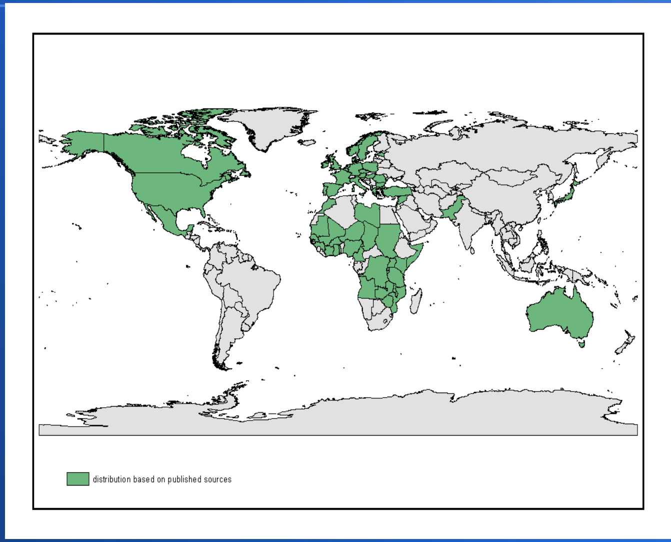
Amaranthus

Description:

- *Amaranthus* is a cosmopolitan genus of annual or short-lived perennial plants, consisting of approximately 65 species growing in tropical, subtropical and warm conditions (see figure).
- *Amaranthus* plants grow annually as an erect, monoecious herb, up to 100–130 cm tall.
- They are being consumed widely as leafy vegetable or grain across the world due mainly to its lower price and rich source of protein, amino acids (especially lysine), carotenoids, vitamin C, dietary fiber and minerals such as calcium, iron, zinc and magnesium.
- Amaranth compared to other grains has the highest amount of protein, twice the content of essential amino acid lysine, more dietary fiber, and 5 to 20 times the content of calcium and iron.



History of *Amaranthus* plants:

- The earliest archaeological record of pale-seeded grain amaranth is that of *A. cruentus*, found in Tehuacan Puebla, Mexico, about 4000 BC (Pal and Khoshoo, 1974; Sauer, 1979), making it one of the oldest known food crops. It probably originated in Central and South America (Grubber and van Sloten, 1981).
- Pale-seeded amaranths were also grown in Germany in the 16th century, India and Ceylon in the 18th century, the Himalayas in the early 19th century, and interior China and Eastern Siberia in the late 19th century (Sauer, 1977).
- Amaranth was important food crop in the Aztec, Mayan, and Incan civilizations. Ancient Mexicans made idols of a dough from seeds of the crop they called huahtli, which has been identified as grain amaranth (Sauer, 1950b; Marx, 1977).
- However, its production has declined remarkably, after collapsing of the Central American cultures.
- In the study of U.S. National Academy of Sciences entitled *Underexploited Tropical Plants with Promising Economic Value,* performed in 1975, amaranth was elected from among 36 of the world's most promising crops and identified as a major potential crop.
- However, very few clinical studies are available and on the following species only: *A. viridis, A. spinosus*, *A. hybridus, A. lividus and A. graecizans.*

Traditional medicinal uses:

- Amaranthae plants are traditionnally used in the treatment of **abdominal pain, chicken pox, dysentry, dysurea, fever, hysteria, malaria, mania infantum, tonsillitis & vomiting.**
- *A. viridis* has also been used in the **treatment of kidney diseases** in China.
- *A. spinosus* has been used as **antiinflammatory, antimalarial, antibacterial, antidiuretic, antiviral** and in **hepatic disorders.**
- The leaves are used as a **laxative.**
- The root is known as an effective **diuretic.**
- Leaves and roots are **applied as emollient poultice** to relief bruises, abscesses, burns, wound, inflammation, menorrhagia,gonorrhoea, eczema and inflammatory swelling.

- There are **almost no relevant reports about the investigation of the bioactivities of *Amaranthus* extracts.**
- However, lower income people in less developed countries comparatively evaded from cancer diseases, **especially prostate and breast cancer,** but they live on nothing but else rice and weedy vegetables including *Amaranthus*.

Chemical composition:

- In species of genus *Amaranthus*, **16 phenolic acids** were identified. The total amount of phenolic acids in *A. caudatus* grains was 16.8 to 59.7 mg/100 g, whereas the proportion of soluble phenolic acids was 7% to 61%.
- Furthermore, it contains alkaloids, glycosides and/or carbohydrates, flavonoids (**rutin and quercetin**), terpenoids, sterols, tannins, saponins, betalains (**amaranthine and isoamaranthine**), sulphates, nitrogen and chlorides.
- Extracts of *A. spinosus* were found to contain **hydroxycinnamates, quercetin** (305 mg/100 g) **and kaempferol glycosides**. In addition **α-xylofuranosyl uracil, β-D-ribofuranosyl adenine** and **β-sitosterol glucoside** have also been isolated for the first time from this species.
- The phytochemical investigation of the *n*-butanol fraction of the methanolic extract of the whole plant of *A. spinosus*, lead to the isolation of **amaranthoside,** a lignan glycoside, **amaricin,** a coumaroyl adenosine along with **stigmasterol glycoside, glycinebetaine** and **trigonelline.**
- The **α-spinasterol and hectriacontane** were isolated from a petroleum ether extract of leaves and stem of *A. Spinosus*. α-spinasterol was also identified in roots. A saponin mixture was isolated in roots.
- Other constituents were **oleanolic acid, D-glucose and D-glucuronic acid.**
- A new aliphatic ester, **α-spinasterol octacosanoate** and a new saponin, **β-D- glucopyranosyl-(1-4)-β-Dglucopyranosyl –(1-4)-β-D-glucuronopyranosyl-(1-3)-oleonolic acid** were isolated from the roots of *A. spinosus.*

Anticancer activity may be the result of lectin activity present in *Amaranthus* plants.

Lectins are a class of glycoprotein present mainly in plant especially in seed in abundant quantity and having carbohydrate binding capacity.

Lectin has been reported to possess remarkable antitumor activity, exerting apoptotic role by preferential binding to cancer cell membranes with subsequent cytotoxicity. Lectin also exhibits growth inhibitory activity by altering the cell cycle and inducing non-apoptotic G1-phase accumulation mechanisms, G2/M phase cell cycle arrest and apoptosis.

Phenolic constituents in different species (table):

Table 4–Phenolic constituents in various *Amaranthus* spp.

Constituent, material	Content	Ref
Phenolic compounds		
A. cruentus seed: raw flours/high-protein flour fraction/cooked/popped/germinated (dried at 30, 60, and 90 °C)	5.16/5.89/3.53/4.46/3.04 to 3.68 g TAE/kg	1
A. caudatus seed: raw flours/high-protein flour fraction/cooked/popped/germinated (dried at 30, 60, and 90 °C)	5.24/6.86/3.96/4.28/3.41 to 4.20 g TAE/kg	1
A. cruentus: treated vegetables	27.4 to 61.8 GAE/100 g fw	2
A. *hypondriacus* grain: raw/extruded flour	56.60/69.50 mg GAE/100 g dw	27
Total phenolic acids		
A. cruentus var. Aztec seeds/sprouts light/darkness	464/380.7/370.3 mg/kg dw	3
A. cruentus var. Rawa seeds/sprouts light/darkness	424.6/392.6/396.1 mg/kg dw	3
Gallic acid		
Methanol extract of hydrolised defatted flour	0.55 ± 0.065 mg/100 g dw	4
A. paniculatus seeds	40.64 ± 1.1 μg/g	5
A. cruentus var. Aztec seeds/sprouts light/darkness	440/370/360 mg/kg dw	3
A. cruentus var. Rawa seeds/sprouts light/darkness	400/360/350 mg/kg dw	3
Vanillic acid		
Methanol extract of hydrolised defatted flour	0.33 ± 0.002 mg/100 g dw	4
A. cruentus var. Aztec seeds	13.5 mg/kg dw	3
A. hypochondriacus seed flour: var. Tulyehualco/DGETA/Gabriela/Nutrisol	1.8/1.7/1.8/1.5 μg/g flour	6
A. cruentus seeds: 7 accessions	109.69-158.43 mg/kg	7
A. seeds: 18 different genotypes	up to 5.2 μg/g*	8
Syringic acid		
Methanol extract of hydrolised defatted flour	0.49 ± 0.028 mg/100 g dw	4
A. cruentus var. Aztec sprouts light/darkness	6.3/4.2 mg/kg dw	3
A. cruentus var. Rawa sprouts light/darkness	4.3/3.7 mg/kg dw	3
A. hypochondriacus seed flour: var. Tulyehualco/DGETA	0.8/0.7 μg/g flour	6
p-Coumaric acid		
Methanol extract of hydrolised defatted flour	0.27 ± 0.002 mg/100 g dw	3
A. paniculatus/A. caudatus seeds	43.57 ± 0.9/5.2 ± 0.5 μg/g	5
A. cruentus var. Rawa seeds/sprouts light/darkness	3.9/28.3/42.4 mg/kg dw	3
A. cruentus var. Aztec sprouts light/darkness	4.4/6.1 mg/kg dw	3
A. hybridus/A. hypondriacus/A. cruentus seeds: methanol extract	1.2 ± 0.1/1.2 ± 0.1/1.4 ± 0.1 μg/g dw	9
A. cruentus seeds: 7 accessions	8.33 to 11.48 mg/kg	7
A. seeds: 18 different genotypes	up to 3.3 μg/g*	8
Ferulic acid		
Methanol extract of hydrolised defatted flour	0.56 ± 0.054 mg/100 g dw	4
A. paniculatus/caudatus seeds	40.05 ± 1.3/18.41 ± 0.8 μg/g	5
A. hybridus/A. hypondriacus/A. cruentus seeds: methanol extract	309.8 ± 26.1/288.5 ± 23.2/345.0 ± 27.2 μg/g dw	9
A. cruentus seeds: 7 accessions	54.30 to 85.80 mg/kg	7
A. caudatus insoluble fiber (*cis/trans*-ferulic acids)	203/620 μg/g	10
Protocatechuic acid		
A. paniculatus/A. caudatus seeds	100.92 ± 8.7/4.65 ± 0.4 μg/g	5
A. caudatus seeds/sprouts	13.6 ± 9.4/14.0 ± 2.1 μmol/100 g dw	11
A. seeds: 18 different genotypes	up to 17.2 μg/g*	8
p-Hydroxybenzoic acid		
A. paniculatus/A. caudatus seeds	15.62 ± 1.3/20.89 ± 0.8 μg/g	5
A. cruentus seeds: var. Aztec/Rawa	8.5/20.7 mg/kg dw	3
A. hypochondriacus seed flour: var. Tulyehualco/DGETA/Gabriela/Nutrisol	1.7/2.0/2.2/1.9 μg/g flour	6
A. seeds: 18 different genotypes	up to 8.8 μg/g*	8
A. cruentus seeds: 7 accessions	88.68 to 141.92 mg/kg	7
Caffeic acid		
A. paniculatus/A. caudatus seeds	51.67 ± 0.45/55.79 ± 0.96 μg/g	5
A. hybridus/A. hypondriacus/A. cruentus seeds: methanol extract	6.41 ± 0.8/6.49 ± 0.9/6.61 ± 0.7 μg/g dw	9
A. cruentus seeds: 7 accessions	3.08 to 5.51 mg/kg	7
***Sinapic acid**: A. paniculatus* seeds	0.48 ± 0.1 μg/g	5
***Salicylic acid**: A. paniculatus/A. caudatus* seeds	2.65 ± 0.2/1.92 ± 0.2 μg/g	5
***Caffeoylquinic acids**: A. spinosus* stems	109.2 ± 15.6/5.5 ± 0.5 mg/100g	12
***Cumaroylquinic acids**: A. spinosus* stems	54.6 ± 6.0/17.5 ± 2.0 mg/100g	12
***Feruoylquinic acids**: A. spinosus* stems	57.4 ± 5.5/6.5 ± 0.2 mg/100g	12

Source: [1] Petras R. Venskutonis and Paulius Kraujalis: Nutritional Components of Amaranth Seeds and Vegetables: A Review on Composition, Properties, and Uses. *Comprehensive Reviews in Food Science and Food Safety.* Vol.12,2013 .

Phenolic constituents in different species (table):

Table 4–Phenolic constituents in various *Amaranthus* spp.

Constituent, material	Content	Ref
Flavonoids		
A. hybridus raw/cooked	16.9 ± 0.8/21.1 ± 0.7 mg QE/100g	13
A. cruentus: treated vegetables	18.6 to 49.4 CE/100 g fw	2
Amaranthus flour	65 ± 8 μg CE/g dw	14
Ethanol extract of hydrolised defatted flour	37.43 ± 2.10 mg RE/100 g dw	4
A. seeds (PI 604671 cultivar)	37.43 ± 0.210 mg RE/100 g dw	15
A. cruentus: dried leaves water extract	275 ± 2.8 10^{-2} g/kg dw	16
A. hypochondriacus seeds 1.2 M/L HCl in 50% methanol:water	18.66 ± 2.10 mg CE/100 g dw	17
Rutin		
A. cruentus v. Aztec sprouts light/darkness	690/300 mg/kg dw	3
A. cruentus v. Rawa sprouts light/darkness	620/460mg/kg dw	3
A. hypochondriacus seed flour: Gabriela/Nutrisol/Tulyehualco/DGETA var.	4.0/4.7/10.1/5.8 μg/g flour	6
A. hypochondriacus leaves/stems/flowers/seeds (full flowering)	13950 ± 566/4543 ± 67/11925 ± 180/70 ± 7 mg/kg dw	18
A. caudatus leaves/stems/flowers/seeds (full flowering)	12010 ± 658/3505 ± 149/6130 ± 226/55 ± 3 mg/kg dw	18
A. hybrid leaves/stems/flowers/seeds (full flowering)	27500 ± 1626/3723 ± 124/15426 ± 532/99 ± 4 mg/kg dw	18
A. retroflexus leaves/stems/flowers/seeds (full flowering)	13050 ± 636/3360 ± 99/8725 ± 50/11 ± 1 mg/kg dw	18
A. tricolor leaves/stems/flowers/seeds (full flowering)	2385 ± 203/932 ± 54/459 ± 30/7 ± 1 mg/kg dw	18
A. seeds: 18 different genotypes	up to 68 μg/g	8
A. spinosus whole plant powder	0.15%	19
A. spinosus stems	36.4 ± 9.8 mg/100g	12
Isoquercetin: *A. hypochondriacus* seed flour freeze dried methanol:water extract (70:30): var. Tulyehualco/Nutrisol/DGETA/Gabriela	0.5/0.5/0.3 μg/g flour	6
Quercetin: total:released from rutin: *A. hypochondriacus* seeds/flowers/stems/leaves (beginning of growth/harvest time/full flowering)	68 ± 3:65 ± 5.77/5155 ± 205:5765 ± 70/3083± 152:3411 ± 76/(6765 ± 191:6531 ± 433/8750± 566:7322 ± 541/7375 ± 262:7704 ± 289) mg/kg dw	18
Quercetin: total:released from rutin: *A. hybrid/A. caudatus/A.tricolor* leaves (full flowering)	15600 ± 424:16913 ± 505/6695 ± 219:7755 ± 685/ 1395 ± 78:1217 ± 105 mg/kg dw	18
Quercetin diglycoside: *A. spinosus* stems	1.9 ± 0.3 mg/100g	12
Quercetin-3-O-glucoside: *A. spinosus* stems	9.0 ± 1.9 mg/100g	12
Nicotiflorin: *A. hypochondriacus* seed flour: var. Tulyehualco/DGETA/Gabriela/Nutrisol	5.5/5.6/7.2/4.8 μg/g flour	6
Nicotiflorin: *A.* seeds: 18 different genotypes	up to 6.1 μg/g*	8
Vitexin: *A. cruentus* v. Rawa seeds	410 mg/kg dw	3
Isovitexin: *A. cruentus* v. Rawa seeds	266 mg/kg dw	3
Kaempferol diglycoside: *A. spinosus* stems	7.0 ± 1.8 mg/100g	12
Tannins		
A. hypochondriacus/A. cruentus seeds	0.060 ± 0.11/0.12 ± 0.04% dw	9
A. caudatus seeds raw/extruded	1305 ± 0.23/1284 ± 0.52 mg CE/100g	20
A. cruentus: treated vegetables	5.4 to 20.4 mg/100 g	2
Amaranthus 8 varieties	0.043 to 0.116% CE	21
Amaranth seeds: dark/light	1.04 to 1.16/0.8 to 1.2 mg/g	22
Amaranthus 10 samples	0.8 to 4.2 mg/g	23
Amaranthus samples	0.4 to 1.2 mg/g	24
Polyphenolics: *A. cruentus* (4genotypes)/*A. hybridus* (1)	4.5 to 5.2/4.1 mg tannic acid/g	25
Total anthocyanins: *A. cruentus* var. Aztec/Rawa seeds	103.6 ± 10.4/90.83 to 9.2 mg CGE/100 g dw	26
Anthocyanins: *A. hypochondriacus* seeds 1.2 M/L HCl in 50% MeOH:W	35.33 ± 1.70 mg/100 g dw	17

dw, dry weight; fw, fresh weight; W, water; MeOH, methanol; E, extract; t/r from R, total/released from rutin; CGE, cyanidine-3-glucoside equivalents; CE, catechin equivalents; RE, rutin equivalents; QE, quercetin equivalents; TAE, tannic acid equivalents; *measured from the bars in the provided figures.

1 Gamel and others (2006b); 2 Adebooye and others (2008); 3 Paško and others (2008); 4 Mošovska and others (2010); 5 Klimczak and others (2002); 6 Barba de la Rosa and others (2009); 7 Ogrodowska and others (2012); 8 Steffensen and others (2011); 9 Gorinstein and others (2008); 10 Bunzel and others (2005); 11 Alvarez-Jubete and others (2010c); 12 Stintzing and others (2004; 13 Adefegha and Oboh (2011); 14 Chlopicka and others (2012); 15 Mošovská and others (2010); 16 Oboh and others (2008); 17 López and others (2011); 18 Kalinova and Dadakova (2009); 19 Suryavanshi and others (2007); 20 Ferreira and Arêas (2010); 21 Lorenz and Wright (1984); 22 Bressani (1994); 23 Becker and others (1981); 24 Breene (1991); 25 Bejosano and Corke (1998a); 26 Paško and others (2009); 27 Milán-Carrillo and others (2012).

Source: [1] Petras R. Venskutonis and Paulius Kraujalis: Nutritional Components of Amaranth Seeds and Vegetables: A Review on Composition, Properties, and Uses. *Comprehensive Reviews in Food Science and Food Safety.* Vol.12,2013 .

Plant composition:

- The total ash, acid insoluble ash, water-soluble ash values and sulfated ash were observed to be 6.33%, 3.60%, 2.44% and 0.80% w/w respectively. Alcohol soluble and water-soluble extracting values of the leaves were observed to be 6.40%, 3.30%, respectively.
- Different extracts from *A. viridis* L. showed different biological activities. Ethyl acetate extract showed higher free radical scavenging and antiinflammatory activities but no anticancer activity. Ethyl ether extracts showed strong anticancer activities.
- Leaves are usually higher in their chemical constituents than the stems and roots.
- Stem extract has been credited with antimalarial activity.
- Seed extract displays comparative higher anti-proliferative outcome over the stem extract.

Table 5: A preliminary phytochemical screening of active constituents of the different organs of *Amaranthus* species

	Amaranthus graecizans			*Amaranthus lividus*			*Amaranthus viridis*		
Test	Leaves	Stems	Roots	Leaves	Stems	Roots	Leaves	Stems	Roots
Alkaloids	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Glycosides and/or carbohydrates	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Flavonoids	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Sterols	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Tannins	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	ve
Saponins	-ve	-ve	-ve	+ve	-ve	-ve	+ve	+ve	-ve
Sulphates	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Chlorides	-ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve

+ve: Present, -ve: Absent

Table 1
Phytochemical screening of various fractions of *A. graecizans* subsp. *silvestris* (Vill.) Brenan.

Test	Methanolic extract	*n*–Hexane fraction	Chloroform fraction	Ethyl acetate fraction	*n*–Butanol fraction	Aqueous fraction
Terpenoids	–	++	–	++		–
Flavonoids	++	–	++	+++	+++	+
Tannins	++	–	++	+++	+++	+++
Alkaloids	+++	+++	+++	++	+++	–
Carbohydrates	+++	–	++	–	+++	+++
Sterols	+++	+	++	+		–
Cardiac glycosides	+	–	+++	+++		–
Saponins	+	+++	+++	+	–	–
Proteins	–	–	–	–	+++	+

+: Presence; –: Absence.

Source: [2] Ziada A, El-Halawany EF, Mashaly IA and Masoud GF: Autecology and Phytochemistry of Genus Amaranthus in the Nile Delta, Egypt. Asian Journal of Plant Sciences 7 (2): 119-129, 2008.

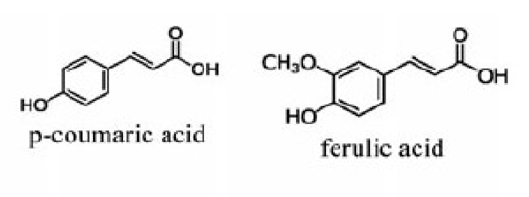
Source: [3] Ishtiaq S, Ahmad M, Hanif U, Akbar S, Mehjabeen5, Kamran SH: Phytochemical and in vitro antioxidant evaluation of different fractions of *Amaranthus graecizans* subsp. silvestris (Vill.) Brenan. Asian Pac J Trop Med 2014; 7(Suppl 1): S342-S347.

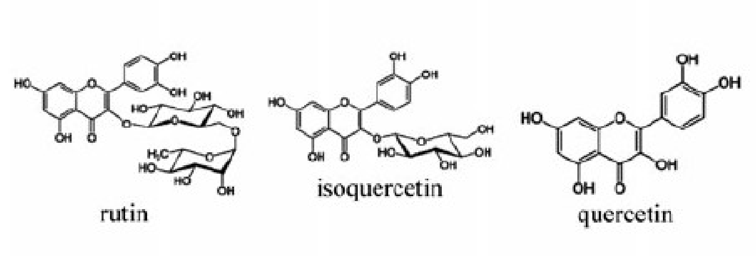
Chemical composition (table and figures):

Table 2: Phyto constituents of *Amaranthus spinosus* Linn.

Therapeutic constituents	Plant part	References
Amaranthine, isoamaranthine, hydroxycinnamates, quercetin and kaempferol glycosides	stems	[49]
7-p-coumaroyl apigenin 4-O-β-D-glucopyranoside, α-xylofuranosyl uracil, β-D-ribofuranosyl adenine and β-sitosterol glucoside.	whole plant	[50]
Rutin and quercetin	whole plant	[51, 52]
Amaranthoside- a lignan glycoside	whole plant	[53]
Amaricin- a coumaroyl adenosine		
stigmasterol glycoside		
α-spinasterol	roots	[54]
hectriacontane	leaves and stem	
oleanolic acid, D-glucose and D-glucuronic acid		
aliphatic ester-α-spinasterol octacosanoate	roots	[55]
saponin-β-D- glucopyranosyl-(1-4)-β-D-glucopyranosyl –(1-4)-β-D-glucuronopyranosyl-(1-3)-oleonolic acid		
Saponin I- β-D- glucopyranosyl-(1-2)-β-D-glucopyranosyl –(1-2)-β-D-glucupyranosyl-(1-3)-α-spinasterol	roots	[56]
Saponin-II- β-D-glucopyranosyl-(1-4)-β-D-glucopyranosyl-(1-3)-α-spinasterol		

<u>Source</u>: [1] Tanmoy G, Arijit M, Tanushree S, Jagadish S, Kumar MT: Pharmacological Actions and Phytoconstituents of *Amaranthus spinosus* Linn: A Review. *International Journal of Pharmacognosy and Phytochemical Research* 2014; 6(2); 405-413.





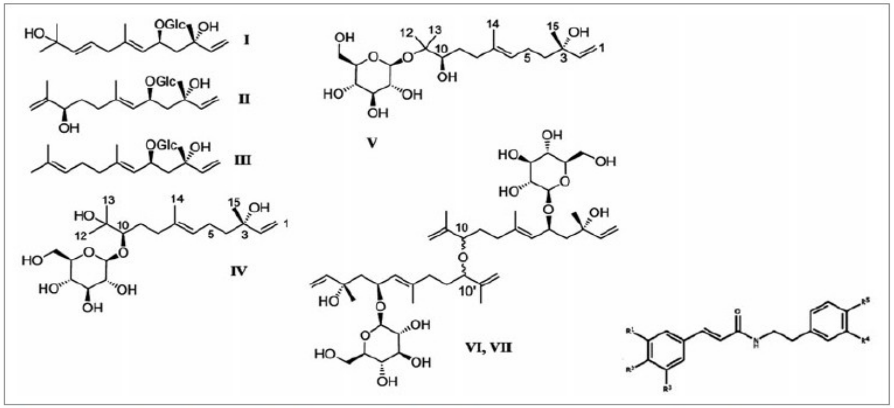


Figure 1–Amaranthölidosides from *A. retroflexus* (Fiorentino and others 2006) and cinnamoylphenethylamines (R = H, OH, OMe) from *A. hypochondriacus* and *A. mantegazzianus* (Pedersen and others 2010).

<u>Source:</u> [1] Petras R. Venskutonis and Paulius Kraujalis: Nutritional Components of Amaranth Seeds and Vegetables: A Review on Composition, Properties, and Uses. *Comprehensive Reviews in Food Science and Food Safety*. Vol.12,2013 .

Recent clinical research:

- Methanolic extract of *A. spinosus* demonstrated **potent anti-diabetic, anti-hyperglycemic, antihyperlipidemic and spermatogenic effects** in alloxaninduced diabetic rats [4].
- The petroleum ether, ethanol extract of whole plant and methanol extract of leaves of *A. spinosus* exhibited **dose-dependant anti-inflammatory effect** in carrageenan induced paw oedema, and produced significant inhibition of acetic acid induced increase in vascular permeability [5]. *A. spinosus* extract also showed a highly specific prostaglandin synthesis inhibitory activity *in-vitro* in an anti-inflammatory model test system [6].
- Different *Amaranthus* species displayed *in vivo* **analgetic activity** in mice [7].
- The *in vivo* **antimalarial activity** of extracts from *A. spinosus* was reported in mice [8].
- The extract of *A. spinosus* displayed **diuretic activity, acting as a thiazide-like diuretic. I**t increased the Na+, K+, Cl^{-} excretion, caused alkalinization of urine, showed strong saluretic activity and carbonic anhydrase inhibition activity. These effects were observed predominantly at 500 mg/kg dose and there was no dose-response relationship [9].
- *A. spinosus* extracts exhibited **moderate to good antimicrobial activity** against a range of gram positive and gram negative bacteria [10].
- The stimulatory effect of wild *A. spinosus* water extract was investigated on spleen cells from female mice *in vitro*. The extract significantly stimulated B-lymphocytes in a dose response manner with subsequent T-lymphocytes proliferation, thus showed **good immuno-stimulating activity** [11].
- Extracts from *A. spinosus, A. hybridus, A. viridis and A. lividus* exhibited **significant antitumor effects** in Ehrlich ascites carcinoma (EAC) bearing mice [12] [14] or in colon and liver cancer cell lines [13].

References:

[4] Sangameswaran B, Jayakar B. Anti-diabetic, antihyperlipidemic and spermatogenic effects of *Amaranthus spinosus* Linn. on streptozotocin-induced diabetic rats. *J Nat Med* 2008; 62: 79-82.

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[12] Samuel Joshua L, Pal VC, Senthil Kumar KL, Sahu RK , Roy A. Antitumor activity of the ethanol extract of *Amaranthus spinosus* leaves against EAC bearing Swiss albino mice. *Der Pharmacia Lettre* 2010; 2: 10-15.

[13] Shan Jin Y, Xuan , Chen M, Chen J, Jin , Piao J, and Tao J: Antioxidant, Antiinflammatory and Anticancer Activities of *Amaranthus viridis* L. Extracts. *Asian Journal of Chemistry*; Vol. 25, No. 16 (2013), 8901-8904.

[14] Al-Mamun A, Husna J, Khatun M, Hasan R, Kamruzzaman M, Hoque KMF, Reza A and Ferdousi Z: Assessment of antioxidant, anticancer and antimicrobial activity of two vegetable species of Amaranthus in Bangladesh. *BMC Complementary and Alternative Medicine* (2016) 16:157.

Pharmacological activities (table):

Table 1: Pharmacological activities of *Amaranthus spinosus* Linn.

Pharmacological activities	Parts use	Extract	Reference
Hepatoprotective Activity			
against carbon tetrachloride (CCl4) induced hepatic damage in rats.	whole plant	ethanolic extract	[12, 13]
against d-galactosamine/ lipopolysaccharide (d-GalN/LPS) -induced liver injury in rats.	whole plant	ethanolic extract	[14]
against paracetamol-induced liver damage in Wistar rat	whole Plant	ethanol extract	[15]
Antioxident activity			
(a) non-enzymatic haemoglycosylation assay	whole Plant	petroleum ether, chloroform, methanol, and water extract	[16, 17]
(b) DPPH assay		methanolic extract	
	leaves	chloroform, n-hexane and ethyl acetate	[17]
	leaves	extract	[20]
Antigenic and allergenic activity	pollen		[18]
Anti diabetic activity			
Alpha amylase enzyme inhibition by CNPG3 (2-chloro-4-nitrophenol a-D-maltotrioside)	leaves	methanol extract	[21]
Streptozotocin-induced diabetic rats			
Alloxan-induced diabetic rats	stems	methanol extract	[22]
	stems	methanol extract	[23]
Anti-inflammatory activity			
carrageenan induced paw oedema	leaves	ethanol extract	[24]
	whole plant		
	leaves	methanol extract	[26]
acetic acid induced	whole plant	petroleum ether and ethanolic extract	[25]
		methanol extract	[27]

Source: [1] Tanmoy G, Arijit M, Tanushree S, Jagadish S, Kumar MT: Pharmacological Actions and Phytoconstituents of *Amaranthus spinosus* Linn: A Review. *International Journal of Pharmacognosy and Phytochemical Research* 2014; 6(2); 405-413.

Pharmacological activities (table):

Table 1: Pharmacological activities of *Amaranthus spinosus* Linn.

Pharmacological activities	Parts use	Extract	Reference
peripheral analgesic activity	leaves		[28]
Anthelmintic activity			
Indian earthworms (*Pheritima Posthuma* & *Tubifex tubifex*)	whole plant	aqueous extracts	[27, 29]
Anti-malarial activity			
Plasmodium Berghei	stem	aqueous extracts	[30]
Heamatologic activity	leaf	ethanol extract	[31]
	whole plant except root	methanolic extract	[32]
Immunomodulatory activity			
Stimulatory effect on spleen cells from female mice.	leaves	water extract	[34]
Dexamethasone (DEX)-induced apoptosis in murine primary splenocytes.	leaves	water extract	[35]
cell-mediated immune response (CMIR)		pet. ether, aqueous, alcoholic extract	
	leaves		[36]
Gastrointestinal activity			
charcoal meal method	leaves	aqueous extract	[37]
Laxative activity	whole plant	aqueous-methanolic extract	[38]
Anti-diarrheal and anti-ulcer activity			
charcoal meal	whole plant	ethanol extract	[39]
Antitumor activity			
Brine shrimp lethality bioassay	leaves	methanol extract	[20]
EAC bearing mice	leaves	ethanol extract	[40]

Table 1: Pharmacological activities of *Amaranthus spinosus* Linn.

Pharmacological activities	Parts use	Extract	Reference
Antitumor activity			
Brine shrimp lethality bioassay	leaves	methanol extract	[20]
EAC bearing mice	leaves	ethanol extract	[40]
Antibacterial activity	leaves	chloroform , n-hexane and ethyl acetate extracts	[20]
		ethanol and aqueous extracts	
	roots	hexane, ethyl acetate,	[41]
	leaves	dichloromethane and methanol extracts	[42]
Diuretic activity	whole plant	aqueous extract	[43]
Other activities			
biochemical role	whole plant except root	methanolic extract	[44]

Source: [1] Tanmoy G, Arijit M, Tanushree S, Jagadish S, Kumar MT: Pharmacological Actions and Phytoconstituents of *Amaranthus spinosus* Linn: A Review. *International Journal of Pharmacognosy and Phytochemical Research* 2014; 6(2); 405-413.

Cytotoxic effect:

- **Potent cytotoxic properties of *A. spinosus* were determined** on brine shrimp nauplii**.** The assay was performed using *A. Salina,* after 24 hours of exposure, and vincristine sulphate was used as a positive control [10].
- The LC50 values for standard vincristine sulphate, chloroform, n-hexane and ethyl acetate extract of *A. spinosus* were **7.55µg/ml, 18.15 µg/ml, 29.15°µg/ml, 18.15 µg/ml,** respectively (see figure).
- The n-hexane extract showed highest cytotoxic activity (LC50 = 29.15 µg/ml).

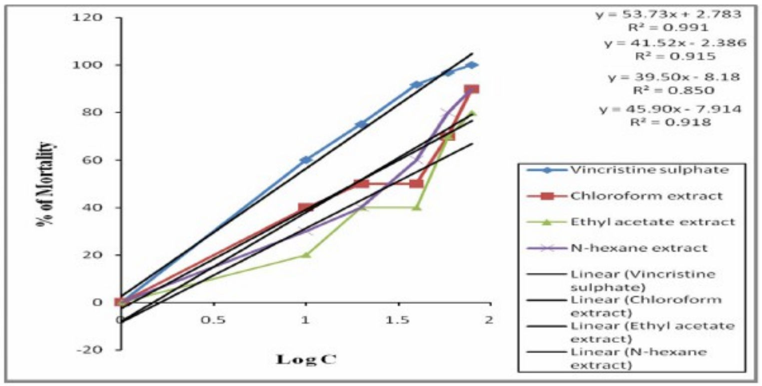

Figure 1: Determination of LC_{50} values for standard and chloroform, n-hexane, ethyl acetate extracts of leaves *Amaranthus spinosus* from linear correlation between logarithms of concentration versus percentage of mortality.

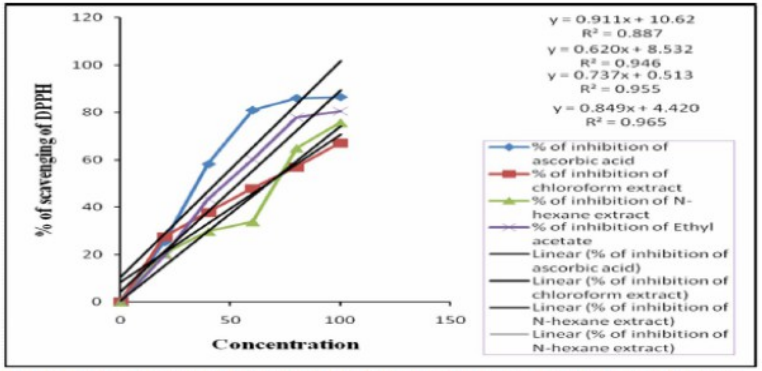

Figure 2: Determination of IC_{50} values for standard and chloroform, n-hexane, ethyl acetate extracts of leaves *Amaranthus spinosus* from linear correlation between logarithms of concentration versus percentage of scavenging of DPPH.

Reference:

[10]Bulbul IJ, Nahar L, Ripa FA, Haque O: Antibacterial, Cytotoxic and Antioxidant Activity of Chloroform, n-hexane and Ethyl Acetate extract of plant Amaranthus spinosus. International Journal of PharmTech Research. Vol.3, No.3, pp 1675-1680, July-Sept 2011.

Anticancer activity:

- Extracts from *A. hybridus* and *A. lividus* [14] and the ethanol extract of *A. spinosus* leaves [12] exhibited **significant antitumor effects** in Ehrlich ascites carcinoma (EAC) bearing mice.
- In one study, the ethanol extract of its leaves, given orally to EAC-bearing mice at the dose of 100 and 200 mg/kg body weight for 16 days, led to **decrease in tumor volume and viable cell count,** and **increase in mean survival time and non-viable tumor cell count** in comparison to the control group [12].
- **Restoration of hematological and biochemical parameters towards normal** was also observed [12].
- Anticancer activity was **dose-dependant.**

Table 1 Antitumor activity of ethanol extract of *A. spinosus* leaves on tumor volume, Viable tumor cells count and Non viable tumor cells count

Treatment	Tumor volume (ml)	Viable tumor cells count (10^6 cells/mouse)	Non viable tumor cells count (10^6 cells/mouse)
EAC Control	4.9±0.91	10.2±0.31	0.4±0.03
EAC+Ethanol Extract (100 mg/kg)	3.8±0.83	7.3±0.03	0.7±0.05
EAC+Ethanol Extract (200 mg/kg)	2.8±0.53*	4.8±0.09*	0.5±0.03
EAC+5FU 20 mg/kg	1.9±0.48*	3.1±0.17*	0.9±0.01

*Values are expressed as mean ± SEM, n = 6 in each group. *P<0.001 compared to EAC control group*

Table 3 Antitumor activity of ethanol extract of *A. spinosus* leaves on hematological parameters

Treatment	Hb content	Total RBC cells/ml×10^6	Total WBC cells/ml×10^6	Differential count		
				Lymphocyte (%)	Neutrophils (%)	Momnocytes (%)
EAC Control	13.2±0.9	1.34±0.2	15±1.0	24.0±8.9	73.0±9.8	3±0.9
EAC+Ethanol Extract (100 mg/kg)	14.8±0.6	1.39±1.2	10.3±0.3	42±5.2	51±4.6	3±0.5
EAC+Ethanol Extract (200 mg/kg)	16.4±1*	1.45±0.2*	6.9±1.0*	62±4.6	33±2.1	2±0.8
EAC+5FU 20 mg/kg	16.3±0.7*	1.40±0.09*	6.7±0.5*	68±3.0	30±2.0	2±0.1*

*Values are expressed as mean ± SEM, n = 6 in each group. *P<0.001 compared to EAC control group.*

Table 2 Antitumor activity of ethanol extract of *A. spinosus* leaves on median survival time and percentage increase in life span

Treatment	Median Survival time (days)	Percentage Increase in Life Span
EAC Control	23.2±1.32	100
EAC+Ethanol Extract (100 mg/kg)	33.8±2.5	143
EAC+Ethanol Extract (200 mg/kg)	40.3±2.2	175
EAC+5FU 20 mg/kg	48.4±1.5	209

Values are expressed as mean ±SEM, n = 10 in each group.

Reference:

[12] Samuel Joshua L, Pal VC, Senthil Kumar KL, Sahu RK , Roy A. Antitumor activity of the ethanol extract of *Amaranthus spinosus* leaves against EAC bearing Swiss albino mice. *Der Pharmacia Lettre* 2010; 2: 10-15.

[14] Al-Mamun A, Husna J, Khatun M, Hasan R, Kamruzzaman M, Hoque KMF, Reza A and Ferdousi Z: Assessment of antioxidant, anticancer and antimicrobial activity of two vegetable species of Amaranthus in Bangladesh. *BMC Complementary and Alternative Medicine* (2016) 16:157.

Anticancer activity:

- In another study on two species of *Amaranthus,* administration of extracts from *A. hybridus* and *A. lividus* extract (100 µg/ml) led to **45% and 43% growth inhibition** of Ehrlich's ascites carcinoma cells (EAC) [14]. Histological analysis showed marked features of apoptosis including cell shrinkage, condensation of cytoplasm and aggregation of apoptotic bodies [14] (see figure).

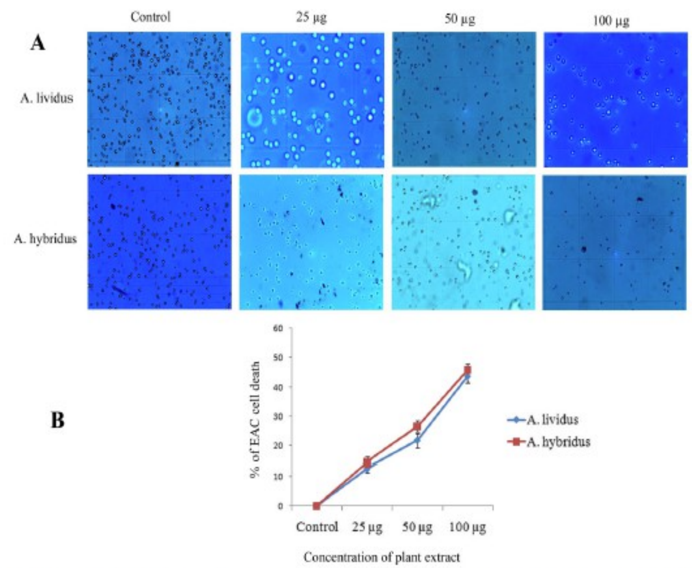

Fig. 3 Growth inhibitory activity of *Amaranthus* extract at different concentration on EAC cells. **a** Hemocytometer counting of EAC cells collected from both control and treated groups was determined using trypan blue dye after six days of EAC cells injection. **b** Percentage of growth inhibition of EAC cells on each concentrations of *Amaranthus* extract (counting the percentage in treated groups by considering zero cell death in control group). Each value represents a mean ± SD (*n* = 6)

Table 2 Half maximal inhibitory concentration (IC_{50}) of the AL and AH along with BHT standard

Samples	IC_{50} values (µg/ml)
A. lividus	93 ± 2.44
A. hybridus	28 ± 1.8
BHT	12 ± 0.5**

Each value is represented as mean ± SD (*n* = 3), significance was set at P <0.01 (**) with respect to BHT standard

Table 3 Growth inhibitory activity of *Amaranthus* extract on EAC cells by hemocytometer counting using trypan blue dye

Plants	Average count of EAC cells per cell of hemocytometer (out of 16 cells)			
	Control	25 µg	50 µg	100 µg
A. lividus	32 ± 0.52	28 ± 0.56	25 ± 0.38*	18 ± 0.33**
A. hybridus	32 ± 0.52	27.37 ± 0.5	23.37 ± 0.58*	17.25 ± 0.41**

Each value is represented as mean ± SD (*n* = 6), significance was set at P <0.05 (*) and P <0.01 (**) with respect to control

References:

[14] Al-Mamun A, Husna J, Khatun M, Hasan R, Kamruzzaman M, Hoque KMF, Reza A and Ferdousi Z: Assessment of antioxidant, anticancer and antimicrobial activity of two vegetable species of Amaranthus in Bangladesh. *BMC Complementary and Alternative Medicine* (2016) 16:157.

Anticancer activity:

- Ethyl ether extract (EEA) from *A. viridis* (400 µg/mL) showed **inhibition of tumor growth rate** on 24h basis **by 96.9 and 85.9%** in HT-29 and HepG2 cells [12] (see figure).
- However, ethyl acetate extract (EAA) from *A. viridis* displayed no significant anticancer activity [12].

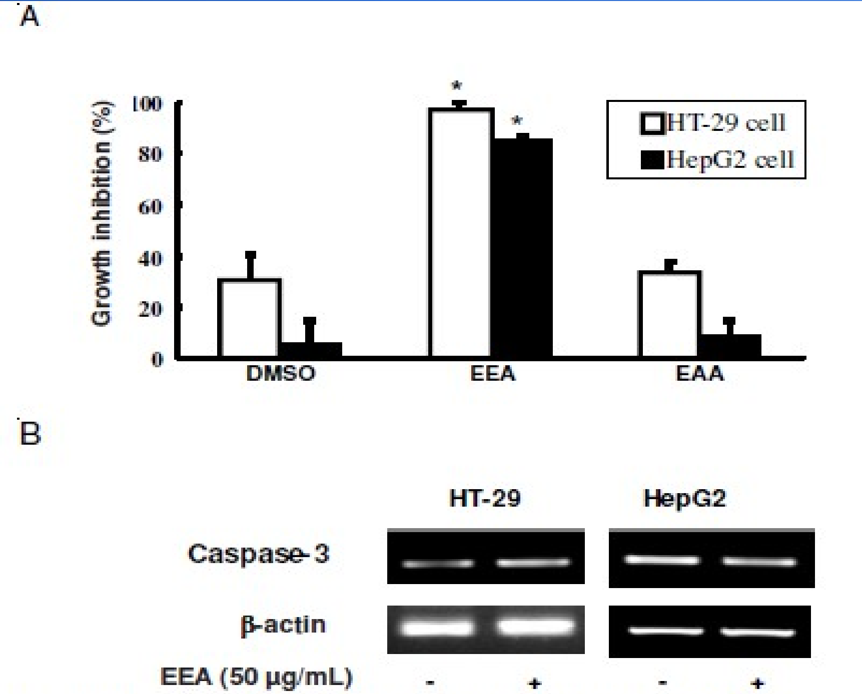

Fig. 3. Anticancer activity. (A) Cell viabilities of HT-29 or HepG2 cancer cells after incubated with 400 µg/mL EEA and EAA for 24h. (B) RT-PCR analysis of caspase-3 mRNA expression on HT-29 and HepG2 cells. Cancer cells were treated with EEA (50 µg/mL) for 24 h

References:

[12] Shan Jin Y, Xuan , Chen M, Chen J, Jin , Piao J, and Tao J: Antioxidant, Antiinflammatory and Anticancer Activities of *Amaranthus viridis* L. Extracts. *Asian Journal of Chemistry*; Vol. 25, No. 16 (2013), 8901-8904.

Molecular pathways and targets:

- Gene expression pattern analysis showed that ethyl ether extract (EEA) from *A. viridis* **up-regulated the expression of caspase-3** in HT-29 cells, however not in HepG2 cells [13].
- In another study on EAC with *A. hybridus* and *A. lividus*, it was established that apoptosis is mitochondria-mediated with **down-regulation of Bcl-2 mRNA and up-regulation of p53, Bax and caspase-3** [14].
- It is postulated that the **mitochondrial-activated apoptosis** pathway is activated by **elevated levels of ROS (reactive oxygen species)** produced by the phytochemicals (lectins, polyphenols, flavonoids) present in *Amaranthus* extract.
- It is unknown what bioactive compound played the key anti-proliferative role on EAC cells [14].

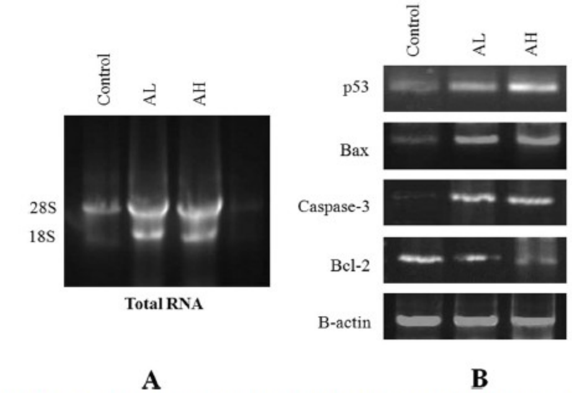

Fig. 5 Apoptotic changes of EAC cells induced by *Amaranthus* extract were found to mediated by mitochondrial pathway: **a** Isolated total RNA exhibited two separate bands as 28S and 18S. **b** The animals treated with *Amaranthus* extract at 100 μg/ml for six days exhibited relatively up-regulation of p53, Bax and caspase-3 and showing bright and bolder band in comparison with control. While the expression of Bcl-2 mRNA was decreased considerably when compared with the control. Beta-actin was used as standard

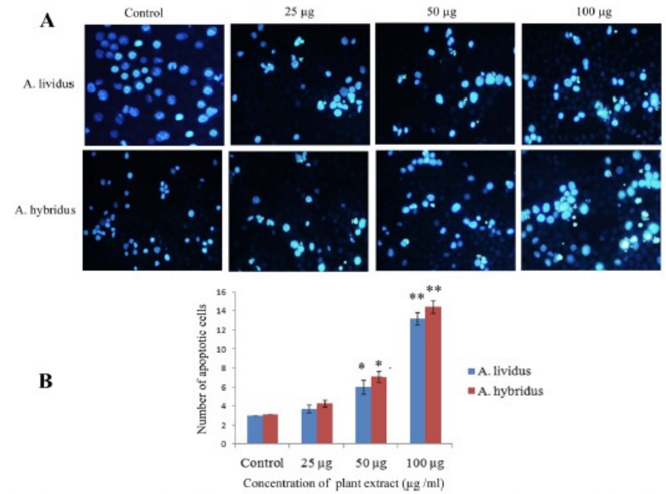

Fig. 4 Detection of apoptotic cells using DAPI staining after six days of treatment. **a** Treatment was started after 24 h of EAC cells injection, at the concentration of 25, 50 and 100 μg/ml. Marked apoptotic features such as membrane blebbing, cell shrinkage, chromatin condensation, aggregation of apoptotic bodies and brightly stained nucleus under blue fluorescence etc were observed in the treated groups, in contrast to round shaped and less brightly stained control cells. **b** Number of apoptotic cells per side was estimated by counting apoptotic cells in five different fields. Each value represents as mean ± SD ($n = 3$). Significance was set at $P < 0.05$ (*) and $P < 0.01$ (**) with respect to control

References:

[13] Shan Jin Y, Xuan , Chen M, Chen J, Jin , Piao J, and Tao J: Antioxidant, Antiinflammatory and Anticancer Activities of *Amaranthus viridis* L. Extracts. *Asian Journal of Chemistry*; Vol. 25, No. 16 (2013), 8901-8904.

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Toxicity:

- *A. spinosus* showed no toxicity or mortality up to a dose of 2000 mg/kg body weight in experimental rats [15]. The LD50 of the ethanol bark extract is greater then **2000 mg/kg** [15]. The aqueous extract of the bark of *A. spinosus* has a **lower toxicity LD50 value of 1450mg/kg** [15].

- *A. spinosus* was reportedly the culprit in cases of spontaneous poisoning of cattle in Brazil during a severe drought. Clinical signs appeared after 30 days in 11 out of 35 adult cows and 8 out of 20 yearling calves which were introduced into a 15 ha maize plantation heavily infested with *A. spinosus*. However, only one calf died within 3-7 days [16].

- The clinical signs of intoxication were: depression, anorexia, marked weight loss, foul smelling diarrhea occasionally tinged with blood, and subcutaneous edema. In post-mortem findings from 5 animals, **the mucosa of the digestive system showed necrotic glossitis, oesophagitis and pharyngitis, abomasal hemorrhages and button-like ulcerations** in the large intestine. The contents of ileum, colon and rectum were blood stained. Hemorrhagic diathesis was apparent by the presence of intra-abdominal hematomas. Histologically, there was **marked tubular nephrosis** associated with epithelial regeneration and hyaline intra-tubular casts. The mucosal lesions consisted of large necrotic areas in the epithelium which extended into the lamina propria and were associated with inflammatory reaction with massive infiltrations of mastocytes [16].

- *A. spinosus* also caused an outbreak of acute poisoning in ewes in southern Brazil. The clinical signs were uremic halitosis, loss of ruminal motility, dispnoea and abortion. The kidneys showed pale red spots, white streaks extending from the cortex to medulla and congestion. Histologically, there was **severe acute tubular nephrosis, dispersed foci of coagulative necrosis in the liver, areas of coagulative necrosis in the myocardium and acute incipient interstitial pneumonia and secondary bronchopneumonia**. Hyperkalemia secondary to renal insufficiency was the underlying cause of myocardial coagulative necrosis observed in seven sheep [17].

References:

[15] D.J. Ecobichon: Fixed Dose Procedure, Guidline 420. *The Basis of Toxicity Testing, 2nd edition*, (CRC Press, 1997) 43.
[16] Cai Y, Sun M, Corke H. Antioxidant activity of betalains from plants of the amaranthaceae. *J Agric Food Chem* 2003; 51: 2288-2294.
[17] Sharma S, Kathuria PC, Gupta CK, Nordling K, Ghosh B, Singh AB. Total serum immunoglobulin E levels in a case-control study in asthmatic/allergic patients, their family members, and healthy subjects from India. *Clin Exp Allergy* 2006; 36: 1019-1027.

Conclusions:

- Data suggest that *Amaranthus* plant extract inhibits the growth of cancer cells by induction of apoptosis.
- However, the complete mechanisms underlying the therapeutic effects such as cytotoxicity need to be investigated as an approach for the development of effective combinational therapy against a range of cancer cell line.