American Journal of Applied Bio-Technology Research (AJABTR)

Vol: 4, Pg:5-29, Yr: 2023-AJABTR

Role of Secondary Metabolites of *Momordica charantia* in combating Copper induced stress

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Abstract:

Plants are widely used for various research purposes, including medicinal, agricultural, and ecological uses. The environment's industrialisation and urbanisation have resulted in a variety of biotic and abiotic challenges that plants must adjust to, such as drought, salt, water deficiency, microbial attack, temperature and pH changes, and heavy metal stress. Many unknown plant species have been tested for their medicinal properties, but many common plants have yet to be fully discovered. Among them is *Momordica charantia*, which belongs to the Cucurbitaceae family also known as Bitter gourd (Karela) is traditionally known for its medicinal and antioxidant properties. It has known to be a hyperaccumulator of toxic metals. *M.charantia* produces a great variety of secondary metabolites/phytochemicals which helps them in scavenging abiotic stresses, these secondary metabolites include terpenoid, alkaloids, phenols. Among abiotic stresses heavy metal stress is major concerns. Heavy metal such as copper when induces stress on hyperaccumulating plants bitter gourd. Copper is one of the essential micronutrients required by plants for Carbon assimilation and ATP synthesis. However excess exposure of Cu can cause oxidative stress an ROS production. *Momordica charantia* plants have developed defense mechanisms against oxidative stress and ROS, which can damage the plants. The secondary metabolites like Phenolics, Terpenes and Steroids, and Alkaloids & Flavonoids are produced by *M. charantia* which help scavenge heavy metal stress. Excess copper exposure in roots induces detoxification strategies such as metal chelation and transport. The most common ROS are O2, H2O2, and OH-, which are known to cause lipid peroxidation and consequently harm cell membranes. *M. charantia* produces flavonoids, which have antioxidant effects due to their high ability to chelate metals. The bitter gourd plant produces enzymes like Cu-SOD, CAT, and POX under copper stress, which help reduce the amount of ROS produced. This increases the activity of

antioxidant enzymes in metal-stressed plants, which is an important part of the plant antioxidant defense mechanism against metal-induced oxidative damage. The relationship between bitter gourd structure and processes of the efficacy of the numerous functional ingredients will likely be explained with much more research on the plants.

1.Introduction

Today, plants are widely used for various research purposes, including medicinal, agricultural, and ecological uses. Plants are the most important means of our survival. Plants need water, energy, carbon and inorganic nutrients to grow. A plant's response to stress depends on the tissue or organ affected by the stress. Any condition or substance that affects plant metabolism, development, or improvement is considered plant stress. Numerous environmental factors that affect plants can be roughly categorised into two categories. biotic stress and abiotic stress. The latter happens as a result of non-living things having a negative effect on the organisms, whereas the former is thought to be damage caused to an organism by other living creatures. Abiotic stress is defined as environmental conditions that reduce growth and yield to suboptimal levels. Abiotic stresses cause significant crop losses worldwide, including radiation, salinity, flooding, drought, temperature extremes, heavy metals, and more. The attack of several diseases, such as fungus, bacteria, oomycetes, nematodes, and herbivores, is a biological stress, on the other hand. Heavy metal stress, notwithstanding all other stresses, is one of the main issues with pronounced detrimental impacts on crop yield and growth. [1] Heavy metal stress causes plants to react in a number of ways, from biochemical processes to yield. Any metallic element with a relatively high density that can damage by inducing toxicity is at very low concentration referred to as a "heavy metal." [1]. Lead (Pb), cadmium (Cd), nickel (Ni), cobalt (Co), iron (Fe), zinc (Zn), chromium (Cr), arsenic (As), silver (Ag), and the platinum group elements are examples of heavy metals [1]. Some of these heavy metals, such as Cu and Zn, either operate as cofactors and activators of enzyme reactions, such as when they combine with substrate metal to form complexes with enzymes, or they have catalytic properties, such as the prosthetic groups found in metalloproteins [1]. Copper (Cu) is said to play two roles in plants. At low concentrations, it acts as a necessary element involved in carbon assimilation, organic matter transport, cell wall growth and metabolism. Excess copper exposure in roots induces detoxification strategies such as metal chelation and transport, activation of signalling mechanisms, hormones, proteins and antioxidant systems.

Vascular plants, of which there are an estimated 400,000 species worldwide, produce hundreds of thousands of metabolites, the structure, function, and utility of which have just been partially elucidated. Plants produce a wide range of structurally and functionally diverse metabolites that have various functions in plant development and growth as well as in how plants react to abiotic and biotic stimuli and the constantly changing environment. Primary and secondary metabolites are the two types of metabolites that plants create. The primary metabolite stress tolerance is principally regulated by the low molecular weight antioxidants ascorbate and glutathione, as well as the conventional antioxidant system comprising ascorbate peroxidase, superoxide dismutase, catalase, glutathione reductase, and monodehydroascorbate reductase. [2]. In contrast, plants have evolved sophisticated defence mechanisms, including a wide range of biomolecules known as secondary metabolites that aid in their ability to adapt to changing environmental conditions and survive under stress. [2], In addition to their use as medications, flavourings and scents, dyes and pigments, insecticides, and food additives, secondary metabolites are also commercially significant..

To increase the synthesis of secondary metabolites in cell cultures, plant cells can be treated with biotic and/or abiotic elicitors [3]. Plant-derived secondary metabolites, or phytochemicals, have notable pharmacological properties including anti-oxidative, anti-allergic, antibacterial, hypoglycaemic, and anti-carcinogenic properties [4]. The class of secondary metabolites consists of terpenoids, alkaloids, and phenolics.. Most plant tissues include phenols, which are frequently found in vacuoles. Flavonoids can appear as monomers, dimers, and higher oligomers. [5] Flavonoids are a broad group of chemicals that serve a variety of purposes. Additionally, some flavonoids have been shown to shield plants from UV-B radiation. [5]. The plant metabolites known as flavonoids include leucoanthocyanidins, catechins, anthocyanins, chalcones, aurones, flavanones, isoflavonoids, flavones, and flavonols [5].

Common sugars, proteins, and chlorophyll are the primary metabolites in *Momordica charantia*, whereas alkaloids, flavonoids, tannins, anthocyanins, coumarins, terpenoids, and saponins are the secondary metabolites [6]. Secondary metabolites are frequently produced from primary metabolites through modified synthetic pathways, or they may share substrates with primary metabolites as their source [6]. In vitro cell cultures can be stimulated to produce secondary molecules via biological, abiotic, and signalling molecules. In plant cells, a significant variety of secondary compounds biosynthesize from primary metabolites. [7] Through careful regulation, the induction of stress may promote the expression or repression of the stress-gene network, which may lead to the production of functional cellular molecules to mitigate the effect of the stress. These molecules may take the form of the biosynthesis of osmoprotectants, detoxification enzymes, transporters, chaperones, and proteases, which act as the first line of defence for the cellular system. Numerous recent studies have demonstrated that plants' SM systems respond to stress and defensive circumstances by increasing metabolite production as part of an integrated defence strategy in dynamic ways. [7]

2. Momordica charantia

I. *SCIENTIFIC IDENTIFICATION*

Scientific name: - *Momordica charantia*

Kingdom: - Plantae

Division: - *Magnoliophyta*

Family: - *Cucurbetaceae*

Genus: - *Momordica*

Species: - *charantia*

Duration: - Annual

Due to its superior culinary and therapeutic qualities, the bitter gourd (Momordica charantia L.), a member of the Cucurbitaceae family, is a well-liked and commonly produced vegetable in Southeast Asia. It is loaded with minerals and vitamins C and E. [8] [9]. Momordica Charantia is a tropical vegetable that is common in Indian cuisine and has a long history of use in traditional medicine as a cure for diabetes. It is also known as bitter melon, balsam, or karela. [10]. For a very long time, bitter melon has been utilised in several Asian traditional medical systems. Like other meals with a bitter flavour, bitter melon encourages digestion. Biologically active plant compounds found in Momordica charantia include triterpenes, proteins, steroids, saponins, flavonoids etc. Thus, the plant has properties that are antifungal, antibacterial, antiparasitic, antiviral, anti-viral, anti-fertility, anti-tumorous, hypoglycemia, and anti-carcinogenic [9] Rheumatism, gout, worms, colic, and ailments of the liver and spleen are only a few of the illnesses that have historically been treated with fruits. It is also effective in the treatment of diabetes and cancer. Alkaloids, peptides that resemble insulin, and a combination of steroidal sapogenins known as charantin make it a strong hypoglycaemic drug. The most popular herb used in complementary treatments for diabetes is *Momordica charantia*. [9].

II. Traditional use

Fruits: Asthma, burning, constipation, colic, diabetes, cough, fever (malaria), gout, helminthiases, leprosy, inflammation, skin conditions, ulcers, and wounds are all treated with *Momordica charantia*. [9]

Leaves: *Momordica charantia* is used as an emmenagogue to cure infections, worms, and parasites as well as measles, hepatitis, and helminthiases. It also treats menstruation problems, burning, constipation, fever (from malaria), colic, and other conditions. [9]

Seeds: *Momordica charantia* is used to treat a variety of conditions, including stomach aches, ulcers, liver and spleen issues, diabetes, high cholesterol, intestinal parasites, and intestinal gas. [9]

Roots: Momordica charantia is used to treat syphilis, rheumatism, ulcers, boils, septic swellings, ophthalmia, and Prolapsus vagenae. Momordica charantia juice helps to stop gum bleeding, or pyorrhoea. [9]

III. Therapeutic Activity

Anti Diabetes: -

Type II diabetes is lessened or prevented by bitter melon. In both healthy and alloxan-diabetic rabbits, oral administration of fresh fruit juice at a dose of 6 cc/kg body weight decreased blood sugar levels [10]. The bitter melon boosts insulin sensitivity, according to a recent scientific study at JIPMER in India. Bitter melon also includes four extremely promising bioactive chemicals. [10] These substances cause the AMPK protein to become active, which is well recognised for controlling glucose absorption and fuel metabolism—processes that are compromised in diabetics. It may be possible to manage adult-onset diabetes by eating bitter melon since it contains a lectin with insulin-like action. This lectin is probably a significant factor in the hypoglycaemic impact that results from eating bitter melon. [10]

Anti-Cancer: -

Clinical tests have shown that a unique phytochemical found in bitter melon has the capacity to block the guanylate cyclase enzyme. Not only leukaemia and cancer but also the pathogenesis of psoriasis is considered to be influenced by this enzyme. [10] A substance known as MAP-30 was created as a chemical analogue of bitter melon proteins, and its creators claimed that it had the ability to stop the growth of prostate tumours. Momordin, a phytochemical, has been shown to have cytotoxic effect against Hodgkin's lymphoma in vivo, and several additional in vivo investigations have shown that the entire bitter melon plant has cytostatic and anticancer activity [10]. Absolutely no proof exists that it can treat cancer. Many in vitro studies have shown that bitter melon has anti-cancerous and anti-leukemic effect against a variety of cell lines, including liver cancer, human leukaemia, melanoma, and solid sarcomas. Bitter melon and bitter melon extracts suppress cancer and tumour growth. [10]

Digestive System: -

Chinese Bitter Melon is the name of a plant called *Momordica charantia* that is also found in China. It has been utilised in traditional Chinese medicine as a digestive infection therapy as well as an appetite stimulant. [10] [11]

Antimicrobial Activity**: -**

The MAP30 protein analogue, obtained from the seeds of Momordica charantia extracts, and bitter melon extracts both exhibit broad-spectrum antibacterial action, according to in vitro investigations. [9] Extracts of the *Momordica charantia* prevent the spread and infection of several viruses, including the Epstein-Barr virus and HIV. *Momordica charantia* therapy resulted in a stabilisation of CD4/CD8 ratios in three HIV patients, according to a preliminary research on the effect of the herb on 2 A and 24 Herpes simplex. [9]Several gram-positive and gram-negative bacteria, such as Salmonella, E. coli, Shigella, Staphylococcus, Pseudomonas, Streptococcus, Streptobacillus, and H. pylori, seem to be inhibited by *Momordica charantia* extracts. [9]

Antimalarial Activity: -

The phenolic component from bitter melon that was isolated has been reported to have antioxidant properties. Momordica charantia seeds have been examined for their antioxidant capabilities in streptozotocin-induced diabetic rats, and the findings strongly imply that they have the potential to normalise the decreased antioxidant status in this condition. [9]

Hypocholesterolemic Activity: -

Conjugated octadecatrienoic fatty acid from Momordica charantia seeds was given to rats fed sunflower for four weeks in a research. These rats demonstrated a considerable reduction in nonenzymatic liver tissue lipid peroxidation, erythrocyte membrane lipid peroxidation, and plasma lipid peroxidation after 4 weeks. [9]

IV. ***Important Metabolites of M.charantia***

The bitter melon's medical benefits come from its bioactive phytochemical components, which are non-nutritive substances that have defined physiological effects on people's bodies and shield them from various ailments. Thousands of them have been found in various groups as secondary metabolites from plants, which are distinct from primary metabolites including nucleic acids, amino acids, carbohydrates, and fats. Flavonoids, a common class of phenolic chemicals, serve as chemotaxonomic indicators. In terms of pharmacology, phytosteroids are crucial for human survival. [11]. All plant components synthesise diosgenin, while the fruit of M. charantia has the highest quantities. Jasmonic acid and salicylic acid operate as endogenous signal chemicals in the interaction between plant pathogens, boosting the synthesis of secondary metabolites via triggering the expression of defense-related genes. [11]. Momordica contains alkaloids and saponins, and volatile ingredients are produced when boiling to improve the flavour. High quantities of iron, beta carotene, calcium, potassium, vitamins, phosphorus, and dietary fibre may be found in bitter melon plants. [11]

2.1 Abiotic Stress

Plants must deal with a wide range of intricate interactions involving many different environmental elements. They have developed certain systems that let them adapt to and endure difficult situations during the course of evolution. Numerous environmental factors that affect plants may be roughly categorised into two groups. biotic stress and abiotic stress. While the latter results from the harmful effects of non-living things on the organisms, the former is thought to be the harm done to an organism by other living species (such as bacteria, viruses, fungus, and nematodes). Abiotic stress refers to any aspect of the environment that might restrict a plant's ability to grow and produce. Abiotic stressors (drought, salt, cold, heat, heavy metal toxicity, UV radiation, etc.) affect a significant amount of arable land, and their frequency is further fueled by anthropogenic activity. These pressures are anticipated to worsen owing to climate change. These abiotic stressors change the physiological and biochemical functioning of plants, which reduces plant growth and output. [12].

When plants are exposed to biotic and abiotic stress, their metabolism is disrupted, indicating physiological costs and eventually lowering fitness and production [13]. Complex reactions to the many stressors are brought on by the combination of biotic and abiotic stress. A plant's reaction to both stressors is favourably impacted by the accumulation of certain metabolites while it is under stress, protecting it against numerous aggressors. [14] [15]. Extreme light (high and low), radiation (UV-B and UV-A), temperature (high and low (chilling, freezing), water (drought, flooding, and submergence), chemical factors (heavy metals and pH), salinity due to excess Na+, a lack of or an abundance of essential nutrients, and gaseous pollutants (ozone, sulphur dioxide) are some of the stressors. [16]. Reactive oxygen and nitrogen species, which alter the regulation of gene expression and enzyme activity, are frequent early warning indicators of abiotic stresses. [17]. A physiological redox state interferes with normal cell processes and has an impact on the plant immune system, which suggests that a threshold level of ROS is required for regular plant functioning in contrast to excessive ROS generation. [12] The formation of ROS, which cause cellular membrane peroxidation and instability, increased exponentially in response to increased ROS synthesis under abiotic stressors. Abiotic stressors upset the equilibrium between ROS creation and scavenge and speed up ROS propagation, damaging essential macromolecules (nucleic acids, proteins, carbohydrates, and lipids), which ultimately results in cell death [12]. The oxidation of amino acids buildups, such as cysteine, for the creation of disulphide bonds, the oxidation of arginine, lysine, and threonine buildups driving to irreversible carbonylation in side chains, and the oxidation of methionine buildup to create methionine sulphoxide are all capable for ROS-induced protein debasement [12]. There has been a detailed analysis of ROS signalling in response to abiotic stressors and its interactions with hormones. Hormones have a significant role in controlling how plants react to abiotic stress. The two most significant ones are ethylene and abscisic acid (ABA). Many plant responses to environmental challenges, especially osmotic stresses, are largely regulated by ABA. [17] Defensive response can be constitutive or induced, with the former occurring always in plants and having secondary compounds that are frequently species-specific and present as stored compounds, precursors of active compounds, or conjugated compounds that can be easily activated in response to injury to the plant's body. The latter begins with the occurrence of physical injury to the plant's body and may entail the creation of protective proteins like lectins and protease inhibitor(s) or poisonous SMs. [7]. But increased phenolic production under metal stress aids in shielding plants from oxidative damage. Phenolic compounds accumulate due to the biosynthesis of phenylpropanoid enzymes such as phenylalanine ammonia lyase, chalcone synthase, shikimate dehydrogenase, cinnamic alcohol dehydrogenase, and polyphenol oxidase, which depends on the modulation of transcription levels of genes encoding metal synthases. [12]

In farmed regions, metal pollution concerns are becoming more prevalent. [18] Metals are necessary for many biological functions, but when present in excess, they can be harmful. [19]. The toxicity of transition metals can significantly influence the onset of illness [19]. Due to their increased use in many anthropogenic activities, which results in their high bioaccumulation and toxicity, heavy metals have emerged as one of the primary abiotic stress factors for living organisms [18] As cofactors of numerous proteins, the transition metals copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) are vital trace elements for plants. Zinc only exists in its stable Zn2+ form within cells; in contrast, the redox active metals Cu, Fe, and Mn exist within cells in a variety of redox states and may thus take part in electron transfer processes. Cu, Fe, and Mn can catalyse the creation of unwanted radicals due to their redox activity. [20]. Copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) are transition metals that are necessary trace elements for plants because they are cofactors of different proteins. In contrast to the redox active metals Cu, Fe, and Mn, which exist in cells in a variety of redox states and may thus take part in electron transfer processes, zinc only exists in its stable Zn2+ form. Cu, Fe, and Mn are redox active metals that can catalyse the creation of harmful radicals. [20]. . Copper (Cu), one of the micronutrients, is a cofactor and essential component for numerous enzymes involved in photosynthetic and respiratory processes. Cu concentrations greater than 0.20 mM led to decreased Fe and Zn content in both leaves and roots, and heavy accumulation of Cu in roots further disrupted the translocation of minerals to the upper plant parts [21]. Active efflux, sequestration, and binding with ligands have all been proposed as techniques to aid in Cu detoxification by lowering Cu2+ concentration to less bioavailable and toxic forms. Cu detoxification by complexation is a crucial defence mechanism.

[21]Several human activities, such as mining, the disposal of industrial waste, and the smelting of ores containing copper, contribute copper to soils. A surplus of copper in the soil is cytotoxic, stressful, and harmful to plants. The result is leaf chlorosis and slowed plant development. [1]Depending on their capacity for scavenging and ability to deposit these metals in the various cellular compartments, some plant species, including Momordica charantia, operate as super accumulators of the metals. These metals go from the root cell membrane to the symplast, where they are transported by membrane metal transporters to the vacuoles, where they are degraded by enzymes, and where they can be deposited with the aid of specialised metal-binding proteins known as metallothioneins. The natural equilibrium of the cellular pigments is meant to be destroyed when heavy metals replace other necessary elements. [22]. The presence of heavy metals has several detrimental consequences on plants, as shown by the numerous study studies. In order to maintain the earth's ecological balance, increased research is needed to better understand the effects of heavy metal toxicity on plants and related ecosystems**.** [1]

2.2 <u>Plant metabolism</u>

The processes and end products of metabolism are metabolites. Usually, only tiny molecules are included when using the word "metabolite." Fuel, structure, signalling, effects on enzymes that are both stimulatory and inhibitory, catalytic activity on their own (often as an enzyme cofactor), defence, and interactions with other species are just a few of the many roles that metabolites play [5] Biochemical, morphological, and molecular processes are used by plants to adapt to a variety of biotic stressors and detrimental environmental circumstances. For cellular growth, development, and reproduction, primary metabolism is crucial. [23]The major metabolites that plants generate are lipids, proteins, and carbohydrates, however only proteins and polysaccharides have antiviral properties. To coordinate insect feeding and the plant's defence response, many signalling pathways rely on primary metabolites. [23]

The vast majority of the organic chemicals that a plant creates appear to have no apparent direct role in growth and development. Within the kingdom of plants, these chemicals, which are typically referred to as secondary metabolites, are frequently distributed differently. Metabolite from commercially available medicinal herbs has been reported in plant cell suspension cultures. The synthesis of plant secondary metabolites has surmounted a number of challenges thanks to the utilisation of plant cell cultures, which are unique sources for medications, food additives, flavours, and other industrial materials. Secondary metabolite synthesis can be significantly aided by organised cultures, especially root cultures. Chemical structure (such as having rings or containing a sugar), composition (such as whether they contain nitrogen or not), solubility in different solvents, or the mechanism by which they are produced (such as the tannin-producing route of phenylpropanoid) may all be used to categorise secondary metabolites. And are often categorised according on their metabolic routes. Typically, three major molecular families are taken into consideration: Alkaloids, flavonoids, phenolics, terpenes, and steroids

Phenylpropanoid pathway in plants

L-phenylalanine is converted into phenylpropanoids, which are natural compounds, by the enzyme L-phenylalanine ammonia-lyase (PAL). All higher plants have the classes of flavonoids and hydroxycinnamic acid, although some genera or species may be more susceptible to specific patterns of substitution in these class members. [24]. Not all plant species include components from all phenylpropanoid chemical classes. The classes of hydroxycinnamic acid and flavonoids are present in all higher plants, however some genera or species may be more vulnerable to particular patterns of substitution in these members of these categories.. [24]. In biotic and abiotic stress situations, a large number of phenylpropanoid pathway genes are reported to be significantly expressed, leading to an increase in the accumulation of the corresponding enzyme and an increase in enzymatic activity [24]. Plant tissues are strengthened by one of the polymeric by-products of the general phenylpropanoid pathway, which is also expected to play a significant role in various disease resistance mechanisms. [25]

The General Phenylpropanoid Pathway:

A shikimate/phenylpropanoid route in plants produces phenolics, whereas a mevalonate system produces terpenoids.. [12] Both of these secondary routes result in a broad range of monomeric and polymeric structures that are used by plants in a wide range of physiological and biochemical functions. The metabolites or phytochemicals produced during secondary metabolism are referred to as "secondary metabolites".. [12]Erythrose 4-phosphate is coupled with phosphoenolpyruvate (PEP) to create phenylalanine during the production of phenolic chemicals. Along with C4H and 4CL, PAL catalyses the first committed step in the biosynthesis of phenylpropanoids, the deamination of phenylalanine to cinnamic acid, and is crucial for the production of all phenylpropanoids.. [26] More than 50 years ago, an ordered pathway involving deamination, 4-hydroxylation, and CoA thioester formation was inferred by exposing plant cells or extracts to radiolabelled candidate intermediates and then monitoring the radioactivity's incorporation into the subsequent metabolites [26]. he catalyst enzymes for these processes were isolated and biochemically characterised, and more recently, the amino acid sequences or antibodies derived from the isolated enzymes were used to clone the genes involved. [26]

The general phenylpropanoid route is launched by the conversion of PAL and Phe's ammonia lyase into cinnamic acid and p-coumaric acid, respectively. Reduction of the carboxylic acid initiates the formation of hydrocyanic alcohols, and the aromatic ring of pine and sinapyl alcohols is methoxylated. This section, which is also referred to as the general phenylpropanoid route, frequently involves PAL, C4H, and 4CL. With p-coumaric acid, ferulic acid, and sinapic acid, hydroxylation and O-methylation of the fragrant ring of antecedents at the level of hydroxycinnamic acids are at that point actuated to deliver their individual CoA thioesters, which are at that point decreased by CCR and CAD to create p-coumaryl liquor, coniferyl liquor, and sinapyl liquor, individually.[24] Hydroxycinnamoyl CoA thioesters are reduced by the enzyme CCR to hydroxycinnam aldehydes, which are subsequently converted by the enzyme CAD to hydroxycinnamoyl alcohols. Overall, the enzymes HCT, CCR, C3H, and CAD produce lignin G units and caffeoyl alcohol. [24] F5H, COMT, and CAD work together to create a different, branching route that results in sinapyl alcohol and S units. Due to its impact on caffeoyl shikimate, the CSE enzyme has recently been included into the well-established lignin production route. [24] on addition to this basic pathway, a recent study on a model grass made a novel monolignol biosynthesis process suggestion. By skipping the stages of C4H catalysis, the tyrosine route creates monolignol more quickly. Tyrosine is transformed into p-coumarate in this process by a bifunctional enzyme called PTAL (phenylalanine and tyrosine ammonolyses). [24].

2.3 Role of Plant Secondary metabolites of *M. charantia* in combating Cu induced stress

As the consequences of climate change intensify, it is anticipated that abiotic stressors like drought, high temperatures, and salinity will occur more frequently in the modern world. Despite all other stressors, heavy metal stress is particularly significant since it has a noticeable negative impact on crop development and yield. Lead (Pb), cadmium (Cd), nickel (Ni), cobalt (Co), iron (Fe), zinc (Zn), chromium (Cr), arsenic (As), silver (Ag), and the elements of the platinum group are examples of heavy metals. [1] The anthropogenic input of heavy metals to the biosphere has grown as a result of industrialization and urbanisation [1]. The physiological functions of respiration, photosynthesis, cell elongation, the interaction between plants and water, N-metabolism, and mineral nutrition are all inhibited by heavy metals. [27].

According to the chemical and physical properties of heavy metals we can divide their harmful action into:

a) ROS (reactive oxygen species) production via autooxidation and the Fenton reaction,

b) blockage of crucial functional groups in biomolecules, such as polynucleotides and proteins (through inactivation of SH-groups in enzyme active centres),

c) replacement of erroneous metal ions for necessary ones. [27]

Free radicals, also known as reactive oxygen species (ROS), are extremely reactive chemical entities whose atomic shells include at least one unpaired electron. Reactive species (RS) are quite unstable, most of them exist in biological systems for a maximum of 10-6 seconds, and to become more stable, they react with biomolecules either by donating or accepting an electron. [28].

Mineral fertilisers for higher plants include important heavy metals including Mn, Cu, Fe, Zn, and Ni. For instance, in normal amounts, Cu and Zn are cofactors in protein and enzyme structure and are essential for plant growth and development. Oxidative stress can be brought on by copper. [29] Cu toxicity may result from redox cycling-induced oxidative damage to biological macromolecules, glutathione depletion, and altered sulfhydryl homeostasis. [29] The development of reactive oxygen species such superoxide radicals, hydroxyl radicals, hydrogen peroxide, and singlet oxygen leads to oxidative stress. Lipids, nucleic acids, proteins, amino acids, carbohydrates, and complex compounds made from all of these in cells are susceptible to damage from reactive oxygen species (ROS). [29] The combined activity of enzymatic antioxidant systems like superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) can shield cells against reactive oxygen species. [29] Chlorophyll and protein content in plants are decreased by heavy metals as Cu, Co, Pb, and Cd. However, an excessive amount of copper in the growing environment has a phytotoxic effect that changes membrane permeability, chromatin structure, protein synthesis, enzyme activity, photosynthesis, and respiratory activities. It also has a phytotoxic impact and causes lipid peroxidation, which starts the ageing process.

When the capacity of antioxidant processes and detoxifying mechanisms is less than the rate of ROS generation, plant damage results. Aerobic organisms have evolved sophisticated defence mechanisms against ROS, which include several enzymes and antioxidants. A staggering variety of secondary metabolites are produced by all plants. Phenolic chemicals, which play a variety of roles in plants, are one of the most significant categories of these metabolites. Under a variety of environmental circumstances and stressors, an increase in phenylpropanoid metabolism and the quantity of phenolic compounds can be seen. [27]. A diverse group of low molecular weight secondary metabolites with a polyphenolic structure, such as flavonoids, are involved in the physiological processes of plants and frequently exhibit protective actions against biotic and abiotic challenges [28]. Flavonoids function as an antioxidant by scavenging reactive oxygen species (ROS), which are formed in plants during biotic and abiotic stresses.[30]. Flavonoids work to reduce ROS by blocking the enzymes that produce them, recycling other antioxidants, and chelating transition metal ions. The increased propensity of phenolic compounds to chelate metals accounts for their antioxidant effect. Instead of specialised chelating groups within the molecule, the universal chelating capacity of phenolic compounds is likely attributable to the strong nucleophilic nature of the aromatic rings. [27]. By hemolytically cleaving the O-O link, metal ions break down lipid hydroperoxide (LOOH), releasing lipid alkoxyl radicals that start the free radical chain oxidation process. By securing the lipid alkoxyl radical, phenolic antioxidants prevent lipid peroxidation. This effect is influenced by the number and position of hydroxyl groups in the molecules and their structure. By changing the lipid packing order, phenolics—especially flavonoids—can change the rate at which peroxidation occurs. [27]They prevent free radical transport, limit peroxidative reactivity, and stabilise membranes by reducing membrane fluidity (in a concentration-dependent manner). However, in vitro research has demonstrated that flavonoids may actively scavenge certain molecular forms of active oxygen: Superoxide, hydrogen peroxide, hydroxyl radicals, single oxygen atoms, and peroxyl atoms are all examples of the compound O2 [27]. Their capacity to transfer electrons or hydrogen atoms accounts for the majority of their antioxidant activity. According to a theory, Phyto phenolics, particularly the flavanols and phenylpropanoids of vacuoles and the apoplast, can detoxify H2O2 by acting as electron donors for phenol peroxidases (guaiacol peroxidases) that are located in these compartments, which causes the generation of the corresponding phenoxyl radicals. [27].

Copper (Cu) is considered a plant trace element and is essential for ATP synthesis and CO2 assimilation. However, when plants are exposed to excessive amounts of Cu, oxidative stress and ROS are produced [1]. Some hyperaccumulator plant species, including M. charantia, which manage to scavenge hazardous heavy metals from polluted soils, prevent copper from reaching stems and leaves by retaining it in their roots. These plants are copper-tolerant [29]. They discovered that following heavy metal dosage, rising MDA concentrations, a byproduct of lipid peroxidation, are a sign of oxidative stress; the rise is correlated with the rise in metal concentrations.

Defence mechanisms adapted by M.charantia against Copper stress

Superoxide dismutase (SOD)-Superoxide anion (•O2) is dismutated by (SOD) to produce H2O2 and O2. SOD contains many isoforms, including Cu/Zn-SOD, Mn-SOD, and Fe-SOD, depending on the metallic co-factors (such as Cu, Zn, Mn, Fe, and Ni) that are connected with it. [31] These co-factors' function is to stabilise the formation of transitory bonds during the metabolising of intermediates. Due to the matching stable electronic arrangement (ions created by losing two electrons), copper has a +2 valency. [31] These co-factors can receive the additional electron from superoxide (•O2) that is delivered during the creation of the reaction intermediate. These extra electrons eventually mix with H to form H2O2, which releases O2 as a by-product. Instead of adding any functional characteristics to the catalytic activity of Cu/Zn-SOD, Zn plays the job of providing structural stability to the enzyme per se [31]

Catalase (CAT)- doesn't need a reductant to function as a catalyst because, in this two-step reaction, H2O2 first oxidises the iron in the CAT to produce compound I, an intermediate iron peroxide. If the H2O2 concentration is low, the enzyme can stay in this resting-state [31]. Higher H2O2 concentrations, however, cause the second molecule of H2O2 to act as a reductant for this intermediate chemical I, renewing the enzyme and releasing water and oxygen in the subsequent step. However, at high H2O2 concentrations, CAT is more active[31].

Plant peroxidases (POX)- The only Class III peroxidase enzymes can scavenge H2O2 in the extracellular environment are plant peroxidases (POX).. [31] By employing H2O2 as an electron acceptor to catalyse the oxidation of phenolic substrates, the POX scavenges the H2O2. MDA (mono-dehydro-ascorbyl radical), ascorbate, DHA (de-hydro-ascorbate), and cross-linking products of phenolic compounds, such as lignin or suberin, were produced as a result of subsequent reactions. [31]

Vacuole Compartmentalization- Vacuole Controlling the distribution and concentration of metal ions may be done relatively successfully by compartmentalization. Another method of detoxification is the accumulation of metals in plant cell walls and vacuoles, which prevents the metal from reaching vulnerable metabolic sites. Vacuole seizes unnecessary metal and limits and squeezes it in a constrained region in order to compartmentalise. This prevents such unnecessary metal ions from entering other sensitive areas of the cell, ensuring cell safety against metal toxicity. [32]

Chelation- One of the most common adaptive mechanisms for metal tolerance and its detoxification has been shown to include the chelation of metal using high-affinity cellular ligands. Through complexation with cellular ligands such organic acids, cysteine phytochelatins, glutathione, etc., metal detoxification activities are facilitated. [32]. Hytochelatins (PCs) and metallothioneins, two types of metal-binding ligands that are frequently present in plants, have been widely explored for their roles in metal detoxification and tolerance. The level of tolerance to exposure to excessive amounts of metal ions, such as copper, increases when metal-binding proteins associated with metallothioneins (MTs) and/or phytochelatins (PCs) (-glutmylcysteinyl-isopeptides) are stimulated. These proteins that bind to metals are cysteine-rich polypeptides that effectively remove heavy metals from vulnerable areas, aid in their detoxification, and accumulate in plant cells. [32]

2.4 Current Research and Future Aspects

The bitter gourd is a significant vegetable from the Cucurbitaceae family that is grown mostly in humid, subtropical Asia. Due to the presence of medicinal substances including charatin, vicine, and polypeptide-p, which are crucial in lowering blood glucose levels, bitter gourd is a vegetable with significant health advantages. Additionally, bitter gourd fruits are very abundant in carotenes, minerals, and vitamin C [33]. Understanding how plants react to severe pressures will help us come up with plans for creating a variety of stress-tolerant agricultural species. [18] Momordica charantia Linn., which has been extensively employed in traditional remedies to treat a variety of diseases and has been primarily utilised for culinary reasons in several nations across the world, including South East Asia. A member of the Cucurbitaceae family is M. charantia. On the fruit, leaves, and seeds of the plant, much research has been done. Most notably, all of these studies have demonstrated their effectiveness against a variety of cancer cell lines, including those from lymphoid leukaemia, lymphoma, choriocarcinoma, melanoma, breast cancer, skin tumours, prostatic cancer, squamous cell carcinomas of the tongue and throat, human bladder carcinomas, and Hodgkin's disease.

A significant abiotic stressor, drought restricts plant development and output, especially in vegetable crops. In this experiment, M. charantia, which requires more water for its regular metabolic function, was used to study the effects of water deprivation on morphological, physiological and biochemical processes. [34]The two kinds of M. charantia have different capacities for tolerating drought stress, according to an analysis of the physiological and biochemical characteristics of drought tolerance in the wild and cultivated forms of the plant. Therefore, the study highlighted the usefulness of physiological and biochemical indicators to assess the wild genotype of M. charantia's potential for drought tolerance, thereby expanding its options for crop development programmes. [34]. This work is the first to describe the relationship between potential genes for phenylpropanoid and flavonoid biosynthesis and the changed levels of these compounds in various bitter melon organs and plantlets.. [35] Flavonoids are found in bitter melon. [35] The findings of this study show that blue light is a useful light source for enhancing the manufacturing of phenylpropanoid and flavonoid compounds. The tactics that are created to boost the output of therapeutic chemicals in bitter melon will benefit from these findings [35]. The findings of this study show that blue light is a useful light source for enhancing the manufacturing of phenylpropanoid and flavonoid compounds. The tactics that are created to boost the output of therapeutic chemicals in bitter melon will benefit from these findings. [35]. Evidence-based research are now required to establish these facts so that these miraculous medications with numerous therapeutic activities can be used historically safely (without the harmful components) to treat the different ailments plaguing humanity's suffering with the greatest degree of efficacy and the fewest possible adverse effects. Many additional medicinal plants, such as M. charantia, are also accessible, and the time has come to investigate their usefulness and optimal utilisation as alternatives to manufactured contemporary medications with harmful side effects. [36].

The number of linked articles that are published on the SCI website each year shows the consistent upward trend in the number of research papers written each year that use the term "Momordica charantia." [37]his plant is used as a traditional herbal remedy and has several pharmacological properties, including laxative, antidiabetic, abortifacient, anthelmintic, and contraceptive. [37]Studies conducted in vitro have demonstrated that the HIV virus is inhibited by the M. charantia proteins (α- and β-momorcarin). Additionally, a broad-spectrum antibacterial drug may be created from its extract to combat infections.[37]The many bioactive elements of M. charantia, which are significant sources of phytoconstituents used to cure a variety of ailments since ancient times, are credited with these beneficial properties. [37].

M. charantia polysaccharides have neuroprotective effects against overall cerebral ischemia/reperfusion injury by scavenging radicals (O2-, NO, and ONOO-), reducing neural cell death in vitro, and inhibiting the release of cytochrome C, phosphorylation of JNK3, and expression of Fas-L in both pre-ischemia and post-ischemia treatment, according to research on a cerebral ischemia-reperfusion injury model in male [37]. ngestion of M. charantia fruit at large doses (equal to 250–500 g) resulted in stomach discomfort and diarrhoea in diabetics, according to clinical investigations. Research on M. charantia's bioactivities has progressed quickly up until this point. While mechanisms in many of the research still need to be explored, the isolation and identification of bioactive components from the plant have received increasing interest and continue to trend higher. The link between structure and mechanisms of the efficacy of the various functional ingredients will be elucidated with much more study on bitter gourd. [37]

In the control of several biological processes, including plant growth, development, and responses to biotic and/or abiotic stimuli, reactive oxygen species (ROS) play a crucial role as signalling molecules. To deal with these challenges, plants like Momordica charantia have evolved a range of adaption techniques [32]. Recent research has shown that the temporal-spatial coordination of ROS and other signals, which depends on the generation of molecules, compounds, and hormones unique to stress, mediates the stress responses in plants.[38]. Several studies have shown that metal partition in apoplastic tissues such as trichomes and cell walls, and metal chelation of certain ligands, followed by sequestration of the metal-ligand complex by vacuolar tonoplast-enabling metal transporters, can help prevent heavy metal toxicity in plants. [32] According to a number of study studies, plants and fission yeast both push phytochelatin-metal complexes into the vacuole, which aids in a variety of plant defence mechanisms against common inorganic contaminants and metals. [32] It's interesting to note that data suggests that oxidative stress, which is brought on by the production of free radicals and a decline in the body's natural antioxidant defences, exacerbates diabetes and other health issues. The existence of the enzyme -glucosidase is a characteristic that suggests that plants may have therapeutic benefit. Although -glucosidase enzyme activity has been shown at high levels in bitter gourd (Momordica charantia), it does not directly increase the antioxidant value of vegetables and fruits. However, the products of these interactions may act as precursors to some non-enzymatic antioxidant chemicals. [39] Due to their significance in producing precursor chemicals required in several functions including hormone control, defence mechanisms, and antioxidant generation, recent studies have discovered that plants with high activity of this enzyme have been effective tools in drug development [39] According to studies, bitter gourd is an antioxidant-rich plant food that may be a significant topic and provide a stronger framework for pharmacological study since it contains much more phenolic compounds than other types of gourd.

Conclusion

In recent years, there has been great progress in the study of medicinal plants and research into treatments for untreated diseases. Many unknown plant species have been tested for their medicinal properties, but many common plants have yet to be fully discovered. Among them is *Momordica charantia*, which belongs to the Cucurbitaceae family and is traditionally known for its medicinal properties. Shown to be a hyperaccumulator of toxic metals. Plants must adapt to a range of biotic and abiotic stresses, such as heavy metal stress brought on by environmental industrialisation and urbanisation. Among heavy metals, copper is one of the essential micronutrients required by plants for CO_2 assimilation and ATP synthesis. However, abundance of Cu cause oxidative stress and ROS which can induce stress and damage the plants. Eventually plants have acquired defense mechanism against these types of stresses. Plants like *Momordica charantia* have adopted mechanisms to counteract these stresses. *M.charantia* produces a great variety of phytochemicals which helps them in scavenging abiotic stresses, these secondary metabolites include terpenoid, alkaloids, phenols. The most effective way to eliminate and mitigate the impact of the free radicals responsible for such oxidative stress are antioxidant defense mechanisms. In general, the cells of the plant try to maintain a concentration of ROS are as low as possible because they are more reactive than molecular oxygen. ROS are known to cause lipid peroxidation, which damages cell membranes. The three most common ROS are OH (hydroxyl radical), H_2O_2 (hydrogen peroxide), and O_2. An increase in the metabolism of phenylpropanoids and the amount of phenolic compounds can be observed under copper-induced stress conditions. Flavonoids belong to the major class of phenols produced under Cu stress in *M. charantia*. The antioxidant effects of flavonoid compounds are due to their high propensity to chelate metals. The plant produces enzymes like Cu-SOD, CAT, POX under copper stress which helps reduces the amount of ROS produced. Increased activity of antioxidant enzymes in metal-stressed plants appears to be an important part of the plant antioxidant defense mechanism against metal-induced oxidative damage. Thus, researchers have found *Momordica charantia* to be of great importance in establishing a major ground for various medicinal and therapeutic findings.

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