

Biological Activities of *Spinacia oleracea* L.

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Abstract:

As the evolution process of microbes continues to attempt to render our antibiotics obsolete, cancer plagues the unfortunate of our population, and the well being of citizens decline, the scientific community has come up with the super food which has the effect of all 3 of the ailments listed above. The *Spinacia oleracea* L. (Spinach) has been reported to have antimicrobial activities, antioxidant activities and anticancer activities along with its other perks such as antiproliferative, central nervous system depressant, antihistaminic, and protection against gamma radiation. This literature review aims to highlight the biological activities that can be beneficial for anyone by compiling and analysing its findings.

Keywords —Antimicrobial activity, anti-oxidation, anticancer, *Spinacia oleracea* L.,

I. INTRODUCTION

Spinacia oleracea L. or Spinach is a leafy green vegetable plant which can be grown in the spring and autumn, yielding a lot in a short time (1). It belongs to the family Chenopodiaceae and is native to Central Asia and Persia (Iran) (2). Spinach produces Leaves annually while it's seeds are produced biennially (1). It can also be divided into two subspecies: ssp. *Glabra* and ssp. *Spinosa* (3). Several types of vitamins and minerals can also be found inside Spinach such as Vitamin a (Lutein), Vitamin c, Vitamin e, Vitamin k, magnesium, manganese, folate, and iron (4). Aside from having a great nutritional value, Spinach is also linked to various biological activities, eg: being a virus inhibitor, anthelmintic, antioxidant, hepatoprotective, and reducing the risks for several forms of cancer. The aim of this review is to highlight the biological activities of *S. oleracea* L. regarding its nutritional value, antioxidants, and anticancer properties (5, 6). This review aims to highlight the biological activities of *S. oleracea* L. and points out the benefit of *S. oleracea* regarding medical purposes.

II OVERVIEW OF *Spinacia oleracea* L.

S. oleracea is a consumable angiosperm plant from the family of Amaranthaceae. Standing at heights of up to 30 cm, it has the resilience to survive over winters within temperate regions (7, 8). The larger leaves are usually around the base of the plants and get smaller the more you move up the spinach's stem (7). The locations of the leaves along the spinach's stem are alternated on each side of the stem while its leaves have an ovate, triangular shape. The leaves can grow up to 30 centimeters long and can grow as broad as 15 centimeters (7). Its flowers are low profiled yellowish white buds at the size of 3-4 mm and mature into small clusters of seeds spanning from 5-10 mm (1). Being high in Magnesium, Iron, Folate, Manganese, and vitamins C, K, and A, Spinach is high in nutritional value (9-12). These biochemical resources are what makes it of great pharmacological and medical importance (13). The natural compounds inside leafy green vegetables are also good for reducing risks for diabetes, cancer and hepatotoxicity, as well as

protecting against oxidative stress, eye disorders, iron deficiencies (14-18).

S. oleracea has been used since ancient times as a source for food since it contains many useful nutrients and minerals essential to maintaining good human vitality (19). Spinach can also be very useful in modern times as a great food source for developing countries especially in times where the world population is still increasing quickly, the increasing demand of food may be a big challenge (19). Spinach can supply many countries with the nutrients that it needs and combat malnutrition (20, 21). Leafy green vegetables can act as great sources of energy being producers themselves (21). They can supply nutrients and micronutrients essential for good vitality. For example, antioxidant activity (22). Some of the vegetables are also reported to be able to relieve more than one ailment while passively recuperating the human well being and health in other areas (19-21). Some of the diseases that these fruits and vegetables can prevent include cardiovascular diseases for example heart attack, high blood pressure, and stroke (23-25). These chemicals inside Fruits and vegetables carry out their functions of protecting us by combining with minerals and vitamins. Phytochemicals which help a human thrive originates from a fruit or vegetable's feature for preventing itself from getting the disease. This can cause the fruit to have a specific color, smell, or flavour. Phytochemicals are non-nutrient compounds inside Vegetables, fruits, grains and have been linked to the reduction of major degenerative diseases in humans (4). So, the capacities of local vegetables need to be evaluated concerning its nutrients and its phytochemicals which will greatly assist nutritionists, food processors, dieticians, and consumers about what they should be eating. Plants which have medical attributes are being used locally to cure infections caused by viruses, bacteria, parasites and fungi (4, 26-29). Many people living in Nigeria have been relying on their own traditional means of treating their diseases ever since ancient times (30-32). Plants have been the starting point of many modern medicines whether it would be in its extract form, or its pure form.

III ANTIMICROBIAL ACTIVITIES

S. oleracea leaf extracts were examined by the random amplification of polymorphic (bacterial) DNA (RAPD) and scanning electron microscopy (SEM) to determine the effects on Gram-negative and Gram-positive bacteria models (33). The best conditions for extraction were 45 °C, 44 per cent ultrasound power, and a 23-minute extraction time (33). The leaf extract of Spinach was antimicrobial to both bacteria, with minimum inhibitory concentrations (MICs) from 60 mg/ml to 100 mg/ml (33). Analysis by SEM revealed that the treated bacterias were damaged, and decreasing in number. Analysis by RAPD of DNA showed that treatments dwindled both the number and size of amplicons. Upon these findings, Spinach leaf extract was concluded to exert bactericidal activity by promoting DNA mutations and destroying cell walls (33). In comparison to controls, extract preparation under ideal circumstances improved antibacterial activity and increased the area of inhibition zones of growth for the treated bacteria (non-treated bacteria). Bacteria cells treated with Spinach (*S. oleracea*) leaf extract were revealed by SEM image to be reduced in the number of viable bacteria cells. Additionally, *E. coli* and *S. aureus* strains treated with spinach extract can be gauged by using DNA polymorphism detected by RAPD as a Biomarker (34, 35). Previously in two separate studies, extracts from *Rhazya stricta* leaves, and *Conocarpus erectus* had demonstrated similar results (36, 37). This indicated that ultrasonicated spinach extracts can both treat and combat bacteria that include both Gram-positive and Gram-negative organisms like *E. coli* and *S. aureus* (36, 37). Iqbal et al. researched antimicrobial properties of n-hexane, sonicated n-hexane, aqueous, and ethanolic extracts of Spoiled and fresh Spinach and discovered through agar disc diffusion testing that both the spoiled and fresh *S. oleracea* extracts exhibit activity against several mammalian pathogens (38). *E. coli*, *Salmonella typhimurium*, *S. aureus*, *Pasteurella multocida*, *Klebsiella pneumoniae*, *Staphylococcus epidermidis*, *Micrococcus luteus*, *Proteus vulgaris*, and *Lactobacillus bulgaricus* were the most frequently

encountered mammalian bacterial strains tested for antimicrobial activity (38). All pathogens tested, except *S. typhimurium*, were inhibited by aqueous extracts of spoiled and fresh spinach (38). Adapa and the team determined the minimum concentration of ethanolic and aqueous extracts of spinach in opposition to *Lactobacillus* and *Streptococcus mutans*. They reported that the MIC of the ethanolic extract of spinach was 25 µg/ml for *Lactobacillus* and 12.5 µg/ml for *S. mutans*. In addition, the ethanolic extract showed more significant bacteriostatic activity toward *S. mutans*(39). Furthermore, *in vitro* antimicrobial activity was demonstrated by the extracts from cooked and uncooked leaves of *S. oleracea* were tested against *S. aureus*. The zone of inhibition between cooked and uncooked *S. oleracea* leaves was similar at 75 and 100 mg/ml (40).

IV ANTIOXIDANT ACTIVITY

The antioxidant activity of *S. oleracea* green leaves exhibited antioxidant capacity performing via several assays including reducing power assay and superoxide anion scavenging activity (40-43). The DPPH radical scavenging activity of uncooked and cooked leaves is given in milligrammes per gramme (mg/g) and is calculated using the equation in 2.7. Cooked and uncooked leaf methanol extracts demonstrate antioxidant activity (40). Since a hydrogen atom, or an electron was transferred, the reduction of DPPH was witnessed. Because of their ability to donate hydrogen, phenol compounds are also excellent antioxidants (44). Antioxidants are found in plants in flavonoids, phenol acids, and tocopherols (45). The decrease of a chemical reaction by the addition of DPPH was utilized to determine the reaction's radical nature. Due to its high absorption capacity, the DPPH radical has a dark violet colour while acting as solute and degrades to colourless (Clear solution) or light yellow when neutralised (40). These activities are beneficial for disease treatment and drug extraction from spinach leaves.

V ANTICANCER ACTIVITY

Research showed that phenolic compounds can hinder the growth of cancerous tumours and have a range of other therapeutic properties, including antifungal, antiviral, anti-inflammatory, antispasmodic, and anti-diarrheic (14, 46, 47). Because of its high levels of phenolic acids, flavonoids, and pigments such as chlorophyll and lutein, it is thought that Soleracea is high in antioxidants, such as tocopherols (48). Many bioactive compounds are found in spinach, like MGDG, DGDG, and SQDG, all located in the chloroplast membrane. It has been demonstrated that MGDG and SQDG have an inhibitory effect on mammalian DNA polymerases, but DGDG does not (49). The tumour growth, angiogenesis, and blood vessel development inhibitors found in these glycolipids were shown to target cancer cells, endothelial cells, and tumour growth via the inhibition of replicative DNA polymerase activities. Glycolipids from subspecies of spinach were influential in developing cancer-fighting foods in research done by Kuriyama et al (49).

Another study, which studied spinach ethanol extract (SE), the three hydrophobic column chromatography fractions, and DNA polymerase activity, demonstrated that these three compounds inhibited calf DNA polymerase (pol) (50). Glycoglycerolipids in spinach completely hindered the activity of pol α at a concentration of 43.0µg/ml (50). Fat-soluble glycolipids were less potent at this concentration and had no additional effect on pol α activity. Water-soluble glycolipids were not observed to affect pol α . However, the extract from spinach does not affect pol α (50). This is because the extract contains a pol-inhibitory glycolipid, a glycolipid that acts as a pol inhibitor (50). Following these experiments, it was found that the spinach glycolipids fraction could hamper mammalian pol activity, human cultured cancer cell growth, and *in vivo* solid tumour proliferation when administered orally. This fraction may contribute to helping stave off cancer and might also be a preventative food with anticancer properties.

CONCLUSIONS

Spinach (*S. oleracea L.*) is a goosefoot-family leafy green vegetable. Numerous pharmacological activities of *S. oleracea* have been reported, including antioxidant, anti-bacterial activity, and anticancer. This plant has been reported to contain various secondary metabolites. Thus, *S. oleracea* warrants additional phytochemical, pharmacological, and clinical studies to develop an effective natural remedy capable of providing therapeutically effective lead compounds or extracts.

REFERENCES

1. Nešković M, Čulafić L. Spinach (*Spinacia oleracea L.*). Crops II: Springer; 1988. p. 370-85.
2. Pandey SC, Kalloo G. Spinach: *Spinacia oleracea L.* Genetic improvement of vegetable crops: Elsevier; 1993. p. 325-36.
3. Murcia MA, Jiménez-Monreal AM, Gonzalez J, Martínez-Tomé M. Spinach. Nutritional Composition and Antioxidant Properties of Fruits and Vegetables: Elsevier; 2020. p. 181-95.
4. Roberts JL, Moreau R. Functional properties of spinach (*Spinacia oleracea L.*) phytochemicals and bioactives. Food & function. 2016;7(8):3337-53.
5. Fiorito S, Preziuso F, Epifano F, Scotti L, Bucciarelli T, Taddeo VA, et al. Novel biologically active principles from spinach, goji and quinoa. Food chemistry. 2019;276:262-5.
6. Ashok TS, Ravindra DV. DETECTION OF IMIDACLOPRID RESIDUES IN SPINACH BY HPLC AND ITS EFFECT ON ANTIOXIDANT ACTIVITY. 2020.
7. Wesche-Ebeling P, Maiti R, García-Díaz G, González DI, Sosa-Alvarado F. Contributions to the botany and nutritional value of some wild *Amaranthus* species (*Amaranthaceae*) of Nuevo Leon, Mexico. Economic Botany. 1995;49(4):423-30.
8. Verma S. A study on medicinal herb *Spinacia oleracea* Linn: *Amaranthaceae*. Journal of Drug Delivery and Therapeutics. 2018;8(4):59-61.
9. Pereira C, Dias MI, Petropoulos SA, Plexida S, Chrysargyris A, Tzortzakis N, et al. The effects of biostimulants, biofertilizers and water-stress on nutritional value and chemical composition of two spinach genotypes (*Spinacia oleracea L.*). Molecules. 2019;24(24):4494.
10. Ogbaji PO, Li J, Xue X, Shahrajabian MH, Egrinya EA. Impact of bio-fertilizer or nutrient solution on Spinach (*Spinacea Oleracea*) growth and yield in some province soils of PR China. 2018.
11. Roupheal Y, Giordano M, Cardarelli M, Cozzolino E, Mori M, Kyriacou MC, et al. Plant-and seaweed-based extracts increase yield but differentially modulate nutritional quality of greenhouse spinach through biostimulant action. Agronomy. 2018;8(7):126.
12. Mirahmadi SF, Hassandokht M, Fatahi R, Naghavi MR, Rezaei K. High and low oxalate content in spinach: an investigation of accumulation patterns. Journal of the Science of Food and Agriculture. 2021.
13. Xu C, Mou B. Responses of spinach to salinity and nutrient deficiency in growth, physiology, and nutritional value. Journal of the American Society for Horticultural Science. 2016;141(1):12-21.
14. Arru L, Mussi F, Forti L, Buschini A. Biological Effect of Different Spinach Extracts in Comparison with the Individual Components of the Phytoextract. Foods. 2021;10(2):382.
15. Milano F, Mussi F, Fornaciari S, Altunoz M, Forti L, Arru L, et al. Oxygen availability during growth modulates the phytochemical profile and the chemo-protective properties of spinach juice. Biomolecules. 2019;9(2):53.
16. Chen J, Xia X, Zhang Z, Wen W, Xi N, Zhang Q. The combination of warming and copper decreased the uptake of polycyclic aromatic hydrocarbons by spinach and their associated cancer risk. Science of The Total Environment. 2020;727:138732.
17. Abdul-Wahab FK, Jalil TZA. Study of iraqi spinach leaves (phytochemical and protective effects against methotrexate-induced hepatotoxicity in rats). Iraqi Journal of Pharmaceutical Sciences (P-ISSN: 1683-3597, E-ISSN: 2521-3512). 2012;21(2):8-17.
18. Ruel MT. Can food-based strategies help reduce vitamin A and iron deficiencies. 2001.
19. Srivastava R, Srivastava V, Singh A. Multipurpose Benefits of an Underexplored Species Purslane (*Portulaca oleracea L.*): A Critical Review. Environmental Management. 2021:1-12.
20. Ebert AW, Hidayat IM, De los Santos EB, editors. Cultivar trials of indigenous vegetables in Indonesia and community-based seed conservation and multiplication in the Philippines 2011.
21. Bucher A, Bucher R. Working with Santal Villagers, West Bengal, India: Moringa and Kitchen Gardens to Combat Malnutrition, 2012-2017. Hidden Hunger: Strategies to Improve Nutrition Quality. 2018;118:77-83.
22. Ferreira JFS, Sandhu D, Liu X, Halvorson JJ. Spinach (*Spinacea oleracea L.*) response to salinity: Nutritional value, physiological parameters, antioxidant capacity, and gene expression. Agriculture. 2018;8(10):163.
23. Linoby A, Wahyudi E, Nasuha H, Zufwan A, Ruslan H. RED SPINACH EXTRACT WITH POTENT NITRIC OXIDE BIOSIGNALING NUTRIENT.
24. Sridhar KR, Pavithra M. Bioactive Compounds of Ceylon Spinach [*Talinum Triangulare* (Jacq.) Willd.] Bioactive Compounds of Ceylon Spinach. Bioactive Compounds in Underutilized Vegetables and Legumes. 2021:151-68.
25. Amir RM, Randhawa MA, Nadeem M, Ahmed A, Ahmad A, Khan MR, et al. Assessing and reporting household chemicals as a novel tool to mitigate pesticide residues in spinach (*Spinacia oleracea*). Scientific reports. 2019;9(1):1-6.
26. Chitra W, Nirmala T. Natural remedies for dengue fever. TNNMC Journal of Community Health Nursing. 2020;8(1):33-5.
27. Subramanian SB, Ramani A, Ganapathy V, Anbazhagan V. Preparation of self-assembled platinum nanoclusters to combat *Salmonella typhi* infection and inhibit biofilm formation. Colloids and Surfaces B: Biointerfaces. 2018;171:75-84.
28. Swathi Krishna S, Thennavan A, Kanthlal SK. Dietary foods containing nitric oxide donors can be early curators of SARS - CoV - 2 infection: A possible role in the immune system. Journal of Food Biochemistry. 2021:e13884.
29. De LC, De T. Protective foods to develop immunity of individuals against COVID 19. Biotica Research Today. 2020;2(5 Spl.):287-90.
30. Babayeju AA, Nmomo IO, Obalowo MA, Adebisi TT, Gbadebo CT. Sensory attributes and consumption of melon-soybean soup blends with Indian spinach vegetables in Ilorin, Kwara State Nigeria. Agrosearch. 2017;17(1):89-100.
31. Ogungbile PO, Ayeku PO, Ajibare AO. Comparative biotolerance of water spinach, *Ipomoea aquatica*, to heavy metal pollution in the Agodi Reservoir and the Ogunpa River, Oyo State, Nigeria. African Journal of Aquatic Science. 2021:1-7.
32. Ibrahim EG, Yakubu N, Nnamonu L, Yakubu JM. Determination of organochlorine pesticide residues in pumpkin, spinach and sorrel leaves grown in Akwanga, Nasarawa State, Nigeria. Journal of Environmental Protection. 2018;9(05):508.
33. Altemimi A, Lakhssassi N, Abu-Ghazaleh A, Lightfoot DA. Evaluation of the antimicrobial activities of ultrasonicated spinach leaf extracts using rapid markers and electron microscopy. Archives of microbiology. 2017;199(10):1417-29.
34. Atienzar FA, Venier P, Jha AN, Depledge MH. Evaluation of the random amplified polymorphic DNA (RAPD) assay for the detection of DNA damage and mutations. Mutation Research/Genetic Toxicology and Environmental Mutagenesis. 2002;521(1-2):151-63.
35. Atienzar FA, Evenden AJ, Jha AN, Depledge MH. Use of the random amplified polymorphic DNA (RAPD) assay for the detection of DNA damage and mutations: possible implications of confounding factors. Biomarkers. 2002;7(1):94-101.
36. Hajar AS, Gumgumjee NM. Antibacterial Efficiency and DNA Impairment in Some Bacteria Strains Treated with *Conocarpus Erectus L.* Extract. 2013.

37. El-Tarras AAE, Hassan MM, El-Awady MAM. Evaluation of the genetic effects of the in vitro antimicrobial activities of *Rhazya stricta* leaf extract using molecular techniques and scanning electron microscope. *African journal of Biotechnology*. 2013;12(21).
38. Iqbal M, Ghous T, Khan AN, Akhtar K. Evaluation of antimicrobial activity of extracts of fresh and spoiled *Spinacia oleracea* against some mammalian pathogens. *African Journal of Microbiology Research*. 2012;6(29):5847-51.
39. Adapa SB, Sushanth VH, Prashant GM, Mohamed I. In vitro antimicrobial activity of *Spinacia Oleracea* against *Streptococcus mutans* and *Lactobacillus acidophilus*. *Journal of Indian Association of Public Health Dentistry*. 2018;16(3):251.
40. Babu NGR, Divakar J, Krishna UL, Vigneshwaran C. Study of antimicrobial, antioxidant, Anti-inflammatory activities and phytochemical analysis of cooked and uncooked different spinach leaves. *Journal of Pharmacognosy and Phytochemistry*. 2018;7(5):1798-803.
41. Gunes G, Dogu - Baykut E. Green Leafy Vegetables: Spinach and Lettuce. *Handbook of Vegetables and Vegetable Processing*. 2018:683-99.
42. Mzoughi Z, Souid G, Timoumi R, Le Cerf D, Majdoub H. Partial characterization of the edible *Spinacia oleracea* polysaccharides: Cytoprotective and antioxidant potentials against Cd induced toxicity in HCT116 and HEK293 cells. *International journal of biological macromolecules*. 2019;136:332-40.
43. Khalid M, Hassani D, Bilal M, Asad F, Huang D. Influence of bio-fertilizer containing beneficial fungi and rhizospheric bacteria on health promoting compounds and antioxidant activity of *Spinacia oleracea* L. *Botanical studies*. 2017;58(1):1-9.
44. Michalak A. Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. *Polish Journal of Environmental Studies*. 2006;15(4).
45. Ali Bahmanyar M. Effects of Long - Term Irrigation using Industrial Wastewater on Soil Properties and Elemental Contents of Rice, Spinach, Clover, and Grass. *Communications in soil science and plant analysis*. 2008;39(11-12):1620-9.
46. Chen Y-S, Li J, Menon R, Jayaraman A, Lee K, Huang Y, et al. Dietary spinach reshapes the gut microbiome in an Apc-mutant genetic background: mechanistic insights from integrated multi-omics. *Gut microbes*. 2021;13(1):1972756.
47. Maeda N, Matsubara K, Yoshida H, Mizushima Y. Anti-cancer effect of spinach glycolipids as angiogenesis inhibitors based on the selective inhibition of DNA polymerase activity. *Mini reviews in medicinal chemistry*. 2011;11(1):32-8.
48. Araújo-Rodrigues H, Santos D, Campos DA, Guerreiro S, Ratinho M, Rodrigues IM, et al. Impact of Processing Approach and Storage Time on Bioactive and Biological Properties of Rocket, Spinach and Watercress Byproducts. *Foods*. 2021;10(10):2301.
49. Kuriyama I, Musumi K, Yonezawa Y, Takemura M, Maeda N, Iijima H, et al. Inhibitory effects of glycolipids fraction from spinach on mammalian DNA polymerase activity and human cancer cell proliferation. *The Journal of nutritional biochemistry*. 2005;16(10):594-601.
50. Gaikwad PS, Shete RV, Otari KV. *Spinacia oleracea* Linn: A pharmacognostic and pharmacological overview. *International Journal of Research in Ayurveda and Pharmacy (IJRAP)*. 2010;1(1):78-84.