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Nutritional and Bioactive Constituents of Antioxidant and Antimicrobial Properties in *Spinacia oleracea*: A Review

(Juzuk Pemakanan dan Bioaktif Serta Sifat Antioksidan dan Antimikrob *Spinacia oleracea*: Suatu Tinjauan)

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ABSTRACT

In recent years, overwhelming studies have recognized the excellent functional and nutritional properties of green leafy vegetables that can be gained through a proper human diet. Among the vegetables studied, *Spinacia oleracea* Linn. or commonly known as spinach is widely being acknowledged for having a diverse range of nutritional composition and bioactive phytochemical compounds. Spinach, which is grouped under the *Amaranthaceae* family, contains various beneficial effects owing to their nutritional compositions, such as carbohydrates, proteins, fats, fibre, minerals, vitamins, and bioactive constituents that are directly linked to various bio-functional properties. The valuable bio-constituent of polyphenols that exist in spinach contributes to its effective antioxidant and antimicrobial properties. Therefore, the antioxidant from spinach extract is a promising source of natural antioxidants to replace the harmful effect of synthetic antioxidants. Moreover, it can inhibit cellular oxidative damage, increase storage stability, and restrict the growth of a wide range of pathogenic bacteria, which offers a huge prospect for potential food application. The main attention of this review was to highlight the effective antioxidant and antimicrobial properties of phytochemical compounds in spinach extract. Additionally, this review provided a comprehensive description of the wide range of food applications with regards to the use of spinach extract.

Keywords: Antimicrobial; antioxidant; spinach compounds; spinach application; spinach propeties

ABSTRACT

Dalam beberapa tahun kebelakangan ini, banyak kajian telah mengiktiraf kebaikan ciri-ciri berfungsi dan ciri-ciri pemakanan sayur-sayuran berdaun hijau yang boleh diperoleh melalui diet manusia yang betul. Antara sayur-sayuran yang dikaji, *Spinacia oleracea* Linn. atau dikenali sebagai bayam oleh masyarakat tempatan diakui secara meluas mempunyai pelbagai komposisi nutrisi dan sebatian fitokimia bioaktif. Bayam yang dikelompokkan di bawah famili Amaranthaceae mengandungi pelbagai kesan berfaedah kerana komposisi pemakanannya seperti karbohidrat, protein, lemak, serat, mineral, vitamin dan juzuk bioaktif yang dikaitkan secara langsung dengan pelbagai sifat bio-fungsi. Bio-konstituen berharga polifenol yang wujud dalam bayam menyumbang kepada sifat antioksidan dan antimikrob yang berkesan. Antioksidan daripada ekstrak bayam adalah sumber antioksidan semula jadi yang berpotensi untuk menggantikan kesan berbahaya antioksidan sintetik. Selain itu, ia boleh merencat kerosakan oksidatif sel, meningkatkan kestabilan penyimpanan dan menyekat pertumbuhan pelbagai bakteria patogen yang menyediakan prospek besar untuk aplikasi makanan yang berpotensi. Oleh itu, perhatian utama ulasan ini adalah untuk menghuraikan sifat antioksidan dan antimikrob yang berkesan bagi sebatian fitokimia dalam ekstrak bayam. Di samping itu, ulasan ini memberikan penerangan menyeluruh tentang pelbagai aplikasi makanan berkaitan dengan penggunaan ekstrak bayam.

Kata kunci: Antimikrob; antioksidan; komponen bioaktif bayam; aplikasi bayam; sifat-sifat bayam

INTRODUCTION

Spinach or scientifically known as *Spinacia oleracea* Linn is a plant that belongs to the *Amaranthaceae* family (Yang, Tan & Zhu 2016). Locally, spinach known as *Bayam Hijau* (Malay) but globally known as the green spinach. It is grown in many countries with other indigenous names, such as Palak (Hindi), Pasalai (Tamil), and Palakh (Kashmiri) (Rao et al. 2015). In fact, the term 'spinach' was initially derived from the Spanish phrase, *Hispania* (Ribera et al. 2020). Historically, spinach was reported to have originated from Central and Western Asia (Yang, Tan & Zhu 2016), where the plant was presumed to be cultivated first by the Arabians and then by the Persians around 2000 years ago (Nešković & Ćulafić 1988; Ribera et al. 2020). The crop was believed to emerge late in the Greek and Roman civilisations, as revealed through the finding of an old documented manuscript from 40 AD in Mesopotamia (Ribera et al. 2020). Spinach was brought to Spain via the Moorland before being spread all around the world.

Spinach can be widely used in versatile ways, either eaten raw as salad, added into processed food, or as an ingredient in vegetable and meat dishes. The plant has been acknowledged ever since as an excellent source of nutrient components and phytochemical content for therapeutic usage (Babu et al. 2018). The benefit of nutritious content in spinach that could provide good health. Apart from being a good source of nutrients, such as carbohydrates, proteins, and fibres, spinach is identified as an excellent source of beneficial phytonutrient components, such as phenolic compounds and carotenoids that prevents the accumulation of Reactive Oxygen Species (ROS) and pathogenic proliferation (Altemimi et al. 2017). The effectiveness of natural antioxidants could also replace synthetic antioxidants that pose potentially adverse chronic effects to human health (Lobo et al. 2010). Therefore, numerous green vegetables with good phytochemical composition have earned significant attention among the scientific community in the past decade to be used as a good supplementary diet. Thus, this review highlighted the nutritional and bioactive constituents in spinach that contributes to its antioxidant and antimicrobial properties and the relevant application of spinach extract in the food industry.

SPINACH AND ITS NUTRIENTS

PLANT DESCRIPTION

Spinach is identified as a dicotyledonous genus with both male and female species are herbaceous plants. Alternatively, it could be found in from ovate to triangular based on its simple leaves (Sabaghnia, Asadi-Gharneh & Janmohammadi 2014). The plant is easily grown by putting a sowed seed of 0.5 to 1 inch deep in medium moist soil with a net covering and requires only a small amount of light penetration. It usually requires fast-ripening optimum growing conditions between 15 and 20 °C. The minimum temperature for seed propagation is approximately 2 °C, while the ideal climate for seed propagation is approximately 21 °C (Sensoy, Turkmen & Gorgun 2011). Generally, spinach is an annual edible and versatile vegetable that is grown worldwide in most temperate climates and can even survive over moderate winter. Moreover, Spinach is a unique plant that could tolerate the cold temperature around -9 °C and survive with a frost condition. Interestingly, this dark green leafy plant with smooth and fleshy leaves (Figure 1(a)) is abundantly grown and harvested in Malaysia due to its average ambient temperature of 27 °C throughout the year.

The size of spinach leaves is usually around 5 to 8 cm. Spinach can be classified into three groups according to the morphological structure of the leaves: curly leaves, broad flat leaves, and smooth leaves, as shown in Figure 1(a)-1(c) (Hu, Mou & Vick 2007). The leaf morphology greatly depends on geographic areas, intense selection of the implemented species, and genetic variation (Hu, Mou & Vick 2007). The smooth spinach leaves exhibit an alternate pattern between bigger leaves, which grows around 2 to 30 cm long at the plant's base, and small leaves that are roughly 1 to 15 cm on the flowering shoot (Olasupo, Aborisade & Olagoke 2018). In addition, the plant produces small flowers in a yellow-green colour combination with a typical size of 3 to 4 mm in diameter. The smooth type leaves are recommended to be used in processed food, whereas semi-savoy can be directly eaten raw.

NUTRIENT COMPOSITION

The nutrient composition in spinach can be divided into major six components, which are carbohydrates, proteins, fats, fibres, minerals, and vitamins. The content of carbohydrates (50.10 to 50.59 %), proteins (14.13 to 14.44 %), and fats (23.02 to 23.11%) were reported in spinach (Ambo, Patience & Ayakeme 2023). Spinach has also been reported to possess an essential source of dietary fibres, essential fatty acids including omega-3 fatty acids, iron, vitamins, and major bioactive antioxidants in the form of carotenoids that could be useful for the preservation, growth, and regeneration of tissue control (Maeda, Yoshida & Mizushina 2010). The fibre content of spinach ranged from 2.52 to 2.63% (Ambo, Patience

FIGURE 1. Morphological structure of spinach leaves comprising (a) wrinkled savoy leaf, (b) semi-savoy with semi-wrinkled crinkled leaves, and (c) smooth flat-leaf with unwrinkled leaves

& Ayakeme 2023). The current Recommended Dietary Allowance (RDA) of fibre for adults (31 to 50 years) is typically around 31 g/day for men, and 25 g/day for women for every 2,000 kcal of diet (Dietary Guidelines for Americans, 2020). Thus, for every 2,000 kcal of diet, spinach could provide sufficient fibre for men and women. Moreover, α-linolenic acid, which is a vital component of omega-3 fatty acids, is comprised of 695 mg/100 in chloroplast-rich fractions of spinach (Gedi et al. 2017).

Approximately, the mineral composition in spinach consisted of 28.4 mg/100 g iron, 827 mg/100 g magnesium, 5840 mg/100 g potassium, 827 mg/100 g sodium, 5.5 mg/100 g zinc, 1036 mg/100 g calcium, and 513 mg/100 g phosphorus (Natesh, Abbey & Asiedu 2017). In Malaysia, Recommended Nutrient Intakes (RNI) 2017 for iron and zinc for adult men and women were 14 and 29 mg/100 g, and 6.5 and 4.6 mg/day, respectively. Furthermore, the Malaysian RNI 2017 for calcium and phosphorus for both men and women were 1000 mg/day and 700 mg/day (National Coordinating Committee on Food and Nutrition 2017). This obtained that spinach providing more than enough daily RNI for these major minerals in a 100 g serving.

Several researchers also reported that spinach is rich in phytochemical constituents, such as β-carotene, as well as a large number of polyphenols, such as phenolic acids, flavonoids, and aromatic compounds (Maeda, Yoshida & Mizushina 2010; Zubairi & Jaeis 2014). Proper consumption of spinach would provide a synergistic effect to control the level of free radicals in the body since spinach contains vitamin C and E, flavonoids, carotenoids, and phenolic compounds (Tiveron et al. 2012). In fact, spinach contains approximately 256 mg/100 g of vitamin C and 18.2 mg/100 g of vitamin E (Edelman & Cotl 2016). In addition to its wide variety of bioactive and phytochemical compounds, spinach has different potential functionalities comprising mainly antioxidant, antimicrobial, and anticancer properties that eventually provides huge beneficial health to human (Tyagi 2017).

PHYTOCHEMICAL PROPERTIES OF SPINACH

Many studies found that flavonoids and polyphenols obtained from green vegetables can be categorised as a natural alternative of antioxidant agent and antimicrobial application (Huda-Faujan et al. 2015; Mohd Azzimi, Mohd Fazil & Zubairi 2018; Xi & Shouqin 2007; Yolmeh et al. 2015). In fact, other phytochemicals present in most green leafy vegetables has been proven to provide various excellent functional properties such as antioxidant, antimicrobial, and antifungal properties (Sengul et al. 2009). In terms of the spinach plant, the existence of essential and non-essential phytochemicals in spinach, such as carotenoids, phenolic acids, and flavonoid compounds, has been widely reported to have antioxidant activity (Alnashi et al. 2016; Bergman et al. 2001; Khairi, Aizad & Zubairi 2017; Yosefi et al. 2010). However, alkaloids, simple phenolics and flavonoids, quinones, and tannins were believed to be associated to have antimicrobial activity in spinach. Table 1 shows the identified phytochemical compounds in spinach that might related to antioxidant and antimicrobial activity.

PHYTOCHEMICAL COMPONENTS AS ANTIOXIDANT AGENTS

The harmful effect of free radicals has ignited the interests of researchers to prevent the undesirable lipid oxidation in the body using natural antioxidants from plant materials instead of synthetic antioxidants that may cause health problems and side effects (Barlow 1990; Hentati et al. 2019; Lobo et al. 2010). The term 'antioxidant' is relatively well-known to describe chemical materials that donate electrons to free radical species and turn them into harmless molecules (Mohd Fazil et al. 2016; Yosefi et al. 2010). Reducing agents in spinach, such as carotenoids and polyphenols that consist of phenolic and flavonoid acids, play an essential role as an antioxidant agent to effectively counteract the harmful free radicals molecules (Alnashi, Hassouna & Dairouty 2016; Ligor, Trziszka & Buszewski 2012).

Carotenoids

Carotenoids are renowned for their scavenging characteristics in decreasing the number of generated ROS (Ochoa Becerra et al. 2020). The two types of carotenoids include xanthophyll (e.g., lutein), and zeaxanthin/carotene (e.g., β-carotene). Previously, β-carotene has been reported to exhibit effective free radical quencher, lipid antioxidant, and is believed to possess high scavenging activity towards many free radicals species (Lobo et al. 2010). Carotenoid-based spinach has been discovered to consist of lutein, β-carotene, violaxanthin, and neoxanthin constituent (Bunea et al. 2008; Jaswir et al. 2011; Jiraungkoorskul 2016). Spinach contains around 8.3 mg/100g of β-carotene (Yang, Tan & Zhu 2016). In fact, the number of carotenoids identified using HPLC analysis in saponified spinach were lutein (0.037 to 0.053 mg/100 g), β-carotene (0.018 to 0.031 mg/100 g), violaxanthin (0.009 to 0.023 mg/100 g), and neoxanthin (0.010 to 0.022 mg/100 g) (Bunea et al. 2008).

TABLE 1. Phytochemical compounds in spinach that contain antioxidant and antimicrobial activity

	Antioxidant activity	Antimicrobial activity	
Carotenoids	Lutein, β-carotene, violaxanthin, neoxanthin (Bunea et al. 2007; Jing et al. 2023)	Alkaloids	quinolones, metronidazole (Othman, Sleiman & Abdel-Massih 2019)
Phenolic compounds	o-Coumaric acid, *p*-coumaric acid, ferulic acid (Bunea et al. 2008), vanillic acid, ellagic acid, caffeic acid, chlorogenic acid, *m*-coumaric acid, trans-cinnamic acid (Khanam et al. 2012)	Simple phenolic compounds	Caffeic acid (Askun et al. 2009), naringenin, *p*-coumaric acid, (Jiraungkoorskul 2016; Kosina et al. 2010), phenol, 2,4-Bis(1,1-dimethyl), ovidin A, 1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester, cinnamic acid (Jha, Subramanian & Sahoo 2014)
Flavonoids	Patuletin, spinacetin, spinatoside, jaceidin (Cho et al. 2008; Wiermann 1981), quercetin, luteolin (Hóvári, Lugasi & Dworschák 1999; Nuutila Kammiovirta & Oksman-Calddentrey 2002), myricetin (Chu, Chang & Hsu 2000; Sultana & Anwar 2008), kaempferol (Nuutila), rutin (Khanam et al. 2012)	Tannins	Condensed tannins (Gupta & Verma 2011)

Phenolic Acids

The phenolic compounds in spinach have been discovered to consist of *o*-coumaric acid, *p*-coumaric acid, and ferulic acid (Harris & Trethewey 2010; Jiraungkoorskul 2016;). The total phenolic compounds in fresh spinach were 2088.9 ± 30.4 GAE/kg FW with *o*-coumaric acid, ferulic acid, and *p*-coumaric acid up to 27.8 ± 2.7 mg/kg FW, 9.9 ± 0.4 mg/kg FW, and 1.3 ± 0.1 mg/kg FW, respectively (Bunea et al. 2008). Phenolic compounds, such as quercetin, patuletin, spinacetin, and jaceidin as well as chlorophyll pigment have been proven significantly contribute to the total phenolic composition in spinach due to their abundant phenolic structure molecules (Naczk & Shahidi 2003; Nejad, Sani & Hojjatoleslamy 2013). Quercetin and catechin have been effectively proven to stabilise phospholipid bilayers structure against ROS via the peroxidation reaction (Gülçin et al. 2010).

Flavonoid Acids and Flavonoids

Flavonoid acids in spinach plants were found to contain anthocyanins, glucuronide, flavone, myricetin, methoxy flavone, methylenedioxy derivatives of 6-oxygenated, and the isolated flavonol structure of patuletin (Dehkharghanian, Adenier & Vijayalakshmi 2010; Naczk & Shahidi 2003; Pandjaitan et al. 2005). In addition, the major flavonoids constituents, which are apeigenin (170 mg/kg), quercetin (50 mg/kg), and kaempferol (30 mg/kg), were also found in fresh spinach plants (Dehkharghanian, Adenier & Vijayalakshmi 2010) apart from other flavone and its derivative structures, including patuletin, spinatoside, spinacetin, jaceidin, lignans lariciresinol, secoisolariciresinol, and pinoresinol (Cho et al. 2008; Roberts & Moreau 2016; Wiermann 1981), quercetin, luteolin (Hóvári, Lugasi & Dworschák 1999; Nuutila, Kammiovirta & Oksman-Calddentrey 2002). Therefore, the abundance of flavonoids in spinach was believed to be effective against oxidative stress by reducing the damaging effects of free radical molecules due to the presence of hydroxyl groups that are responsible for the antioxidant properties (Shivaranjani et al. 2014).

The interest in bioactive antioxidant component from vegetable sources are mainly associated with its free radical scavenging effect and the potent antioxidant properties that are highly reactive as hydrogen or electron-donating agents are significantly related to the reduction potential (Afanas'ev et al. 1989; Ligor, Trziszka & Buszewski 2012). Flavonoids in spinach act as a vital secondary metabolite plant phenolic in the antioxidant and chelating properties to induce desirable health effects (Pandjaitan et al. 2005).

PHYTOCHEMICAL COMPONENTS AS ANTIMICROBIAL AGENTS

Spinach extract has been reported to possess antibacterial and antifungal activities (Alnashi, Hassouna & Dairouty 2016; Altemimi et al. 2017; Olasupo, Aborisade & Olagoke 2018). The phytochemical screening of secondary metabolites from spinach leaves has been proven to inhibit the growth of *Escherichia coli* and *Bacillus subtilis* (Adeniran, Olajide & Orishadipe 2013). The secondary metabolites (e.g., phenols, flavonoids, saponins (non-carotenoid terpenes), and alkaloids) that are present in spinach leaves could also contribute to the protection against pathogenic infections as these components were proven to demonstrate antimicrobial properties (Shivaranjani et al. 2014). The studies by Hintz, Matthews and Di (2015) and Olasupo, Aborisade and Olagoke (2018) reported that alkaloids, simple phenolic, flavonoids, quinones and tannins obtained from spinach extract contribute to the antimicrobial properties and are effective against specific pathogens.

Alkaloids

Alkaloids is one of the secondary metabolites found in spinach that consist of heterocyclic nitrogen atoms. These alkaloid constituents possess antimicrobial and antidiarrhoeal properties (Hintz, Matthews & Di 2015). The quantitative analysis of 100 g of spinach contained approximately 4.82 g of alkaloid, which accounted for 4.82% (w/w) of the dried powdery sample (Shivaranjani et al. 2014). The presence of alkaloids in spinach can be determined qualitatively through the formation of red coloured precipitate using Dragendroff's reagent (Singh, Tailang & Mehta 2016). Furthermore, spinach extract containing alkaloids could also inhibit various bacterial growth, such as *E. coli, Streptococcus pneumoniae, B. subtilis, Bacillus anthracis,* and *Staphylococcus aureus* (Ranjitha & Sudha 2015). Alkaloid components that have antimicrobial activity included quinolones, and metronidazole react through inhibiting enzyme activity or other mechanisms such as affecting cell division and disrupt bacterial membrane cell. In fact, many of alkaloids in plants have not been identified and scientists are currently racing to search for new antimicrobial compounds within this family that may help fight against bacteria (Othman, Sleiman & Abdel-Massih 2019).

Simple Phenolic Compounds and Flavonoids

Simple phenolic compounds comprise of a single substituted phenolic ring, while flavonoids are composed of one carbonyl of phenol with a 3-hydroxyl group (Hintz, Matthews & Di 2015; Othman, Hasan & Zubairi 2017). Furthermore, phenolic acid of caffeic acid compounds in spinach and white grapes could be effective to inhibit the growth of *Bacillus* species (Askun et al. 2009). The findings of these polyphenol-based phytochemicals in spinach, in particular caffeic acid, naringenin, and *p*-coumaric acid, exhibit a huge potential application to immobilise pathogens, especially Gram-positive bacteria (Jiraungkoorskul 2016; Kosina et al. 2010). Meanwhile, the phytochemical composition of spinach leaves consists of 45.24 g of phenolic compounds and 27.34 g of flavonoids in every 100 g of dried powder sample (Shivaranjani et al. 2014). Additionally, the polyphenols in spinach extract can be determined using the Folin Ciocalteau reagent in which a light pink colour appears gradually, while the addition of ferric chloride solution for the screening of flavonoids exhibits green blue or violet colour profiles (Babu et al. 2018).

The mechanism of antimicrobial activity in spinach is mostly based on the polyphenol adsorption on the bacterial membrane that affects the membrane stability, leading to subsequent rupturing of the cellular contents (Negi 2012). Flavonoids can interact with extracellular and soluble proteins of the bacterial cell walls, which could interrupt the microbial membrane with its lipophilic flavonoids (Cowan 1999; Mohd Azzimi, Mohd Fazil & Zubairi 2018). Other valuable bioactive components that exhibit potential antibacterial properties in spinach are summarised in Table 2. Hence, the scientific evidence presented in this review paper firmly verifies the antioxidant and antimicrobial properties of variousphytochemical compounds in spinach.

Quinones

Quinone is an aromatic ring consisting of two carbonyl groups that are capable to stabilise free radicals and providing an antimicrobial effect, while tannin is formed through the polymerisation of quinones (Hintz, Matthews & Di 2015). The presence of quinones in spinach has been evaluated qualitatively with 5 mL of benzene and 10% (v/v) ammonia solution that turns the solution into pink, red, and violet in a low-phase appearance. The presence of quinones in spinach extract is beneficial as a food substrate, which prevents the growth of undesirable pathogens, as they can deactivate specific proteins in pathogens and make them immobilised (Olasupo, Aborisade & Olagoke 2018).

TABLE 2. Bioactive antioxidant and antimicrobial constituents in spinach plant based on recent studies

Tannins	Phenolic and flavonoids	Saponins	Coumarins	Steroids	Terpenoids	Alkaloids	Reference
/	■	■	■	/	■	■	Babu et al. (2018)
/	/	/	■	/	■	■	Olasupo, Aborisade & Olaguke (2018)
/	■	■	■	/	■	/	Alnashi, Hassouna & Dairouty (2016) total phenolic compounds(TPC
/	/	■	■	■	■	/	Singh, Tailang & Mehta (2016)
/	/	■	/	■	■	/	Hintz, Matthews & Di (2015)
/	/	/	■	/	/	/	Adeniran, Olajide & Orishadipe (2013)

Note: (/) = Present; ■ = Unavailable

Tannins

On the other hand, tannins can be detected in spinach by treating the sample with 1% (w/v) ferric chloride solution, which forms blue-black, green, or blue-green precipitate in the presence of tannins (Babu et al. 2018). The antimicrobial activity of tannins is quite similar to that of quinones and was reported to effectively restrict the proliferation of bacteria, yeast, and certain fungi (Cowan 1999). In fact, some of the tannins components that have demonstrated antimicrobial activity include ellagitannins, condensed tannins (proanthocyanidins), gallotannins, catechins, punicalagins, and epigallocatechin gallate (EGCG) (Reddy et al. 2007). In spinach, tannins content was 5.5% and the condensed tannins was 0.293% (Gupta & Verma 2011).

ANTIOXIDANT ACTIVITIES OF SPINACH

The formation of harmful ROS and free radicals that could harm proteins, lipids, and nucleic acids, such as oxidative, can be slowed down by increasing the rate of antioxidant activity (Alnashi, Hassouna & Dairouty 2016). Ligor, Trziszka and Buszewski (2012) reported that fresh spinach extract exhibit the highest antioxidant activity among green vegetables in the study due to its high amount of polyphenols, including phenolic acids and flavonoids. The polyphenolic acids from the fresh spinach extract recorded the highest concentration of 1.823 mg GAE/g compared to that of lutein (0.830 mg GAE/g). Furthermore, it was found that the assay demonstrated a positive correlation between the number of polyphenols and the radical scavenging activity. In addition, Chew, Goh and Lim (2009) reported that the main components of polyphenols in spinach extract was able to neutralise free radicals and break down peroxide species based on the determination of the total phenolic content using the Folin-Ciocalteu technique.

The total antioxidant activity of spinach extract depends heavily on the range of solvent polarity during the solvent extraction procedure, which normally employs water, methanol, ethanol, or ethyl acetate. Spinach extract using water as the solvent consists of two main fractions: water-soluble fraction and insoluble water fraction (Bergman et al. 2001). This finding proved that spinach consisted of hydrophobic and hydrophilic components. The solubility of antioxidant components from spinach extract can be evaluated using aqueous (water) spinach extracts using different types of solvent ratio combination, such as water and acetone combination at a ratio of 1:9 (Bergman et al. 2001; Zubairi, Sarmidi & Aziz 2014). The amount of antioxidant-based polyphenol compounds in spinach extract using the water-soluble extraction method recorded the highest value of 54.02% (w/w) compared to that of the alcohol-soluble extraction method at 45.02% (w/w) (Sah et al. 2017). Meanwhile, three different solvents comprising petroleum ether, ethanol, and aqueous ethanol were used to obtain the antioxidant compounds in spinach extract (Alnashi, Hassouna & Dairouty 2016). The aqueous ethanol extract recorded the highest total antioxidant activity of 112.2 mg GAE/g with 63.7% of total phenolic compound concentration, while petroleum ether extract showed the lowest antioxidant activity of 67.9 mg GAE/g with 31.1% of total phenolic compound concentration. The result was in line with the study by Stanković (2011) in which the highest total phenolic extract was obtained using methanol as the solvent extract, followed by acetone, water, petroleum ether, and ethyl acetate.

In addition, several studies have demonstrated the effect of leaf type on the antioxidant activity of spinach extract. The smooth leafy type spinach demonstrated the highest antioxidant activity (44.8 mmol Trolox/g) compared to that of the savoy leaf type (39.67 mmol Trolox/g) and semi-savoy leaf type (35.35 mmol Trolox/g), which corresponded with the total phenolic content of 11.43 mg GAE/g, 10.83 mg GAE/g, and 10.50 mg GAE/g, respectively (Yosefi et al. 2010). The results indicate that the antioxidant activity was highly correlated with the increased total phenolic contents. Table 3 summarises other assays that have been used to detect antioxidant activities in spinach extract. Overall, the results indicate that the free radical uptake and antioxidant activity in spinach extract with different levels of antioxidant properties enhanced the scavenging capacity and simultaneously facilitated the reduction of lipid peroxidation. The presence of gallic acid, caffeic acid, and p-coumaric acid as antioxidant agents in spinach extract was assumed to be the major reason for the effective antioxidant properties of spinach extract (Askun et al. 2009). Furthermore, the findings demonstrate that the total phenolic content in spinach with high antioxidant compounds also showed strong antimicrobial properties, where the reduction of Gram-positive bacteria, Gram-negative bacteria, and faecal coliform were successfully reported (Agüero et al. 2016).

ANTIMICROBIAL ACTIVITIES OF SPINACH

Previous studies reported that polyphenols and flavonoids obtained from green plant vegetables can be categorised as a natural alternative antimicrobial agent (Xi & Shouqin 2007; Yolmeh et al. 2015). Likewise, spinach extract has been reported to possess antibacterial and antifungal activities as shown in Table 4 (Alnashi, Hassouna & Dairouty 2016; Altemimi et al. 2017; Olasupo, Aborisade & Olagoke 2018). The antimicrobial activity essentially depends on the extract composition and its affinity to restrict pathogenic growth. Babu et al. (2018) reported that the Kirby-Bauer method is an effective approach to evaluate the inhibition zone diameter of spinach leaf extract and identify the potential growth of pathogenic through a positive screening. The inhibition test results (Figure 2) demonstrate the inhibition zone diameter of 24.95 ± 0.10 mm on *S. aureus* and 20.93 ± 0.13 mm on *E. coli*, respectively, indicating that the presence of spinach leaf extract rapidly inhibited the bacterial growth (Altemimi et al. 2017). Moreover, the Minimum Inhibitory Concentration (MIC) assay was conducted to determine the susceptibility of antimicrobial agents in spinach leaf extract against the growth of pathogens (Figure 3). The antimicrobial activity of spinach leaf extract (60 mg/mL of MIC value) was more susceptible towards the Gram-positive *S. aureus* compared to the Gram-negative *E. coli* (70 mg/mL MIC value) (Altemimi et al. 2017). The result was in line with the definition of MIC, which determines the lowest concentration of an antimicrobial agent to inhibit bacterial growth (Bonjar 2004).

Many studies found that spinach leaf extract was more effective against Gram-positive bacteria (Ali, Ayub & Ali 2017; Altemimi et al. 2017). Typically, Gram-negative bacteria possess a hydrophilic surface on their outer membrane, are rich in lipopolysaccharide molecules, and exhibit a unique periplasmic space that is resistant to antimicrobial substances (Shan et al. 2007). In contrast, Gram-positive bacteria lack the outer membrane that serves as an additional barrier, hence, are more susceptible to antimicrobial agents. Similar to the antioxidant properties of spinach leaf extract, previous studies reported that the successful antimicrobial activity of spinach leaf extract was associated with the type of solvent used during the extraction process (Nasim et al. 2012). For instance, ethanol extract has a higher antimicrobial activity consistency than water extract due to the low polarity of ethanol. Additionally, ethanol was the best solvent for the extraction of antimicrobial compounds due to its high affinity towards specific bio-constituents (Ahmad & Aqil 2007). In comparison, water is not a suitable solvent to solubilise antimicrobial compounds (e.g., phenolic and flavonoids) since the phytochemicals exhibit low solubility in aqueous solutions (Nasim et al. 2012). Table 4 summarises the variety of polar solvent extraction approaches based on the prior works that demonstrate the antimicrobial activity of spinach leaf extract against Gram-positive and Gram-negative bacteria.

TABLE 3. Determination of free radical scavenging capacity and antioxidant activity in spinach extract in various studies

Reactive oxygen species/free radical	Antioxidant assay	Basis	Scavenging capacity	Reference
Peroxyl (LOO^-)	DPPH	The radical DPPH present in the UV-Vis spectrum showed a decrease in antioxidant level	Medium	Zhou & You (2006)
Peroxyl (LOO^-)	DPPH	The DPPH measures the relative ability of antioxidants with ascorbic acid. The result recorded low concentration compared to ascorbic acid	Moderate	Sah et al. (2017)
Total antioxidant capacity	FRAP	The sample was measured at 593 nm, reducing Fe^{2+} to Fe^{3+} at acidic pH to give a coloured complex	High	Xie et al. (2015)
Hydroxyl ion (OH^-)	Deoxyribose	(OH^-) attack on deoxyribose was determined by thiobarbituric acid and measured at 532 nm	Medium	Kaur, Bains & Kaur (2012)
Antioxidant capacity	TBARS	The technique depends on the colour reaction of polyunsaturated lipids involving TBA and oxidation component in which the antioxidant activity was observed at 532 nm	High	Castenmiller et al. (2002)

Note: DPPH = 1,1-diphenyl-2-picrylhydrazyl; UV-Vis = Ultraviolet-visible; FRAP = Ferric Reducing Antioxidant Power Assay; TBARS = Thiobarbituric Acid-Reactive Species

Furthermore, past studies have acknowledged the antifungal constituent in spinach leaf extract that effectively inhibit fungal and bacterial growth. Defensins are one of the antifungal elements in spinach that also exhibit antimicrobial activities (Altemimi et al. 2017; Stotz, Thomson & Wang 2009). The properties of defensins were initially revealed through the discovery of a new group of defensins in the spinach plant, which was effective against Gram-positive and Gram-negative bacteria as well as fungi (Segura et al. 1998). Generally, defensins are categorised into three classes according to their potential antimicrobial activities. The first class of defensins can inhibit both bacterial and fungal growth. In contrast, the second class of defensins is capable of preventing fungal growth but is less effective towards bacteria. Meanwhile, the third class of defensins is effective in inhibiting insect-feeding by preventing the amylases and proteinase. Previously, Hintz, Matthews and Di (2015) introduced the fourth class defensin, which could hamper the growth of Gram-positive bacteria, Gram-negative bacteria, and fungi. Besides their effective microbial inhibition activities, defensins were reported to be harmless and non-toxic to animals or plant cells, which can be safely used as a natural bio-preservative. Hence, the ability of spinach plant constituents to restrict the growth of foodborne pathogens can be essentially applied to enhance the stability of food products.

The antimicrobial activity of phenolic compounds is thought to be different from antioxidant activity mechanism. This includes enzyme inhibition by the oxidised compounds, possibly through reactions with proteins through SH- groups or through non-specific interactions (Mason & Wasserman 1987). Highly oxidised phenols (Scalbert 1991) or those with more hydroxyl groups are more inhibitory than those less oxidised are. Moreover, flavonoids with more hydroxyl groups had a greater antimicrobial activity (Sato et al. 1996). However, flavonoids lacking hydroxyl groups on their β-rings were more active in membrane disruption in microbial targets (Chabot et al. 1992). However, quinones act as a source of free radicals' stability and bind irreversibly with proteins leading to its loss of function. Other targets are inactivating enzymes, binding to adhesins on the microbial cell surface, binding to cell wall proteins, and interacting with substrates, rendering them unavailable to the microorganism, and complexing with metal ions (Cowan 1999).

FOOD APPLICATION

The novel product-based spinach supplement has gained the attention of the food industry as an alternative source of natural antioxidants and antimicrobial agents. This reason could be related to the fortification process of spinach extract that improves the quality of foods with phenolic compounds and other nutrition (Mandal et al. 2013). For example, the addition of spinach in yoghurt recorded an increased DPPH scavenging capacity from 13.00 ± 0.05 GAE/g to 22.00 ± 0.05 GAE/g of lipid peroxidation inhibition due to the high percentage of polyphenols in the formulation (Nejad, Sani & Hojjatoleslamy 2013). The restricted lipid oxidation enhanced the shelf-life of the product by inhibiting or slowing down the formation of ROS and free radicals that

TABLE 4. Major antimicrobial activity inhibition towards Gram-negative bacteria with a different type of solvent extraction used to demonstrate the microbial activity present in spinach leaf extract

Type of solvent	Gram-positive bacteria	Gram-negative bacteria	Reference
Ethanol	*S. aureus*	*E. coli* *Salmonella typhimurium*	Nasim et al. (2012)
Ethanol	*Lactobacillus acidophilus* *Streptococcus mutans*	N.A	Adapa et al. (2018)
Ethanol	*S. aureus* *Staphylococcus epidermidis* *S. typhimurium*	*E. coli* *Pasteurella multocida*	Shimaa et al. (2016)
Methanol	*Bacillus pumilus* *Staphylococcus citreus* *Corynebacterium xerosis*	*Klebsiella ozaenae*	Ali, Ayub & Ali (2017)
Methanol	*S. aureus* *B. subtilis* *Bacillus cereus*	*E. coli* *Pseudomonas aeruginosa*	Dubey, Mishra & Singh (2010)
Ethyl acetate	*S. aureus*	*P. aeruginosa*	Olasupo, Aborisade, & Olagoke (2018)

Note: N.A = Not available

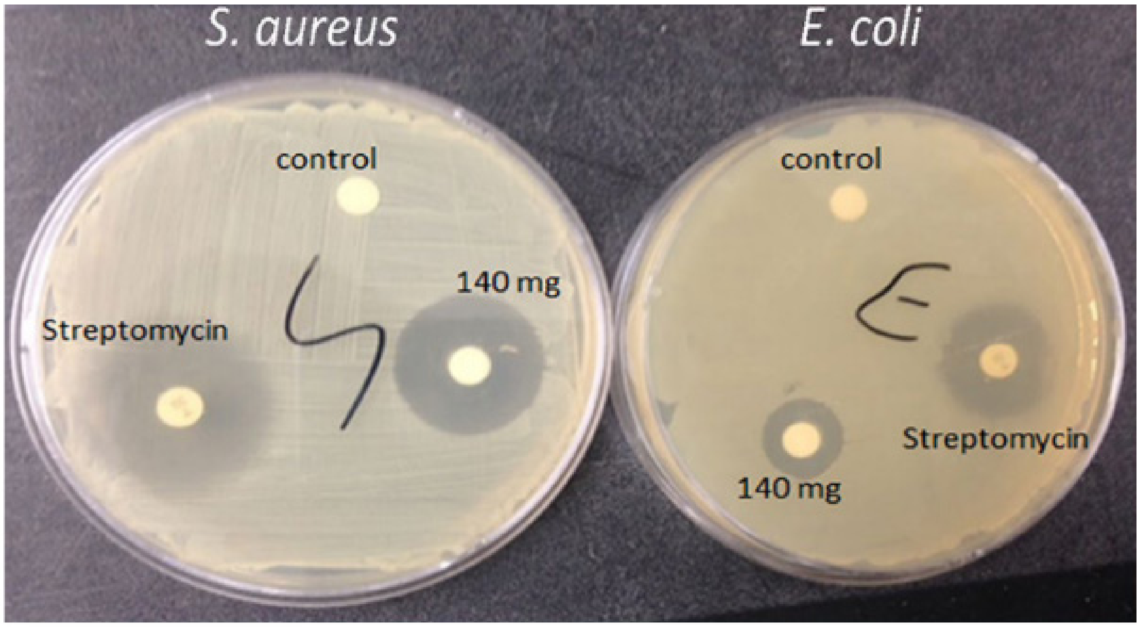

FIGURE 2. Comparison of microbial inhibition of spinach extract (140 mg) and standard streptomycin under optimised conditions. The halo zone indicates the inhibition of the pathogenic growth (Altemimi et al. 2017)

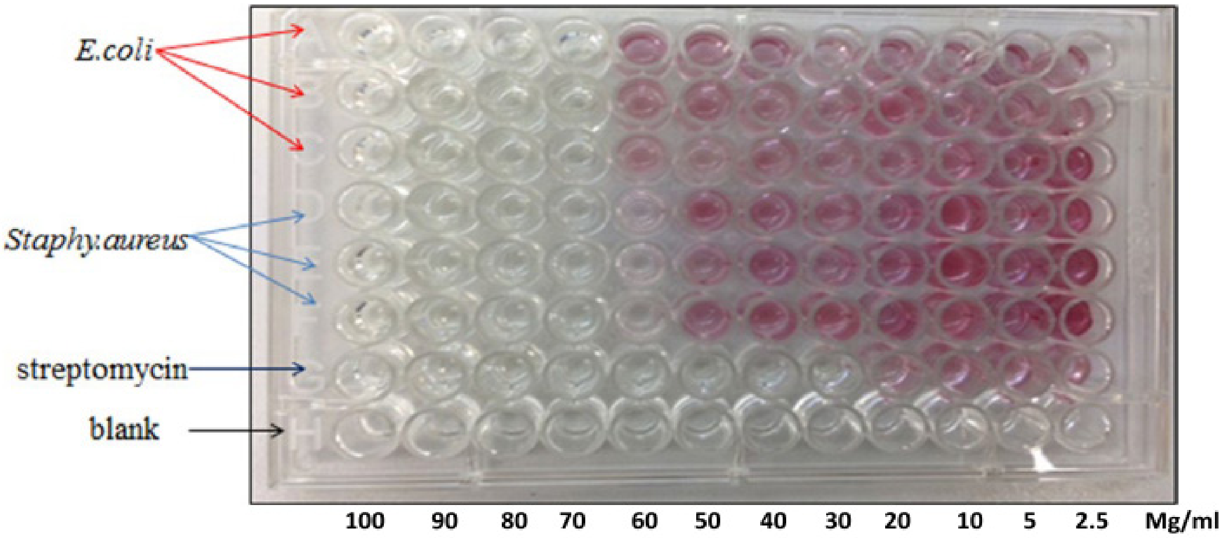

FIGURE 3. The purple colour indicates microbial growth. The colour intensity of *S. aureus* from the right side reduced earlier compared to that of *E. coli*, which indicate that spinach extract was more effective towards Gram-negative bacteria Altemimi et al. 2017)

could potentially harm proteins, lipids, and nucleic acids, as well as microbial growth (Alnashi, Hassouna & Dairouty 2016). The addition of spinach in yoghurt products with an initial Lactic Acid Bacteria (LAB) culture of 106 CFU/mL showed a gradual decrease of the LAB culture from day 1 to day 21 of the storage period due to the reduction in pH and microbiota acidity microenvironment (Nejad, Sani & Hojjatoleslamy 2013). Moreover, it was also showed that the yeast and mould growth count during the 15-day storage period were lower than 5 CFU/g, which was in accordance with the Codex standard.

Apart from that, the incorporation of spinach extract in chicken sausage increased the total phenolic content from 14.37 GAE/100 g to 17.23 ± 0.33 GAE/100 g and simultaneously decreased the microbial count of 104 CFU/g towards a 21-day storage period (Ahmad et al. 2020). The microbial growth profiles throughout the 21-day storage period were below 106 CFU/g, which was in accordance with the Maximal Permissible Limit (MPL) of the Malaysian Food Regulation. Furthermore, the peroxide value of the product was also below 25 meg of active O2/kg of fat, which followed the acceptable limit of fatty foods. Similarly, the addition of spinach extract in turkey meat patties improved the stability and the shelf-life of the product due to the antioxidant properties of the spinach extract that inhibited the proteinic and lipidic oxidation in the product (Duthie et al. 2013). Additionally, the spread plate method was employed to test the antimicrobial activity of Namakparas Indian traditional food added with spinach extract. The results showed no microbial growth for up to 15 days of storage period (Tyagi 2017). Other studies have successfully proven the correlation between the antioxidant and antimicrobial properties of spinach extract in cheese and wheat pasta, which showed highly effective inhibition against food pathogens (Abrol et al. 2017; El-Sayed 2020). Previously, the natural antioxidant of spinach has been found to be nonmutagenic and it has not shown any target-organ toxicity or side effects (Lomnitski et al. 2003).

Conclusion and Recommendations

In conclusion, spinach extract has been widely proven for its excellent natural source of nutrients and phytochemical compounds that provides synergistic interaction of antioxidant and antimicrobial bioactive components to enhance the quality of food products. The antioxidant activity restricts lipid oxidation and increases the shelf-life of food products, while the antimicrobial activity inhibits the growth of potentially harmful food pathogens. Given all the aforementioned findings, there is an increasing need to develop spinach-based food products that would likely possess improved qualities. Hence, further studies on the bioavailability of spinach as a functional natural source of antioxidant and antimicrobial agent should be scaled up continuously to impede nutritional deterioration in food products and potentially replace synthetic antioxidants that pose high toxicity and carcinogenic effects to the consumers. Besides the food industry, the potential application of spinach extract offers a huge prospect in the pharmaceutical industry as it can be developed as a promising antimicrobial drug for the treatment of various pathogens. In line with the expanding application of spinach in the food industry, a clear guideline and regulation should be formulated by the responsible authorities to ensure the safety and usage of spinach-based products either in food-based applications or pharmaceutical products. Therefore, such findings on phytochemical and pharmacological studies from the spinach plant would open a huge possibility of discoveries that is safely and clinically effective.

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