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Nutritional and phytochemical evaluation of *A. lividus* L. syn. *Amaranthus blitum* subsp. *oleraceus* (L.) Costea leaves

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The determination of the proximate composition of *A. lividus* showed the extract to be rich in protein (17.28 ± 0.42 gm/100 gm and dietary fiber (8.35 ± 0.16 gm/100 gm) but very low in fat content (0.69 ± 0.05 gm/100 gm). The phytochemical screening of the various extracts revealed the presence of phenolics ranging between 1.38 to 9.07 µg GAE/mg sample, flavonoid in the range 0.88-5.04 µg RE/mg sample, alkaloids (8.16 %) saponins (29.44 %) and tannins (4.27 %). The bioactive compounds were explored through GC-MS which showed presence of compounds like β-sitosterol, phytol and tetradecane in high amounts. Methanolic soxhlet extract showed highest radical scavenging activity against ABTS (IC_{50} - 1.61±0.01 mg/ml), DPPH (IC_{50} - 3.18±0.04 mg/ml), FRAP (161.39±0.64 TE/g extract) and reducing power assays. The plant extracts screened against various bacterial strains showed good to moderate antibacterial activity. Findings of the study justify the traditional usage of *A. lividus* and provide evidence of its potential use as antioxidants and antimicrobials.

Keywords: *A. lividus*, Proximate, Phytochemical, Antioxidant, GC-MS, Antibacterial activity

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FAO's (Food and Agriculture Organization) recent estimates of population suffering from chronic undernourishment across the world (842 million; 12 % of the global population) and in developing regions (827 million)[1] indicates towards finding diet-based ways of combating such nutritional disorders. One such remedy can be the exploration of underutilized foods like indigenous leafy vegetables which are inexpensive sources of nutrition and can be used to eradicate micronutrient malnutrition and degenerative diseases owing to their allelopathic properties[2]. Moreover, the growing interest in exploration of dietary phytochemicals with rich antioxidant activity is paving the way towards the commercial development of new, natural, cost-effective and safe sources of antioxidants[3].

Amaranthus grows in the wild of the Indian subcontinent and is often considered a weed. The *Amaranthus* genus and its different genotypes are divided into vegetable *Amaranthus* (two major species, *A. tricolor* and *A. lividus*) and grain *Amaranthus*. In contrast to grain *Amaranthus* which is a multipurpose high-potential crop for agriculture, food, feed and forage uses; vegetable *Amaranthus* has received less research attention[4]. Vegetable *Amaranthus* has been rated equal to or superior in taste to spinach and is considerably higher in calcium, iron, and phosphorous[5]. *A. lividus* has two synonyms *Amaranthus blitum* L. (1753), *Amaranthus oleraceus* L. (1763). This species of *Amaranthus* is available in various parts of the world and has common names such as wild Amaranth, pigweed and purple *Amaranthus*. In India, *amaranthus* plant is indigenously known as *Chaulai* or *lalsaag*. Leaves and stem are prepared with other vegetables as a *di*, especially potato. In North and South India, *lal bhaji* is a favourite dish and eaten with rice[6]. The *Amaranthus* being resistant to varied soil and agroclimatic conditions, has low production cost and is one of the cheapest vegetable in the tropical market (esp. North India), therefore, often described as the poor man's vegetable[7]. Earlier studies have emphasized the use of *Amaranthus* as a vegetable and grain crop which can be a cheap alternative rich source of protein, carotenoid, vitamin C, dietary fiber[8] and nutrient for poor people, particularly in developing countries.

In Nigeria, *A. lividus* is used as a medicine against lung disorders. The fluid extract of the plant is used as an astringent internally in the treatment of ulcerated

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mouths and throats; and externally as a wash for ulcers and sores[9]. It is also recommended in diarrhea, dysentery and hemorrhages from bowels. *A. lividus* also finds application in *Ayurveda* (known as *Marisa, Vaspaka*, etc., in Sanskrit) and is used to relieve vata, cough and cold, and control excessive bile secretion. The whole plant acts as an excellent cooling agent for urinary troubles, as a lotion for external use, in relieving pain during pregnancy and as a remedy for skin diseases. Its root extracts along with amla and bark of Ashok and *Daru Haldi* are used to treat leucorrhea[6]. In the *Unani* system of medicine, the juice of the *Amaranthus* root is considered a remedy for gastric problems, leucorrhoea, menorrhagia, boils, burns and nausea. It is popularly used as antipyretic, appetizer, diuretic, febrifuge, galactogogue, haematinic, laxative and stomachic effects and as treatment for hallucination, leprosy, eczema and piles[10]. Thus, the present study has been carried out to explore the various aspects (nutritional, phytochemical, antioxidant, antimicrobial) of underutilized plant *Amaranthus lividus.*

Material and methods

Plant material

The plant specimen, *Amaranthus lividus* was collected from a local market of Dwarka, New Delhi, India. The collected sample was identified (Ref. No. NISCAIR/RHMD/Consult/2013/2219/225) by Dr Sunita Garg at National Institute of Science Communication and Information Resources, Council of Scientific and Industrial Research, New Delhi. *Amaranthus lividus*, an annual plant species belonging to the plant family Amaranthaceae is commonly called purple *Amaranthus*. This plant is native to Mediterranean region and is now available in other parts of the world.

Sample preparation and extraction

The aerial portion of the plant specimen, *Amaranthus lividus* (leaf and stem) was chopped and solvent extracted with methanol. Two methanolic extracts [by soxhlet extraction (MSE) and modified methanolic extraction (ME)] and one aqueous extract (AE) were prepared, and stored at 4 °C for further analysis.

Proximate composition analysis

The nutritional constituents of *A. lividus* were determined for moisture, ash, protein, fat, carbohydrate and crude fiber content (AOAC, 2005). Mineral content was analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES)[11].

Phytochemical analysis

The total phenolics content of the various extracts of *A. lividus* were determined by Folin-Ciocalteu method. Gallic acid (10-50 µg/ml) was used as the standard and the total phenolic content of the extracts were expressed in terms of µg of gallic acid equivalents (GAE) per mg of dry weight. Aluminium chloride colorimetric method was used to estimate total flavonoids content of the sample. The results were expressed in µg of Rutin equivalents (20-100 µg) per mg of dry weight of the extracts based on the linear equation using the calibration curve of Rutin as the standard. The crude alkaloid and saponin content in the extract was determined on dry weight basis. Assessment of crude tannin content was performed by determining the optical density measured at 605 nm using tannic acid as the standard to plot the calibration curve[12].

Determination of secondary metabolites composition

Secondary metabolites were identified by GC-MS (Agilent Technologies; Agilent 6890 GC and 5975B MSD) by split injection (1:20) at 280 °C[13]. Screening of volatiles and semi-volatiles were performed using NIST'05 library.

Free radical scavenging activity

The 1,2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS); 2,2-diphenylpicryl- hydrazyl hydrate (DPPH); ferric reducing antioxidant power (FRAP) free radical-scavenging activity and reducing power of various extracts were also determined[12].

Anti-microbial assay

Agar Well-diffusion method

The various extracts of *A. lividus* were tested for their antibacterial activity[14]. Antimicrobial activity was determined by measuring the diameter of the zone of inhibition.

MIC (Minimum inhibitory concentration)

Plant extracts were screened to determine MIC against bacterial microorganisms, using broth microdilution susceptibility assay. For detecting growth, 40 µl of 70 % alcoholic solution of INT (conc. 0.1 mg/ml) was added to each well where microbial growth was detected by the change in the color of INT dye from yellow to purple. MIC values were defined as the lowest concentration which completely inhibited microbial growth. The results were expressed in mg/ml. Tetracycline was used as the positive control.

Results and discussion

Proximate analysis

Moisture content and dry matter analysis during nutrition reporting is very important because it directly affects its nutritional content, stability and storage. The result of the proximate composition of *A. lividus* leaves show high moisture content (85.01 %). Ash content, which is an index of mineral contents preserved in the food material, was low, 13.81 % for *A. lividus* leaves as compared to that of other green leafy vegetables. The variability is dependent upon the species and also on parameters like environmental temperature, relative humidity during growth and relative amount of metabolic water produced in the plant. The crude protein content of *A. lividus* was found to be 17.28 % . According to Pearson[15], plant food that provides more than 12 % of its calorific value from protein is considered good source of protein. *A. lividus* showed negligible crude fat content (0.69 %), thus an ideal component in several diets and more palatable, as dietary fats function to increase palatability by absorbing and retaining flavours. The carbohydrate content of *A. lividus* was found to be very low, i.e., 4.13 % as compared to other green leafy vegetables. The crude fiber content in *A. lividus* was recorded as 8.35 % which was significantly high. Crude fiber helps in digestion, prevention of colon cancer, controlling cholesterol metabolism and regulation of blood sugar[16]. Low calorific value of any food makes it good for the diet of obese people. The energy value of *A. lividus* was estimated to be 91.92 kcal/ 100 gm and found to contain macro-minerals (K: 33410.10 ppm, Ca: 19295.00 ppm, Na: 5138.20 ppm, Mg: 19101.00 ppm, P: 3964.90 ppm), micro-minerals (Fe: 309.60 ppm, Mn: 24.30 ppm, Zn: 1.20 ppm, Cu: 6.30 ppm) and negligible amount of toxic heavy metals (Pb: 1.40 ppm, Cd: 0.30 ppm, Cr: 0.00, As: 0.00, Hg: 0.00)

Phytochemical analysis

Phenols and phenolic compounds have been used as disinfectants and remain the standard when compared to other bactericides. The total phenolic content of the various extracts of *A. lividus* was found to be highest for MSE (9.07 ± 0.05), followed by ME (6.41 ± 0.11) and then, AE (1.38 ± 0.038). The content of flavonoid expressed in µg of Rutin equivalents (RE) per mg of dry weight of the extracts was estimated as 5.04 ± 0.04µg RE/mg sample (MSE), 4.81 ± 0.05µg RE/mg sample (ME) and 0.88 ± 0.03 µg RE/mg sample (AE). Flavonoids are known for their biological functions such as protection against allergies, inflammation, free radicals, platelet aggregation, microbes, ulcers, hepatoxins, viruses and tumours[17,18]. Secondary metabolites like alkaloids are known to control development in living system and have a protective role in animals[19]. *A. lividus* was found to be an abundant source of alkaloids (8.16 ± 0.41 %) indicative of its therapeutic values. The results reveal the crude sample to have high levels of saponin content (29.44 ± 0.62 %) suggesting its pharmacological and commercial use. Saponins are known for their medicinal uses, as they exhibit antispasmodic, antidiabetic and anticancerous activity[20]. Tannins (often considered as antinutrients) was found under acceptable limits (4.27 ± 0.69 %) which can easily be detoxified by soaking, boiling or frying. Tannins also have astringent properties, hastens wound healing and inflamed mucous membranes[21]. The above results of the various biologically significant chemical constituents of *A. lividus* leaves demonstrate its potential use in therapeutics.

GC-MS analysis

GC-MS analysis showed presence of various bioactive compounds in methanolic crude extract (ME). β-sitosterol was present in maximum amount (27.44 %) followed by phytol (10.68 %) and eicosamethyl-cyclodecasiloxane (8.63 %). Phytol possesses anticancer, antioxidant, anti-inflammatory, antitumor, antimicrobial, diuretic, chemopreventive properties and often used in vaccine formulations[22]. Sitosterol is reported to show antihyperlipoproteinaemic, antibacterial, antimicotic and antitumor activity *in vivo*[23]. Presence of tetradecane was also detected which exhibits antibacterial activity. Earlier studies have reported heptadecane to possess antioxidant and antimicrobial properties[24]. Ergost-5-en-3-ol, (3, β) is a steroid and found to be biologically active against liver diseases, jaundice and artherosclerosis[25]. Hexadecanoic acid, methyl ester has been reported to show bioactivities like antioxidant, hypocholesterolemic, pesticide, 5-α reductase inhibitor and also imparts flavor[26]. The presence of such prevailing bioactive compounds shows that *A. lividus* can be a potential source of pharmaceutical raw material (Table 1).

Free radical scavenging activity

The antioxidant activity *A. lividus* extracts was evaluated in accordance with the decolorization of the ABTS to its radical cation $ABTS^{+}$. The ABTS radical scavenging activity was found to be linearly

Table 1—Secondary metabolites profiling of *A. lividus* MeOH extract

Compound Name	Molecular formula	RT	Cas#	% Area
Tetradecane	$C_{14}H_{30}$	17.532	000629-59-4	0.25
Valeric acid	$C_5H_{10}O_2$	18.631	109717-37-5	0.97
Phytol	$C_{20}H_{40}O$	21.761	000150-86-7	10.68
Cyclodecene	$C_{10}H_{18}$	19.506	066633-38-3	2.51
Nonadecane	$C_{19}H_{40}$	19.663	000629-92-5	1.42
Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	19.932	000112-39-0	1.40
Cyclodecasiloxane, eicosamethyl-	$C_{20}H_{60}O_{10}Si_{10}$	20.370	018772-36-6	8.63
Eicosane	$C_{20}H_{42}$	20.650	000112-95-8	0.91
Ergost-5-en-3-ol, (3.β.)-	$C_{28}H_{48}O$	21.334	004651-51-8	5.35
Heptadecane	$C_{17}H_{36}$	21.581	000629-78-7	6.31
Cyclooctasiloxane, hexadecamethyl-	$C_{16}H_{48}O_8Si_8$	21.873	000556-68-3	7.96
Rolicyprine	$C_{14}H_{16}N_2O_2$	22.030	002829-19-8	1.41
Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	23.174	000556-68-3	5.94
1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy) tetrasiloxane	$C_{16}H_{48}O_6Si_7$	24.374	038147-00-1	4.16
3,6-Dioxa-2,4,5,7-tetrasilaoctane, 2,2,4,4,5,5,7,7-octamethyl-	$C_{10}H_{30}O_2Si_4$	25.495	004342-25-0	2.31
β-Sitosterol	$C_{29}H_{50}O$	25.753	000083-46-5	27.44
5-Methyl-2-phenylindolizine	$C_{15}H_{13}N$	29.421	036944-99-7	1.50
N-Methyl-1-adamantaneacetamide	$C_{13}H_{21}NO$	30.968	031897-93-5	1.77
1,5-Dioxaspiro[5.5]undecan-9-one, 3,3-dimethyl	$C_{11}H_{18}O_3$	31.226	069225-59-8	7.67

increasing with the increasing concentration of the extracts (Fig. 1). Ascorbic acid and BHT were used as standards. The inhibitory concentration (IC_{50}) of various extracts for ABTS radical scavenging ability can be ranked as MSE (1.61 ± 0.01) > ME (1.89 ± 0.02) > AE (83.91 ± 0.52).

Among the various extracts of *A. lividus*, MSE exhibited the highest DPPH radical scavenging activity in a dose-dependent manner (Fig. 2). Present free radical scavenging results showed the IC_{50} (mg/ml) ranking order as MSE (3.18 ± 0.04) > ME (3.22 ± 0.03) > AE (51.91 ± 0.62).

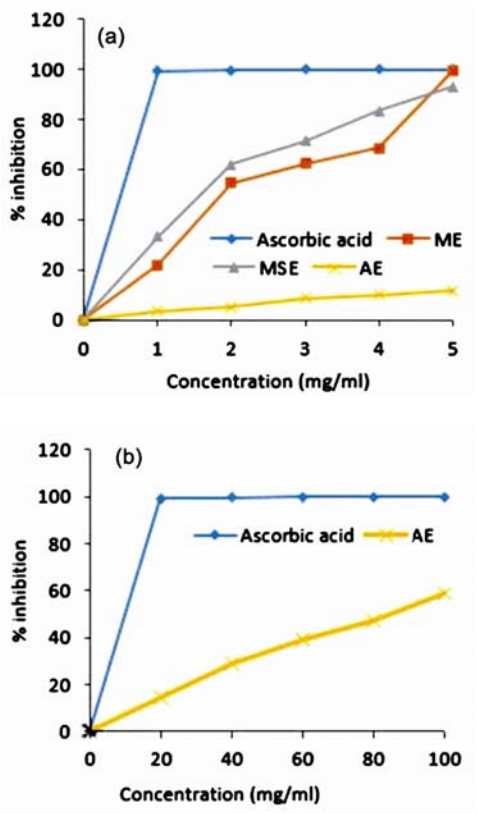

Fig. 1—ABTS scavenging activity of various extracts of *A. lividus*, a) ME-methanolic solvent extract, MSE- methanolicsoxhlet extract, b) AE- aqueous extract

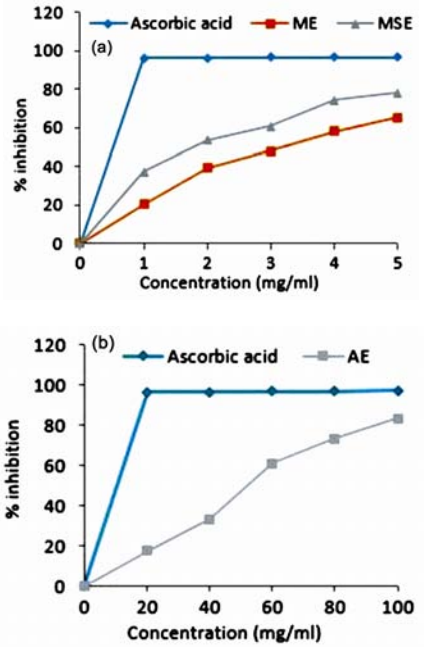

Fig. 2—Free radical scavenging activity of various extracts of *A. lividus* by DPPH antioxidant assay; a) ME-methanolic solvent extract, MSE- methanolicsoxhlet extract, b) AE- aqueous extract

Table 2—Antibacterial activity of various extracts of *A. lividus* against bacterial strains

Bacterial strains	Diameter of Zone of inhibition (mm)			
	Agar Well-Diffusion method			MIC values
	ME	MSE	AE	MSE
	Gram positive			
B. cereus	10.0 ± 0.00	11.3 ± 0.56	_	7.0
B. subtilis	12.7 ± 0.49	11.5 ± 0.63	_	12.5
M. luteus	10.7 ± 0.53	7.0 ± 0.0	10.0 ± 0.0	_
S. aureus	_	_	_	6.25
S. epidermis	8.0 ± 0.0	7.0 ± 0.0	8.0 ± 0.0	6.25
	Gram negative			
E. coli	8.3 ± 0.39	_	_	1.25
P. aeruginosa	_	_	_	_
S. typhi	9.3 ± 0.58	8.8 ± 0.56	_	6.8
S. flexneri	_	_	_	_

The ferric reducing activity of the various extracts were found to be 161.39 ± 0.64, 132.86 ± 0.80, 7.50 ± 0.04 µg TE/mg extract for MSE, ME and AE, respectively. In comparison to the aqueous extract, the methanolic extracts (MSE and ME) had shown better antioxidant activity, being highest for MSE. Increase in absorbance was noticed in a concentration-dependent manner for standard and various extracts of *A. lividus* (Fig. 3).

Antimicrobial activity

All the 6 bacterial strains used to demonstrate antibacterial activity by broth dilution method showed some degree of sensitivity against the 3 extracts ranging from 1.25-12.5 mg/ml. The quantitative estimation for antimicrobial activity for *A. lividus* extracts against food-borne and pathogenic microorganisms are shown in Table 2.

Comparatively, MSE exhibited good inhibition against the test microorganisms which can be attributed to the phenolic content of the sample. MIC values reflected *E. coli* to be the most sensitive (1.25 mg/ml) to the extracts while *B. subtilis* exhibited least inhibition with MIC values of 12.5 mg/ml. The antibacterial activity demonstrated by well diffusion method was not in accordance with MIC results. These problematic results can be attributed to differences in microbial growth, exposure of micro-organisms extracts, non-uniform diffusion of extracts in the agar medium.

Antibacterial activity of *A. lividus* extract has not been reported yet. However, a comparison of the current values with previously reported other *Amaranthus* species (*A. hybridus*, *A. spinosus* and *A. caudatus*) reflect that *A. lividus* is more potent in antibacterial action than others[27]. *A. viridis* on the other hand shows better inhibition against *E. coli* and *S. aureus* than *A. lividus*[28]. In order to confirm the validity of the results obtained in the study and its use infood preservation or medicinal purposes, *in vivo* studies may be required to address issues of safety and toxicity[29].

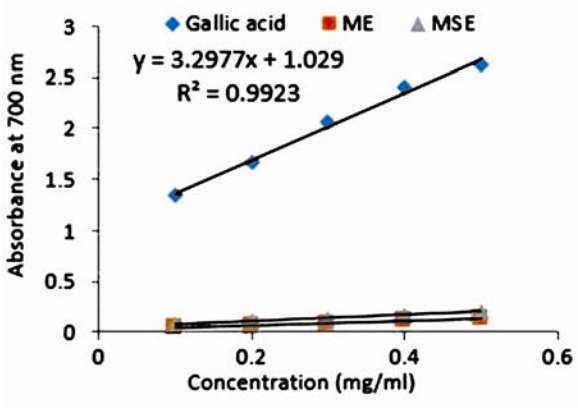

Fig. 3—Reducing power estimation of *A. lividus* extracts; ME- methanol solvent extract, MSE- methanol soxhlet extract

Conclusion

The present study has shown the presence of phenolics, flavonoid, alkaloids and saponins in high amount in *A. lividus*. The plant was found to be nutritionally adequate due to the high protein and low fat content. Present findings show that this vegetable plant can be used as a functional food. The presence of several therapeutically important bioactive compounds makes *A. lividus* a potential source of food as well as biopharmaceuticals. Further, effective scientific study is needed for isolation of such compounds before being put to commercial use in the drug industry.

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