

Review Article

An overview on *Amaranthus cruentus* L.: Alternative source of crop, its economical uses and associated beneficial microbes

ABSTRACT

Amaranthus cruentus L. is a medicinally important locally available tropical plant belonging to the family Amaranthaceae. In present scenario, this plant is designated as 'alternative source of crop' or 'Pseudocereal' having various nutritional properties (Protein, carbohydrate, fatty acid, Vitamins, Minerals). Moreover, this plant has various medicinal properties also i.e. antioxidant, antihypertensive, antidiabetic, immune stimulation, anthelmintic, hepatoprotective, antimalarial etc. This plant has Worldwide distribution and short growing period of time. It is also regarded as Fodder crop and other industrial uses. The microbes associated with this plant help to protect the host plants from both pathogenic microorganisms and adverse climatic conditions by secreting different types of bioactive secondary metabolites i.e. phenolics, flavonoids, terpenoids, terpenes, tannins, alkaloids, quinones, anthocyanin, saponin, steroids and promote growth and development of the plant. Some unique features of this plant are also described here. This review intends to expand the understanding of taxonomic characteristics of this plant, economic uses, associated beneficial microbes and their application for sustainability of this alternative source of crop. The cultivation of *A. cruentus* possess a good attraction among both the researchers and farmers and cultivation procedure is more easy than other species. Some species of this genus are not cultivated yet, they are under process. Additionally, this species is more adorably cultivated due to the high nutritional value of its seeds.

Keywords: *Amaranthus cruentus*, alternative source of crop, secondary metabolites, microbes.

1. INTRODUCTION

Amaranthus mainly consists of 400 species, which are under cultivation. But, the total number of species under this genus are needed to be determined. The genus includes leafy vegetable amaranth, grain amaranth, ornamental, and weedy amaranth. Three major seed amaranth types: *A. caudatus* L., *A. hypochondriacus* L., and *A. cruentus* L., with *A. tricolor* grouped as an ornamental. *A. blitum* (slender amaranth), *A. lividus*, *A. dubius* (spleen amaranth), *A. spinosus*, *A. thunbergia*, and *A. graecizans* are produced for their leaves (leafy amaranth). Typical types common in Sub-Sahara are *A. graezianus* & *thumbergii* (Mukuwapasi et al., 2024).

Amaranthuscruentus is a tropical, endemic, invasive, short lived perennial herbaceous plant in Africa and South America (Gresta et al., 2020). Nevertheless, now this plant is distributed all over the world and contains approximately 70 species (Gresta et al., 2020). It is cultivated mainly in tropical and temperate region (Letchamo et al., 2017). This species has greater variability (Ma et al., 2021; Gins et al., 2024). Phenotypic plasticity, Introgression and hybridization are profoundly observed in this species (Gresta et al., 2020; Prajitha & Thoppil, 2018; Jimohetal., 2022; Amosova et al., 2024). To protect from pathogenic microorganism, this plant has some advanced physiological features (Jimohetal., 2022). In Africa, this plant is recognized as source of food and constructive and traditional medicine (Letchamo et al., 2017). In present scenario, food scarcity is a major problem and food security is necessary to protect the future generations. So, this plant is used as 'alternative source of crop' and also considered as 'Pseudocereals' (Fletcher, 2015; Mburu, 2011; Gresta et al., 2020). Moreover, this plant is cultivated for using both the Grains and leaves in Africa and Asia (Hoidal et al., 2019). The cultivation procedure is followed by firstly, 2-4 inches shoot with 2-4 leaves cut and then they are deeply buried into the soil and regularly watered. When the root is optimum for its growth and when the plants are 14-20 days old, the leaves are harvested (Achigan et al., 2014). It has various medicinal principles i.e. antioxidant, anti-diabetic, anti-inflammatory properties, antihypertensive properties, stimulation of immune system, anthelmintic properties (Torane et al., 2017) etc. Presence of 3.1% phenolics and 11.6% flavonoid contribute to the antioxidant and anti-inflammatory property to scavenge the free radicles and protect from Reactive Oxygenic Species (ROS) (Ali, 2021). 3.6% alkaloids present in it possessing antimicrobial property and antidiabetic property is shown due to the presence of 1.3% saponin. (Ali, 2021). This plant harbor endophytic and others microflora. Endophytic

microbes secrete active secondary metabolites i.e. phenolics, flavonoids, terpenoids, terpenes, tannins, alkaloids, quinones, anthocyanin, saponin, steroid etc. These metabolites are potential agents for antimicrobial, anticancerous and many other therapeutic properties which are beneficial to protect the host plant from both biotic and abiotic stresses (Mousavi&Karami,2022). This plant is an example of C4 photosynthetic ones (Achigan et al., 2014, Gins et al., 2024) and reported as high-water using efficiency (WUE) (Wolosik&Markowska,2019) and grow in any types of soil i.e. acidic, alkaline, saline or nutrient-poor soil (Park et al., 2014). This species is highly resistant to drought (Gresta et al., 2020; Achigan et al., 2014) and salinity(Gins et al., 2024;Emam et al., 2024) heat, oxidative stress (Jimohetal., 2022). and UV radiation also(Castrillón&Frier, 2016 and;Omami&Hammes, 2006). It has very good nutritional properties as it contains vitamins, proteins (Lysine, Tryptophan), unsaturated fatty acid, minerals, tocopherols and tocotrienol etc. and essential antioxidant properties (Wolosik&Markowska,2019). Its short growing period give expectancy and provide a source of employment to the farmers (Makinde&Ojeniyi, 2010). According to genomic studies, *A. cruentus* contains 17 chromosomes (Sammour&Mitra,2012).The species are diploid ($2n=32$) sometimes chromosome no. varies ($2n=34$). So, the chromosome no. $n=17$ sometimes $n=16$ (Hricovaetal., 2016, Ma et al., 2021). Previous report shows that it is hybridized easily (Lanta&Ondrej,2003). In South Africa, it is considered as potential alternative cereal having minerals, phytochemicals (Manyelo et al., 2022). Several species of *Amaranthus* have a role in CO₂ sequestration (Jimoh et al., 2022; Malik et al., 2023). This is short day plant and flowering takes place in 44 days and harvested in 102 days (Ogwu,2020). Microbes present in the plant significantly help the host to increase growth, protect from both biotic and abiotic stresses and also stimulate the medicinal activities without causing any disturbance or damage. In this review, taxonomic characteristics, secondary metabolites, economic importance of this plant and lastly the microbes (PGPR, PGPF, fungal and bacterial Endophytes and Mycorrhizae) associated with this plant and how they promote the plant growth is discussed here.In this review, we discussed mainly the beneficial features and also the exceptional features of this plant *A. cruentus* (Red Amaranth). It distributed all over the world and can grow any type of soils. This C4 photosynthetic plant can tolerate adverse climatic conditions. Short growing period are very advantageous for agriculture and farmers are also benefitted. As India and most of the countries are suffered from Malnutrition, this Red Amaranth provide high amount of nutrition (amino acid, carbohydrate, fatty acid, vitamins, minerals, fibres, antioxidant). Financial expenditure of the maintenance of this plant is comparatively low.

2.TAXONMIC DESCRIPTION OF AMARANTHUS CRUENTUS L.

2.1Taxonomic classification: (Wolosik&Markowska,2019)

Kingdom: Plantae

Subkingdom: Tracheobionta

Subdivision: Spermatophyta

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Caryophyllidae

Order: Caryophyllales

Family: Amaranthaceae

Genus: *Amaranthus*

Species: *Amaranthuscruentus* L.

2.2Habit:

This plant is herbaceous, tropical and belongs to the family Amaranthaceae. The common name of the plant is red or blood amaranth. In Bengali, it is popularly known as 'Lal note sak'. It reproduces by seeds and have very short (4-6 weeks) growth period (Wolosik&Markowska,2019).

2.3 Phenological Characteristics:

One dominant large tap root is present which is soft, 6-7 cm long (Wolosik & Markowska, 2019; Mukuwapasi et al., 2024).

Stem is straight, branched, thick, ribbed, 60-65 cm in height, reddish in colour, surface minutely haired (Wolosik and Markowska, 2019).

Leaves are simple, reddish, 5-7.5 cm length and 2.1-4.2 cm breadth, margin entire, spiral arrangement, shape ovoid, exstipulate, leaf surface hairy. Petiolate, petiole 0.5 to 1 cm long, leaf apex retuse (Wolosik and Markowska, 2019; Mukuwapasi et al., 2024).

Inflorescence is numerous concentrated spikes, axillary arrangement, ended with racemes and spikes. More than 50 cm long, reddish or pinkish colour. Branches are 40-45 cm long, perpendicular with numerous laterals. Monoecious bearing both male and female flower (Wolosik and Markowska, 2019).

Flowers are numerous, unisexual, greenish and form a terminal spike (Wolosik and Markowska, 2019).

This plant produces thousands of seeds which are round or lenticular, dark brown or shiny, *Campylotropus ovule* (Wolosik and Markowska, 2019). Endosperm present in the micropylar region (Wolosik and Markowska, 2019)

Dissected parts of the plant under the simple microscope are represented in figure 1.

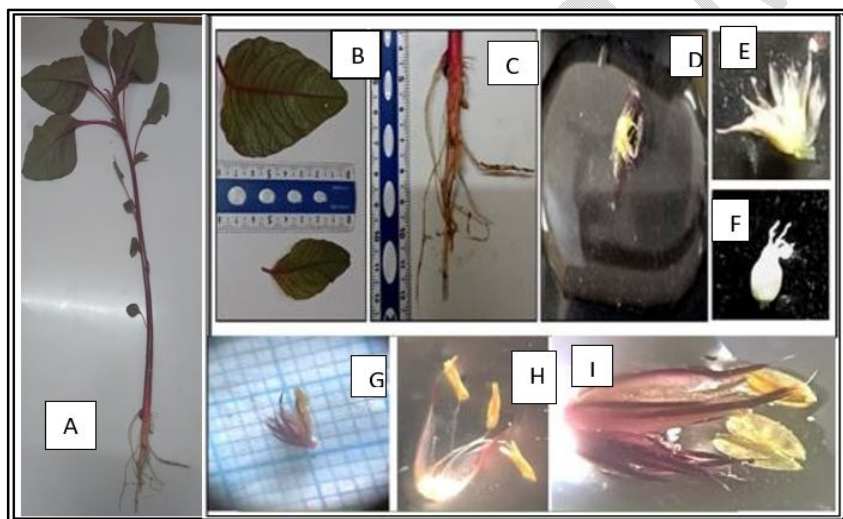


Figure 1. Morphological characters of *Amaranthus cruentus* (where A, B, C, D, E, F, G, H, I represent full plant, dorsal and ventral surface of the leaf, root morphology, Dissection of female flower, male flower)

3. ECONOMICAL USES

3.1 Food: *Amaranthus cruentus* is a leafy vegetable (Toraneetal., 2017; Mukuwapasi et al., 2024) and useful food supplement for human. This vegetable is similar to the well-known Barley, Wheat and Bazra crop for its nutritional properties and contain high amount of amino acids. So, this plant is considered as 'Alternative Source of Crop' or 'Pseudocereal' (Fletcher, 2015; Mburu et al., 2011) and it protects the disease Malnutrition or Undernutrition. It has essential nutritional properties i.e. amino acid glutamine (Motta et al., 2019) proteins (Lysine and Tryptophan), vitamins, essential minerals, saturated and unsaturated fatty acid (Gresta et al., 2020), tocopherol, tocotrienol etc. and also contain high amount of fiber (Ali, 2021). Powdery dry leaves are used in sauces during the dry weather (Aderibigbe et al., 2022; Toraneetal., 2017). In Nigeria, leaves are mixed with the condiments to

prepare the soups (Mepba&Banigo, 2007; Ali, 2021). Boiled leaves are mixed with groundnut flower and consumed as relish (Ali, 2021). Leaves are consumed as spinach or green vegetable (Dhellotetal., 2006). Mixture of boiled leaves and groundnut sauce are prepared for salad in West Africa and Mozambique (KauffmanandWeber,1990). Seeds are also used as nutritious food which enriched in minerals, vitamins and proteins (Emam et al., 2024;Mukuwapasi et al., 2024).In America, this plant is cultivated as Grain seed or grain crop. Seeds are boiled and then eaten as rice. Laddoos are made from Grain Amaranth in India. Flour is made from the seeds which is gluten free, so it is fed to the Cealic patient or Gluten allergic patient comfortably and popularly used in baking Industry and producing snack bars (Mukuwapasi et al., 2024, Wolosik and Markowsa, 2019). The steps are followed to prepare flour from seeds are Flaking, Extruded and finally Grinding.

3.2Fodder: This plant is used not only for food but also as fodder. It has been planted as leafy vegetables for cattle, rabbit, pigs and chicken food across many countries. Dietary fiber present in the plant have positive impact on animal gut by improving behavior on digestive tract (Manyeloetal., 2022; Alegbejo 2013).*A. cruentus* plant have high energy source and have not any negative effect on cattles i.e., Ships, Rabbits and Pigs. It is a substitute of Oil cake which is very essential for animal food. But, oil cake is too much costly. The clustered inflorescence of this plant have potential nutritional properties which acts as a substitute of Oil cake in Rabbits. In case of pig, this Amaranth grain is also helpful because it fulfils the dietary criteria but not fatter the pigs. It has no negative effect on growth of lambs also (Grestaet al.,2020).

3.3Cosmetics Industry: Amaranth oil contains high amount of unsaturated fatty acid, tocopherols, Squalene which act as hair and skin conditioner (Baraniak&Kania-Dobrowolska2022). This seed oil also cures aging effect, wounds, skin diseases and moistening the skin. It is efficiently used as strong antioxidant and photoprotector in herbal medicine industry (Baraniak&Kania-Dobrowolska,2022).

It is also used as lubricant in computer industry (Achiganetal., 2014).

Oil extraction: Amaranthseeds were grinded for 30 seconds in a micro mill and oil extractedwith hexane in a Soxhlet apparatus for 7 hour (Leonet al., 2001). Antioxidant extraction:Powdered seed samples and blended grains (1 g) were extracted with40 mL 8:1:1 (v/v) methanol–0.16 molar hydrochloric acid–water for 2 h. Theextracts were then separated by decantation and the residues were extracted with 40 mL 70% acetone again for 2 h. The extracts were combined, separatedby decantation, centrifuged, and stored in dark condition in a refrigerator at –20°C (Pasko et al., 2008).

3.4Dye: In some countries, this plant is cultivated or grown only for making different dyes (Toraneetal., 2017) Dried plant is burnt for the preparation of potash in Benin countries (Toraneetal., 2017). Plants are used to extract red dye or coloring corn-based foods in South Western United states (Allemann,2016).Red dye obtained from this plant is utilized to colour textile materials, medicines, food product and beverages (Ogwu,2020).

3.5Ornamental value: *A. cruentus* is cultivated as an ornamental plant in some countries (Toraneetal., 2017) specially in Nigeria (Ogwu, 2020; Mukuwapasi et al., 2024) valued for its feather-like flowering plumules and also have aesthetic value also (Hricovaetal., 2016). And sometimes it used as 'potherb' (Das & das, 2016). The *A. cruentus* isgreatly valued as ornamental because of its bright colour flowers(Amosovaetal., 2024).

3.6Medicinal properties: It has various medicinal properties i.e. antioxidant (Osei et al., 2023), anti-inflammatory properties, antihypertensive properties stimulation of immune system, cardioprotective, antidiabetic, antimalarial, hepatoprotective (Peter & Gandhi,2017), anthelmintic (Torane et al., 2017), antiandrogenic (Alegbejo, 2013) etc. Bioactive secondary metabolites and protein hydrosylates present in the plant are potential anticancerous, antimicrobial agents (Ramkisson et al., 2020). Presence of high amount of fiber reduces cholesterol levels as well as risk of cardiovascular diseases (Jimoh et al., 2022) and inhibits the intake of starchy food decreases the chances of diabetes (Adeleke &Babalola, 2021). Prevention of diuretic and hypertension problem is revealed by potassium content (George, 2015). Oils or fats decreases the lipid content of blood and chances of coronary diseases have been reduced (Chatepa& Masamba,2020)

Traditional Methods: According to report of Azab 2020, the cooked leaves of the plant have laxative properties. Seeds have some medicinal properties also i.e. hypocholesterolemic effect. For infants,

treatment of constipation, roots of this plant is boiled with honey and feed it (Aderibigbe et al., 2022). Its water extract is used for the treatment of limb pain and as expellant of tapeworm also for wound dressing and tumors (Aderibigbe et al., 2022). This leafy vegetable is also important for lactating women to treat kidney complaints, constipation, anemia etc. (Torane et al., 2017; Aderibigbe et al., 2022; Achigan et al., 2014). Decoction of leaves is utilized as sore throat, ulcers and extraction of inflorescence swallowing by women for relieving from periodic pain (Ali et al., 2021).

Industrial Methods: Ethanolic leaf extract have protective effect such as restoring RBC, WBC and hemoglobin levels in blood (Azab, 2020). So, it is used in Anemia treatment. *A. cruentus* extract administrated to rats receive Cyclophosphamide which is an immunosuppressant to treat nephritis and arthritis and also used in Chemotherapy in combination with other drugs (Pandey et al., 2016). Ethanolic leaf extract of mixture of *Amaranthus cruentus* and *Cleome gynandra* have blood glucose lowering effect (Siwale, 2018).

4. BIOACTIVE SECONDARY METABOLITES PRESENT IN THE PLANT

A number of different bioactive secondary metabolite present in the plant is graphically represented in Figure 2 (Peter&Gandhi,2017). which helps to promote plant growth and protect from pathogens. These metabolites also possess antioxidant, biochemical and pharmacological properties and beneficial for human health (Omale & Okafor,2008).

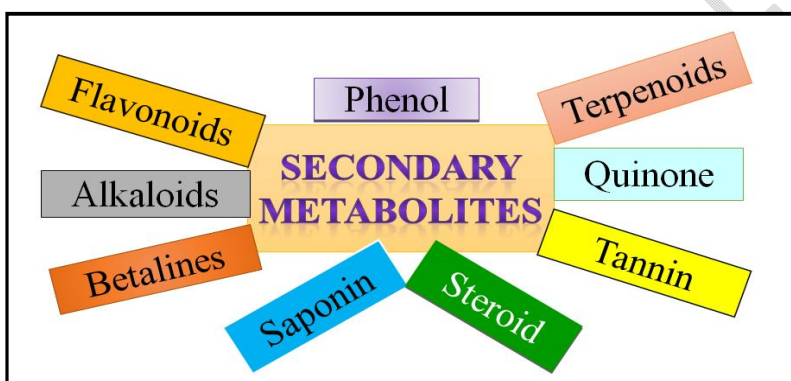


Figure 2. Lists of Secondary Metabolites of red Amaranth (Peter&Gandhi, 2017)

4.1 Betalines: Betacyanin (red violet pigment) and Betaxanthin (yellow pigment) present in different parts (root, stem, leaf, flower) of the plant (Castrillón&Frier, 2016), water soluble vacuolar pigment used in Folk medicine and also as anticancerous, anti-inflammatory and cardiovascular agent (Nana et al., 2012). The Betacyanin and Betaxanthin content were 7.74 and 19.34 mg/100 g dry weight respectively (Nana et al., 2012). Extraction and testing method of Betalines followed by the method Nana et al., 2012. 5gram fresh plant material was homogenized in mortarpestle and extracted with 25 ml distilled water containing 250 milimolar ascorbate for 15 minutes. Then centrifuged at 6000 rpm for 15 minute and stored the supernatant at 4°C. Betalines content was done by spectrophotometric method 536 nm for betacyanins and 475 nm for betaxanthins with molar extinction coefficient.

4.2 Phenolics: Anthocyanin produce excessive radicles which surpass the physiological antioxidant properties (Alvarez et al.,2010). Salicylic, cinnamic, ferulic, p-coumaric, gallic, sinapic acids are also present (Peter&Gandhi,2017;Castrillón&Frier, 2016). Premature grain of this plant contains a huge amount of phenolic acids i.e., ferulic acid, caffeic acid, galic acid, p-coumaric acid and anthocyanin) than mature grain (Manyeloetal., 2022, Osei et al., 2023). It has been reported that phenolics have beneficial role in antioxidative property serving as the free radical scavengers and lower the heart diseases and inflammation (Omale&Okafor,2008; Iwuagwueta., 2019). Biosynthetic pathway of phenolics is diagrammatically represented in figure 3(Buchanon, 2015).

Extraction procedure is done according to the method of Torane et al., 2017.First, plant material was collected and then air dried. Afterthat,10g plant material was extracted with 50 ml of solvent which have different polarity. Then they are kept at room temperature for 24hrs.To obtain the crude extract,

the solvent was recuperated under low pressure. Quantity of each phenolics is represented in Table 1.

Table1. Quantity of each phenolics in premature and mature grain (Manyelo et al.,2022)

Phenolics	Premature Grain (mg/kg)	Mature Grain(mg/kg)
Ferulic acid	310	345.20
Gallic acid	41	43.48
Caffeic acid	6.5	9.93
p-Coumaric acid	1.2	2.94
Anthocyanin	35.2	35.92

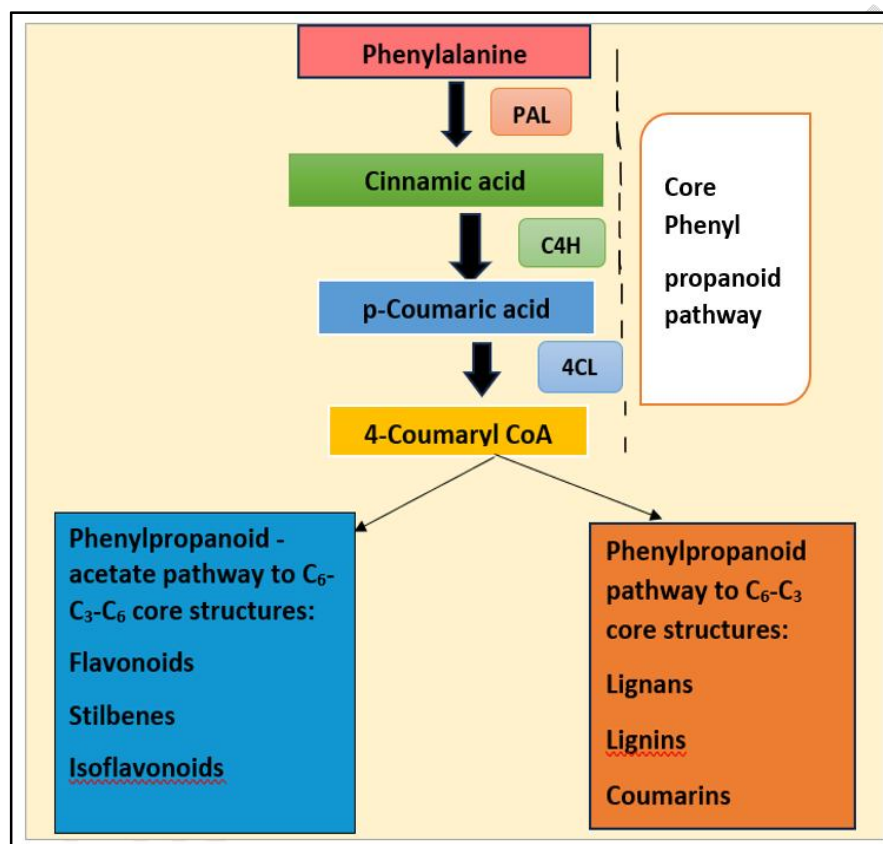


Figure 3. Overview of the biosynthetic pathways contribute to the production of several major phenolic compounds in plants. The synthesis process starts with Phenylalanine and diverge into phenylpropanoid – acetate and phenylpropanoid pathways. First three steps are common to both pathways. The core phenylpropanoid pathway ends with the formation of 4-Couaryl CoA where PAL- Phenylalanine ammonia lyase, C4H- Cinnamate-4- hydroxylase, 4CL- Coumarate CoA ligase. adapted from Buchanon (2015).

4.3Flavonoids: Rutin is one of most important flavonoids reported to have various beneficial effects on human and protective effect on memory disfunction (Koda et al., 2009). Quercetin is also reported. It serves as antimicrobial agent and showing potential antioxidant property (Castrillón&Frier, 2016) and reduce the oxidation level of triglyceride, cholesterol, low density lipoprotein (Erdtmanetal., 2007).

4.4Terpenoids: Terpenoid showing antimicrobial, antifungal, antiparasitic, antiviral, anticancerous, antispasmodic, antiallergic, anti-inflammatory, immunomodulatory properties (Rabi&Bishayee,2009).

4.5 Alkaloids: It is one of the important metabolites in living organisms help in development of the plant and served as useful repellent for any pathogen or parasite and possesses antimicrobial properties (Usunomena&Ngozi,2016;Cherian&Sheela,2016).

4.6 Quinones: According to the result of phytochemical screening, ethanolic extract contains flavonoid, phenolics and quinones which are used in glucose tolerance test i.e., antihyperglycemia (Nofianti,2015).

4.7 Saponin: It limits the growth of cancerous cells and has beneficial effects on antiinflammation (Prohp&Onoagbe,2012; Rao&Sung,1995) and stimulate release of insulin and lower blood glucose level (Siwale, 2018).

4.8 Steroid: Steroid is present at vegetative and flowering stage which protect from harmful effects of microbial pathogens and also have anti-inflammatory and anti-aging properties (Okunlolaetal., 2017). Amaranth grain and leaf extract contain high amount of Squalene (He&Corke,2003).

4.9 Tannin: It has been reported to possess potential antiviral (Cheng& Lin, 2002) and anticancerous activity (Narayanan etal., 1999).

The Extraction procedure is discussed here. Collected plant material was air dried and grinded and then filtrate to obtain fine powder. 30 g dried plant powder was soaked in 300 ml of ethanol and n-hexane respectively and kept this mixture for 2 days. Then the mixtures were filtered by using the Whatman filter paper. The filtered material was dried in the vacuum by utilizing the rotary evaporator. Dried-out residue was used as extract (Edo and Onoharigo,2024).

This procedure confirms the presence of alkaloids, tannins, steroids, terpenoids and quinones in ethanol and n-hexane extract.

Saponin extraction begins with the dried plant extract mixed with methanol or water and then used organic solvent chloroform to remove the lipid substance. Soxhlet extraction done, after evaporation and most often dissolution in water and phase separation into n-butanol. Saponins with higher polarity could found in aqueous phase (Mroczek, 2015).

The quantity of each secondary metabolites is represented in Table 2.

Table 2. Quantity of each secondary metabolites (Ali,2021)

SecondaryMetabolite	Quantity (%)
Phenolics	3.10
Flavonoids	11.60
Terpenoids	1.8
Alkaloids	3.6
Saponin	1.3
Steroid	4.3
Tannin	2.40

5. BENEFICIAL MICROBIAL ASSOCIATION

5.1 Endophytes: Numerous studies have shown that symbiotic (mostly Endophytic) fungi enhance abiotic stress tolerance mainly water deficit i.e. Drought and protect the host plant from critical environmental condition and plant survive (Rodriguezetal., 2009; Singhetal., 2011). Endophytic microbes reside within living plant tissues and can interact with roots, stem and leaves without causing symptoms of diseases in their host plants (Schulz& Boyle, 2006). Commonly found fungal endophytes belong to different classes of Ascomycota, mostly Dothidiomycetes, Leotiomyces, and Sordariomycetes, and in some hosts Basidiomycetous fungi are also observed (Rodriguez et al., 2009). Endophytic fungi form a symbiont with the host plant and can protect from insect herbivore by changing the chemical composition of the plant and form a chain between the parasitism and mutualism (Rodriguez et al., 2009). The endophyte infected plants express a range of adaptations to biotic stress (Morelietal., 2020) and abiotic stresses mainly drought tolerance. Some previous reports demonstrated that many grass species showed vegetative growth improvement in the presence of their fungal symbionts and these endophytic fungi enlarge plant fitness by the discharge of plant

growth advancing secondary metabolites (Priti et al., 2009; Hardoimet al., 2015). Endophytes have both the horizontal and vertical mode of transmission (Frank et al., 2017). Endophytic microbes are mainly bacteria and fungi and some reports revealed that it may be algae also (Rit et al., 2023). Fungal endophytes mainly belong to the Ascomycetes, Zygomycetes and Deuteromycetes group and Basidiomycetous fungi is rarely reported (Rajamanikyamet al., 2017; Stone et al., 2000). Endophytes showing mutualistic behaviour i.e., it receives nutrition and shelter from host plant and give the competitive ability to the host (Rajamanikyamet al., 2017). After isolation, pure culture and characterization of each specific endophyte, Maximum individual possessing antibiotics, anticancerous, antimycotics and immunosuppressant property (Alvin et al., 2014). Treatment of various types of endophytes upon *Amaranthus* plant showing enhancement of growth due to discharge of different plant growth regulator i.e. IAA, GA etc and increased SAR by decreasing diseases by direct antagonistic behaviour (Uppala et al., 2010). Fungi Isolated from Phyllosphere and Rhizosphere of *A. cruentus* (Cladosporium, *Talaromyces*, *Gibberella*, *Penecillum*, *Alternaria*, *Trichoderma*, *Rhizopus*, *Fusarium*) increase the soil quality of the plant and plants health and produce antibiotic compound against pathogens (Puszetal., 2015). According to Uppala et al., 2010, several bacterial endophytes promotes biometrics of Amaranth. Some endophytic bacteria isolated from plant roots and promote plant growth, survival and protect from biotic stress (Enebe&Babalola, 2018). Endophyte protects the host plant from oxidative damage by producing ROS scavenging enzymes i.e. Superoxide dismutase (SOD), Glutathione peroxidase (GR) etc. (Choudhury et al., 2023). It was reported in Red Amaranth that the application of an endophytic strain *Sphingomonaspanaciterrae* not only increases the morphological parameter i.e., root length, shoot length, leaf number etc. of the host plant but also increase the chlorophyll and mineral content of the plant(Sultana et al., 2024).

Endophytic fungi are isolated(Shilpa&Shikha, 2015) from different partsof the plant *A. cruentus* is represented in figure 4.

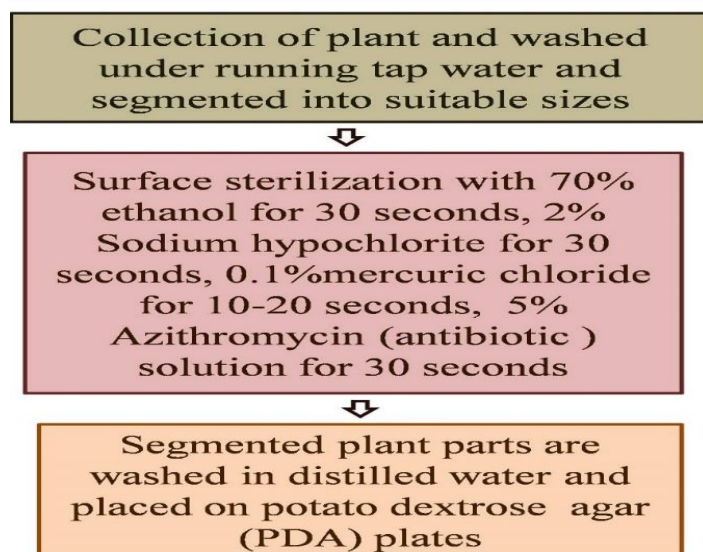
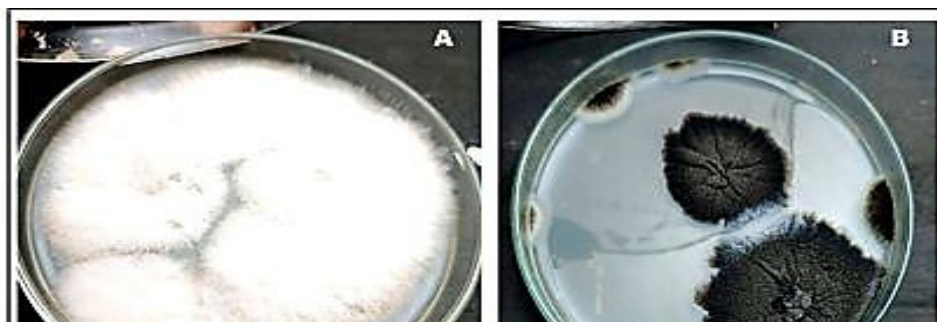


Figure 4. Protocol of Isolation of endophytic fungi from *A. cruentus* (Shilpa and Shikha, 2015)



The plates containing pure culture of the isolates are represented in figure 5.

Figure 5. Fungal isolates after pure culture

According to standard protocol, leaf tissues of red Amaranth were dissolved in organic solution and used to detect endophytes under microscope after staining with Trypan blue (Vahabi et al., 2011).

5.2 PGPR and PGPF: Plant Growth Promoting Rhizobacteria (PGPR) are a group of bacteria that enhance phytoremediation (Muthukumar et al., 2017). PGPR are actually the beneficial, non pathogenic rhizobacteria which colonize the root surfaces but not inside the plant tissues and promote the growth and development of the host plant (Rit et al., 2023, Choudhury et al., 2022). It colonizes plant root surface efficiently and promote plant growth. PGPR may facilitate plant growth either by promoting the acquisition of nutritional resources such as Nitrogen, Phosphorus or Iron; preventing or decreasing the damage to plants by pathogenic organisms (mainly fungi and bacteria) or by directly stimulating plant growth by either providing plant hormones such as Auxin, Cytokinin or GA or lowering plant ethylene levels through the action of the enzymes 1 amino-cyclopropane 1 carboxylic acid deaminase (ACC) (del Carmen Orozco-Mosqueda et al., 2020, Choudhury et al., 2022, Rit et al., 2023). Other mechanisms involved i.e. solubilization of minerals, production of siderophores, increases in leaf area, chlorophyll and soluble protein content. These can also produce antioxidant, enzymes to protect plants from environmental stresses that lead to generation of ROS which cause cell damage (Choudhury et al., 2022). Rhizobacteria may reduce the level of disease by synthesizing hydrolytic enzymes, antibiotics, introduction of systemic acquired resistance (SAR) and competition for nutrients and niches (Sindhu & Sharma, 2019). PGPR significantly decrease the use of chemical fertilizer, pesticides, insecticide and herbicides in modern agricultural practices (Choudhury et al., 2022).

PGPR *Pseudomonas sp.*, isolated from rhizosphere soil improve plant growth and crop production and survival of *Amaranthus* species and protect from pathogen attack (Enebe & Babalola, 2018; Ikhajagbe et al., 2021). They have the ability to produce siderophore, hydrogen cyanide (HCN) and phosphate solubilization property (Sagar et al., 2018).

PGPF are mainly rhizospheric fungi which promote plant growth and protected from diseases by colonizing the root. But, endophytic fungi (inside the plant tissues), phyllospheric fungi are also included in PGPF (Rit et al., 2023; El Maraghy et al., 2021). Plant Growth Promoting Fungi (PGPF) maintain plant growth through the generation of a number of significant enzymes like ACCD (1-aminocyclopropane-1-carboxylate deaminase), urease, catalase etc, phosphate solubilization, siderophores and IAA production, antagonism to pathogens and resistant to antibiotics and take a crucial role in plant growth. PGPF are non-pathogenic, soil borne fungi enhance plant growth and survival (HyAKuMAcHi, 1994; Debashis et al., 2020). It also enhances soil fertility, productivity, nutrient uptake and Defence mechanism (Yapa et al., 2022). It triggers induced systemic resistance (ISR) against pathogenic microorganism by modifying cell wall accumulating callose, phenol, lignin (Yapa et al., 2022). Different PGPR and PGPF strains when combined and applied to soil of

Amaranth, then morphological parameters i.e., shoot height, root height, fresh weight all are increased and induced SAR (Debashis et al., 2020).

5.3 VAM: Mycorrhiza (VAM) is the symbiotic association between higher plants and fungi (Giovannini et al., 2020). The fungus grows internally inside the root cortical cells where they form specialized structures called vesicles and arbuscules (Holland & Roth, 2023). Vesicles are swollen, sac like, unbranched, modified structures of the fungal hyphae. The major function is the storage of reserve food whereas arbuscules are branched, finger like projected hyphal modifications of the endomycorrhiza and the major function is the absorption of food nutrients and water from the host cytoplasm (Holland & Roth, 2023). VAM efficiently extend plant roots making the uptake of water much more efficient, better mineral nutrition especially Phosphate, alteration in root architecture, modification of some physiological and enzymatic activities, induction of the plant hormones ABA (Feng et al., 2020). VAM inoculation increases stomatal conductance under drought condition. The VAM fungi are obligatory biotrophs in nature (Giovannini et al., 2020) and also act as bioprotectants against pathogen and toxic stresses. Application of VAM as fertilizer to the Amaranth plant significantly increase the shoot diameter, root length, leaf number etc. (Dada et al., 2017). According to the study of Safeena & Sasmina, 2018, AM (*Glomus fasciculatum*) were inoculated into the pesticide treated soil of *A. cruentus*, the result revealed that growth of the plant is increased and pesticide toxicity in the soil is decreased and soil-microbe growth are not inhibited. Report of Kalaikandhan et al., 2012, revealed that when VAM (*Glomus hoi*) is inoculated in soil of *A. cruentus*, heavy metal concentration of soil is reduced because of the VAM fungi having capacity to sequester it in their mycelia and make the soil less toxic. In semiarid and arid regions of India, occurrence of VAM was reported in some members of Amaranthaceae mainly *Amaranthus*. Vesicles, arbuscules and intracellular hyphae was observed in root cortical zone of the host plant (Neeraj et al., 1991)

7. CONCLUSION

This plant has huge role in food, fodder, dye, cosmetic and medicinal industry. Secondary metabolites extracted from this plant possesses important role in pharmacy Food quality is more important to prevent malnutrition than food quantity. Major food crops are deficient in proper nutrient. By consuming these crops, many peoples are susceptible to diseases. This Red Amaranth is considered as 'Alternative source of Crop' or 'Pseudocereal'. It contains high percentage of nutritional compounds, bioactive secondary metabolites and medicinal properties. This plant is not only used for human food but also very efficient Fodder crop. This leafy vegetable is rich source of dietary fibre which helps in digestion of animals. In some countries, it, regarded as 'Ornamental' and also used in dye and cosmetic industry. Bioactive secondary metabolites (Phenolics, Flavonoids, Terpenoids, Saponins, Alkaloids, Quinone, Tannin) present in this plant prevent the entry of pathogenic microorganisms and have several medicinal activities i.e. antioxidant, anti-inflammatory, immunomodulatory etc. Microbes are a noble and consistent source of unique natural medicines with a high level of biodiversity and may also yield several pharmaceutically important compounds. This symbiotic association at the molecular and genetic levels will be helpful for enhancing secondary metabolite production and can be an active area for future investigations. In recent years, there have been a large number of studies, both in the laboratory and in the field, that have provided information about the use of plant-associated microbes to the benefit of plant growth and development. Bacteria, fungi, mycorrhiza provide resistance to plants against various environmental (abiotic) stresses. Fungi isolated from this plant secret antibiotic compounds and protect plant from abiotic stresses (Pusz et al., 2015). PGPF are non-pathogenic soil borne fungi enhance nutrient uptake and soil quality of the plant have different antagonistic mechanisms. VAM have the capacity to decrease the heavy metal toxicity and devastating effect of pesticides of the soil. In the recent time, we are hopefully determined that the purposeful use of microorganisms will be commenced on to replace the use of energy-intensive and often pollution causing chemicals in agricultural practices. Application of microbes and microbial inoculants as biofertilizers and biocontrol agent is an integral component in organic farming practices. Lastly, improved understanding of

'mycorrhizosphere' interactions is crucial in order to preserve crop yield stability under an increasingly unstable climate change. To think about the food scarcity of our near future, we should protect this plant as a Crop for alternative source and try to increase the production of the plant by using the associated microbes and secondary metabolites extracted from this plant.

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Competing interests

Authors declared that no competing interests exist.

Author Contribution:

Main concept was given by Sikha Dutta. Literature review was performed by Chandrama Mukherjee. Editing of figures, arrangement of references and formatting of total manuscript was done by Debapriya Choudhury. Arrangement of references were done by Shinjan Dey and Sayani Datta. Whole manuscript was discussed by all the authors. Finally, all authors read and approved the manuscript.

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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