Antioxidant and Antibacterial Activities of Phytochemicals in Methanolic Extracts of Five Underutilized Leafy Vegetables

Raj Adarsha[1], Sikdar Bratati[1], Roy Alokesh[1], Mukhopadhyay Asish Kumar[2] and Roy Sudipta[1*]
1. Department of Botany, University of Kalyani, Kalyani, Nadia, 741235 West Bengal, INDIA
2. Division of Bacteriology, National Institute of Cholera and Enteric Diseases, P-33, C.I.T. Road, Kolkata-700010, INDIA
*dr.sudiptaroy@gmail.com

Abstract

Leafy vegetables are important for their nutritive and medicinal values. However, many of them are less explored and underutilized. The present study evaluates the antioxidant and antibacterial activities present in five underutilized leafy vegetables such as Alternanthera philoxeroides, Boerhavia diffusa, Enydra(=Enhydra) fluctuans, Glinus oppositifolius and Suaeda maritima. Methanolic extracts from leafy edible parts of these plants possessed diverse phytochemicals. Of these plants, the polyphenol contents like total phenolic (TPC), total flavonoid (TFC) and total tannin (TTC) were the highest in E. fluctuans with significant variations concerning other plants. Also, the antioxidant activities of E. fluctuans were greater comparing other plants; the parameters were the total antioxidant activity (TAA), ferric reducing power assay and ability to scavenge DPPH, ABTS and superoxide radicals.

The effect of varying extract-quantities revealed the highest antibacterial activity of the E. fluctuans extracts with substantial inhibition zones against six bacterial strains. Accordingly, the E. fluctuans extracts showed the lowest MIC (2.75±0.35 mg ml^{-1}) and MBC (3.50±0.71 mg ml^{-1}), albeit with significant variations with ampicillin. The study highlights that these plants are the untapped repertoires of natural antioxidants and antimicrobials for dietary and therapeutic uses.

Keywords: Antimicrobial, *E. fluctuans*, Leafy species, Phenolics, Radical scavenging.

Introduction

Leafy vegetables are a rich source of nutrition. Humans have used these from the early days of civilization as food and other well-beings.[43] Among these leafy species, a large number of plants are still underutilized. The possible reasons are their restricted dietary usage in a particular area, lack of global recognition and limited exploitation of health benefits.[51]A vast majority of underutilized leafy species grow naturally as weeds in the fallow lands. These are cheaper in market value compared to commercially grown vegetables.[8] Due to the higher nutritional values of many such leafy vegetables, the underprivileged ethnic groups in rural areas traditionally use these plants, especially during scarcity of food.[18]

Apart from their higher nutrient and micronutrient contents, these plants also possess an ample amount of natural antioxidants like vitamins, polyphenols etc. which minimize oxidative damage.[47] A good number of studies also highlighted the health benefits of these leafy species in nutritional deficiency, digestive disorders, microbial infections, diabetic complications, hepatoprotection and cardiovascular diseases.[22,25,31]

Antioxidant molecules scavenge reactive oxygen species (ROS) during oxidative stress of an organism is maintaining a balance between oxidation and anti-oxidation.[13] The natural antioxidants primarily come from different plant parts. The leaves contribute a major share of it. The natural antioxidants of plant origin are mainly polyphenols (phenolic acids, flavonoids, tannins, anthocyanins, lignans and stilbenes), carotenoids and vitamins.[16] Plant-derived antioxidants manifest diverse biological effects that include antibacterial, antiviral, anti-inflammatory, anti-analgesic, anticancer, anti-ageing etc.[45]

Pharmacologically important plant-derived compounds, secondary metabolites, play an important role in plant defence against many bacterial diseases.[17] Antibiotics are the prime therapeutic agents in combating bacterial infections for several decades. However, their indiscriminate use led to the development of various antibiotic-resistant bacterial strains.[15] The situation has worsened in recent times due to the emergence of multidrug-resistant bacteria. [16]

It prompted the researchers to explore novel antimicrobials from plant origin. The phytomedicines from phytochemicals have emerged as the new therapeutic targets against microbes. These are safe, reliable and cheaper than synthetic drugs. Most importantly, these have few or no side effects.[21]

The study includes five underutilized leafy plant species namely *Alternanthera philoxeroides* (Mart.) Griseb. (Family: Amaranthaceae), *Boerhavia diffusa* L. (Family: Nyctaginaceae), *Enydra fluctuans* DC. (Family: Asteraceae: = *Enhydra fluctuans* Lour.), *Glinus oppositifolius* (L.) Aug. DC (Family: Molluginaceae) and *Suaeda maritima* (L.) Dumort. (Family: Amaranthaceae), which are commonly used as green vegetables in different locales and are known for their medicinal values.

**** Author for Correspondence***

A. philoxeroides, a native of South America, grows profusely in Gangetic plains mostly as a marshy weed.[9] *B. diffusa* is a creeping herb and the leaves are used as a vegetable as well as herbal medicine in many parts of India. The Indian vernacular name of the plant is 'punarnava', which reflects its rejuvenating activity as mentioned in Ayurveda.[2] *E. fluctuans* is an aquatic or marshy herb with wider distribution in India and is used for its edible leaves. The plant also possesses immense therapeutic potential with folkloric uses to cure digestive complications and nervous disorders.[24] *G. oppositifolius* is a much-branched annual herb that grows in dry areas as well as dried-up ditches, pools and rice fields. The leaves and young stems of the plant are slightly bitter and used for both food and medicine.[28] *S. maritima*, an annual herb, grows as a salt marsh mangrove associate in Sundarbans and other coastal areas of India. The plant is edible as a leafy vegetable with a pleasant salty flavour and is often used in salads and seasonings.[38]

The present work represents a detailed comparative study of these five plant species. It includes phytochemical analyses, diverse antioxidant assays and antibacterial properties using dose-dependent methanolic extract (3, 6 and 12 mg) treatments against three Gram-negative and three Gram-positive bacteria. The work aims to screen lesser-known vegetables with potent antioxidant and higher antibacterial activities which can be exploited in the future for the development of natural antioxidants and novel antimicrobials.

Material and Methods

Plant specimens: Among the five studied plant species, *A. philoxeroides*, *B. diffusa*, *E. fluctuans* and *G. oppositifolius* were collected from the Kalyani University campus and adjoining area, Nadia, West Bengal (22.989132° N and 88.447412° E, 11 m above sea level). We collected *S. maritima* from the salt marshes of Sundarbans at Bakkhali (21°33′47″N and 88°15′34″E, 4 m above sea level), South 24-Parganas. We identified the plant specimens following the standard taxonomic manual and authentication from the Botanical Survey of India (BSI), Kolkata, West Bengal, India. The voucher specimens were deposited in the herbarium at the Department of Botany, University of Kalyani.

Preparation of methanolic extracts: The aerial parts of the plants containing healthy leaves and young twigs were collected for the extraction of plant samples. The plant parts (10 g) were cleaned with water and soaked with tissue paper to remove the adherent water. The extractions were made with 90% methanol (v/v) keeping the sample to solvent at 1:20 (w/v) ratio for 48 h. The extractions (cold maceration method) were performed at room temperature (RT; 30±1ºC) with shaking (40 rpm). The solvent extracts were filtered (Whatman No. 1), concentrated and dried using a rotary vacuum evaporator at 50ºC (Rotavapor R-II-HB; Büchi, Switzerland) and the resulting crude extracts were kept at 4ºC until further use.

Phytochemical analyses

Qualitative tests: Qualitative tests for different phytochemicals like sugars, phenolics, flavonoids, tannins, terpenes, alkaloids, saponins and iridoids in the crude extracts were carried out following different tests like Benedict's test for sugars[46], ferric chloride test for phenolics and tannins[52], sodium hydroxide test for flavonoids[52], p-anisaldehyde test for terpenes[19], Dragendorff's test for alkaloids[19], froth test for saponin[20] and Trim-Hill test for iridoids[53].

Quantitative determination of phenolic components: Estimation of the phenolic components in the extracts like total phenolic content (TPC), total flavonoid content (TFC) and total tannin content (TTC) was performed following previously described methods of Ojha et al.[33] Briefly, TPC was estimated by mixing 0.5 ml extract solution, 1 ml FC reagent (Folin-Ciocalteu reagent) and 2 ml Na_2CO_3 (0.7 M) incubated for 45 min in dark and absorbances were measured at 765 nm. The amounts of TPC were expressed as mg gallic acid equivalent (GAE)/ g extract.

TFC was determined by sequential addition of 0.5 ml test sample, 1.5ml ddH_2O, 0.2ml $NaNO_2$ (5%), 0.2ml $AlCl_3$ (10 %) and 0.6 ml NaOH (1 N), mixed and incubated for 10 min in dark. The absorbances were measured at 510 nm and the flavonoid contents were represented as mg quercetin equivalent (QE)/ g extract.

TTC contents were detected by preparing reagent mixtures consisting of 0.5 ml sample, 8 ml ddH_2O, 0.5 ml $FeCl_3$ (0.1 M) and 0.5 ml potassium ferricyanide (8 mM). The mixtures were incubated at RT for 10 min, the absorbances were recorded at 720 nm and expressed as mg GAE/ g extract.

Antioxidant assays

Total antioxidant activity (TAA): The assay was performed[33] based on the phospho-molybdenum method in which 0.5ml extract sample was mixed with 3 ml reagent solution containing 28 mM sodium phosphate, 600 mM H_2SO_4 and 4 mM ammonium molybdate and incubated at 90±1ºC for 1½ h in a water bath. The absorbances were recorded at 695 nm and represented as mg ascorbic acid equivalent (AAE)/ g extract.

Ferric reducing antioxidant power (FRAP): The reducing power assay by Benzie and Strain[7] was modified[33] with the preparation of assay mixture consisting of 0.2 ml extract solution (5mg/ml) and 1.8 ml FRAP reagent, containing 0.3 M sodium acetate buffer (pH 3.6); 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) and 20 mM $FeCl_3$. The mixtures were kept for 5 min at 37°C, absorbances were recorded at 594 nm and the FRAP values were expressed as mM Fe^{2+}/ mg of sample. Ascorbic acid was used as the reference standard (positive control) in the assay.

DPPH radical scavenging assay: This assay was performed by the previously documented method of Ojha et al.[33]

Different concentrations of extract samples (100 µl) were mixed with 1.2 ml 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution (6×10^{-5} M), incubated at RT for 15 min in dark and the absorbances were monitored at 517 nm. The percent scavenging of the DPPH$^{\bullet}$ radical was represented by:

$$\text{Scavenging activity (\%)} = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100$$

where $A_{control}$ = absorbances of the control and A_{sample} = absorbances of test samples.

The IC_{50} values of the radical (representing 50 % scavenging) were determined from the percent scavenging activities of increasing extract concentrations and reference control ascorbic acid.

ABTS radical scavenging assay: The $ABTS^{\bullet+}$ scavenging assay described by Re et al[41] was also modified by Ojha et al[33] in which increasing concentrations of extract samples (100 µl) were added with 900 µl of the 2,2´-azino-bis-3ethylbenzothiazoline-6-sulphonic acid (ABTS) solution (generated by mixing 7 mM ABTS in water with 2.45 mM potassium persulfate; diluted with ethanol to obtain absorbance of 0.70) and the absorbances were recorded at 734 nm. The percent scavenging activity and IC_{50} value of the extracts and reference (ascorbic acid) were calculated from the obtained data.

Superoxide (SO) scavenging activity: The SO assay[33] based on the previously described method of Beauchamp and Fridovich[6] was performed by adding 1 ml sample solution, 1.8 ml sodium phosphate buffer (50 mM; pH 7.6), 20 µl riboflavin (2.66 mM), 80 µl ethylenediaminetetraacetic acid (EDTA) (12 mM) and 100 µl nitro blue tetrazolium (NBT) (1.22 mM). The reaction was induced by light, kept for 90 seconds at RT and the absorbances were measured at 590 nm in comparison with a blank (non-illuminated reaction). Results were represented as IC_{50} values from the percent scavenging of the varying extract concentrations and quercetin as reference.

Antibacterial assay

Bacterial strains, media and growth conditions: The bacterial strains used as test organisms are *Escherichia coli* MTCC 443, *Salmonella enterica* serovar Typhi C-6953, *Vibrio cholerae* N16961, *Bacillus subtilis* MTCC 121, *Staphylococcus aureus* ATCC 25923 and *Staphylococcus epidermidis* MTCC 3086. All the strains were preserved in glycerol (40%) supplemented with NB (HiMedia) at -80ºC. Before commencement of experiments, bacteria were cultured aerobically either in NB or TSB (HiMedia) at 37ºC with agitation (60 rpm) or on nutrient agar (NA) / tryptone soya agar (TSA) plates. During antibacterial assays, the strains were grown in Mueller-Hinton agar (MHA) media or in Mueller-Hinton broth (MHB) at 37ºC.

Disc diffusion assay: The assay was performed essentially following Kirby-Bauer method[5] with minor modifications. Bacterial suspension (100 µl) of each strain containing approximately 2×10^8 colony forming units (CFU)/ml (prepared from overnight cultures) was uniformly spread on MHA plates. In the meantime, plant extracts of different concentrations (3, 6 and 12 mg) were prepared from a stock solution in methanol (200 mg ml^{-1}; w/v), applied on sterile paper discs (5 mm) and allowed to dry. Methanol (60 µl) was used as vehicle (negative) control while ampicillin (2, 4 and 6 µg in 20µl double distilled water) was used as a positive control. Finally, the sample and control discs were aseptically placed over the bacteria seeded MHA plates and incubated for 24 h at 37ºC. The diameter of the inhibition zones was measured at 24 h. All the treatments were determined in triplicate.

Determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC): A modified micro-well dilution method[23] was used for the determination of MIC and MBC of plant extracts against six bacterial strains. The extract solutions were serially diluted with MHB (0.5-15 mg ml^{-1}) and dispensed in the wells of a micro-well polystyrene plate (Tarsons, India). Overnight grown bacterial cultures (10 µl) were added in each well to reach the final volume of 200 µl in each well containing inoculum of 1×10^7 CFU/ml. Different concentrations of antibiotic (ampicillin) were used similarly as a positive control. The extract-control (medium with extracts of different dilutions) and inoculum control (medium with the only inoculum) were used as negative controls.

Following the treatments (in triplicate), the micro-plates were covered by a lid and incubated for 18 h at 37°C with gentle shaking (40 rpm). MIC was determined by estimating the lowest concentration of the extract or antibiotic that inhibited the apparent growth of the bacteria. The bacterial growth was assessed by their absorbances at 620 nm using a microplate reader (ELx800, BioTek, Switzerland).

MBC was estimated using the same setup for MIC determination and a loop full of bacterial suspension (10 µl) from each well was taken and streaked on properly labelled MHA plates.[29] The plates were incubated for 24 h at 37°C and the presence or absence of bacterial growth on the streaked area was observed and recorded. MBC was calculated by determining the lowest concentration of the test sample at which all the inoculated microorganisms were killed.

Statistical analyses: The experiments were carried out in triplicate and represented as mean ± standard error (SE). Results obtained for quantitative analysis of phenolic components, antioxidant assays, MIC and MBC were analyzed using One-way Analysis of Variance (ANOVA) with post hoc Tukey's multiple comparison test ($p<0.05$).

Critical difference (CD) was performed to determine a significant level ($p<0.05$) if any, between/among the different extract concentrations in disc diffusion assay. All

statistical analyses were performed with Microsoft Excel and SPSS version 20 (IBM, USA).

Results and Discussion

Phytochemical analyses: The qualitative analyses of phytochemicals (Table 1) from methanolic extracts reveal the presence of total phenolics, flavonoids, tannins and alkaloids in all the plants at high to moderate levels. *E. fluctuans* extract contains higher phenolics and flavonoids while higher tannin and alkaloid contents are observed in *B. diffusa*. Terpenes manifest higher presence in *E. fluctuans* and *S. maritima*, however, it is low in *G. oppositifolius* and absent in the other two species. Iridoid glycosides and sugars are present in all the species except *S. maritima* whereas saponin is detected only in *A. philoxeroides* and *G. oppositifolius* at moderate and higher levels respectively.

Methanol is used as an extraction solvent in the study due to its higher polarity index (5.1) which is better suited for extracting polar phytoconstituents.[49] Occurrence of diverse phytochemical groups in these plants is affirmed by the previous reports[2,14,28,40,44] in different solvent extracts. However, methanolic extractions are reported to yield higher amounts of phytochemicals in different plants like *Paederia foetida*[33] and *Cajanas cajan*[27].

The quantitative estimation of total phenolics (GAE/g), flavonoids (QE/g) and tannins (GAE/g) from plant extracts is presented in figure 1. Results reveal that the highest estimates of TPC (4.65±0.29), TFC (1.92±0.07) and TTC (4.75±0.31) are observed in *E. fluctuans* with significant ($p<0.05$) differences from other studied plant species. In comparison, the TPC and TTC estimates in different plants are in the order of *E. fluctuans*>*B. diffusa*>*A. philoxeroides*>*G. oppositifolius*>*S. maritima* while the order in TFC alters as the estimate of *B. diffusa* is lower than *A. philoxeroides* and *G. oppositifolius*.

All the plant species under study are reported to contain TPC, TFC and TTC in varying amounts.[9,24,28,34,35] However, the present study highlights the comparison of polyphenol contents among the five leafy vegetables. It represents the superiority of *E. fluctuans*. The richness of phenolics in leaves is associated with its photosynthetic ability as evidenced by the up-regulation of phenolic compounds in photosynthetic tissues.[55] This observation may justify the lower yield of phenolics in *G. oppositifolius* and *S. maritima* with reduced leaf area than the other three plants. Furthermore, the mangrove associate herb *S. maritima* grows under salinity stress[28] with special adaptive growth parameters which may account for lower phenolic yield.[1]

Table 1
Qualitative estimation of phytochemicals in methanolic extracts of five leafy vegetables.

Plant Species	Phytochemicals							
	Sugars	Phenolics	Flavonoids	Tannins	Terpenes	Alkaloids	Saponins	Iridoids
A. philoxeroides	++	+	+	+	–	+	+	++
B. diffusa	+	+	+	++	–	++	–	++
E. fluctuans	+	++	++	++	++	+	–	+
G. oppositifolius	++	+	+	+	+	+	++	+
S. maritima	–	+	+	+	++	+	–	–

'++' High, '+' Moderate, '–' Absent

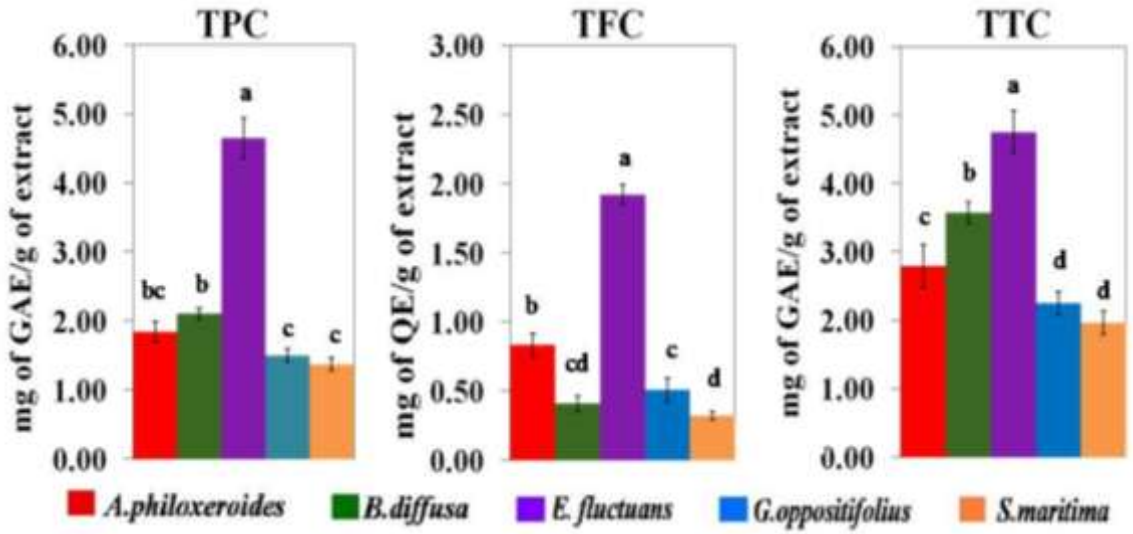

Figure 1: Quantitative estimation of Total Phenolic Content (TPC), Total Flavonoid Content (TFC) and Total Tannin Content (TTC) in five leafy vegetables.
Different alphabets used in figure represent significant differences ($p<0.05$) as calculated by Tukey's test

Antioxidant activities: The antioxidant activities of the plant species assessed by five different assays are presented in table 2. TAA activity (µg AAE/mg) is maximum in *E. fluctuans* (5.99±0.63) followed by *A. philoxeroides* (3.72±0.31), *B. diffusa* (3.34±0.28), *G. oppositifolius* (2.92±0.14) and *S. maritima* (2.27±0.18). Among the five plants, TAA activity of *E. fluctuans* is noticeable (1.6, 1.8, 2.2 and 2.6-fold more than *A. philoxeroides*, *B. diffusa*, *G. oppositifolius*, *S. maritima* respectively) and shows significant variations ($p<0.05$) from other studied species.

Among the different extracts, maximum reducing power activity by FRAP (mM Fe^{2+}/ mg) assay is also found in *E. fluctuans* (28.23±2.80) which is much closer to the activity of reference control ascorbic acid (34.46±1.04), though differing significantly. The FRAP activity of *S. maritima* is also noteworthy with a sufficiently higher estimate (19.37±1.91) than *A. philoxeroides* (14.73±1.23), *G. oppositifolius* (10.38±1.45) and *B. diffusa* (8.72±1.05) with significant variations ($p<0.05$).

The DPPH, ABTS and SO radical scavenging assays are represented with their IC_{50} (µg ml^{-1}) values. In all scavenging assays, lower IC_{50} values of *E. fluctuans* among all methanolic extracts reflect its high antioxidant potential. The IC_{50} value of *E. fluctuans* (113.84±2.73) detected in DPPH assay is closer to the standard antioxidant ascorbic acid (28.69±1.50) than the other leafy vegetables like *S. maritima* (444.80±48.89), *A. philoxeroides* (758.55±46.42), *G. oppositifolius* (869.41±24.49) and *B. diffusa* (981.17±14.59). However, the estimates are significantly ($p<0.05$) different from each other. A similar trend is observed in ABTS assay with a minimum IC_{50} value in *E. fluctuans* (98.84±8.85) followed by *S. maritima* (446.55±28.77), *A. philoxeroides* (586.34±38.17), *G. oppositifolius* (712.26±19.39) and *B. diffusa* (747.80±10.19) showing significant variations ($p<0.05$) between each other excepting between *G. oppositifolius* and *B. diffusa*.

In SO assay, the highest activity is observed in *E. fluctuans* extract. The lower IC_{50} values of *E. fluctuans* (146.91±13.14) and *S. maritima* (252.98±22.85) reflect their greater antioxidant activities when compared with the reference standard quercetin (267.35±11.33). It is worth mentioning that significant variations ($p<0.05$) exist between *E. fluctuans* and quercetin though the variation between *S. maritima* and quercetin is nonsignificant ($p>0.05$). The IC_{50} values of *A. philoxeroides* (659.70±33.91), *G. oppositifolius* (716.48±10.46) and *B. diffusa* (801.42±3.95) also vary significantly ($p<0.05$) from *E. fluctuans* and *S. maritima*.

The study highlights the significant antioxidant potential of five leafy vegetables using total antioxidant, reducing power and radical scavenging assays as it has been evidenced that the efficacy of antioxidant is better judged through different assays based on distinct principles.[39] Presence of antioxidant activities at varying amounts in *E. fluctuans*[42], *S. maritima*[28], *A. philoxeroides*[9], *G. oppositifolius*[3] and *B. diffusa*[34] has been reported earlier. However, a comparative account of antioxidant activities among five leafy greens in this study reveals the superiority of *E. fluctuans* in different antioxidant assays. In radical scavenging assays like DPPH and ABTS, the efficacy of *E. fluctuans* extract is much closer to the reference standard (ascorbic acid) and in SO assay, even the activity is greater than the reference (quercetin).

It suggests the potent antioxidant nature of *E. fluctuans* among the five plants. Such robust activity in *E. fluctuans* can be attributed to their rich phenolic contents as polyphenols are considered as one of the major contributors of antioxidants in plants.[36,50] Apart from *E. fluctuans*, the antioxidative efficacy of *S. maritima* is also notable. In reducing and in radical scavenging assays, the activity is substantially high. However, in TAA it is not at par with the other extracts.

Table 2
Antioxidant activities of methanolic extracts from five leafy vegetables

Antioxidant assays	Plant species					Reference standard*
	A. philoxeroides	***B. diffusa***	***E. fluctuans***	***G. oppositifolius***	***S. maritima***	
TAA mg AAE/g of extract	3.72±0.31^{b}	3.34±0.28^{bc}	5.99±0.63^{a}	2.92±0.14^{bc}	2.27±0.18^{c}	-
FRAP mM Fe^{2+}/ mg of extract	14.73±1.23^{c}	8.72±1.05^{d}	28.23±2.80^{b}	10.38±1.45^{d}	19.37±1.91^{b}	34.46±1.04^{a}
DPPH IC_{50} value (µg ml^{-1})	758.55±46.42^{d}	981.17±14.59^{f}	113.84±2.73^{b}	869.41±24.49^{e}	444.80±48.89^{c}	28.69±1.50^{a}
ABTS IC_{50} value (µg ml^{-1})	586.34±38.17^{d}	747.80±10.19^{e}	98.84±8.85^{b}	712.26±19.39^{e}	446.55±28.77^{c}	34.92±3.97^{a}
SO IC_{50} value (µg ml^{-1})	659.70±33.9^{c}	801.42±3.95^{e}	146.91±13.14^{a}	716.48±10.46^{d}	252.98±22.85^{b}	267.35±11.33^{b}

Different alphabets within a row represent significant differences ($p<0.05$) as calculated by Tukey's test.
* Ascorbic Acid (FRAP, DPPH, ABTS); Quercetin (SO)

The antioxidative activities of *S. maritima* are implicated in polyphenolics[1] and an increase in antioxidant phenolics like quercetin during salt stress is exemplified.[26] The plausible explanation for greater reducing and radical scavenging activities in *S. maritima* may be due to an enrichment of antioxidant phenolics and their synergy in the extract[35] albeit with lower phenolic contents.

Antibacterial activities: The antibacterial activities of methanolic extracts (3, 6 and 12 mg) from five leafy vegetables measured by disc diffusion assay against six bacterial strains are depicted in table 3. The plant extracts are inhibitory to the test organisms at varying degrees, though foreseeably manifestation of inhibition is maximum at their highest concentration (12 mg). The effect is dose-dependent and the variations among the extract concentrations are most significant ($p<0.05$).

E. fluctuans represents the highest inhibitory activity (Table 3, Figure 2) among the five plants with maximum inhibition zone (mm) against *B. subtilis* (21.30±0.66) followed by *E. coli* (19.10±1.43), *S. epidermidis* (16.20±0.53), *S. aureus* (13.20±0.89), *V. cholerae* (11.90±0.98) and *S.* Typhi (10.20±0.43). The effect is visible from the starting concentration (3 mg) and shows significant ($p<0.05$) increase in activity with increments in extract concentrations.

The efficacy of *S. maritima* is also marked (Table 3) and represents the second-highest among the five plants. It shows a maximum inhibition zone against *B. subtilis* (21.30±0.66) and a minimum against *V. cholerae* (8.20±1.43). However, significant variations ($p<0.05$) of inhibition zones are only evident at higher extract concentrations (6 and 12 mg). The other three plant species *G. oppositifolius*, *B. diffusa* and *A. philoxeroides* show significant inhibitory effect only at their highest treatment concentrations (12 mg). However, at lower concentrations, the inhibition zones are either undetected or insignificantly present.

The MIC and MBC of the leafy species extracts against six bacteria are determined to assess their antimicrobial potency and presented in figure 3. The minimum inhibitory effect of plant extracts ranges from 2.75±0.35 mg ml^{-1} to 12.00±0.71 mg ml^{-1} and bactericidal efficacy ranges from 3.50±0.71 mg ml^{-1} to 13.00±0.71 mg ml^{-1}. Among the five plants, *E. fluctuans* extract demonstrates the highest activities against both *B. subtilis* and *E. coli* (MIC/MBC: 2.75±0.35/ 3.50±0.71) followed by *S. epidermidis* (MIC/MBC: 4.25 ± 0.35/5.25 ±0.35), both *S.* Typhi as well as *V. cholerae* (MIC/MBC: 5.25±0.35/6.25±0.35) and *S. aureus* (MIC/MBC: 5.75±0.35/6.75±0.35).

These estimates show significant variation ($p<0.05$) among the other plant extracts as well as with reference antibiotic ampicillin. The ampicillin as positive control demonstrates the lowest MIC and MBC with significant differences ($p<0.05$) among all the treatments.

Table 3
Antibacterial activities of methanolic extracts of five plant species against six bacteria by disc diffusion assay

Bacterial strains	**Inhibition zone (mm) at different extract concentration (mg) in plant species**															
	A. philoxeroides			***B. diffusa***			***E. fluctuans***			***G. oppositifolius***			***S. maritima***			**CD ($p<0.05$)**
	3	**6**	**12**	**3**	**6**	**12**	**3**	**6**	**12**	**3**	**6**	**12**	**3**	**6**	**12**	
E. coli	6.30 ± 0.90	6.80 ± 1.10	7.70 ± 0.23	5.80 ± 1.12	8.20 ± 1.56	10.10 ± 0.81	11.20 ± 0.31	13.90 ± 0.89	19.10 ± 1.43	5.80 ± 1.11	7.80 ± 0.67	10.80 ± 0.23	9.10 ± 0.78	9.50 ± 0.33	12.30 ± 0.65	**1.66**
V. cholerae	ND	ND	5.80 ± 0.32	ND	ND	7.20 ± 0.56	5.80 ± 0.43	8.80 ± 0.54	11.90 ± 0.98	ND	ND	6.20 ± 0.32	ND	6.20 ± 1.05	8.20 ± 1.43	**1.21**
***S.* Typhi**	ND	ND	7.30 ± 0.65	ND	ND	6.30 ± 0.76	6.00 ± 0.65	7.80 ± 0.54	10.20 ± 0.43	ND	5.90 ± 0.26	7.20 ± 0.45	5.80 ± 0.75	6.70 ± 0.54	9.90 ± 0.78	**0.81**
B. subtilis	7.20 ± 0.75	7.30 ± 0.76	9.20 ± 0.43	ND	5.80 ± 0.84	7.20 ± 1.12	13.10 ± 0.34	16.20 ± 0.67	21.30 ± 0.66	ND	5.80 ± 0.98	6.30 ± 0.89	7.30 ± 0.52	12.20 ± 0.56	16.20 ± 0.78	**1.36**
S. epidermidis	ND	ND	7.30 ± 0.78	ND	ND	8.20 ± 0.65	6.20 ± 0.78	11.20 ± 0.67	16.20 ± 0.53	ND	ND	8.30 ± 0.56	ND	7.30 ± 1.14	9.30 ± 0.89	**1.01**
S. aureus	ND	ND	6.80 ± 0.56	ND	ND	7.90 ± 0.23	6.40 ± 0.98	10.20 ± 0.87	13.20 ± 0.89	ND	6.20 ± 0.56	9.20 ± 0.67	ND	7.20 ± 0.67	10.20 ± 0.56	**0.94**

ND: Not detected; CD: Critical difference

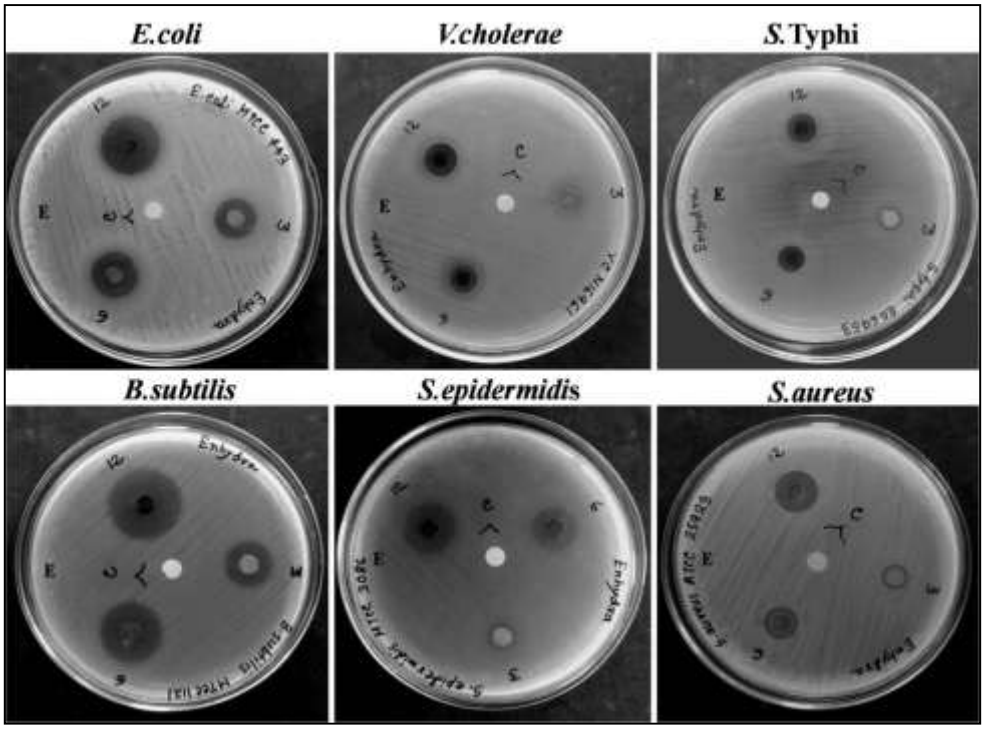

Figure 2: Growth inhibition zones of different bacterial strains by varying methanolic extract concentrations (3, 6 and 12 mg) of *E. fluctuans* (E); C represents vehicle control.

In *S. maritima*, the activities are substantial but lower than *E. fluctuans* showing significant variation ($p<0.05$) and differ in the order of bacterial susceptibilities like *B. subtilis* (MIC/MBC: 5.00±0.71/6.00±0.71), *E. coli* (MIC/MBC: 5.25±0.35/6.25±0.35), *S. epidermidis* (MIC/MBC: 6.00±0.71/7.00±0.71), *S. aureus* (MIC/MBC: 8.25±0.35/ 9.00±0.71) and both *S.* Typhi and *V. cholerae* (MIC/MBC: 8.50±0.71/9.50±0.71). The extracts of the other three leafy species show reduced activities and comparisons among them ($p<0.05$) reveal a better activity of *B. diffusa* than *G. oppositifolius* and *A. philoxiroides*. The least activity representing the highest MIC/MBC among all the treatments is observed in *A. philoxiroides* against *V. cholerae* (12.00±0.71/13.00±0.71).

The methanolic extracts of five leafy vegetables manifest substantial antibacterial efficacy against Gram-positive and Gram-negative bacteria. The inhibitory activity of plant microbials is reported to be conferred by a different group of secondary metabolites.[11] In this study, *E. fluctuans* extract shows significant activity representing the highest among the five plants which indicates higher amounts of bioactive phytochemicals in the extract.[44]

Studies on microbial inhibition by *E. fluctuans* are meagre, however, available reports indicate moderate inhibition with methanolic extract[24] and toluene extract[18] against various microorganisms. The superior activity of *E. fluctuans* in this study may be attributed to the phenolic contents as it possesses the highest TPC, TFC and TTC estimates than the other tested plant extracts. The role of phenolic components in antibacterial activity is also substantiated from various plants.[4,12]

The activity of *S. maritima* is also remarkable with the second-highest MIC and MBC values among the studied plants; however, its phenolic contents are significantly low. The effectiveness of the methanolic extracts[30] and its hexane fraction[32] against various bacteria and even non-polar solvent extracts[54] of the species is documented for antibacterial activities. It points towards diverse antimicrobials in *S. maritima* with distinct solubility in different solvents.

The enrichment of methanol extractable bioactive compounds, particularly phenolics, may be attributed to the observed activities in the present study against the variety of bacteria. The role of polyphenols in inhibiting bacterial growth has also been found in different studied plants.[10]

The other three species *B. diffusa*, *G. oppositifolius* and *A. philoxiroides* represent better antibacterial activities only at higher concentrations against the bacteria under study. Relatively lower activities by these plants may be due to the reduced amount of the bioactive antimicrobials in the solvent extracts which is in conjunction with the studies showing low to moderate antimicrobial activities using methanolic extracts of these three species.[2,28,40]

Comparison among bacterial strains highlights a higher degree of susceptibility towards normal laboratory strains like *B. subtilis* and *E. coli* followed by Gram-positive pathogens *S. epidermidis* and *S. aureus* while Gram-negative clinical isolates like *S.* Typhi and *V. cholerae* are less susceptible. The cell wall architectures of the Gram-positive organisms make them more susceptible to antimicrobial compounds than the Gram-negative ones.[37]

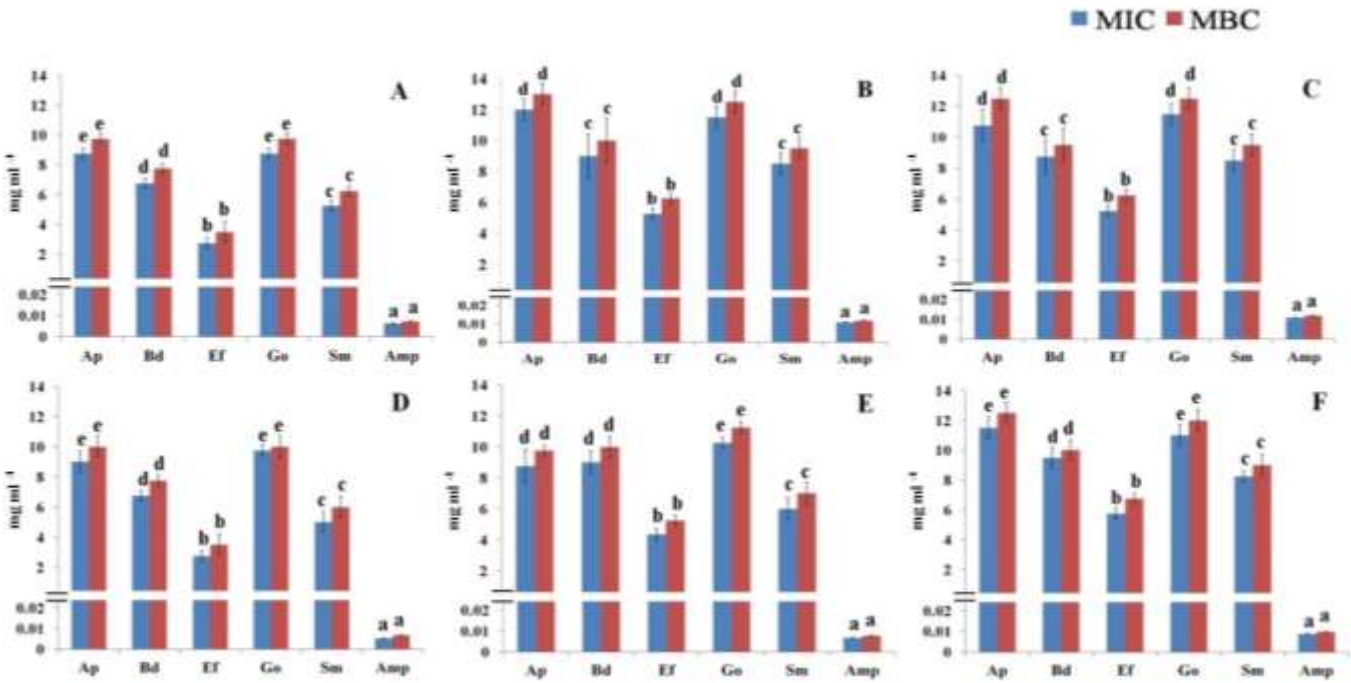

Figure 3: MICs and MBCs of methanolic extract of five leafy vegetables (Ap = *A. philoxeroides*, Bd = *B. diffusa*, Ef = *E. fluctuans*, Go = *G. oppositifolius*, Sm = *S. maritima*) and ampicillin (Amp) against six bacterial strains (*E. coli*: A, *V. cholerae*: B, *S.* Typhi: C, *B. subtilis*: D, *S. epidermidis*: E, *S. aureus*: F)."
Different alphabets used in figure represent significant differences ($p<0.05$) calculated by Tukey's test.

However, the greater susceptibility of laboratory strains than clinical isolates may develop due to repeated freeze-thaw cycles during their recurrent culture over a longer period.[48] Thus, comparisons among five plants for their phytochemical contents, antioxidant and antibacterial activities highlight the pre-eminence of *E. fluctuans*.

Conclusion

The study manifests the phytochemical diversity and phenolic contents in five leafy vegetables from their methanolic extracts. Antioxidant efficacy of these plants measured by total antioxidant, reducing power and radical scavenging assays reveals substantial activities in *E. fluctuans* and *S. maritima*. These five plants also represent antibacterial activities against Gram-positive and Gram-negative bacteria showing maximum efficacy in *E. fluctuans*.

The superiority of *E. fluctuans* in antioxidant and antibacterial activities is evident among the five leafy vegetables which may be corroborated with the higher phenolic contents of the plant. The potent antioxidant and higher antibacterial efficacy of *E. fluctuans* can be utilised for the development of cost-effective natural antioxidants and novel plant-derived antimicrobials for effective dietary and therapeutic uses.

Acknowledgement

The authors are grateful to DST-PURSE, University of Kalyani and UGC for financial support. The authors are thankful to Dr. Sudha Gupta, Department of Botany, University of Kalyani and Prof. Gaurab Gangopadhyay, Division of the Plant Biology, Bose Institute, Kolkata for their valuable suggestions during the manuscript preparation.

References

1. Alhdad G.M., Seal C.E., Al-Azzawi M.J. and Flowers T.J., The Effect of Combined Salinity and Water Logging on The Halophyte *Suaeda maritima*: The Role of Antioxidants, *Environ. Exp. Bot*., **87**, 120-5 **(2013)**

2. Apu A.S., Liza M.S., Jamaluddin A.T., Howlader M.A., Saha R.K., Rizwan F. and Nasrin N., Phytochemical Screening and *in vitro* Bioactivities of The Extracts of Aerial Part of *Boerhavia diffusa* Linn., *Asian. Pac. J. Trop. Biomed*., **2(9)**, 673-8 **(2012)**

3. Ashokkumar K., Umamaheswari M., Sivashanmugam A.T., Saradadevi V., Subhashini N. and Ravi T.K., Free Radical Scavenging and Antioxidant Activities of *Glinus oppositifolius* (carpet weed) Using Different *in vitro* Assay Systems, *Pharm. Biol*., **47(6)**, 474-82 **(2009)**

4. Bahri-Sahloul R., Fredj R.B., Boughalleb N., Shriaa J., Saguem S., Hilbert J.L., Trotin F., Ammar S., Bouzid S. and Harzallah-Skhiri F., Phenolic Composition and Antioxidant and Antimicrobial Activities of Extracts Obtained from *Crataegus azarolus* L. var. aronia (Willd.) Batt. Ovaries Calli, *J. Bot*., **2014**, 1-11 **(2014)**

5. Bauer A.W., Kirby W.M.M., Sherris J.C. and Turck M., Antibiotic Susceptibility Testing by a Standardized Single Disk Method, *Am. J. Clin. Pathol*., **36**, 493-6 **(1966)**

6. Beauchamp C. and Fridovich I., Superoxide Dismutase: Improved Assays and an Assay Applicable to Acrylamide Gels, *Anal. Biochem*., **44(1)**, 276-87 **(1971)**

7. Benzie I.F. and Strain J.J., The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power: the FRAP Assay, *Anal. Biochem*., **239(1)**, 70-6 **(1996)**

8. Bhatt L., Samota M.K. and Nautiyal M.K., Potential of Underutilized, Neglected or Untrapped Vegetables, *J. Pharmacogn. Phytochem*., **8(2)**, 1650-3 **(2019)**

9. Bhattacherjee A., Ghosh T., Sil R. and Datta A., Isolation and Characterisation of Methanol-soluble Fraction of *Alternanthera philoxeroides* (Mart.) - Evaluation of their Antioxidant, α-Glucosidase Inhibitory and Antimicrobial Activity in *in vitro* Systems, *Nat. Prod. Res*., **28(23)**, 2199-202 **(2014)**

10. Bouarab-Chibane L., Forquet V., Lantéri P., Clément Y., Léonard-Akkari L., Oulahal N., Degraeve P. and Bordes C., Antibacterial Properties of Polyphenols: Characterization and QSAR (Quantitative Structure–Activity Relationship) Models, *Front. Microbiol*., **10**, 829 **(2019)**

11. Compean K.L. and Ynalvez R.A., Antimicrobial Activity of Plant Secondary Metabolites: A Review, *Res. J. Med. Plant*, **8(5)**, 204-13 **(2014)**

12. Cushnie T.T. and Lamb A.J., Antimicrobial Activity of Flavonoids, *Int. J. Antimicrob. Agents*, **26(5)**, 343-56 **(2005)**

13. Das K. and Roychoudhury A., Reactive Oxygen Species (ROS) and Response of Antioxidants as ROS-Scavengers During Environmental Stress in Plants, *Front. Environ. Sci*., **2**, 53 **(2014)**

14. Dinesh P., Arunprabu S. and Ramanathan T., Phytoconstituents, Antioxidant, Antimicrobial and Haemolytic Activity of *Suaeda maritima* and *Suaeda monoica* a Natural Halophyte World, *J. Pharm. Pharm. Sci*., **5(11)**, 1002-13 **(2016)**

15. Fair R.J. and Tor Y., Antibiotics and Bacterial Resistance in the 21st Century, *Perspect. Med. Chem*., **6**, 25-64 **(2014)**

16. Forni C., Facchiano F., Bartoli M., Pieretti S., Facchiano A., D'Arcangelo D., Norelli S., Valle G., Nisini R., Beninati S. and Tabolacci C., Beneficial Role of Phytochemicals on Oxidative Stress and Age-related Diseases, *Bio. Med. Res Int*., **2019**, 1-16 **(2019)**

17. Gorlenko C.L., Kiselev H.Y., Budanova E.V., Zamyatnin A.A. and Ikryannikova L.N., Plant Secondary Metabolites in the Battle of Drugs and Drug-Resistant Bacteria: New Heroes or Worse Clones of Antibiotics?, *Antibiotics*, **9(4)**, 170 **(2020)**

18. Gupta S., Srivastava A. and Lal E.P., Indigenous Leafy Vegetables for Food and Nutritional Security in two District of Jharkhand, India, *J. Pharmacogn. Phytochem*., **6(6)**, 901-9 **(2017)**

19. Harborne J.B., Phytochemical Methods, a Guide to Modern Techniques of Plant Analysis, 2nd ed., GB, Chapman and Hall Ltd., London **(1984)**

20. Harborne J.B., Phytochemical methods, a guide to modern techniques of plant analysis, GB, Chapman and Hall Ltd., London **(1988)**

21. Karimi A., Majlesi M. and Rafieian-Kopaei M., Herbal versus Synthetic Drugs; Beliefs and Facts, *J. Nephropharmacol*., **4(1)**, 27-30 **(2015)**

22. Khanam U.K., Oba S., Yanase E. and Murakami Y., Phenolic Acids, Flavonoids and Total Antioxidant Capacity of Selected Leafy Vegetables, *J. Funct. Foods*, **4(4)**, 979-87 **(2012)**

23. Klančnik A., Piskernik S., Jeršek B. and Možina S.S., Evaluation of Diffusion and Dilution Methods to Determine the Antibacterial Activity of Plant Extracts, *J. Microbiol. Methods*, **81(2)**, 121-6 **(2010)**

24. Kuri S., Billah M.M., Rana S.M.M., Naim Z., Islam M.M., Hasanuzzaman M., Ali M.R. and Banik R., Phytochemical and *in vitro* Biological Investigations of Methanolic Extracts of *Enhydra fluctuans* Lour, *Asian. Pac. J. Trop. Biomed.*, **4(4)**, 299-305 **(2014)**

25. Lagnika L., Amoussa A.M., Adjileye R.A., Laleye A. and Sanni A., Antimicrobial, Antioxidant, Toxicity and Phytochemical Assessment of Extracts from *Acmella uliginosa*, a Leafy-vegetable Consumed in Bénin, West Africa, *BMC Complement Altern. Med*., **16**, 34 **(2016)**

26. Li Q. and Song J., Analysis of Widely Targeted Metabolites of the Euhalophyte *Suaeda salsa* under Saline Conditions Provides New insights into Salt Tolerance and Nutritional Value in Halophytic species, *BMC Plant Biol.*, **19**, 388 **(2019)**

27. Mahitha B., Archana P., Ebrahimzadeh M.H., Srikanth K., Rajinikanth M. and Ramaswamy N., *In vitro* Antioxidant and Pharmacognostic Studies of Leaf Extracts of *Cajanus cajan* (l.) Millsp, *Indian J. Pharm. Sci.*, **77(2)**, 170-7 **(2015)**

28. Martin-Puzon J.J. and Rivera W.L., Free-radical Scavenging Activity and Bioactive Secondary Metabolites from Various Extracts of *Glinus oppositifolius* (L.) Aug. DC. (Molluginaceae) Roots, Stems and Leaves, *Asian Pac. J. Trop. Dis.*, **5(9)**, 711-5 **(2015)**

29. Mohammad H., Reddy P.N., Monteleone D., Mayhoub A.S., Cushman M. and Hammac G.K., Antibacterial Characterization of Novel Synthetic Thiazole Compounds against Methicillin-resistant *Staphylococcus pseudintermedius*, *PloS ONE*, **10(6)**, 1-19 **(2015)**

30. Moon Y.G., Song C.Y., Yeo I.K., Kim G.Y. and Heo M.S., Antibacterial Activities of *Suaeda maritima* Extract, *J. Life. Sci*., **18(6)**, 776-81 **(2008)**

31. Moyo M., Amoo S.O., Ncube B., Ndhlala A.R., Finnie J.F. and Van Staden J., Phytochemical and Antioxidant Properties of Unconventional Leafy Vegetables Consumed in Southern Africa, *S. Afr. J. Bot*., **84**, 65-71 **(2013)**

32. Nayak B., Roy S., Roy M., Mitra A. and Karak K., Phytochemical, Antioxidant and Antimicrobial screening of *Suaeda maritima* (dumort) against Human Pathogens and multiple Drug Resistant Bacteria, *Indian J. Pharm. Sci.*, **80(1)**, 26-35 **(2018)**

33. Ojha S., Raj A., Roy A. and Roy S., Extraction of Total Phenolics, Flavonoids and Tannins from *Paederia foetida* L. Leaves and their Relation with Antioxidant Activity, *Pharmacogn. J*., **10(3)**, 541-7 **(2018)**

34. Olaleye M.T., Akinmoladun A.C., Ogunboye A.A. and Akindahunsi A.A., Antioxidant Activity and Hepatoprotective Property of Leaf Extracts of *Boerhaavia diffusa* Linn Against Acetaminophen-induced Liver Damage in Rats, *Food Chem. Toxicol.*, **48(8-9)**, 2200-5 **(2010)**

35. Oueslati S., Trabelsi N., Boulaaba M., Legault J., Abdelly C. and Ksouri R., Evaluation of Antioxidant Activities of the Edible and Medicinal *Suaeda* species and related Phenolic Compounds, *Ind. Crops Prod.*, **36(1)**, 513-8 **(2012)**

36. Pandey K.B. and Rizvi S.I., Plant Polyphenols as Dietary Antioxidants in Human Health and Disease, *Oxid. Med. Cell Longev.*, **2(5)**, 270-8 **(2009)**

37. Pasquina-Lemonche L., Burns J., Turner R.D., Kumar S., Tank R., Mullin N., Wilson J.S., Chakrabarti B., Bullough P.A., Foster S.J. and Hobbs J.K., The Architecture of the Gram-positive Bacterial cell Wall, *Nature*, **582(7811)**, 294-7 **(2020)**

38. Patra J.K., Dhal N.K. and Thatoi H.N., *In vitro* Bioactivity and Phytochemical Screening of *Suaeda maritima* (Dumort): A Mangrove associate from Bhitarkanika, India, *Asian. Pac. J. Trop. Med.*, **4(9)**, 727-34 **(2011)**

39. Pisoschi A.M., Pop A., Cimpeanu C. and Predoi G., Antioxidant Capacity Determination in Plants and Plant-derived Products: a Review, *Oxid. Med. Cell Longev.*, **2016**, 1-36 **(2016)**

40. Pulipati S. and Babu P.S., *In-vitro* Antibacterial Potential of *Alternanthera philoxeroides* (Mart) Griseb against multi-Drug Resistant Uropathogens, *Int. J. Pharm. Sci.*, **11(8)**, 3834-40 **(2020)**

41. Re R., Pellegrini N., Proteggente N., Pannala A., Yang M. and Rice-Evans C., Antioxidant activity Applying an Improved ABTS Radical Cation Decolorization Assay, *Free Radic. Biol. Med.*, **26(9)**, 1231-7 **(1999)**

42. Sannigrahi S., Mazuder U.K., Pal D.K., Parida S. and Jain S., Antioxidant Potential of Crude Extract and different Fractions of *Enhydra fluctuans* Lour, *Iran J. Pharm. Sc.*, **9(1)**, 75-82 **(2010)**

43. Sarkar P., Kumar D.H.L., Dhumal C., Panigrahi S.S. and Choudhary R., Traditional and Ayurvedic Foods of Indian Origin, *J. Ethn. Foods*, **2(3)**, 97-109 **(2015)**

44. Sarma U., Borah V.V., Saikia K.K. and Hazarika N.K., Screening of *Enhydra fluctuans* for Phytochemical Composition and Broad-spectrum Antibacterial activity against Clinical Bacterial Isolates, *J. Herbs Spices. Med. Plants*, **22(4)**, 300-8 **(2016)**

45. Shah M., Parveen Z. and Khan M.R., Evaluation of Antioxidant, Anti-inflammatory, Analgesic and Antipyretic activities of the Stem Bark of *Sapindus mukorossi*, *BMC Complement Altern. Med.*, **17**, 526 **(2017)**

46. Simoni R.D., Hill R.L. and Vaughan M., Benedict's Solution, a Reagent for Measuring Reducing Sugars: the Clinical Chemistry of Stanley R. Benedict, *J. Biol. Chem.*, **277(16)**, 485-7 **(2002)**

47. Singh A., Dubey P.K., Chaurasiya R., Mathur N., Kumar G., Bharati S. and Abhilash P.C., Indian Spinach: an Underutilized Perennial Leafy Vegetable for Nutritional Security in Developing World, *Energ. Ecol. Environ.*, **3(3)**, 195-205 **(2018)**

48. Sleight S.C., Wigginton N.S. and Lenski R.E., Increased Susceptibility to Repeated Freeze-thaw Cycles in *Escherichia coli* following long-term evolution in a benign environment, *BMC Evol. Biol.*, **6**, 104 **(2006)**

49. Snyder H.L. and Kirkland J.J., Introduction to Modern Liquid Chromatography, 2nd ed., John Wiley & Sons, Inc., Canada **(1979)**

50. Souza J.N., Silva E.M., Loir A., Rees J.F., Rogez H. and Larondelle Y., Antioxidant Capacity of Four Polyphenol-rich Amazonian Plant Extracts: A correlation Study using Chemical and Biological *in vitro* Assays, *Food Chem.*, **106(1)**, 331-9 **(2008)**

51. Srivastava A., Pan R.S. and Bhatt B.P., Antioxidant and Nutritional potential of some Underutilized Leafy Vegetables Consumed by Tribals of Jharkhand, India, *Curr. Sci.*, **114(6)**, 1222-33 **(2018)**

52. Trease G.E. and Evans W.C., Pharmacognosy, 15th ed., Saunders Publishers, London **(2002)**

53. Trim A. and Hill R., The Preparation and Properties of Aucubin, *Biochem. J.*, **50**, 310-9 **(1952)**

54. Umar M.I., Javeed A., Ashraf M., Riaz A., Mukhtar M.M., Afzal S. and Altaf R., Polarity-based Solvents Extraction of *Opuntia dillenii* and *Zingiber officinale* for *in vitro* Antimicrobial Activities, *Int. J. Food Prop.*, **16(1)**, 114-24 **(2013)**

55. Zhang T.J., Zheng J., Yu Z.C., Huang X.D., Zhang Q.L., Tian X.S. and Peng C.L., Functional Characteristics of Phenolic compounds Accumulated in young Leaves of two Subtropical Forest Tree species of different Successional Stages, *Tree Physiol.*, **38(10)**, 1486-501 **(2018)**.

(Received 18th March 2021, accepted 19th May 2021)