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Research Article

Phytochemical Profile of Two Greens: Amaranthus tristis and Basella alba, for their In Vitro Antioxidant and Antimicrobial Properties- A Comparison

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ABSTRACT
Green leafy vegetables occupy an imperative place among the food crops as they provide adequate amounts of carbohydrates, proteins, vitamins and minerals for consumers. Despite their nutritive value, nowadays greens remains underutilized due to lack of awareness and promotion of appropriate technologies for their effective utilization, modernization. Aerial parts of Amaranthus tristis and Basella alba were chosen to investigate their phenolic, flavonoid, carbohydrate and protein contents tagged with essential in vitro antioxidant assays viz. Radical scavenging activities (DPPH°, OO°, H2O2), reducing power, inhibition of lipid peroxidation (TBARS), to make them potentially accessible source of natural antioxidant. By employing sequential solvent extraction, an overall significant activity was established by acetone extract of Amaranthus tristis stem for majority of the assayed experiments, in particular phenolic (1.730 mg), carbohydrate (149.109 mg), DPPH (0.166 mg). In competence, better significance was reported by methanol fractions of Amaranthus tristis, Basella alba leaves and acetone fraction of Basella alba stem, which articulated to assess its antibacterial and antifungal activities. At the outset, Amaranthus tristis stem demonstrated its potent effect and an interest to study the constituents present in it through GC-MS analyses was analyzed to study the major constituents present. This piece of work to endow the scientific validation of these plants for their medicinal, therapeutic, dietary applications etc.

Keywords: Amaranthus tristis, Basella alba, flavonoid, phenolic, DPPH

INTRODUCTION
Traditional medicine is still widespread and plants serve as a potential source of natural antioxidants that might serve as leads for the development of novel drugs. Plant natural products have provided an unparalleled source of chemical diversity for discovery of interesting bioactive molecules that are responsible to act against many chronic and dreadful diseases. Herbs contain antioxidants which are chemical substances that reduce or prevent oxidation is continuously advancing chapter. They have the ability to counteract the damaging effects of free radicals in tissues and thus are believed to protect against cancer, arteriosclerosis, heart disease and several other diseases [1]. Naturally there is a dynamic balance between the amount of free radicals produced in the body and antioxidants to scavenge/ quench them to protect the body against deleterious effects. These natural antioxidants are considerably used in food, cosmetics and medicinal materials to replace synthetic antioxidants, which are being restricted due to their carcinogenity [2]. Unlike synthetic antioxidants, which are phenolics with varying degrees of alkyl substitution, natural antioxidants can be phenolic compounds (flavonoids, phenolic acids and tannins), nitrogen containing compounds (alkaloids, chlorophyll derivatives, aminoacids, peptides and amines), carotenoids, tocopherols or ascorbic acid and its derivatives. This has reinforced the search for antioxidant principles, the identification of natural resources, isolation of active antioxidant molecules, and as an alternative to preventive deterioration of foods.In this context, it has been attempted to tap-out the utmost possible and beneficial bioactive constituents present in the aerial parts of two green leafy vegetables viz. *Amaranthus tristis* (AT) and *Basella alba* (BA) which were used in traditional and dietary medicine, since time immemorial. These green leafy vegetables constitute a major part of balanced diet to this date in India and they were good sources of minerals and vitamins. Leafy vegetables most often come from short-lived herbaceous plants such as lettuce and spinach. In general, they cover enormous medicinal properties which include antidiabetic, anticarcinogenic, antibacterial, antihistaminic, antioxidant, anticancer etc [3]. Hence, *in vitro* biochemical assays will be priceless to exude the scientific approach to understand the influence of the compounds on the prospective therapeutic utilization of these plant parts. Owing to the above fact, *in vitro* enzymatic and non-enzymatic antioxidant and antimicrobial assays have been carried out, which will be accompanied with GC-MS analyses to reveal the crucial role that these plants may play in several therapeutic and dietary formulations. Therefore,

worthy results of the free radical scavenging properties will be correlated with the spectral data to obtain the levels of the selected greens as antioxidant supplements. To the best of knowledge, there are still underexploited scientific evidences on these species based on their phytochemistry to be beneficial for the modern society. Thus to explore the properties of their leaf and stem parts, exhaustive research work has been of interest to open-up new avenues in the field of life sciences.

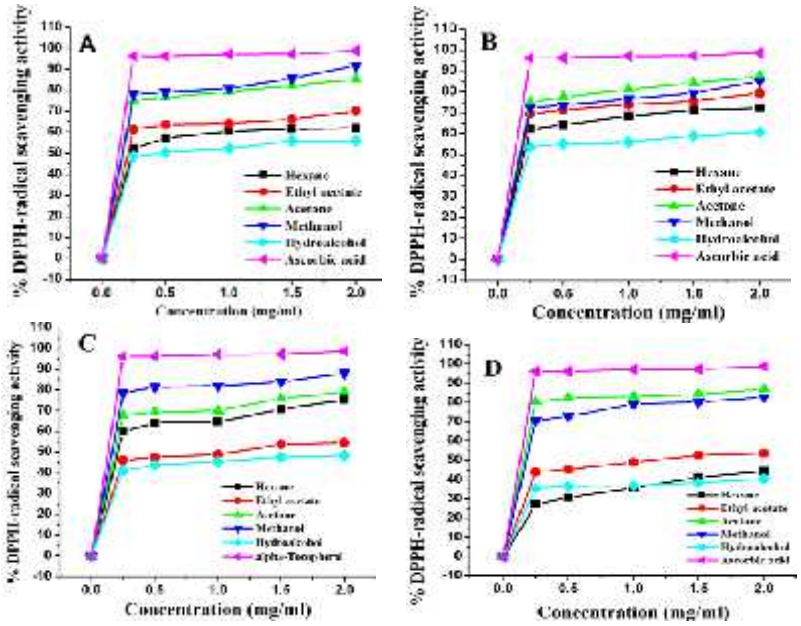

Fig. 1. DPPH- scavenging activity of AT L [A], AT S [B], BA L [C] and BAS [D]

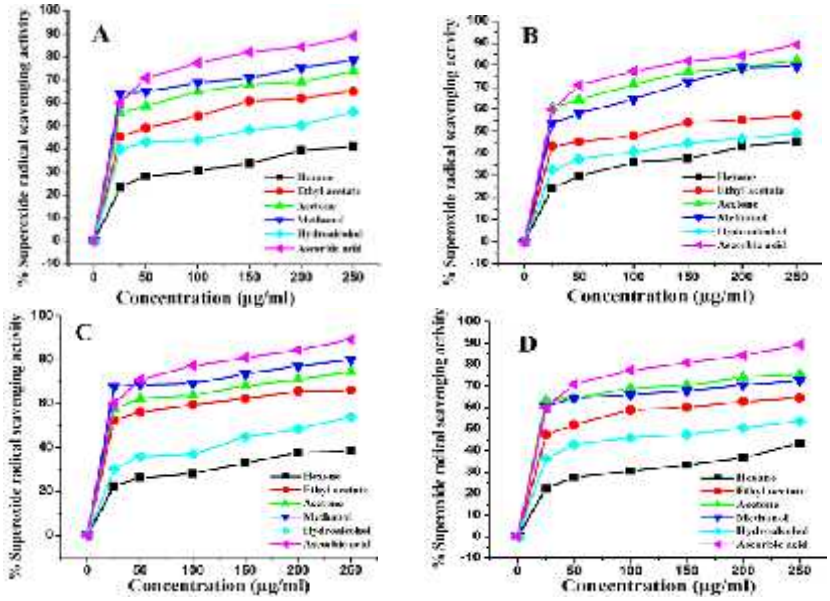

Fig. 2. Superoxide scavenging activity of AT L [A], AT S [B], BA L [C] and BAS [D]

Materials and Methods

Chemicals

1,1-diphenyl-2-picrylhydrazyl (DPPH), butylated hydroxyanisole (BHA), gallic acid, (+)-catechin and solvents like hexane, ethyl acetate, acetone, methanol were purchased from Sigma Chemicals Pvt. Ltd.

Plant selection and authentication

A primary approach by consulting with experts, collection (during the specific August- October), identification, extraction and the phytochemical screening based on their antioxidant activity, derived primarily from a random selection of commonly occurring native plants. At the time of collection, a pressed specimen was prepared and authenticated [BSI/SRC/5/23/2012-13/Tech-586] by a Botanist,

Botanical Survey of India, Southern Circle, TNAU, Coimbatore.

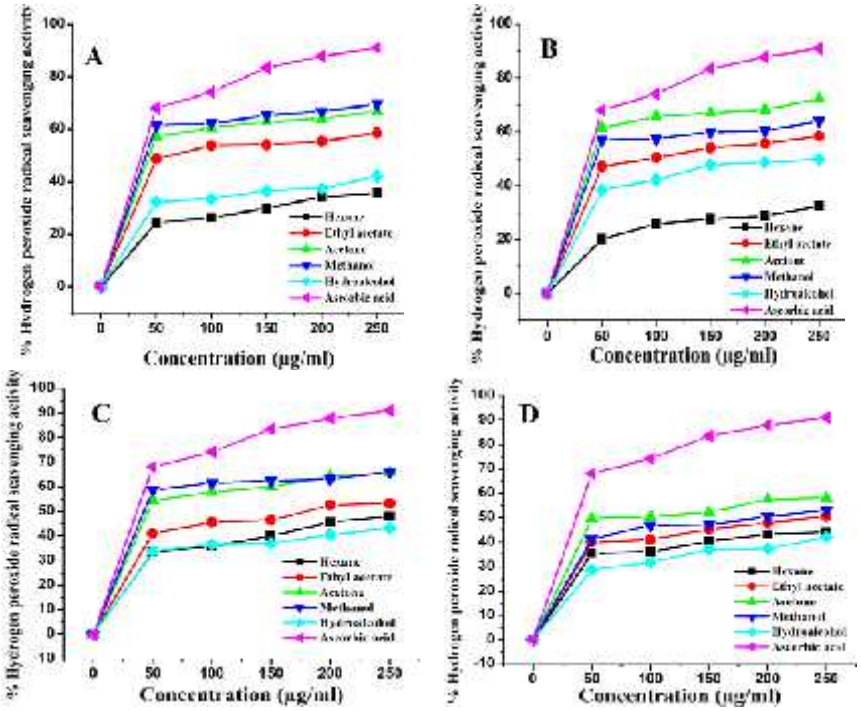

Fig. 3. Hydrogen peroxide scavenging activity of ATL [A], AT S [B], BAL [C] and BAS [D]

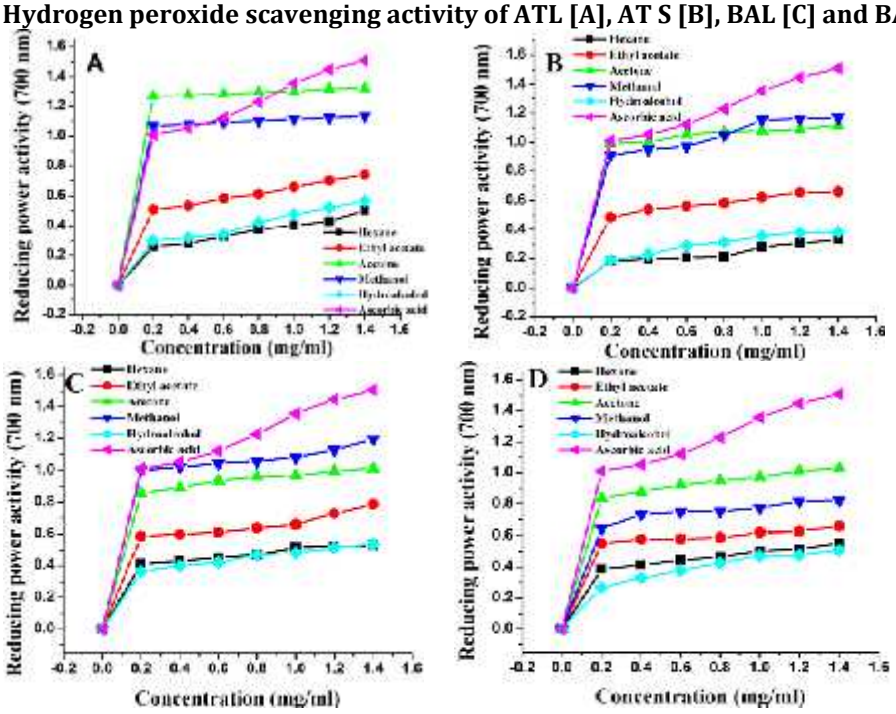

Fig. 4. Reducing power activity of ATL [A], ATS [B], BAL [C] and BAS [D]

Extraction procedure

The old, insect-damaged, fungus-infected spots were removed and only the fresh, healthy samples were selected and washed well with dechlorinated water prior to distilled water, deprived of dusts. It was then chopped, air-dried, and coarsely powdered in a mixture grinder. Soxhlet extraction of the powdered flower samples was carried out using each of the following solvents in increasing polarity: *n*-hexane (HX) (defatting), ethyl acetate (EA), chloroform (CH), methanol (ME) and finally with water (HA) which is highly polar comparatively. The soxhelation process was carried out until the solvent was found to be colorless. Then the solvent was filtered and distilled, till the extract turns off into a syrupy consistency. All the crude solvent extracts were stored at 4–5 °C until further use.

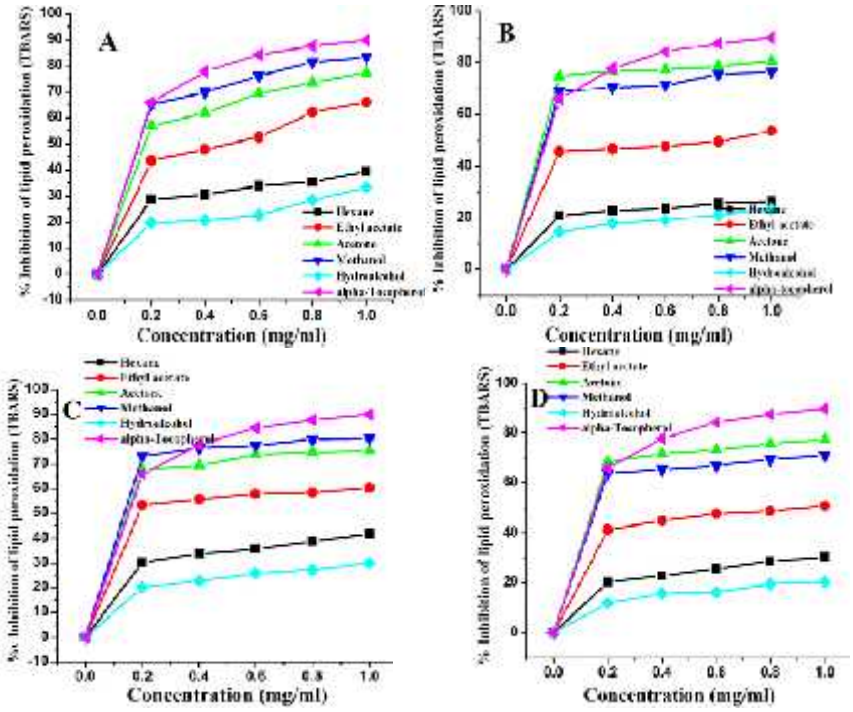

Fig. 5. Inhibition of TBARS of ATL [A], ATS [B], BAL [C] and BAS [D]

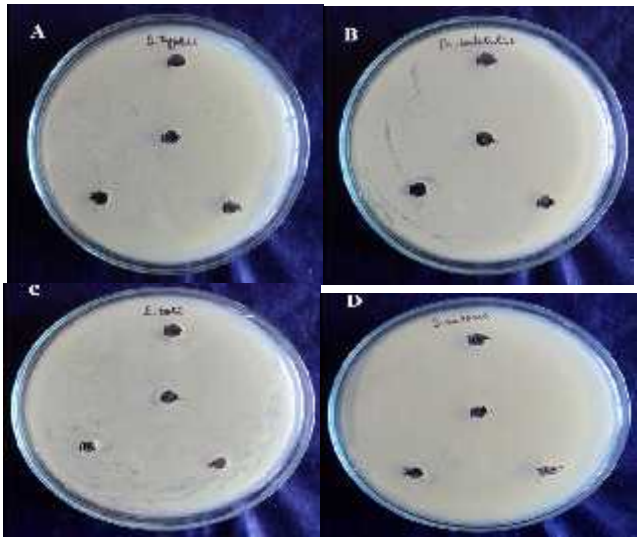

Fig. 6. Antibacterial properties of ATS against Salmonella typhi (A), Bacillus subtilis (B), Escherichia coli (C) and Staphylococcus aureus (D)

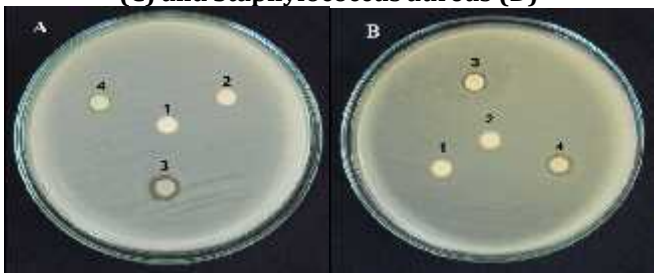

Fig.7. Antifungal properties of ATS against *Aspergillus niger* (A) and *Aspergillus fumigatus* (B)

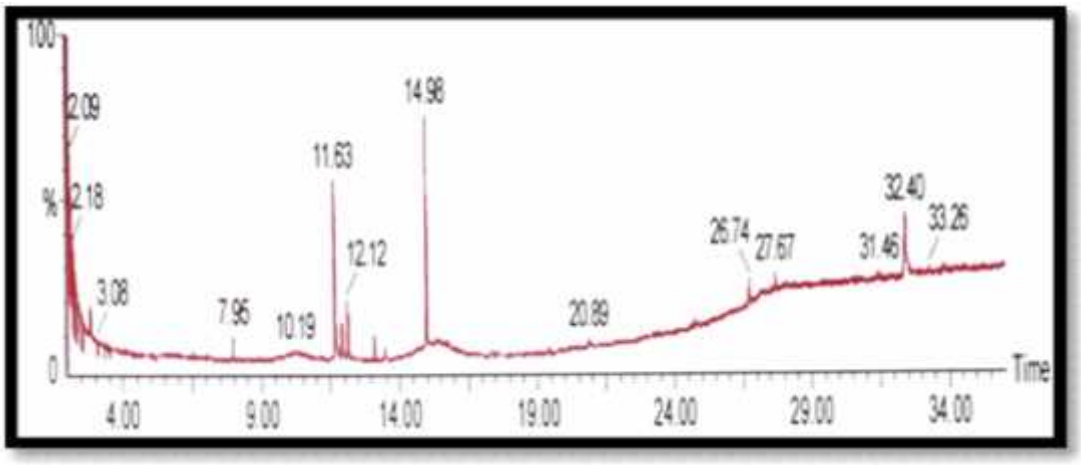

Fig. 8. GC-MS Chromatogram of ATS

Phytochemical screening

The leaves and stem extracts were diluted in their respective solvents and subjected for qualitative preliminary phytochemical screening to identify the presence of the secondary metabolites according to the standard methods [4]. From the intensity of the color inferred for the tests, they will be rated for their presence.

Non-Enzymatic Assays

Total phenolic contents

Total Phenolic Contents (TPC) in the extracts was estimated by a colorimetric assay based on procedures.[5] Basically, 1 mL of sample was mixed with 1ml of Folin and Ciocalteu's phenol reagent. After 3 min, 1 mL of saturated sodium carbonate solution was added to the mixture and it was adjusted to 10 mL with distilled water. The reaction was kept in the dark for 90 min, after which the absorbance was read at 725 nm (Analytik Jena 200–2004 spectrophotometer). Gallic acid was used for constructing the standard curve (0.01–0.4 mM; y = 2.94848 x -0.09211; R^2 = 0.99914) and the results were expressed as mg of gallic acid equivalents g^{-1} of extract (GAEs).

Total flavonoid contents

Total Flavonoid Contents (TFC) in the extracts was determined by a colorimetric method [5]. The plant extract (250 µL) was mixed with 1.25 mL of distilled water and 75 µL of a 5% $NaNO_2$ solution. After 5 min, 150 µL of 10% $AlCl_3.H_2O$ solution was added. After 6 min, 500 µL of 1 M NaOH and 275 µL of distilled water were added to prepare the mixture. The solution was mixed well and the absorbance was read at 510 nm. (+)-Catechin was used to calculate the standard curve (0.250–2.500 mM; Y = 0.2903; R^2 = 1.0000) and the results were expressed as mg of (+)-catechin equivalents (CEs) per g of extract.

Total carbohydrate contents

Total carbohydrate contents were estimated by anthrone method [6]. Glucose was used to calculate the standard curve (20-120 µg/mL, Y= 0.0263x+0.0532, R^2= 0.9992) and the results were expressed has µg of glucose equivalents per mg of extract.

Total protein contents

Total proteins were estimated by Lowry's method [7]. Bovine serum albumin was used to calculate the standard curve (20-160 µg/mL, Y = 0.0159x + 0.0319, R^2 = 0.9569) and the results were expressed as µg of bovine serum albumin equivalents per mg of extract.

Enzymatic assays

DPPH radical-scavenging activity

Various concentrations of the plant extracts (0.3 mL) were mixed with 2.7 mL of methanol solution containing DPPH radicals (6 x 10^{-5} mol/L). The mixture was shaken vigorously and left to stand for 60 min in the dark (until stable absorbance values were obtained). The reduction of the DPPH radical was determined by reading the absorbance at 517 nm. The radical scavenging activity (RSA) was calculated as a percentage of DPPH discoloration, using the equation: %RSA= $[(A_{DPPH} - A_S)/A_{DPPH}] \times 100$, where A_S is the absorbance of the solution when the sample extract is added at a particular level, and A_{DPPH} is the absorbance of the DPPH solution [5]. BHA and α-tocopherol were used as standards.

Superoxide anion radical scavenging assay

The assay for superoxide anion radical scavenging activity was based on a riboflavin – light –NBT system [8]. The reaction mixture contain 0.5 mL of phosphate buffer (50 mM- pH-7.6), 0.3 mL riboflavin (50 mM),0.25 mL PMS (20 mM), 0.1 mL NBT (0.5 mM), prior to the addition of 1 mL sample solution at varying concentrations (25-250 µg/mL). Reaction was started by illuminating the reaction mixture with different concentrations of the methanolic extracts using a fluorescent l amp. After 20 min of incubation, the absorbance was measured at 560 nm. Ascorbic acid was used as standard. The percent inhibition of Superoxide anion generation was calculated using the following formula:

$$\text{Scavenging activity (\%)} = 1 - \frac{\text{absorbance of sample}}{\text{absorbance of control}} \times 100$$

Hydrogen peroxide scavenging assay

The ability of the extracts to scavenge hydrogen peroxide was determined according to the method of Ruch, Cheng and kalunig (1989) [9]. A solution of hydrogen peroxide (2 mM) was prepared in 50 mM phosphate buffer (pH 7.4). Aliquots (0.1 mL) of the extracted samples were transferred into the test tubes and their volumes were made upto 0.4 mL with 50 mM phosphate buffer (pH 7.4). After addition of 0.6 mL hydrogen peroxide solution, tubes were vortexed and absorbance of the hydrogen peroxide at 230 nm was determined after 10 min, against the blank. The abilities to scavenge the hydrogen peroxide were calculated using the following equation:

$$\text{Hydrogen peroxide scavenging activity} = 1 - \frac{\text{absorbance of sample}}{\text{absorbance of control}} \times 100$$

Reducing power

Various concentrations of the plant extracts (2.5 mL) were mixed with 2.5 mL of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. After 2.5 mL of 10% trichloroacetic acid (w/v) was added, the mixture was centrifuged at 1000 rpm for 8 min (Centorion K24OR-2003 refrigerated centrifuge). The upper layer (5 mL) was mixed with 5 mL of deionised water and 1 mL of 0.1% of ferric chloride, and the absorbance was measured spectrophotometrically at 700 nm [5]. BHA and α-tocopherol was used as standards.

Inhibition of lipid peroxidation using thiobarbituric acid reactive substances (TBARS)

The procedure using a Fenton reaction-induced lipid peroxidation has been adapted for this assay [10]. The extracts of all species in concentration of 100 µg/mL have been mixed with 300 µL Tris- HCl buffer, pH=7.5, 500 µL of 20 mM linoleic acid and 100 µL of 4 mM $FeSO_4$. The peroxidation was started with the addition of 100 µL of 5 mM ascorbic acid. The reaction mixture was incubated for 60 min at 37°C. Thereafter, 2 mL of 10% ice cold trichloroacetic acid was added and 1 mL aliquot of the samples was added with 1 mL of thiobarbituric acid. The TBA/sample mixture was heated in the water bath at 95 °C for another 60 min. the absorbance was read at 532 nm and the percentage of linoleic acid peroxidation inhibition was calculated using appropriate controls. α-tocopherol was used as positive control.

Antibacterial assay

The antibacterial activity assay was performed by agar disc diffusion method [11]. Muller Hinton agar medium was seeded with 100 µL of inoculum (1× 108 CFU/mL). The impregnated discs containing the test sample (100 µg/mL) were placed on the agar medium seeded with tested microorganisms. Standard antibiotic discs (Kanamycin 30 µg/disc, Neomycin 10 µg/disc) and blank discs (impregnated with solvent and water) were used as positive and negative control. The plates were then incubated at 37 °C for 24 h to allow maximum growth of the microorganisms. The antibacterial activity of the test samples was determined by measuring the diameter of zone of inhibition expressed in millimeter. The assay was repeated twice and the mean of the three experiments were recorded.

Antifungal assay

The antifungal assay was performed by the agar dilution method using 100 mm × 15 mm petri dishes (Falcon) [12]. Final inoculums of 105 cell/mL for the *Aspergillus niger, Aspergillus fumigatus* and 106 spore/mL for the filamentous fungi was spouted on top of the solidified agar with a loop calibrated to deliver 0.010 mL. Experiments were triplicated. The plates were incubated at 29 °C. The fungal growth was checked, first in control plates prepared without any test sample, after 24, 48 and 72 h, depending on the period of incubation time required for a visible growth; 24 h for *Aspergillus niger,* 48 h for *Aspergillus fumigatus* and 72 h for the dermatophytes. The zone of inhibition in mm was defined as the lowest concentration of extract that inhibited visible growth on agar.

GC-MS

GC-MS was performed with GC Clarus 500 Perkin Elmer equipment; conditions modified from a method published by Frankel et al [13]. Compounds were separated on a 30 mm x 0.25 mm capillary column coated with a 0.25 μm film of HP-5-MS. Samples were injected with a split ratio of 50:1; helium was used as carrier gas at 1 mL min^{-1}. The column temperature was maintained at 100 °C for 1 min after injection then increased at 10 °C min^{-1} to 275 °C which was sustained for 20 min. the time required for chromatography of one sample was 36 min.

Results

Phytochemical screening

A detailed and systematic chemical analysis for the presence of essential phytoconstituents was conducted using one or more tests for individual constituent Table 1(a&b). Compounds like alkaloids, flavonoids, phenolics, carbohydrates, tannins, proteins, glycosides were found to be ubiquitously present in almost all the extracts.

Total flavonoid contents

The flavonoid compositions of both the species were notable as their parts showed a competence among their own extracts which varied widely from 6 to 28 μg. AT (L) showed the highest levels (21.675 μg) followed by the ME extract (25.014 μg) of BA and AC extract (21.675 μg) of AT (S).

Total phenolic contents

Highest TP contents were measured using Folin-ciocalteu's reagent which gave optimum amounts in AC extract (1.730 μg) of AT (S) rather than their L

(1.728 μg). Also there has been a wide distribution of TP content in all the extracts of BA too, in which the highest was ME extract (1.715 μg) of L. For a note, a close competence was provided by the EA extract for both the parts of the two species viz. AT (L)~(1.45 μg), AT (S)~(0.118 μg), BA (L)~(0.116 μg), BA (S)~(0.107 μg).

Total carbohydrate contents

An excellent or above the expectations, a fortuitous event was flaunt by the AC extract of AT (S), with a content of about 149.109 μg. No other extracts, except ME of AT (S) (147.122 μg), recorded such a lasting content which was significant than the standard glucose used for this estimation. For a comparison, the carbohydrate content of BA (L) were for 96.92 μg ME, 92.528 μg for AC, 66.056 μg for EA, 52.397 μg HA and BA (S) were 62.842 μg for ME, 101.324 μg for AC, 54.046 μg for EA, 17.763 μg for HA. It can be inferred that the highest content in BA was seen in the AC extract of S part, thus being in correlation with AT. Though carbohydrates are water soluble components, the HA extracts of both the species have not recorded a significant content compared with the highest.

Total protein contents

BA (L) extended its highest level of protein content of about 17.509 μg for its ME extract. Next ranged AT (L) (17.162 μg of ME extract) and AC extract (16.441 μg) of BA (S), as listed in Table 2 for a clear understanding. From the above results, it can be grasped that richest contents assayed for AT and BA showed their significance in AC extracts for their S and ME extracts for L. HX and HA extracts for all the parts exhibited satisfactory contents and were expected the same for the enzymatic assays too.

DPPH- radical scavenging study

The antioxidant potency through DPPH- radical scavenging assay were interesting in correlation analysis of this study whose order of activity accompanied with SC_{50} values obtained as follows:

AT (L): ME (0.16 mg)> AC (0.166 mg)> EA (0.206 mg)> HA (0.466 mg)> HX (0.24 mg)

AT (S): AC (0.166 mg)> ME (0.173 mg)> EA (0.164 mg)> HA (0.24 mg)> HX (0.2 mg)

BA (L): ME (0.159 mg)> AC (0.186 mg)> EA (0.213 mg)> HX (1.153 mg)

BA (S): AC (0.153 mg)> ME (0.18 mg)> EA (1.186 mg)

Superoxide anion radical scavenging assay

The results of superoxide scavenging action of various extracts are illustrated in Figs. 2a-d, wherein all the significant extracts given below have presented excellent SC_{50} value greater than the standard (20.22 μg). The differences in the concentration/constituents at various extracts are likely to be responsible for their varied scavenging effects. Obviously, AC extracts for S parts [AT= 17.81 μg; BA= 19.41 μg;] and ME extracts of L [AT= 19.41 μg; BA= 20.22 μg] were significant in line with the above assays. For a note, except for AT (S) HA extract, all the other WA extracts had recorded a minimum activity with SC_{50} value and whereas, HX extract has not produced its SC_{50} value even once. EA has developed a satisfactory content with SC_{50} values of 64.23 μg and 97.05 μg for AT (L) and AT (S), 23.28 μg and 39.42 μg for BA (L) and BA (S).

Hydrogen peroxide scavenging assay

Greens neutralized the peroxides in a concentration-dependant manner as projected in Figs. 3a-d. Among the extracts, the highest scavenging effect of 68.21% was produced by AC extract of AT (S) at 200 μg/mL, 63.08% by ME extract of BA (L) on the other hand. As per superoxide scavenging activity, both HX and HA extracts was not much effective in scavenging free radicals, as they never recorded SC_{50} values. Indeed, these extracts have been found to exhibit such SC_{50} values in DPPH and superoxide scavenging activity. None of the extracts exhibited higher SC_{50} values than the standard (37.82 μg), unlike the above assays.

Ferric reducing antioxidant power assay (FRAP)

Highest FRAP value of 0.08 mg and 0.095 mg was obtained from the ME extract of AT and BA (L) respectively. Also the S parts have showed their reducing activity among which the best was AC extract (0.1001 mg) of AT against the standard ascorbic acid whish produced 1.001, EC_{50} value. It can be inferred from the EC_{50} values, the potency of the plant extracts, especially ME extract of AT, greater than the standard. Figs. 4a-d shows the concentration-dependant curves for the reducing powers of all the extracts from aerial parts of AT and BA, with and increasing reducing power as the concentration increased. From the EC_{50} values, a serious attention shall be applied for the strong correlation between the FRAP capacity and TP content.

Inhibition of lipid peroxidation using thiobarbituric acid reactive substances (TBARS)

An effective inhibition was exhibited by the ME extracts of AT (L) (0.157 μg) and BA (L) (0.137μg), whereas the S parts showed 0.145 μg for AT (ME) and 0.148 μg for BA (AC). It was observed that except HX and HA extracts of BA (S) and AT (S), all the other fractions seem to lower the degree of lipid peroxidation induced by hydroxyl radical generated by iron/ascorbate system, present in 1 mg/mL of the crude. The results were clearly portrayed in Figs. 5a-d.

Antibacterial and Antifungal study

As inferred from the above results, this particular assay was shortlisted to perform only for the part that produced significant effects. Thereby AC fraction of AT (S) has recorded its better inhibition against the pathogens viz., *Bacillus subtilis* (NA), *Escherichia coli* (0.3 mm), *Salmonella typhil* (NA), *Staphylococcus aureus* (NA), *Aspergillus niger* (1 mm) and *Aspergillus fumigatus* (0.7 mm). Whatsoever, the zone of

inhibition (mm) were not satisfactory in comparison to the standard. In many cases, fractions other than ME and AC fell short to record their inhibition. Compared to antibacterial, antifungal activities were recorded well with satisfactory inhibitions.

GC-MS

GC-MS, a relative tool was employed to identify the possible number of compounds. To achieve them, the major peaks with significant retention time were compared with NIST library and the constituents were listed in Table 3 along with their structures and spectrum (Fig. 8).

Discussion

Due to the complicated constituents and pharmacological diversities of plant materials, *in vitro* bioassay-guided fractionation has been effectively applied to screen the biological activities that provide important indications for investigating the characteristics of active components [14]. Hence, the study focused here, has exhibited good results with excellent correlation among their contents and assays. As reported by Masuda et al 1999, [15] plant leaves are subject to illumination and has efficient antioxidant system consequently and consistently. Contrastingly, from the results it can be predicted that alike leaves, stem parts also produce excellent antioxidant activity enclosing richer contents. Various plant and herbal extracts possess different phytochemicals with certain biological activity that can exhibit valuable therapeutic index. Biochemical and phytochemical screening of various crude extracts (HX, EA, AC, ME and HA) revealed the ubiquitous presence of the constituents as laid in Table 1(a&b). The immense presence of the constituents has provided the possibilities to estimate their contents and activities through *in vitro* biochemical assays. Table 2 has listed various contents of the non-enzymatic assays for all the parts and extracts of AT and BA for a better understanding. The content of TF, TP, carbohydrate and protein were found to be higher in L than S. An exceptional case is the carbohydrate and TP content of AC extract of AT (S). It was surprising to record such contents on S rather than L. In agreement with some earlier reports, it was portrayed that high yields of extracts contained high levels of phenolic compounds and antioxidant properties, thereby supporting the content of AT (S) [16-18]. On the other hand, BA has also recorded its significant contents in AC extract for S and ME extract for L, whose yield were higher for that respective extracts. In protein content, L has taken up their place of being potent, among which ME extract of BA (L) were highest than AT (L). This was the only content where BA has preceded AT, though they have justified contents when stood alone, has provided a great competence too. Next to the ME extracts of AT (L) and BA (L), richer TF contents were recorded by AC extracts of S parts. AT (L) are expected to be a potential radical scavenger with appropriate antioxidant activity with such a level of TF content. Moreover, flavonoids being highly polar have got extracted in ME and thus the higher content in ME extracts as reported earlier [19,20]. In order to quantify more precisely about relationships, flavonoids and phenolics are widely distributed in all parts of plants that have the ability to scavenge free radicals, by single electron transfer and are the major sources of human diet, though at low levels in most [21]. An appreciable content were projected by other extracts especially EA extracts of both the species, among which the notable was the protein content for AT (L) of about 11.607 μg, AT (S)-10.218 μg, BA (L)-11.259 μg, BA (S)-14.705 μg. There was not found a uniformity of the order between the HX and HA extracts. For TP and TF contents, the highest were seen in HX extracts and the rest two contents were procured by the HA extract, and in fact this order was similar for both the parts of the species, except BA (S). Knowingly or unknowingly both these greens were used in common dietary systems, especially India, with tremendous nutritional and neutraceutical benefits, as claimed from the results discussed so far in the present investigation. Our results of various *in vitro* free radical scavenging assays have established the antioxidant potential of each plant extracts which has its own unique arsenal of numerous phytoconstituents. The following segment throws light on the biological roles in the observed antioxidant and antimicrobial activities. For acceptable evaluations, the antioxidant capacities of a combined assay of two or more different methods rather than a single one, and a comparative analysis of the results are recommended because the assay results are affected by various factors including the concentration of radical source, incubation time, reaction solvent, pH, light and O_2. Reasonable amounts of the constituents estimated above has produced such an efficient activities as expected from their SC_{50} values calculated as the concentration of sample required to scavenge 50% of radical species. DPPH-assay, a considered method which acts rapidly and directly in a simple manner with the antioxidants producing a decrease [purple DPPH' to pale yellow] in colour with increasing concentration. Superoxides are the reactive oxygen species causing damage to the cells and DNA leading to various diseases. It could be essential to measure the comparative interceptive ability of the plant extracts to scavenge the superoxide radical. Hydrogen peroxide, though not very reactive, at times can be toxic to cells to give rise hydroxyl radicals producing diseases. From the results obtained, it can be seen that the ME extract of AT (L) were predominant than any other extracts. BA (L) has showed their equal activity, wherein their SC_{50} values revealed this fact. S part has higher activity on their AC extract for both the species. This difference between the parts of the plants showing potent activity at a particular extract may be due to the

compounds differing at the polarity and got extracted in the differential solvent extraction. Moreover, the absorption values decreased with increasing concentration as the free radicals getting attenuated with the intensity of antioxidant action, for all the above scavenging assays. Rather than DPPH assay, for both superoxide and hydrogen peroxide assay, HX and HA extracts were not much effective ($p<0.01$) whose SC_{50} values were $>>250\mu g$. The scavenging capacities (SC_{50}) on DPPH°, H_2O_2°, for all the respective extracts of the aerial parts demonstrated the same order of activity for the two species, as dealt in Table 3. Deliberately, EA in all the above *in vitro* assays, has evidenced its competing activity, though being less potent, for a comparison and cannot be considered for degency. For instance, in reducing power assay, the EC_{50} value of EA extract for AT (L) were 0.196 μg which was found to be very potent than ME extract (1.118 μg), whereas the ascorbic acid was 0.1001 μg on the other hand. This was not the case in BA too. Seemingly ME and AC extracts have sorted the significant scavenging effects revealing the synergistic potential of the high polar compounds that had go extracted. There has not been a remarkable difference observed between the effective SC_{50} values of the parts, except their solvent extracts. Thus the scavenging abilities of the plant extracts at each concentration can be monitored in Figs. 1a-3d for assessing their strong antioxidant property which deserves a derailed attention. Reducing power activity, one of the important antioxidants *in vitro* assay, determined to measure the ability of extracts to reduce Fe^{3+} to Fe^{2+} by the formation of Perl's Prussian blue. Hence, can reduce free radicals to convert them into more stable non-reactive species and could terminate the chain reaction, by donating electrons or protons. This trend of reducing ferric-ion has been recorded by all the submitted extracts among which the significant being the AC and ME extracts, thereby being consistent with above discussed activities. In stem, there can be observed the EC_{50} values of the AC extracts being closer to the ascorbic acid for both AT (0.1001 mg) and BA (0.119 mg). Thus, an equal reducing activity provided by S was the striking evidence, which challenges the ME extract of L. None of the HX extracts showed potent EC_{50} values showing the least activity of the non-polar compounds got extracted in them. Overall order is as follows: ATL (AC) >BAL (ME)> ATS (AC) > BAS (AC) > BAL (EA) > ATL (EA) > BAS (EA) > ATS (EA) > ATL (HA). In an attempt of relating the reducing power activity with that of the phenolic and flavonoid content, the results are pertinent with the literature [22]. In fact, these particular correlations are in good accordance with the report of Liu et al [21].Fe^{2+} induced lipid peroxidation is an excellent system for assessing antioxidant action of the test extracts, due to chelation of Fe^{2+} or by scavenging free radicals [23]. TBARS is a highly sensitive method the results being fully peroxidation being proportional to the extracts with respect to concentration. It shall be worth discussing the activity at highest concentration 0.8 to 1.0 mg/mL. It can be noted from the results that HX and HA fractions of S were not active against peroxidation, whereas the L does the same. Based upon the above variations in the activities produced by the plant parts, the antibacterial and antifungal activities were performed as inferred from the results (Fig. 6a-7b). It was convincing that AT (S) presented a minimum one of inhibition at the most for *Escherichia coli* in antibacterial assay and both the pathogens in antifungal assay. This could also been correlated with the TP, TF and carbohydrate content of AT (S) reasoning this antimicrobial activity, importantly attributing to its nutritional property [23-25].Hence from the implications of the present study, it has been procured that the ME and AC extracts has played a massive role in extracting possible amounts of antioxidants/ constituents responsible for such antioxidant activity. This can evidence from all the *in vitro* enzymatic and non-enzymatic assays. None of the extracts had given an equal competence. Thus it serves as a reason for the polarity of the solvents (AC and ME) being capable of extracting constituents that directly attribute to such radical scavenging and still it is surprising that a uniform manner of significance has been observed for both the species respective to their parts viz. ME fraction significant in L, AC fraction significant in S. Amongst, AC fraction of AT (S) being elevated, hence the GC-MS analysis analyzed for it has identified many major compounds specially antioxidants, vitamin-E, phytol, decanoic acid, for instance. Overall it can be revealed that AT is one step ahead than BA, both nutritive and chemically.

Conclusion

AT and BA leaves and stem evaluated in this present investigation has explored their new dimension of acting as an antioxidant agent. Contrastingly, AT (S) AC has showed its excellence for almost all the enzymatic and non-enzymatic *in vitro* assays, carbohydrate content to point out. Pronounced consumption of these greens in daily dietary basis will strengthen up and add to the immunity power, above and beyond antioxidant capacity of body. If these two species if established with advanced technological and marketing strategies, it would help in furthering the value addition, promotion, pricing and distribution of many other medicinal plant products too.

Conflict of interests

The authors declare no conflict of interest

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Table 1. Qualitative phytochemical screening of AT and BA

Constituents	Name of the test	*Amaranthus tristis*										*Basella alba*									
		Leaves					Stem					Leaves					Stem				
		HX	EA	AC	ME	HA	HX	EA	AC	ME	HA	HX	EA	AC	ME	HA	HX	EA	AC	ME	HA
Alkaloids	Wagner's	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
	Meyer's	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+
	Dragendrfs	+	+	-	-	-	-	-	+	-	+	+	+	+	+	-	+	+	+	+	-
Flavonoids	Shinoda's	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
	$Fecl_3$	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-	-	-	+	+
	Alkaline	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	+	-
	Con.H_2SO_4	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-
Phenolics	$Fecl_3$	-	-	-	-	+	-	-	-	-	+	-	-	-	+	+	-	-	-	+	+
	Dichromae	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+
	Lead acetate	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	+	+	+	+	-
Tannins	Gelatin	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	+	+	-	-	-
	KOH	-	-	-	+	-	-	-	-	-	-	+	+	+	-	-	+	+	+	+	-
Saponins	Foam	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	+	+	+	+	-
Carbohydrae	Molisch's	-	-	-	-	-	+	+	-	-	+	+	+	+	+	-	+	+	+	-	+
	Fehling's	-	+	-	-	+	-	-	-	-	+	-	-	-	-	+	-	-	-	-	+
	Barfoed's	-	-	-	-	+	-	-	+	-	+	-	-	-	-	+	-	-	-	-	+
	Borntrage's	-	-	-	-	+	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-
Proteins	Biuret	+	+	-	-	-	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+
	Ninhydrin	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+
Steroids	Liberman's	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
	Salkowski's	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Terpenoids	Hager's	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Glycosides	Keller-killiani	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	-	-	-	-
Fats /Oils	Biuret's	+	+	-	-	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+

Table 2. Non-enzymatic assays

Species	Content	Crude Extracts				
		Hexane	Ethyl acetate	Acetone	Methanol	Hydroalcohol
Amaranthus tristis (leaves)	Phenolic	1.247	1.450	1.715	0.606	1.512
	Flavonoid	13.342	18.898	14.598	28.967	15.079
	Carbohydrate	59.281	92.613	118.11	134.90	65.549
	Vitamin	9.871	11.607	13.343	17.262	11.259
Amaranthus tristis (stem)	Phenolic	0.998	1.403	1.730	1.512	1.107
	Flavonoid	10.218	13.690	21.675	14.251	11.580
	Carbohydrate	64.449	110.077	149.109	147.122	73.456
	Vitamin	8.484	10.218	10.913	11.259	8.829
Basella alba (leaves)	Phenolic	0.905	1.309	1.527	1.730	1.123
	Flavonoid	6.986	12.007	14.091	25.014	10.805
	Carbohydrate	17.384	66.056	92.528	101.324	52.397
	Vitamin	8.135	11.259	12.996	17.509	10.913
Basella alba (stem)	Phenolic	0.718	1.138	1.325	1.527	0.920
	Flavonoid	7.093	12.461	14.037	13.343	8.348
	Carbohydrate	14.465	54.046	96.926	62.842	17.763

Table 3. Compounds identified in AT (S) through GC-MS

RT	Peak Area	Name of the compound	Mol formula	MW
7.95	1.38	Phend, 2,6-bis (1,1-dimethylethyl) -4-methyl-methyl carbamate	$C_{17}H_{27}NO_2$	277
11.63	21.08	3,7,11,15-Tetramethyl -2-hexadecen-1-ol	$C_{20}H_{40}O$	296
11.90	3.38	1-Tridecyne	$C_{13}H_{24}$	180
12.12	5.85	3,7,11,15-Tetramethyl -2-hexadecen-1-0l	$C_{20}H_{40}O$	296
13.09	2.00	Ethane peroxoic acid, 1-Cyano-1-(2-(2-phenyl-1,3-dioxolan-2-yl) ethyl] pentyl ester	$C_{19}H_{25}NO_5$	347
13.47	1.08	Pentadecanoic acid, 2,6,10,14-tetramethyl, methyl ester	$C_{20}H_{40}O_2$	296
14.98	30.64	Phytol	$C_{20}H_{40}O$	296
26.74	7.23	Vit-E-acetate	$C_{31}H_{52}O_3$	472
27.67	10.15	1,2-Benzenediol, 3,5-bis(1,1-dimethyl ethyl)	$C_{14}H_{22}O_2$	222
32.40	17.38	Androstane-11,17-dione, 3-[trimethylsilyl)oxy]- 17-[O-(phenylmethyl oxime], 3a,5a	$C_{29}H_{43}NO_3Si$	481