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Antibacterial Effect of Successive Extracts of Leaves of *Spinacia oleracea* Linn

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ABSTRACT

The present study was undertaken with a view to evaluate the successive extracts of leaves of *Spinacia oleracea* Linn., for its antibacterial activity against various strains of gram positive and gram negative bacteria. The shade dried, powdered, defatted plant material was subjected to successive soxhlet extraction with Ethyl acetate and Ethanol. The plant powder and the extracts were subjected to preliminary phytochemical screening which revealed the presence of flavonoids, saponins, tannins, phenols, sugars, lipids, alkaloids, steroids. The *in vitro* antibacterial assay of successive extracts revealed variable degrees of antibacterial activity against different microorganisms. The ethylacetate extract showed effective inhibition against *Aeromonas and Salmonella species* whereas ethanolic extract showed relatively higher inhibitory potency against *Bacillus subtilis*. Both the ethylacetate and ethanolic extracts were found to be ineffective against *Staphylococcus aureus*.

Keywords: Spinach, successive extracts, antibacterial.

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INTRODUCTION

Worldwide, pharmacological industries and research institutions have launched a number of new antibiotics in the past few decades, yet resistance to these drugs by microorganisms has increased gradually. The genetic ability of the microorganisms to transmit and acquire resistance to drugs is the major concern especially for the patients with suppressed immunity and further new infections leading to high mortality[1]. The increasing prevalence of multi-drug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raised the specter of 'untreatable' bacterial infections and adds urgency to the search for new infection-fighting strategies[2, 3].Plants provide the major source of natural products for human health. Enormous antimicrobial studies were carried out worldwide on various plant extracts and many of them turned out to be therapeutic alternatives[4]. The practice of indigenous healing is now on the raise in developing countries in response to World Health Organization directives culminating in several pre-clinical and clinical studies that were scientifically proven to treat infections[5, 6]. Therefore, there is an everlasting need from natural sources with proven antimicrobial activity. This has led to the search for more effective lead compounds of plant origin, which could serve as source and template for various antimicrobial infections[7, 8]. Apart from its nutritional value, *Spinacia oleracea* Linn. of family Chenopodiaceae also possess wide medicinal properties. Though Spinach is most often used as a food, it is rich in flavonoids with anti-oxidant, antiproliferative, antiinflammatory, antihistaminic, CNS depressant, antibacterial, hepatoprotective, anthelmintic properties in addition to its many other benefits[9, 10]. The present study aims at exploring the antibacterial effects of successive ethyl acetate and ethanolic extracts of the leaves of *Spinacia oleracea* Linn., against various gram positive and gram negative bacteria.

MATERIALS AND METHOD

Selection of bacterial strains, growth medium and standard drug

Authentic bacterial strains of *Staphylococcus aureus, Bacillus subtilis, Salmonella species, Aeromonas species* were selected for the study. Mueller Hinton agar was selected as growth medium for bacterial strains, while Ampicillin (20µl/disc) was used as the standard drug to compare the efficacy of the selected plant parts.

Preparation of Inoculum

Stock cultures were maintained at 4°C on nutrient agar slant. Active cultures for experiments were prepared by transferring a loop full of culture from the stock cultures into the test tubes containing

nutrient broth, that were incubated at 24hrs at 37ºC. The Assay was performed by agar disc diffusion method

Plant Material

Fresh leaves of *Spinacia oleracea* Linn., were collected from Uthiramerur, Chengalpattu District, Tamil Nadu, during October. Fully grown leaves were collected in a fine dry weather and dried in a shed for 3 weeks. The plant specimen was identified and authenticated by Prof. Dr. Jayaraman, Plant Anatomy Research Centre, Tambaram.

Preparation of Plant Extracts

The shade dried and powdered leaves of *Spinacia oleracea* Linn., were first defatted with n-hexane and subjected to successive extraction using Ethyl acetate and Ethanol by continuous percolation process in Soxhlet apparatus. The successive extracts were concentrated and stored in airtight containers.

Qualitative & Quantitative Phytochemical Analysis

The successive ethylacetate and ethanolic extracts were subjected to preliminary phytochemical screening for the presence of bioactive compounds.

Antibacterial activity[11]

Antibacterial efficacy was determined by disc diffusion method on Muller Hinton agar (MHA) medium. Muller Hinton Agar (MHA) medium is poured in to the petriplate. After the medium was solidified, the inoculums were spread on the solid plates with sterile swab moistened with the bacterial suspension. The discs were placed in MHA plates and 20 µl of ethylacetate and ethanol extracts at 250µg/ml, 500µg/ml and 1000µg/ml were placed in the disc. The plates were incubated at 37ºC for 24 hrs. The antimicrobial activity was determined by measuring the diameter of zone of inhibition.

RESULTS AND DISCUSSION

Medicinal plants form the potential source of lead molecules for the development of various chemotherapeutic agents[12].Various research reports are available on the antibacterial, antifungal, anthelmintic, antiviral, antimolluscal and anti-inflammatory properties of plants [13,14,15,16,17,18]. But only few observations resulted in identifying the active compound responsible for the activity. Infections attributed to *Bacillus subtilis* include food poisoning, localized infections related to trauma (e.g. ocular infections), deep seated soft tissue infections, and systemic infections including meningitis, endocarditis, osteomyelitis, bacteremia, pneumonia and septicemia. However, these infections were found in patients in immune compromised states[19]. Various species of *Aeromonas*

were reported to cause gastrointestinal illness, soft tissue infections, pneumonia, meningitis, endocarditis, osteomyelitis, and septic arthritis [20]. In the present study, the defatted leaves of *Spinacia oleracea* Linn., were subjected to successive soxhlet extraction using ethylacetate and ethanol. Preliminary phytochemical screening was carried out to detect the presence of phytoconstituents and the results were tabulated (Table 1). Ethyl acetate extract was found to possess proteins, lipids, steroids, alkaloids, flavanoids, phenols and tannins whereas carbohydrates, proteins, saponins, glycosides, flavanoids, alkaloids, phenols and tannins were found to be present in the ethanolic extract of *Spinacia oleracea* Linn.

Table 1: Qualitative Phytochemical Screening of the Leaves of *Spinacia oleracea* Linn

S.No	Plant constituent	Powder	Ethylacetate	Ethanol
1	Carbohydrates	+	-	+
2	Flavonoids	+	+	+
3	Glycosides	+	-	+
4	Alkaloids	+	+	+
5	Phenols	+	+	+
6	Tannins	+	+	+
7	Terpenoids	-	-	-
8	Saponins	+	-	+
9	Proteins	+	+	+
10	Lipids	+	+	-
11	Steroids	+	+	-
12	Anthroquinones	-	-	-
13	Iridoid glycosides	-	-	-

The *in vitro* antibacterial assay of both ethylacetate and ethanol extracts of leaves of *Spinacia oleracea* Linn., was carried out by simple agar disc diffusion method against *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella species*, *Aeromonas species* and their activity potentials were qualitatively evaluated by the presence or absence of inhibition zones. The diameters of inhibition zones were measured in mm and results are presented in Table 2 and Table 3. Both the ethylacetate and ethanolic extracts showed a significant zone of inhibition against *Salmonella* species of which ethylacetate extract (7.2mm) was found to be effective compared to ethanolic extract (5.4mm).The ethylacetate extract showed effective inhibition against *Aeromonashydrophila* where as ethanolic extract (6mm) showed relatively higher inhibitory potency against *Bacillus subtilis*. Also the zone of inhibition was found to be dose dependent and showed the maximum at 1000µg/ml compared to the standard. However the extracts were ineffective against *Staphylococcus aureus*. Some of the phytochemical compounds viz., glycosides, saponins, tannins, flavonoids, terpenoids, alkaloids, have been reported to be responsible for antimicrobial activity [21, 22, 23]. In our study, the two

extracts showed varied response to bacterial strains. The degree of variation in antibacterial activity of the successive extracts can be attributed to the distribution of antimicrobial substances in the solvents of varying polarity [24].

Table 2: Zone of Inhibition (mm) of Ethanolic extract of leaves of *Spinacia oleracea* Linn

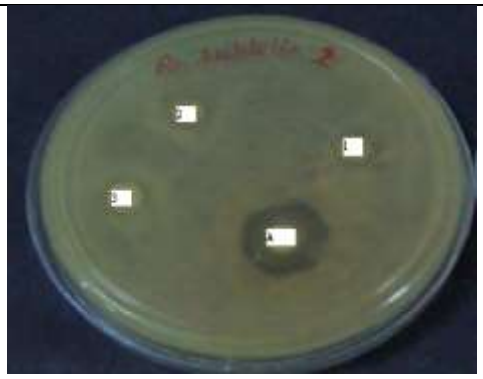
Organisms	Zone of Inhibition (mm) Concentration(µg/ml)			Antibiotic (mg/ml)
	1000	750	500	
Staphylococcus aureus	-	-	-	9.54±0.26
Bacillussubtilis	6±0.57	3.50±0.42	2.04±0.96	12.04±0.48
Aeromonas spp.	-	-	-	8.5±0.42
Salmonella spp.	5.40±2.40	3.68±0.24	-	8.64± 1.22

Values are mean inhibition zone (mm) ± S.D of three replicates

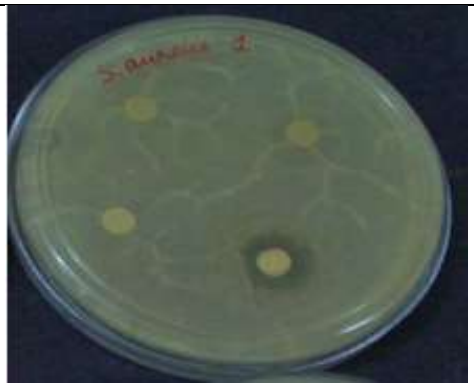
Table 3: Zone of Inhibition (mm) of Ethyl acetate extract of leaves of *Spinacia oleracea* Linn

Organisms	Zone of Inhibition (mm) Concentration(µg/ml)			Antibiotic (mg/ml)
	1000	750	500	
Staphylococcus aureus	-	-	-	9.16±0.83
Bacillussubtilis	-	-	-	9.16±0.74
Aeromonas spp.	5.64±*0.62*	3.64±0.10	1.24±0.1	7.28±0.88
Salmonella spp.	7.24±1.45	3.8±0.34	-	10.83±0.70

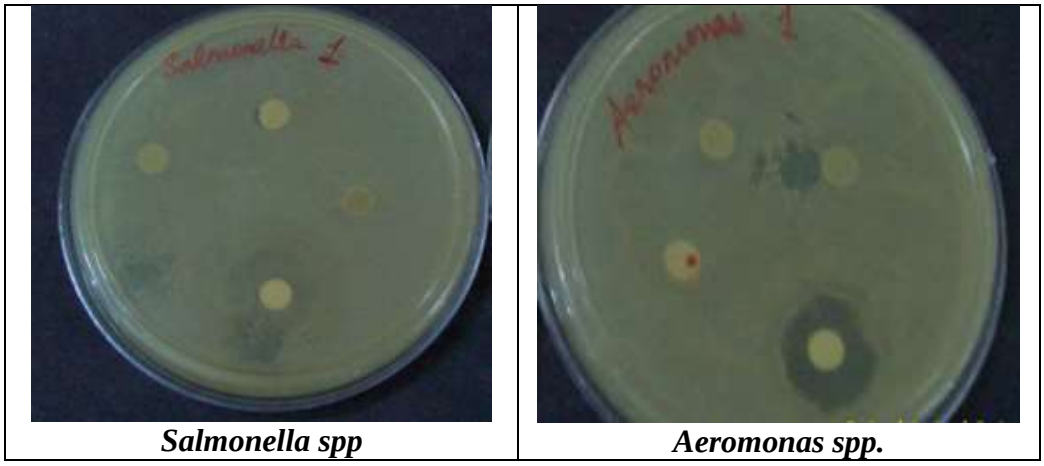
Values are mean inhibition zone (mm) ± S.D of three replicates



B.subtilis



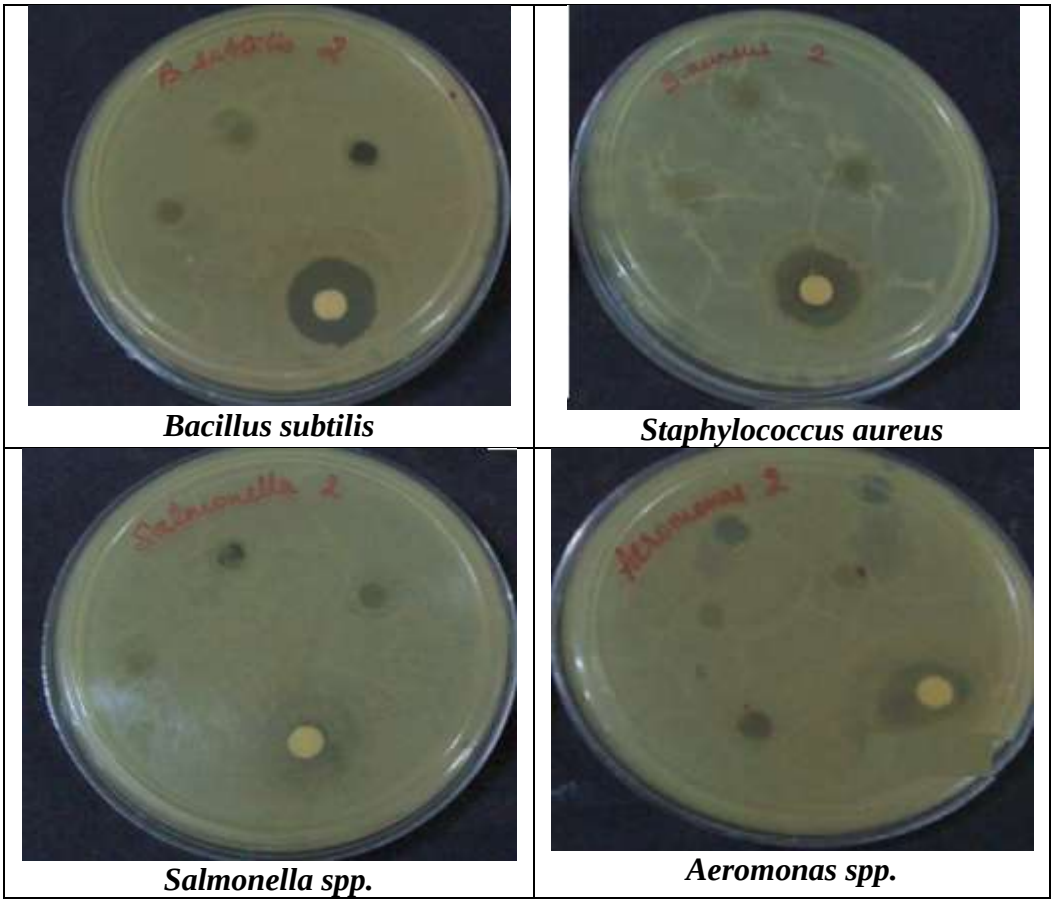
S.aureus



Salmonella spp ***Aeromonas spp.***

Figure 1: Zone of inhibition exhibited by ethanol extract

A-Ampicillin,1-1000µg/ml, 2-500µg/ml, 3-250µg/ml



Bacillus subtilis ***Staphylococcus aureus***

Salmonella spp. ***Aeromonas spp.***

Figure 2: Zone of inhibition exhibited by ethyl acetate extract

CONCLUSION

The present study was conducted to develop lead molecule for better and safer chemotherapeutic agents. Further studies are required to isolate and identify the pure component and establish the exact mechanism of action for antibacterial action of the plant extract.

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