti-inflammatory activities, respectively.

Collection and Preparation of Samples

Random samples of spring onion samples were collected from local retail markets of Multan, Pakistan. Samples were then washed thoroughly with tap water to remove dust and dirt particles. Afterwards, the outer skin of the samples were removedand then divided into small sections and they were placed into hot oven, Memmert GmbH UNB 200, for drying at 40°C. The dried samples were grinded into fine powder by using a grinder and then wereput in glass bottles [20**].**

Preparation of extracts

Ten grams of spring onion's leaves or bulb were soaked in 100 mL of methanol, ethanol, or water, respectively. The prepared samples were shake using orbital shaker for 7 hrs followed by centrifugation for 15

min at 7000 rpm. The extracts were then filtered using vacuum filtration assembly and the solvents were recovered at 40°C by Rotary Evaporator, Heldoph G1, Germany, [21]. The extracts were assessed for different assays including ferric reducing antioxidant power (FRAP), free radical scavenging activity and total phenolic contents (TPC), by DPPH (1,1-diphenyl-2-picrylhydrazyl) and as below.

Ferric reducing antioxidant power

Ferric reducing antioxidant power of spring onion leaves and bulb extracts in water, ethanol and methanol extracts was evaluated using the method of Sun *et al*. [22]. 0.5 mL of each sample has been mixed with 1.25 mL of 0.2 M phosphate buffer (pH 6.6) and 1.25 mL of 1% (v/v) potassium ferricyanide. After incubation, 0.1% ferric chloride along with 1.25 mL of 10% trichloroacetic acid [TCA] were added to the mixture and then left at RT for 10 min. The absorbance was measured at 700 nm using UV-Vis spectrophotometer.

Total Phenolic contents

Total phenolic contents (TPC) for the resultant extractsof ethanol, methanol, and water extracts of spring onion samples were assessedusing Folin-Ciocalteu method [23]. A calibration/standard curve for gallic acid was plottedat concentrations of 0.05 - 0.30 mg/mL.

DPPH scavenging activity

Muller et al.[24] method was used for the determination of DPPH scavenging activity of spring onion leaves and bulb extracts in water, methanol, and ethanol extracts. 300 µg/mL of freshly prepared methanolic solution of DPPH was added to 1 mL DPPH solution. The reaction mixtures were gently shaken and allowed to stand for 30 min at R.T.The absorbance was measured at 520 nmvia UV-Vis spectrophotometer.

Determination of AntimicrobialActivity

Strains

Pure ATCC strains were used. The strains were inoculated on nutrient media plates and they were incubated at 37°C for 24 hours, andstored at 4°C for up to 3-weeks. The following strains were used for detection of the antibacterial activity of spring onion.

Escherichia coli	(ATCC25922)
Bacillus cereus	(ATCC11778)
Pseudomonas aeruginosa	(ATCC27853)
Staphylococcus aureus	(ATCC33591)

Preparation of Media Plates

Antibacterial activity of spring onion Diagnostic Susceptibility Test was determined using agar. 28 grams of nutrient agar powder was left to soak in 1 L of distilled water for around 10 minutes. The media was swirled using a magnetic stirrer. The media was swirled via a magnetic stirrer prior of being autoclaved at 121°C for 21 min, poured under aseptic conditions into sterilized petri dish and left to solidify at 47°C.

Diagnostic Susceptibility Test Agar (DSTA)

Antibacterial activity of spring onion was determined using Diagnostic Susceptibility Test Agar (DSTA). The preparation of DSTA media was done according to the instructions provided by the manufacturer. First of all, 40 g of DST agar was added to 1 liter of distilled water. The mixture was placed on hot plate with magnetic stirrer and brought to boiling. Then media was put in the autoclave at the temperature of 121°C for 15 min. After autoclaving, the media was allowed to cool to 45-50°C and was poured into the plates. These plates were stored at 4°C and were used within a week.

Preparation of Broth Culture

Preparation of Nutrient Broth

Nutrient Broth was prepared by addition of yeast extract (5g), beef extract (5g) and sodium chloride (5g), in the same proportions in a conical flask. Ten milliliter of the broth was poured into test tubes before it was autoclaved at the temperature of 121°C for 21 min. Then after autoclaving, the nutrient broth was permitted to cool down to 40-45°C.

Inoculation

For the preparation of broth culture, a single colony was picked from the pure culture plate with a wire loop and was inoculated in the sterilized nutrient broth present in test tubes. The inoculated broth was then placed for incubation at 37°C for 16-18 hours.

Preparation of McFarland Solution

McFarland standards were made by dissolving barium chloride to sulfuric acid sequentially to get a barium sulfate precipitate and they were used to standardize the amount of bacteria in a liquid suspension. Using the suitable McFarland standard, turbidity of the McFarland Standard was visually compared with turbidity of a bacterial suspension. The turbidity of the test suspension was adjusted until it matched the standard. The turbidity was adjusted by adding nutrient broth. McFarland Solution was prepared as described by Andrews [25] and Deshmukh & Deshmukh [26]as follows; solution of 1.17% barium chloride and solution of 1% sulfuric acid

In this work, 0.5, 1 and 2 McFarland solutions were prepared as follows. For the preparation of 10 ml of 0.5 McFarland solution, 9.95 mL of 1% H_2SO_4 was transferred to a screw cap test tube followed by adding 0.05 mL of 1.17% barium chloride solution and the mixture was thoroughly mixedafter putting on the screw cap. Similarly 1 and 2 McFarland's solutions were prepared by transferring 9.90 mL and 9.80 mL of 1% H_2SO_4, respectively. The meniscus in all of the McFarland standards was marked with a marker to avoid any chances of usage after evaporation of liquid, as that will increase the turbidity of the solution. The McFarland Standards were thoroughly shaken every time before usage.

Procedure using the Disc Diffusion Method

The procedure used was based on microbiological inhibition zones as described by different authors [27:30] with few modifications.

The media plates were inoculated with the test strains through swabbing using sterilized cotton swabs. Before swabbing, the density/turbidity of broth cultures was set to McFarland 0.5 standards. Diluting the broth culture with sterile nutrient broth until its turbidity matched the 0.5 McFarland standards. The adjustment of turbidity to 0.5 Mcfarland's Standard provided a concentration of $1x10^8$ cfu/ml in the resultant broth culture. After swabbing, sterile filter paper disc of 10 mm diameter were impregnated with 0.5mg, 1mg and 2mg of plant extracts were placed on culture plates. The 50 µl of extract was absorbed in a filter paper disk. The plants were Incubated (Memmert GmbH + Co.KG D-91126) at 37ºC for 24 h. Antimicrobial action was recorded by the weight of clear hindrance zone around the plates. The measure was rehashed thrice and mean of three investigations was recorded.

Antifungal Activity

The antifungal activity of methanol and ethanol extracts of spring onion bulb and leaves part were monitored against various fungal strains, *Microsporum canis, Aspergillus flavus, Trichophyton longifusis, Fusarium solani, Candidaalbicans* and *Candida glaberata*. Agar tube dilution method was utilized for the assessment of activity against fungal strains. The agar used for the growth of fungus was SDA (Sabouraud dextrose agar) and 24 mg of each spring onion extract of the tested sample was dissolved in 1 ml of sterile DMSO (dimethyl sulfoxide) to prepare stock solutions. Acidic media of pH of 5.5-5.6 was prepared by mixing high amount of maltose or glucose (32.5 g) in 500 ml of distilled water. In screw caped tubes, media was kept and autoclaved at temperature of 121°C for 15 min and then they were left to cool down to the temperature

of 50°C. Non-solidified SDA was loaded with 66.6µL of spring onion extract and was left to solidify at room temperature. The tubes were then inoculated with 4 mm piece of inoculums and incubated at temperature of 27 - 29°C for 7 - 10 days. The relative humidity of incubation room was maintained at 40-50%. After incubation, percentage growth inhibition was computed with reference to the negative control through applying the given equation:

$$\% \, Inhibition = \frac{Linear \ growth \ in \ test \ (mm)}{2Linear \ growth \ in \ control \ (mm)} * 100$$

Amphotericin B and miconazole were used as standard drugs, whereas DMSO, amphotericin B and miconazole were used as negative and positive controls [31].

Quantification of polyphenols through High Performance Liquid Chromatography

One gramof each dried spring onion powder was extracted with 10 mL methanol (95%) for 2 hours at room temperature. The plant extract was then hydrolyzed with 1.2N HCl by refluxing on a water bath at 85°C for one hour (The hydrolysis was prepared and subjected to quantitative investigation by utilizing HPLC system). The extract was then cooled, filtered and made to 5 ml with methanol then filtered again with Whatman membrane filter 0.45 µm before injected into the HPLC.

Chromatographic analysis was carried out by Shim-Pack CLC-ODS (C-18), 25cm × 4.6mm, u5µm. The mobile phase gradient was: A (H_20: AA-94:6, pH2.27), B (ACN 100%), 0-15min=15%B, 15-30=45%B, 30-45=100%B. Then through a 0.45 µm membrane filter, the mobile phase was filtered. Then before use it was ultrasonically de-aerated. HPLC separation of standards 4-hydroxy 3 methoxy benzoic acid quercitin, trans 4 hydroxy 3 methoxy cinamic acid, sinapic acid, gallic acid, *p*-coumeric acid, and chlorogenic acid at 280 nm. Flow rate and injection volume were 1 ml /min and 10µL. The chromatographic peaks of the analytes were recorded by comparing UV spectra and their retention time with their reference standards. Gallic acid quercitin, trans 4 hydroxy 3 methoxy cinamic acid, chlorogenic acid, 4-hydroxy 3 methoxy benzoic acid, sinapic acid and *p*-coumeric acid were carried out by the integration of the peak using external standard method. Every chromatographic operation was done at its optimum temperature[32].

Anti-Inflammatory Activity

Before the experimentation male wistar rats were fasted for12 hours. 0.5 ml extract of spring onion plunged in 0.5% CMC (carboxymethylcellulose) were given to a group of 5 rats. Similarly, 5 mg per kg standard medication indomethacin in 0.5% CMC was given to one group of 5 rats. Avehicle of 0.5 ml of 0.5% CMC was given to the control group of 5 rats. After one hour, drug was given and rats were anesthetized with diethylether. 0.1 mL of 1% carrageenan was injected in rats and that caused paw edema. To measure the volume of paw edema, water plethysmometer was used to followthe progress instantaneously before the carrageenan was injected and after every five hours. For every group, the percent of inhibition of edema was recorded (group of standard drug-treated and group of spring onion extracts-treated) against the group vehicle-treated control [33].

Anticancer activity

By utilizing the standard MTT (3-[4, 5-dimethylthiazole-2-yl]-2, 5-diphenyl-tetrazolium bromide) colorimetric method, cytotoxic activity of spring onion bulb and leaves were tested in 96-well level bottomed small scale plates [34].According to this method, MCF-7 cells (Breast Cancer) were grow in medium of Dulbecco's Modified Eagle, supplemented with 100 IU/mL of penicillin, 100 µg/mL of streptomycin and 5% of fetal bovine serum (FBS), in 75 cm in two flasks, then kept in incubator having 5% $Co_2$and temperature was set at 37°C. Exponentially growing cells were collected, by using haemocytometer cells were counted and with specific medium they were diluted. The prepared cell culture having the concentration of $1x10^5$ cells/mL and introduced (100 µL/well) into 96-well plates. And plates were incubated for overnight, and then medium was removed and fresh medium of 200 µL was added with various concentrations of bulb and leaves extracts of spring onion (1-30µM). Following 48 hrs, from MTT, 200 µL of 0.5 mg/mL was taken and then added to each well, after which they were incubated for 4 hours. After that in each well, 100µL of DMSO was added. The extent of MTT reduction to formazan within cells was observed by measuring the absorbance at 570 nm,

utilizing a micro plate reader (Spectra Max plus, Molecular Devices, CA, USA). The cytotoxicity was calculated as concentration causing 50% growth inhibition (IC_{50}) for MCF-7 cells. The percent inhibition was recorded by using the given formula:

$$\%\ Inhibition = 100 - \frac{(mean\ O.D.\ test\ sample - Mean\ O.D.\ negative\ control)}{(mean\ O.D.\ positive\ control - mean\ O.D.\ negative\ control)} * 100$$

Statistical Analysis

The collected data were subjected to statistical analysis using complete randomized design (CRD) through statistical software Cohort version 6.1 (Co Stat, 2003). Furthermore, analysis of variance (ANOVA) technique was used to determine the level of significance [35].

RESULTS AND DISCUSSIONS

Extraction Yields

The extract yield of bulb in methanol, ethanol and water was 10.42±1.08, 12.28±0.86 and 15.44±0.65%, respectively. Whereas it was 8.28 ± 1.05, 14.29 ± 1.07 and 10.28±1.09%, respectively in leaves as shown in Table 1.

Table 1: Extract yield % in bulb and leaves parts of spring onion

Solvent	Bulb	Leaves
Methanol	10.42±1.08c	8.28±1.05d
Ethanol	12.28±0.86b	14.29±1.07a
Water	15.44±0.65a	10.28±1.09c

Antioxidant indices of spring onion leaves and bulb

Methanol extract of leaves showed the highest TPC of 116.90±15.27mg/100g GAE, followed by107.45±9.96mg/100g GAE ethanol, and 97.70±5.34mg/100g GAE water, respectively. Likewise, resultant extracts of the bulb part were reported TPC contents as 28.59±4.55 mg/100g GAE, 24.16±3.69 mg/100g GAE, and 14.41±3.11 mg/100g GAE, respectively. Similarly, DPPH and FRAP activities in methanol, ethanol and water extracts of leaves and bulb were observed as 84.00±2.74 & 80.69±4.54 and 1285.5 ± 128.55 & 1905.5 ± 190.55, 73.18±2.14 & 70.68±2.98 and 810.50 ± 81.050 & 1825.5 ± 182.55, 64.49±2.46 & 61.79±1.85 and 715.50 ± 71.55 & 775.25 ± 77.525 µmol/g, respectively (Table 2).

Table 2: Antioxidant indices of bulb and leaves parts of spring onion

Solvents	TPC (mg/100g GAE)		DPPH (%)		FRAP(µmol/g)	
	Bulb	Leaves	Bulb	Leaves	Bulb	Leaves
Methanol	28.59±4.55	116.90±15.27	80.69±4.54	84.00±2.74	1905.5 ± 190.55	1285.5 ± 128.55
Ethanol	24.16±3.69	107.45±9.96	70.68±2.98	73.18±2.14	1825.5 ± 182.55	810.50 ± 81.05
Water	14.41±3.11	97.70±5.34	61.79±1.85	64.49±2.46	775.25 ± 77.52	715.50 ± 71.55

The current results are in line with the findings of Eshak *et al.* [36]. They investigated the total phenols contents of two spring onion varieties such as leaves (15.82) and bulb (8.82) mg/100g FW. In another study, Issa *et al.*[37] investigated the total polyphenol content (TPC) of leaves and bulb using Folin-Ciocalteu reagent and the results were ranged from 1665.7 ± 13.6 to 2288.9 ± 12.5 mg GAE/100g DW, and 390.9 ± 11.3 to 479.4 ± 1.5 mg GAE /100g DW , respectively. A group of researches, [38-39], determined that phenolics contents were varied from 4.6 to 74.1 mg GAE/g FW in onion whereas spring onion comprised 94±2.30 mg/100g GAE FW. Likewise, Santas et al. [40], investigated the phenolic contents in Spanish onions which were varied from

0.51 ± 0.22 to 6.53 ± 0.16 mg gallic acid GAE/g DW. Furthermore, Malla *et al.* [41] observed that the phenolic contents varied from 15.7 to 34.7 mg GAE/g FW in spring onion bulb.

On the other hand, a group of peers, Tsai*et al*. [42]haveexplored that TPC contents were varied from 11.6±1.2 to 948 ± 130.8 mg/100g FW in varieties of spring onion. Prakash *et al*[39] and Nuutila *et al.,*[43]reported that DPPH antioxidant activity for onion bulb varied from 13.6% to 90%. Similarly, Eshak *et al*. [36] observed that leaves of spring onion extract had DPPH (15.86%) and 25.61% for the bulb. Likelywise, Issa *et al.* [37] determined the antioxidant activity of bulb and leaves to be 70.3% and 83.1%, respectively.

The reducing antioxidant power in onion was investigated by Yan *et al*. [44]and it was found to be 0.30 µM to 2.29 µM. Malla *et al*. [41]found that FRAP values were 0.9 µM to 3.3 µM Fe (II) in different onions.Similarly, Nicoletta *et al*., [45] found that the FRAP showed 5.28 mM Fe^{+2}/kg FW activity in yellow onion. Sakiko and Yakiko[46] reported that the FRAP values of green welsh onion to be 1130±16 µmol Fe^{2+}/kg FW in water soluble fraction and 1554±56 µmol Fe_2^{+}/kg FW in lipid soluble fraction. However, in white welsh onion, it was 616±36 µmol Fe^{2+}/kg FW in water soluble fraction and 494±34 µmol Fe^{2+}/kg FW in lipid soluble fraction. Szeto*et al.*[47] reported the FRAP values of spring onion to be 8040±1440 mmol/kg FW in acetate buffer and 5900±540 mmol/kg FW in water.

Quantification of Spring Onion Polyphenols

As shown in Table 8, the mean values for quercetin and gallic acid in bulb portion and leaves were reported as 15.06±1.56 & 99.57±9.55 and 23.42±2.34 & 16.96±1.69, respectively. Likelywise, sinapic acid was quantified as 3.14±0.31 and 2.11±9.21 in leaves and bulb parts of spring onion. Similalry, chlorogenic and *p*-coumaric acid were quantified in leaves as 10.37±1.03 and 12.33±1.23. Furthermore, Trans 4 Hydroxy 3 methoxy cinamic acid and 4 Hydroxy-3 Methoxy Benzoic acid were quantified as 5.01±0.50 and 737.30±73.7, respectively. Tsanova [48] quantified the querecitin and gallic acid in leaves and bulb parts of spring onion and they were found to be 103.2 mg/kg FW & 179.4 mg/kg FW, respectively.

Table 8: HPLC quantification of spring onion polyphenols (bulb and leaves)

Compound	Bulb	Leaves
Quercitin	16.96±1.69	23.42±2.34
Gallic Acid	99.57±9.95	15.60±1.56
Cholorogenic acid	N.D	10.37±1.03
p-Coumaric acid	N.D	12.33±1.23
Sinapic acid	2.11±0.21	3.14±0.31
4 Hydroxy-3 Methoxy Benzoic acid	737.30±73.7	N.D
Trans 4 Hydroxy 3 methoxy cinamic acid	5.01±0.50	N.D

Antimicrobial Activities

The different methanolic concentrations of leaves and bulb were recorded at the zone of inhibition for *S. aureus*. Likelywise, the mean values for *E. coli* 25922 and *P.aeruginosa* 27853 of bulb and leaves in methanolic extracts were observed. The mean values of antibacterial activity of bulb water extracts for zone of inhibition at 1.0 and 2.0 g/ml concentrations for *S. aureus 25923, B. cereus 11778,* and *P. aeruginosa 27853* were also reported. The antifungal activity of ethanolic extracts of leaves and bulb parts for *Aspergillusniger* were evaluated as 2.0 ± 2.0 and 40.0 ± 4.0 and %, respectively as in Table 3-6. Eltaweel, 2013 concluded that Allium cepa extract has antimicrobial activity against Staphylococcus aureus.

Similarly, Jeffrey *et al.*[12] and Purseglove *et al.*[11] found that the highest inhibition zone against microbes can be achieved at higher concentrations of spring onion extracts. Hendrich [49] stated that onion has antibacterial activity due to the presence of flavonoids and polyphenols. Similarly, Irkin *et al.* [50], found that *Aspergillusniger* has been inhibited using onion extract with ethanol. Bakht *et al.*[51] found that chloroform petroleum, ethyl acetate, and ether extracts of *Allium cepa* inhibited the growth of *Staphylococcus aureus* at all concentrations. Kirilov *et al*. [52]showed that green onion bulb had no antibacterial activity against *E.coli* (9 mm), *Bacillus subtilis* (9 mm) and leaves of green onion. He also reported that the frail

antibacterial effect of green onion could be regarded to more water content and less amount of sulfur compounds in the plant, which accumulate during the onion maturation.Vamshi *et al*. [53]found that,hexane, ethanol anddiaxon, extracts of scale leaves of onionat 1000µg/ml exhibited an inhibition zone of 8.0 mm against gram positive bacteria.It was reported that the inhibitory effects of basil, onions, hot peppers,Chinese parsleygarlic andginger showedgrowth inhibition against*A. flavus*and*A. niger*.It was also reported that ethanolic extracts of spring onion delayed the growth of *A. parasiticus* in liquid culture[54].Similarly,Kim (2010) found that concentrations of 0.1 – 1.0% of green onion decreased the growth of different fungal.

Anti-inflammatory Properties

The results as shown in Table 7 has shown that bulb of spring onion extracted using ethyl alcohol has shown the highest inhibition percentage of 10.45± 1.04% followed by by methanol at 8.53 ± 0.85%. Similalry, the inhibition percentages of bulb of spring onion extracted in ethyl alcohol and ethanol were 2.03 ± 0.20 & 5.23 ± 0.29%, respectively. This finding were supported by Tsanova [49] who reported that spring onion's polyphenols have a positive effect on lowering the pain in osteoarthritis and rheumatic arthritis as well as showing positive effect on common asthma and influenza.Furthermore, Wagner *et al*. [55] reported that spring onions have anti-inflammatory effect on prostaglandins and leukotrienes,where they showed subsequent histamine release and anti-asthmatic activity. Furthermore, Chisty *et al.*[56] and Dorsch et al. [57]reported that thiosulfinates and capaenes bioactive compounds have inhibition effect and anti-inflammatory activities on the immune system.

Table 3: Antibacterial activity (mm) using methanol extract of bulb and leaves part of spring onion

Inhibition of ATCC Strains (mm)	Methanol Extract					
	Bulb			Leaves		
	0.5 g/mL	1 g/mL	2 g/mL	0.5 g/mL	1 g/mL	2 g/mL
S. aureus 25923	9.00 ± 0.90	10.00 ± 1.00	14.00 ± 1.40	11.00 ± 1.10	11.00 ± 1.10	13.00 ± 1.30
B. cereus 11778	N.D	N.D	11.00 ± 1.10	8.00 ± 0.80	11.00 ± 1.10	12.00 ± 1.20
E. coli 25922	8.00 ± 0.80	14.00 ± 1.40	17.00 ± 1.70	N.D	N.D	N.D
P.aeruginosa 27853	8.00 ± 0.80	12.00 ± 1.20	13.00 ± 1.30	N.D	N.D	16.00 ± 1.60

Table 4: Antibacterial activity (mm) using ethanol extract of bulb and leaves part of spring onion

Inhibition of ATCC Strains (mm)	Ethanol Extract					
	Bulb			Leaves		
	0.5g/mL	1 g/mL	2 g/mL	0.5 g/mL	1 g/mL	2 g/mL
S. aureus 25923	N.D	12.00 ± 1.20	14.00 ± 1.40	11.00 ±1.10	13.20 ± 1.32	15.00 ± 1.50
B. cereus 11778	N.D	8.00 ± 0.80	15.00 ± 1.50	N.D	12.00 ± 1.20	16.00 ± 1.60
E. coli 25922	11.00 ± 1.10	12.00 ± 1.20	16.00 ± 1.60	N.D	8.00 ± 0.80	12.00 ± 1.20
P.aeruginosa 27853	N.D	12.00 ± 1.20	16.00 ± 1.60	N.D	8.00 ± 0.80	14.00 ± 1.40

Table 5: Antibacterial activity (mm) using water extract of bulb and leaves part of spring onion

Inhibition of ATCC Strains (mm)	Water Extract					
	Bulb			Leaves		
	0.5 g/mL	1 g/mL	2 g/mL	0.5 g/mL	1 g/mL	2 g/mL
S. aureus 25923	N.D	10.00 ± 1.00	10.00 ± 1.00	N.D	N.D	12.00 ± 1.20
B. cereus 11778	N.D	6.00 ± 0.60	10.00 ± 1.00	N.D	N.D	N.D
E. coli 25922	N.D	N.D	11.00 ± 1.10	11.00 ± 1.10	N.D	10.00 ± 1.00
P. aeruginosa 27853	N.D	11.00 ± 1.10	14.00 ± 1.40	N.D	8.00 ± 0.80	10.00 ± 1.00

Table 6: Antifungal activity (% inhibition) of bulb and leaves part of spring onion

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Name of strain	Methanol Extract		Ethanol Extract		Standard
	Bulb part	Leaves Part	Bulb part	Leaves Part	
Trichphyton rubrum	N.D	N.D	N.D	5.00 ± 0.50	Miconazole 97.8 µg/ml
Aspergillus niger	N.D	N.D	40.00 ± 4.00	2.00 ± 2.00	Amphotericin B 20.70 µg/ml
Fusarium lini	N.D	50.00 ± 5.00	N.D	N.D	Miconazole 73.50 µg/ml
Microsporum canis	N.D	N.D	N.D	N.D	Miconazole 98.1 µg/ml
Candida albicans	N.D	N.D	N.D	N.D	Miconazole 13.1 µg/ml

Table 7: Anti-inflammatory and anticancer activities of bulb and leaves part of spring onion % inhibition

Solvents	Anti-inflammatory		Anticancer (MCF-7 cell line)	
	Bulb	Leaves	Bulb	Leaves
Ethanol	10.45± 1.04	2.03 ± 0.20	10.03 ± 1.00	49.61 ± 4.96
methanol	8.53 ± 0.85	5.23 ± 0.29	31.00 ± 1.77	28.68 ± 2.86

Anticancer Properties

As shown in Table 7, the ethanolic and methanolic extract of leaves showed inhibition activities as 49.61 ± 4.96 and 28.68 ± 2.86%, respectively. Similarly, the extracts of bulb spring onion revealed inhibition activities as 10.03 ± 1.0 & 31.0 ± 1.77%, respectively. These results are in agreement with that of Mohammadi-Motlagh *et al.* **[13]**

CONCLUSION

In this study, different extracts of spring onion's leaves and bulb were explored using HPLC. Water, methanol and ethanol were used as solvents. The leaves extracted in methanol showed the highest TPC of 116.90 ± 15.27 mg/100g GAE. Both ethanol and methanol have shown powerful extraction results as solvents. For example, ethanol extract of bulb portion exhibited the highest percentage of inhibition of 10.45± 1.04% against anti-inflammatory activity, whereas bulb methanolic extracts of spring onion showed the highest inhibition activities as anticancer at 31.0 ± 1.77%. In conclusion, spring onion was found to havegood anti-microbial, anti-inflammatory and anticancer properties and a preventive role as a diet against many human diseases and disorders.

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