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ASSESSMENT OF INHIBITORY ACTIVITIES OF MONOAMINE OXIDASE ENZYME IN EULOPHIA GUINEENSIS

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Abstract: Signal conduction via synapses between neurons is essential for the central nervous system (CNS) to process and relay information from the peripheral nervous system. Neurotransmitters (NTs) play a crucial role in this process, affecting a range of bodily functions including emotions, cognition, memory, movement, and sleep. Dysregulation of NT levels can lead to various neurological and psychiatric disorders. Monoamine oxidase (MAO), crucial in NT metabolism, exists in two isoforms: MAO-A and MAO-B, with distinct roles in neurotransmitter degradation. This study investigates the inhibitory effects of Eulophia guineensis extracts on monoamine oxidase (MAO) enzymes, which are involved in the catabolism of neurotransmitters and implicated in conditions like depression and Parkinson's disease. Methanolic extracts demonstrated significant inhibition of both MAO-A and MAO-B, suggesting potential as natural MAO inhibitors. Phytochemical analysis identified various phenolic compounds and alkaloids as potential bioactive components responsible for the observed MAO inhibition. These findings suggest that Eulophia guineensis could serve as a source of natural MAO inhibitors, offering a basis for developing new therapeutic agents for neuropsychiatric and neurodegenerative disorders. Further in vivo studies and clinical trials are necessary to confirm these results and assess the safety and efficacy of these extracts in humans. This research highlights the therapeutic potential of Eulophia guineensis and underscores the importance of biodiversity conservation and sustainable use of medicinal plants.

Keywords: Neurotransmitters, monoamine oxidase, MAO-A, MAO-B, Eulophia guineensis, natural inhibitors, phytochemicals, neuropsychiatric disorders, depression, Parkinson's disease, Alzheimer's disease, medicinal plants, biodiversity conservation, therapeutic agents.

INTRODUCTION

Signal conduction via synapses between neurons allows the central nervous system (CNS) to process information from the peripheral nervous system and send it to it. Neurotransmitters (NTs), which are endogenous chemical messengers that convey and amplify nerve-to-nerve signaling or signals between nerves and other cell types, play a crucial role in information transmission throughout the central nervous system (CNS) and peripheral nervous system. These tiny molecules play a critical role in the transmission of sensory, motor, and integrative neural signals that impact a wide range of bodily processes, including emotions, cognition, memory, movement, and sleep cycles. Because they are crucial regulators of how the brain functions, the signals are transmitted between neurons via synapses in the central nervous system (CNS), which also analyses and distributes information to the peripheral nervous system. The central nervous system (CNS) can thereby regulate smooth, skeletal, and cardiac muscles, body fluids, and organ functioning via synaptic transmission, also referred to as neurotransmission [1]. Neurotransmitters (NTs) are endogenous chemical messengers that transfer and amplify signals between neurons and other cell types, as well as carry information between nerves throughout the central nervous system (CNS) and peripheral nervous system. These substances are basic regulators of neuronal development, differentiation, and survival, and they play vital roles in brain function. As a result, aberrant NT levels cause deregulation of brain activity, which in turn causes a range of neurological, psychiatric, and physical illnesses [2].

These days, neuropsychiatric conditions like depression, Parkinson's disease (PD), and Alzheimer's disease (AD) are gaining international attention as major socioeconomic issues. However, due in large part to a lack of knowledge regarding the intricate etiology, there is now no viable treatment or other methods to cure these disorders. The aberrant expression of the mitochondrial enzyme monoamine oxidases has been identified as a prominent cause among the well-studied etiologies. The premise behind this is that overexpression of MAO leads to the production of excessive monoamine metabolites, which in turn causes neurodegenerative disorders. Because of both these physiological and pathological traits, MAO is a prospective biotarget for the development of therapeutic MAOIs (MAO inhibitors) for neuropsychiatric diseases [3].

Functions of neurotransmitters

Acetylcholine, glutamate, GABA, glycine, dopamine, norepinephrine, and serotonin are just a few of the neurotransmitters that the body uses for various purposes. The primary excitatory neurotransmitter in use in the brain is glutamate. Furthermore, the nervous system's plasticity is mostly mediated by it. Modifiable synapses—which may be the brain's memory-storing components—have been linked to glutamate. On the other hand, the main inhibitory neurotransmitters are glycine and gamma-aminobutyric acid (GABA). For example, GABA may be responsible for about 40% of the brain's inhibitory processing. The spinal cord is the main location for glycine. Dopamine is a significant neurotransmitter that is involved in a number of brain processes, such as emotion, learning, reward, motor control, and executive functions. Additionally, dopamine has been linked to neurological and mental conditions. A neurotransmitter that controls a variety of cognitive processes and neural activity, serotonin is the target of several medications used in neurology and psychiatry. Additionally, serotonin affects cardiovascular health, bladder control, and bowel motility, among other gastrointestinal systems. The sympathetic nervous system and central nervous system both produce the monoamine norepinephrine. The brain's locus cerulean is essential for norepinephrine signaling. Numerous processes in the brain are impacted by norepinephrine release, including stress, sleep, focus, attention, and inflammation. It also has an impact on how the autonomic nervous system responds. Another neurotransmitter that influences the body's homeostatic processes, encourages wakefulness, modulates feeding behaviour, and controls motivational behaviour is histamine [4].

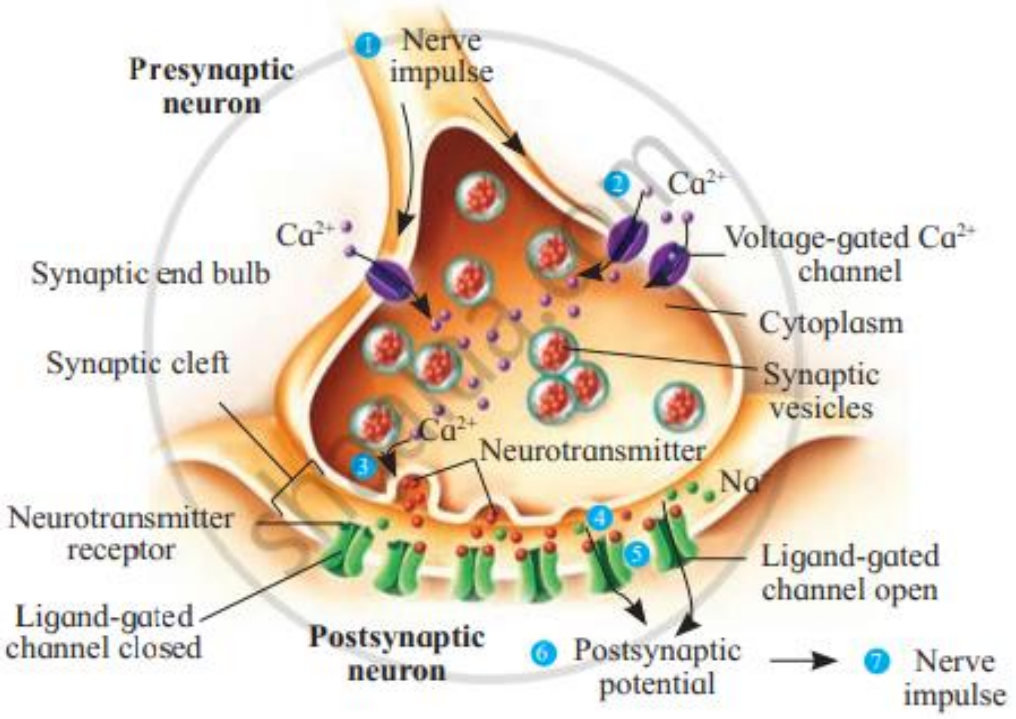

Fig 1. Steps of synaptic neurotransmission

Monoamine oxidase(MAO)

The oxidative deamination of xenobiotic amines and monoamine neurotransmitters is catalyzed by monoamine oxidase. Based on its selective affinity for both substrate and inhibitors, MAO can be classified as either type A or type B (MAO-A, MAO-B). Each type of MAO exhibits unique roles within a particular population of brain neurons. Whereas serotonergic and histaminergic neurons, astrocytes, and ventricular cells exhibit MAO-B, catecholaminergic neurons'

presynaptic terminals express MAO-A. While dopamine (DA) is a common substrate of both MAOs, MAO-A is primarily involved in the degradation of serotonin (5-hydroxytryptamine, 5-HT) and norepinephrine (NE). The brain's regulation of these monoamine levels is essential for controlling mood and emotion as well as motor, perceptual, and cognitive processes. Neuropsychiatric conditions and behavioral characteristics, including as major depressive disorder (MDD), bipolar depression (BD), attention-deficit hyperactivity disorder (ADHD), aggression, panic disorders, and antisocial behaviours, as well as Parkinson's (PD) and Alzheimer's disease (AD), are linked to MAO-A [5]. Two distinct MAO isoforms (MAO-A, MAO-B) exist in humans, and they have distinct but overlapping substrate and inhibitor specificities. Low dosages of clorgyline permanently block MAO-A, which preferentially oxidizes serotonin (5-hydroxytryptamine, 5-HT), norepinephrine (NE), and epinephrine (EN). In contrast, low dosages of deprenyl (selegiline) irreversibly inactivate MAO-B, which prefers phenylethylamine (PEA) as a substrate. Both MAO-A and MAO-B share tyramine and dopamine as common substrates. The brain involvement of the two enzymes has been the subject of previous study on MAO isoforms due to their ability to metabolize monoamines that serve as neurotransmitters. Nevertheless, more recent expression analyses have shown that peripheral organs express many MAO isoforms, many more than the brain does. Human MAO-A, for example, is mostly expressed in the lung, the thyroid gland, the placenta, and adipose tissue; expression in other areas of the brain is very modest. On the other hand, MAO-B is mostly expressed in the spinal cord, uterus, kidney, liver, and heart in addition to the central nervous system (hypothalamus, prefrontal cortex, amygdala, and spinal cord). MAO-B is more prevalent in serotonergic and histaminergic neurons as well as glial cells in the central nervous system, whereas MAO-A is mostly found in catecholaminergic neurons. Many neurological and behavioral conditions, including depression and social anxiety, have been linked to abnormal MAO activity [6]. These Flavin adenine dinucleotide (FAD) dependent enzymes were identified and categorized as the ones in charge of the oxidation of aromatic neurotransmitters over 65 years after the first crystal structure of mammalian monoamine oxidases (MAOs) was solved in 2002. The Rossmann fold, which interacts with dinucleotide cofactors and is closely linked to a substrate-binding domain, epitomizes the two-domain architecture shared by MAO A and MAO B. This globular body is endowed with a C-terminal α-helix that anchors the protein to the outer mitochondrial phospholipid bilayer. Since MAOs are monotopic membrane proteins, determining their structural makeup has been a difficult endeavour that has involved testing various detergent conditions to purify and crystallize the proteins. The oligomerization architecture and active site features of MAO-A and MAO-B structures are different. It is thought that the detergent procedures used to remove the protein from the membrane are the reason why human MAO-A was discovered to be monomeric, while purified human MAO-B and rat MAO-A are dimeric. A hydrophobic cavity that faces the flavin cofactor and extends to the protein surface makes up the active site of MAOs [7].

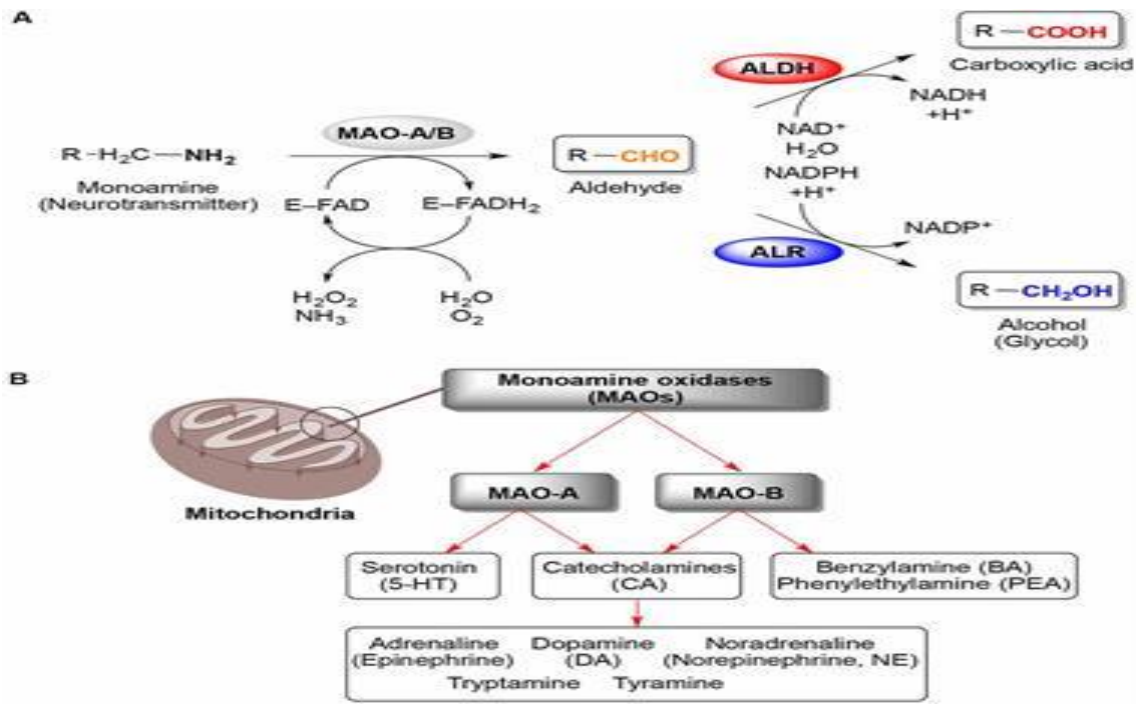

Fig 2. Oxidative deamination of monoamines catalyzed by MAOs A & B.

Structure of monoamine oxidase (MAOs)

The enzymes known as monoamine oxidases (MAOs) are in charge of catalyzing the body's oxidation of monoamines. These enzymes are essential for controlling neurotransmitter levels, especially those of norepinephrine, dopamine, and

serotonin, which are important for mood regulation, thought processes, and other physiological functions. Numerous methods, such as cryo-electron microscopy and X-ray crystallography, have been used to thoroughly investigate the structure of monoamine oxidase. The highest-resolution structures that are most well-known are those of human MAO-A and MAO-B. The homodimer that makes up MAO's crystal structure has a non-covalently bonded isoalloxazine ring—the location of monoamine oxidation and a covalently bound flavin adenine dinucleotide (FAD) cofactor in each monomer. During the catalytic reaction, the FAD cofactor serves as the main electron acceptor [8].

It consists of:

1. **Homodimeric Enzyme:** Made of two identical subunits, MAO is an example of a homodimer. Every subunit adds something to the enzyme's overall structure and functionality.
2. **Cofactor Binding:** A flavin adenine dinucleotide (FAD) cofactor is covalently attached to each MAO subunit. As the main electron acceptor during the oxidation of monoamine substrates, the FAD cofactor is crucial to the catalytic activity of MAO.
3. **Multiple Domains:** The structure of MAO includes several domains within each subunit, including:
   - N-terminal Domain: May recognize substrates and aid in membrane anchoring.
   - Flavin-binding Domain: Houses the FAD cofactor and catalyzes the oxidation of monoamines.
   - C-terminal Domain: Contributes to specificity and substrate binding.
4. **Active Site:** Significant amino acid residues for substrate recognition and catalysis are found in the active site of MAO, which is situated at the interface of its domains. This location helps monoamine neurotransmitters undergo oxidative deamination, which yields their respective aldehydes and ammonia. [9][10]

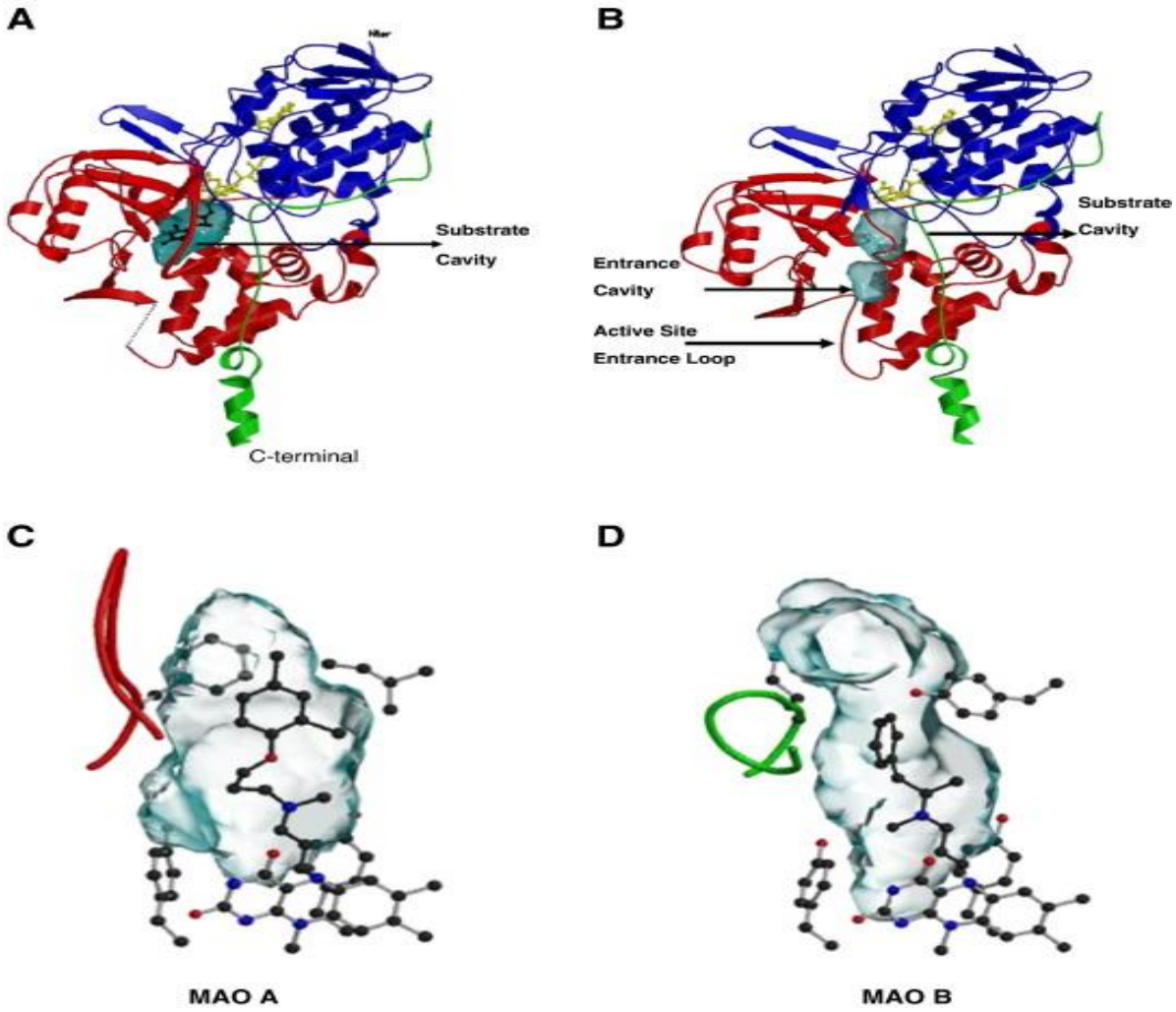

Fig 3. The chemical composition of the amine binding sites and the MAO-A and MAO-B enzymes. Human MAO-A (A) and MAO-B (B) monomeric units are shown in ribbon diagrams in the upper portion. The yellow ball and stick model illustrates the covalent flavin moiety. Blue represents the flavin binding domain, red the substrate domain, and green the mitochondrial membrane binding domain. Below are the active site cavities of human MAO-A (left) with clorgyline and MAO-B (right) containing selegiline. Covalent N(5) flavocyanine adducts are formed by both inhibitors and the flavin coenzymes. Red represents MAO-A and green represents MAO-B in the active site "shaping loop" structure [11].

Functions of MAO

- MAO catalyzes the oxidative deamination of monoamine neurotransmitters.
- This enzymatic reaction converts monoamine neurotransmitters (such as serotonin, dopamine, and norepinephrine) into their respective metabolites.
- The process involves the removal of the amine group, resulting in the production of aldehyde and ammonia.
- MAO's primary role is to regulate the levels of monoamine neurotransmitters in the brain, maintaining their proper balance crucial for normal brain function.
- Dysregulation of monoamine neurotransmitters is associated with various psychiatric and neurological disorders.
- MAO also contributes to the metabolism of dietary amines and drugs.
- MAO inhibitors are used in pharmacology, particularly as antidepressant medications, to increase the availability of monoamine neurotransmitters in the brain.
- Overall, MAO's function is essential for neurotransmitter regulation, impacting brain function, behaviour, and mental health.

Role of MAO Inhibitors

Monoamine oxidase inhibitors have garnered a lot of attention lately due to their purported neurorescue and/or neuroprotective qualities.

The oxidative deamination of monoamines is catalyzed by MAO. MAO-A and MAO-B are the two forms of MAO found in humans. While MAO-B deaminates phenethylamine and benzylamine, MAO-A principally deamines serotonin, melatonin, noradrenaline, and adrenaline. Tryptamine, tyramine, and dopamine are all similarly broken down by both types. MAOIs prevent neurotransmitters from being metabolized or deaminated. MAO was irreversibly suppressed by the early MAOIs. They interact with MAO to permanently deactivate it; replacement of the enzyme is required to restore the enzyme's function. A class of newly developed MAOIs known as reversible MAOIs, including moclobemide, restores activity when the inhibitor separates from the enzyme. Selectivity has a role in defining MAOIs. While certain inhibitors, like moclobemide, exclusively inhibit MAO-A, others, like pargyline and selegiline, selectively inhibit MAO-B, and some, like phenelzine and tranylcypromine, are non-selective and inhibit both A and B. At high doses or concentrations, selegiline is non-selective; selectivity is frequently concentration dependent [12]. Selenium is genuinely selective only at low doses.

Increases in the synaptic levels of "trace amines" (b-phenylethylamine, tryptamine, and tyramine) and other neurotransmitters, such as dopamine, serotonin, and norepinephrine, are brought on by inhibition of MAO activity in the brain. These substances have been linked to a number of neurologic and psychiatric disorders. Due to the potential for additional side effects and the hepatotoxicity of certain hydrazine-based inhibitors, the usage of MAOIs has been restricted. Hypertensive response following consumption of foods high in the sympathomimetic amine tyramine is one of the more serious side effects experienced by patients taking irreversible MAO-A inhibitors (e.g., phenelzine and tranylcypromine). This is because the ingested tyramine, which is normally metabolized by MAO-A in the gastrointestinal tract, enters the circulation and is actively taken up by peripheral adrenergic neurons, dislodging stored norepinephrine and causing a range of symptoms that may culminate in a hypertensive response. Due to the high tyramine content of some cheeses, especially older ones, this side effect of MAOIs has been named the ‘‘cheese effect.’’ Because intestinal MAO-A efficiently metabolizes tyramine and the intestines contain minimal amounts of MAO-B, selective irreversible MAO-B inhibitors do not induce any such side effects at regular dosages. Since dietary tyramine can displace the inhibitor from peripheral MAO-A, allowing for tyramine metabolism, reversible MAO-A inhibitors, like moclobemide, can block enough MAO-A in the central nervous system to produce an antidepressant effect, avoiding the cheese effect. As a possible antidepressant that would reduce the possibility of the cheese effect, the selective MAO-B inhibitor (-)-deprenyl (selegiline) was also initially developed. However, it was shown to exhibit poor antidepressant properties except at high doses, at which it also inhibits MAO-A (more recently, it has been reported to have antidepressant properties if provided as a patch20). Nonetheless, studies have demonstrated the efficacy of (-)-deprenyl in the pharmacotherapy of Parkinson's disease [13].

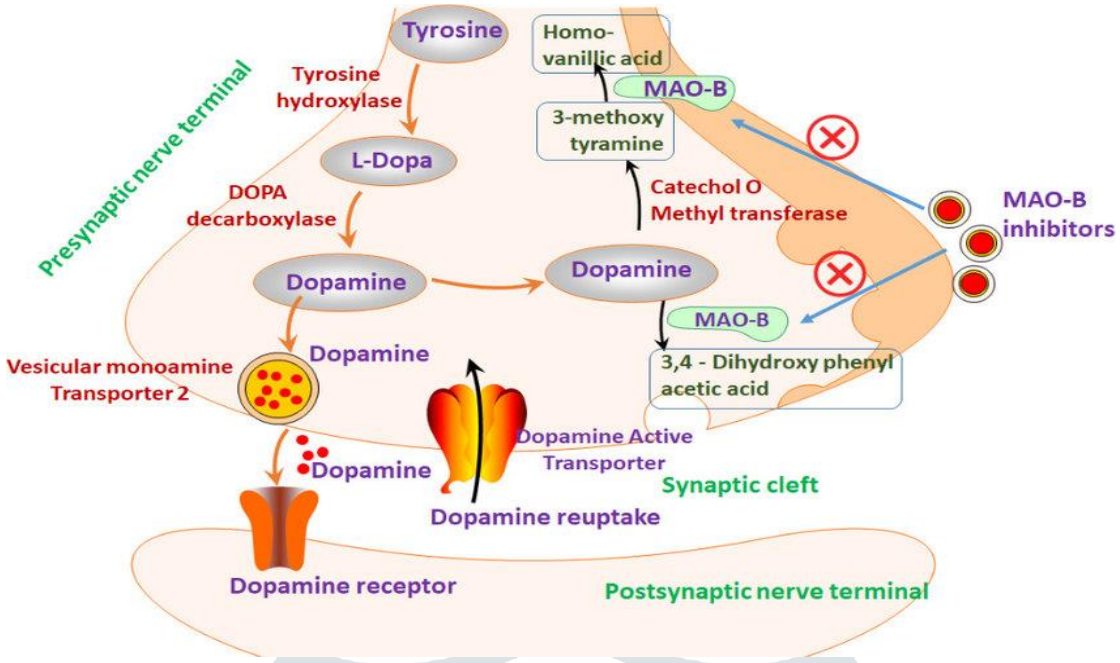

Fig 4. The production of dopamine and its metabolism by MAOB and MAOA. Tyrosine hydroxylase (TH) catalyzes the conversion of tyrosine to levodopa (L-dopa), which is then decarboxylated by dopa decarboxylase (DDC) to produce dopamine. Intraneuronal monoamine oxidase A (MAOA) and the glial and astrocyte MAOA and MAOB metabolize dopamine. The steady-state striatal dopamine levels are not changed by selective inhibitors of MAOA (such as moclobemide) and MAOB (such as selegiline, rasagiline, and safinamide). However, long-term use of these medications increases dopamine release, perhaps as a result of increased endogenous brain amine levels or receptor modulation. Non-selective MAOA/B inhibitors, however, do cause a very noticeable rise in dopamine levels in the striatum and other areas. The brain can access dopamine more readily when it is paired with DDC inhibitors like carbidopa and benzerazide, which do not cross the blood–brain barrier (BBB) but can pass it with L-dopa. Additionally, L-dopa availability is increased by COMT inhibitors like entacapone, which likewise stop COMT from inactivating dopamine. D1, D2 dopamine receptors; 3-OMD, 3-O-methyl dopa [14].

Table 1. Monoamine oxidase inhibitors.

Tranylcypromine	Non-selective	Irreversible
Phenelzine	Non-selective	Irreversible
Nialamide	Non-selective	Irreversible
Isocarboxazid	Non-selective	Irreversible
Clorgyline	MAO-A selective	Irreversible
Moclobemide	MAO-A selective	Reversible
Brofaromine	MAO-A selective	Reversible
Toloxatone	MAO-A selective	Reversible
Cimoxatone	MAO-A selective	Reversible
Befloxatone	MAO-A selective	Reversible
Pargyline	MAO-B selective	Irreversible
Selegiline (deprenyl)	MAO-B selective	Irreversible

Pharmacological Effects of MAOI

1. **Phenelzine (Nardil)**
   Primary Use: For the treatment of a typical depression and major depressive disorder.
   Antidepressant Effects: Suppresses MAO-A and MAO-B, raising serotonin, norepinephrine, and dopamine levels.
   Anxiolytic Effects: Because it affects serotonin and norepinephrine levels, it is useful in treating panic disorder and social anxiety disorder.
   Side Effects: Weight gain, orthostatic hypotension, and sexual dysfunction are common side effects. If dietary limitations are not adhered to, there is a risk of hypertensive crisis [15][16].

2. **Tranylcypromine (Parnate)**
   Primary Use: Treating major depressive disorder, particularly in cases where non-effective therapies have been tried.
   Antidepressant Effects: Suppresses both MAO-A and MAO-B, increasing the amounts of neurotransmitters. Has a comparatively quick start of action in contrast to other MAOIs.
   Stimulant Effects: May result in greater alertness and energy, which may alleviate exhaustion associated with depression, but may also induce sleeplessness.
   Side Effects: Possible side effects include weight gain, sleeplessness, orthostatic hypotension, and hypertension. Tyramine-rich diets increase the likelihood of a hypertensive crisis [17][18].
3. **Isocarboxazid (Marplan)**
   Primary Use: Treatment of major depressive illness is the main use.
   Antidepressant Effects: Inhibits both types of the MAO enzyme, which raises serotonin, norepinephrine, and dopamine levels.
   Anxiolytic Effects: As with other MAOIs, this medication can lessen anxiety symptoms.
   Side Effects: Dizziness, dry mouth, sleeplessness, weight gain, and dysfunctional sexual behaviour are some of the side effects. If improper food choices are made, there is also a danger of hypertensive crisis [19][20].
4. **Selegiline (Eldepryl, Emsam)**
   Primary Use: Transdermal patches are mostly used to treat major depressive disorder and Parkinson's disease.
   Neuroprotective Effects: Raises dopamine levels and is useful for Parkinson's disease by primarily inhibiting MAO-B at lower doses. It is helpful for depression because, at larger doses, it suppresses both MAO-A and MAO-B.
   Stimulant Effects: Boosts dopamine, which enhances energy, mood, and cognitive performance.
   Side Effects: Adverse effects that may arise from the medication include nausea, vomiting, sleeplessness, and stomach pain. Comparing the transdermal patch formulation to oral MAOIs lowers the possibility of dietary tyramine interactions [21][22].
5. **Moclobemide (Aurorix, Manerix)**
   Primary Use: For the treatment of social anxiety disorder and serious depressive illness.
   Reversible Inhibitor of MAO-A (RIMA): In contrast to conventional MAOIs, moclobemide is reversible and selectively inhibits MAO-A, lowering the risk of dietary limitations and hypertensive crises.
   Antidepressant Effects: Elevates norepinephrine and serotonin levels, which boost mood.
   Anxiolytic Effects: Good for reducing social anxiety disorder symptoms.
   Side Effects: Usually well-absorbed with few negative consequences. Headache, nausea, and sleeplessness are among the potential adverse effects [23][24].
6. **Rasagiline (Azilect)**
   Primary Use: Parkinson's disease treatment.
   Neuroprotective Effects: By specifically inhibiting MAO-B, dopamine levels in the brain are raised, alleviating Parkinson's disease symptoms.
   Cognitive and Stimulant Effects: Higher dopamine levels can enhance motor coordination and mental acuity.
   Side Effects: Generally well-tolerated, but may result in indigestion, headaches, and joint pain. reduced incidence of hypertensive crisis in contrast to MAOIs that are non-selective [25][26].

Pharmacokinetics of MAOI

- Data currently available suggest that phenelzine is similarly absorbed quickly. The first 6 to 8 weeks of treatment have been seen to see a modest increase in steady-state plasma concentrations. Phenylethylamine and phenylacetic acid are produced by the biotransformation of phenelzine.
- Almost all of the brofaromine is absorbed; it takes 2 to 4 hours to achieve Cmax. Between Cmax and the area under the plasma concentration time-curve (AUC), there is a linear dosage proportionality. Recurring administration had no effect on the elimination half-life. There is substantial metabolism of brofaromine [27].
- Tranylcypromine has a short half-life of 2 hours for plasma elimination and is readily absorbed. Both ring-hydroxylation and N-acetylation occur in tranylcypromine. Major changes due to aging may not be expected because tranylcypromine is mostly digested and only 4% of a dosage is eliminated unaltered in the urine.
- Arylalkylamines, such as amphetamine, N-methylamphetamine, and N-propargylamphetamine, are produced when the propargylamine selegiline undergoes N-demethylation and N-depropargylation. Subsequent metabolism of these compounds is possible. Cytochrome P450 (CYP) 2D6 (CYP2D6) and CYP3A4 mediate the synthesis of these metabolites. Selegiline has a half-life of l.7 hours for plasma elimination following a single dosage. Oral form: Rapidly absorbed from the digestive system, Transdermal patch: Enables continuous

skin absorption. Widely distributed, including within the brain. Eliminated as metabolites in the urine. Half-life: Transdermal form: 18–25 hours; oral form: 10 hours.

- The gastrointestinal tract absorbs moclobemide quickly and nearly entirely, and it goes through a significant first-pass hepatic metabolism. As a result, moclobemide's systemic availability rises from 40% following a single dosage to 85% following several doses; hepatotoxicity is not known to occur. Moclobemide undergoes aromatic hydroxylation and morpholine ring C- and N-oxidation as biotransformations. Ninety-five percent of the medication is eliminated by the kidneys in a day. Only over 50% of moclobemide is protein bound [28].

NEED OF WORK

The assessment of the inhibitory activities of monoamine oxidase (MAO) enzymes by Eulophia guineensis presents a significant area of interest in neuropharmacological research. Eulophia guineensis, a traditional African orchid, has been used in herbal medicine across several African communities for the treatment of various ailments. However, its potential in treating neurological disorders through the inhibition of MAO, an enzyme critical in the catabolism of neurotransmitters in the brain, has yet to be fully explored. This research is particularly vital given the increasing prevalence of neurodegenerative and mood disorders, alongside the necessity for safer and more effective treatments.

Rationale for the Study: Monoamine oxidase inhibitors are pivotal in the management of depressive disorders, anxiety, and Parkinson's disease due to their role in preventing the breakdown of key neurotransmitters such as serotonin, dopamine, and norepinephrine. However, current synthetic MAO inhibitors are often associated with numerous side effects and dietary restrictions, driving the need for the discovery of new inhibitors that are both effective and have a better safety profile. Natural products, like those derived from Eulophia guineensis, offer a promising alternative due to their bioavailability and typically lower incidence of adverse effects.

Significance of the Study: This research could significantly contribute to the fields of neuropharmacology and ethnopharmacology by providing insights into the therapeutic potentials of Eulophia guineensis. By establishing a scientific basis for its traditional use in neurological conditions, this study may facilitate the development of novel MAO inhibitors that are more acceptable and effective for patients. Furthermore, it may open avenues for the conservation of this plant species and similar ethnomedicinal plants, promoting biodiversity and sustainability in pharmaceutical development.

Methodological Approach: The study would employ both qualitative and quantitative research methods. Qualitative analysis will focus on ethnobotanical surveys and historical usage data, while quantitative methods will involve laboratory-based biochemical assays and analytical chemistry techniques. This dual approach ensures a comprehensive understanding of both the traditional context and the scientific potential of Eulophia guineensis.

In conclusion, investigating the MAO inhibitory activity of Eulophia guineensis not only fills a crucial gap in current research but also potentially offers a new pathway for treating neurological diseases with fewer side effects. This study stands to contribute significantly to our understanding of natural products in neuropharmacology, encouraging further research and development in this vital area of human health.

LITERATURE REVIEW

The primary healthcare system continues to rely heavily on traditional medicines, which have been used for therapeutic purposes since ancient times. 70–80% of people on the planet are thought to get their primary medical care from traditional herbal remedies [29]. Modern scientific testing has confirmed the therapeutic usefulness of a number of ethnobotanical medicinal plants in addition to traditional medicine. As food and medicine, some of these ethnobotanical plants are gaining recognition [30], with Amarkand being among the better examples.

Fig 5. Amarkand tubers.

The tribes people of India frequently use the tubers of the Amarkand group of medicinal plants as both food and medicine. The terms ***"Amar"*** (for immortality) and ***"kand"*** (for tubers) combine to form the word *"Amarkand."* Thirty ***Eulophia*** **(Family-Orchidaceae)** and Dioscorea bulbifera (Family-Dioscoreaceae) plant species are together referred to as "Amarkand." Of these, 21 are known to have culinary and medicinal uses. According to a study on the way of life of the tribes in the Indian state of Maharashtra, amarkand is a prominent part of the diets of the tribal people. Ayurvedic practitioners have reported using Eulophia species for astringent, digestive, expectorant, anabolic, tonic, diuretic, and gentle purgative purposes [31]. Ayurveda praises amarkand for its rasayana qualities and capacity to promote health. Eulophia species are perennial terrestrial orchids (herbs) that grow one to three feet tall and have fleshy tubers shaped like a chain. They are primarily found in the tropical regions of the Himalaya and the Deccan peninsular in India, but they can grow anywhere in the world along water channels on steep soil slopes [32]. From an ethnopharmacological perspective, Amarkand's many applications are a benefit and a sign of its therapeutic worth.

Distribution

The family Orchidaceae includes the extremely diversified genus *Eulophia*, which can be found in a variety of environments. From its subterranean tubers, this plant generates two shoots: vegetative and reproductive. The genus *Eulophia* is widely distributed, with over 230 species found in tropical and subtropical regions of Asia, Australia, Madagascar, and Southern and Tropical Africa. Of these, one species is found in tropical America. This genus is primarily found in the tropical Himalaya and Deccan peninsula regions of India. Within the International Plant Name Index, *Eulophia* has around 723 records. But many of the 500 synonyms are decorative [33]. Approximately 20 species with therapeutic significance are reported from across India. Different regions of India employ different species of eulophia for therapeutic purposes. Across all Eulophia species in India, Amarkand is the most commonly used name; but, these species are also referred to by a number of colloquial names, including Balakand, Manakand, Munjatak, Amrita (Sanskrit), Ambarkand, Salam (Hindi), Budbar (Bengali), Salab (Gujarati), Amarkand and Salibmisri (Marathi) [34].

Morphology

The terrestrial herb species in the genus *Eulophia* are either autotrophic or infrequently heteromycotrophic [Figure 7a]. Perennating organs might resemble tubers or pseudobulbs. These pseudobulbs can be born above ground or underground, resembling corms, and are either tuberous or rhizomatous. They often have numerous nodes and thin or thick fibrous roots at the base. A network of subterranean tubers is developed by Eulophia [Figure 7b]. At or after anthesis, leaves emerge. They are one to many, basal, have petiole-like leaf bases, are stiff yet thin, grass-like, or lanceolate and plicate. Occasionally, they overlap and form a pseudostem. Certain species are saprophytic they don't have green leaves. The inflorescence is laxly to sub-densely many flowered, erect, lateral, racemose, or sporadically paniculate, and it is occasionally reduced to a single flower. Most Eulophia species can be identified primarily by their flowers. Within Eulophia, there are two varieties of flowers. The size, shape, and color of the sepals and petals are comparable in the first type, while they are smaller and frequently recurved in the second. The lip of both kinds continues into a spur, which can have a quite different shape [34].

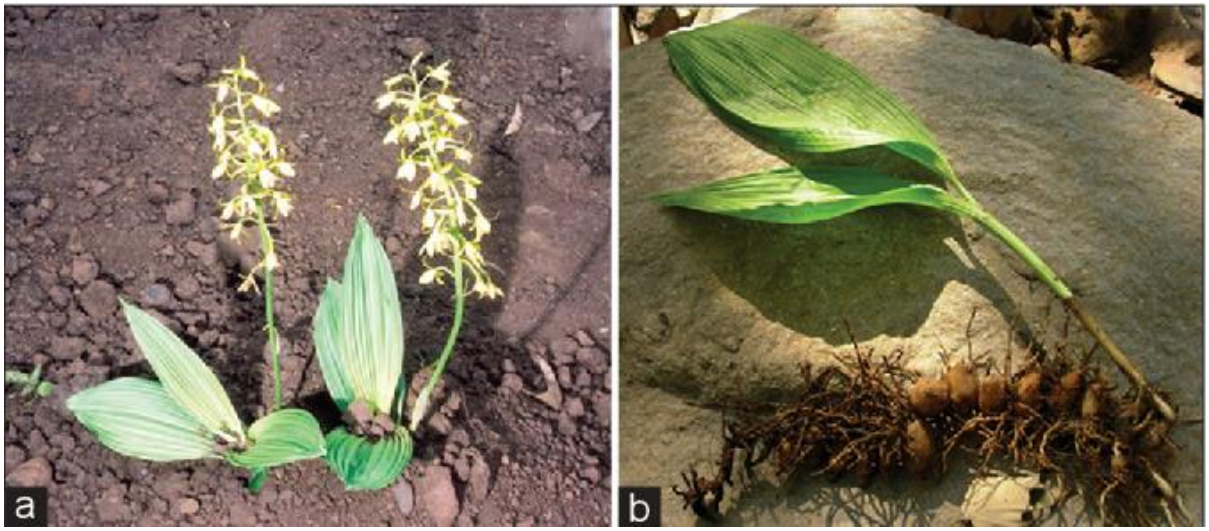

Fig 6. Representative photograph of (a) whole plant of Eulophia species and (b) chain of underground tubers of Eulophia species.

Traditional and ethnobotanical uses

Amarkand is commonly used as an expectorant, anabolic, tonic, diuretic, astringent, digestive, and mild purgative in Ayurvedic medicine. It is also suggested for the treatment of blood clotting, joint edema, debility, and ear discharge syndromes. Moreover, it is thought to be a general tonic that balances all three "doshas" and encourages vigor. These are also used to treat stomatitis, purulent cough, heart issues, dyscrasia, scrofulous diseases of the neck, bronchitis, blood disorders, and vermifuge treatments. In many countries, the traditional medical system makes considerable use of various species of Eulophia [35].

Eulophia guineensis

One type of orchid is called *Eulophia guineensis*. Known by most as the broad-leaved ground orchid or Guinea Eulophia, it is the type species of the genus Eulophia. E. congoensis and E. quartiniana are examples of synonyms. It is a perennial terrestrial orchid that can be found almost everywhere in tropical Africa, including the Cape Verde Islands, Senegal, Angola, Ethiopia, Somalia, Kenya, Tanzania, and much of the east, up to Zambia, Malawi, Zimbabwe, and Botswana, and all the way to the South Arabian Peninsula, which includes Saudi Arabia, Yemen, and Oman [36]. Its underground stem, or rhizome, grows to an average height of 67 cm. The tubers of Eulophia guineensis are ovoid to ellipsoid, light to dark brown on the outside, and white to creamy inside. They store nutrients and water, aiding in the plant's survival and vegetative reproduction, and are found underground at the base of the stem. The pseudobulbs grow upward from the rhizome and are thicker, conical structures. The pseudobulbs have culinary and medicinal uses. According to tradition, the pseudobulbs are crushed, cooked, and sieved. The liquid that is left behind is then used to make the regional delicacy *Akamu pap*, which is well-liked throughout West Africa (Personal communication). Without any supporting scientific data, several civilizations have historically used its pseudobulbs to treat conditions like obesity, hypertension, inflammations, colds, and sexually transmitted infections [37]. When the plant is in flower, it may have three or four elliptic, upto 35 cm long leaves with separate petioles. Lax inflorescence; typically has seven blooms. With a purplish-brown sepals and petals, a whitish base and spur, and a pinkish-purple lip, they are striking. The pedicel and ovary are thin, reaching a maximum length of 25 mm (1.0 in), while the floral bracts can reach upto 20 mm (0.8 in) in length and are ovate-lanceolate. The fragrant, waxy flowers bloom in the autumn and early winter [38].

Fig 7. Eulophia guineensis.

Taxonomical classification

Table 2. Taxonomical classification

1.	Kingdom	Plantae
2.	Phylum	Tracheophytes (Vascular plants)
3.	Subphylum	Angiosperms (Flowering plants)
4.	Class	Liliopsida (Monocotyledons)
5.	Order	Asparagales
6.	Family	Orchidaceae
7.	Subfamily	Epidendroideae
8.	Genus	Eulophia
9.	Species	Eulophia guineensis

Reported activities of E. guineensis

Antioxidant activity

Eulophia species are cultivated worldwide; it is one of the largest orchid's genera in Africa. They are believed to cure many diseases. Eulophia guineensis Lindl (Orchidaceae) is a multipurpose plant, native to West Africa and currently grown in many parts of the world. Traditionally, its pseudobulbs are exploited in the treatment of diseases like hypertension, obesity, inflammations, cold, sexual ailments by various cultures without scientific evidence. The aim of the research was to carry out phytochemical test, antioxidant potentials and acute toxicity test on the pseudobulbs of E. guineensis [37].

Antimicrobial Activity

Extracts of Eulophia guineensis have been reported to exhibit antimicrobial properties against a range of bacteria and fungi. This makes it potentially useful in treating infections [39].

Anti-inflammatory Activity

The plant has been used traditionally to treat inflammatory conditions, and scientific studies have shown that extracts can reduce inflammation [40].

MATERIALS & METHODS

➢ Materials needed

- ✓ Fresh or dried tubers of E. guineensis
- ✓ Solvent (ethanol, methanol, water, or a mixture)
- ✓ Blender or grinder
- ✓ Weighing balance
- ✓ Soxhlet extractor (optional)
- ✓ Filtration apparatus (filter paper, funnel)
- ✓ Distilled water
- ✓ Glasswares (beakers, flasks, etc.)
- ✓ Drying oven

➢ Preparation of extract

The tubers of E. guineensis were thoroughly cleaned to remove dirt and impurities. The cleaned tubers were cut into small pieces, shed dried and grind to fine powder with mixer. Then, this fine powder was extracted with methanol using cold maceration method for 5-6 days in a separating funnel. Allow the mixture to stand at room temperature, shaking it occasionally to ensure thorough mixing and extraction. Finally, the extract was filtered & evaporated at room temperature. The dried extract is then collected and preserved in desiccator [41].

Fig 8. Extraction of E. guineensis tubers.

➢ Phytochemicals tests

Phytochemical screening was performed using standard procedure to test for Flavonoids (NaOH Test), Saponins (Foam test), Alkaloids (Dragendroff's Test) and Cardiac glycosides (Keller killani test). A combined knowledge of chemical constituents is desirable for supportive evaluation of natural drug or drug component as well as phyto-pharmaceuticals for knowledge of phytopharmacognostic and phyto-pharmacological for screening. The plant may be considered as biosynthetic laboratory for multitude of components like alkaloids, glycoside, volatile oils, tannins and Flavonoid etc., which are termed as secondary metabolites and a responsible for therapeutic effects [42].

Sr.no	Test performed	Observation	Inference
(A)	**Test for alkaloids**		
1.	**Dragendroff's test:** Treat test solution with Dragendroff's reagent (potassium bismuth Iodide).	Orange brown precipitate was formed.	Alkaloid present
2.	**Hager's test:** Treat test solution with Hager's reagent (Saturated picric acid solution).	Yellow ppt	Alkaloid present
3.	**Mayer's test:** Treat test solution with Mayer's reagent (potassium mercuric iodide).	Cream coloured ppt	Alkaloid present
4.	**Wagner's test:** Treat test solution with Wagner's reagent(Iodine Potassium Iodide).	Reddish brown ppt	Alkaloid present
(B)	**Test for flavonoids**		
1.	**Alkali test:** Treat test solution with increasing amount of NaOH.	Yellow colour decolourised after addition of acids.	Flavonoid present
2.	**Lead acetate test:** Mix test solution with lead acetate.	Yellow precipitate	Flavonoid present
(c)	**Test for cardiac glycosides**		
1.	**Baljet test:** Mix 2-3 mg of sample in 2ml Sodium picrate solution.	Yellow, orange to deep red Colour.	Cardiac glycosides present.
2.	**Keller killani test:** 2ml of extract, add glacial acetic acid, one drop 5% ferric chloride and conc. sulphuric acid.	Reddish brown color appears at the junction.	Cardiac glycosides present.
(D)	**Test for steroids & triterpenoids**		
1.	**Liebermann-burchard Test:** Mix 2ml of extract with few drops of acetic anhydride, boiled and cooled and Concentrated sulphuric acid was then added from the sides of the test tube.	Formation of a brown ring at the junction of two layers. Green coloration of the upper layer and the formation of deep red color in the lower layer would indicate a positive test for steroids and triterpenoids respectively.	Steroid & Triterpenoids are present.
(E)	**Test for saponins**		
1.	**Foam test:** Shake the drug extracts or dried powder vigorously with water.	No Persistent foam observed.	Saponins are absent
(F)	**Test for tannins**		
1.	**Iodine test:** Mix test solution with dilute Iodine solution.	Red colour	Tannins present
2.	**Ferric chloride test:** Mix 2ml of solution with Ferric chloride solution.	Formation of Blue, bluish black colour observed.	Tannins present

➢ Phytochemical screening

The methanolic extract of Eulophia guineensis was subjected to chemical tests to check the presence of various phytoconstituents like, alkaloids, flavonoids, saponins, carbohydrates, steroids, triterpenoids, tannins, phenolics, coumarins. The results of the phyto-chemical screening are as described in Table, given below.

Table 3. Phytochemical screening of tubers of E. guineensis.

Phyto-chemical screening	Eulophia guineensis (Tubers)
Alkaloids	+
Carbohydrates	+
Flavonoids	+
Cardiac glycosides	+
Steroids	+
Triterpenoids	+
Tannins	+
Phenolics	+
Saponins	-
Coumarins	-

Where, (+) = present & (-) = absent

RESULT

The Monoamine Oxidase (MAO) inhibition assay is a useful and efficient test for evaluating the inhibition of the MAO enzyme, which is implicated in the pathogenesis of psychiatric diseases. In this assay, a EG (Eulophia guineensis) extract was found to inhibit both MAO-A and MAO-B enzymes, with IC50 values of EG extract(100mg) are 4.5µM & 1.4µM and for EG extract(150mg) are 4.9µM and 1.6µM, respectively. This suggests that the EG extract is a more potent inhibitor of MAO-B compared to MAO-A.

Table 4. Effects of the MeOH extracts of E. guineensis on the MAO inhibition assay.

MATERIAL	IC50 (mg/mL)	
	MAO-A	MAO-B
Control	**<10**	**>10**
Standard	**5.6**	**5.8**
EG extract (100mg)	**4.5**	**1.4**
EG extract (150mg)	**4.9**	**1.6**

Among the tests samples 100mg & 150mg demonstrated significant inhibitory activity against MAO enzymes. This suggests that these particular samples contains the bioactive compounds responsible for the MAO inhibition observed in the methanol extract.

➢ **Graphical representation :**

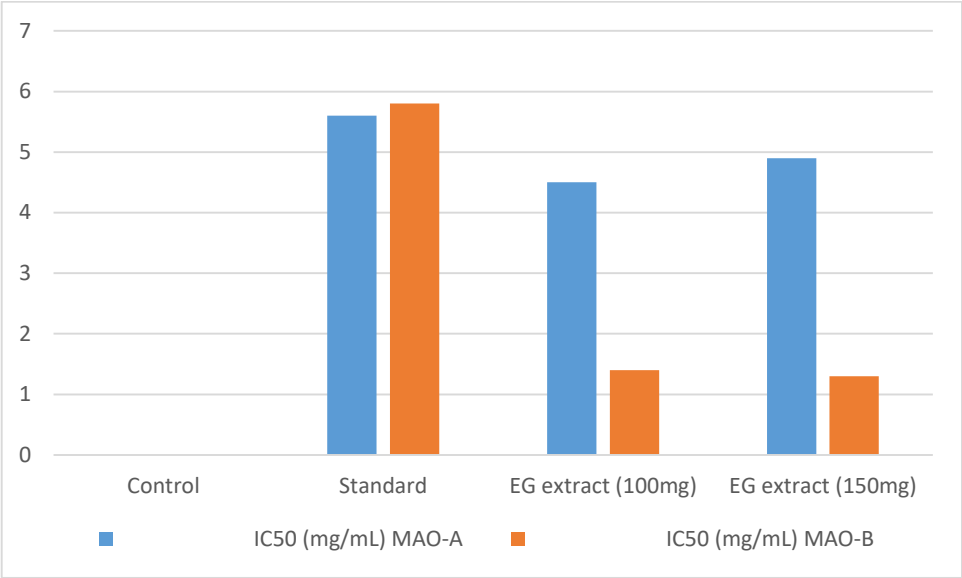

Fig 9. MAO enzyme inhibition assay of Eulophia guineensis.

DISCUSSION

The assessment of inhibitory activities of monoamine oxidase (MAO) enzymes in Eulophia guineensis reveals significant potential for developing new therapeutic agents from this plant. Monoamine oxidases, particularly MAO-A and MAO-B, are critical in the catabolism of neurotransmitters like dopamine, norepinephrine, and serotonin. Inhibiting these enzymes has been a successful strategy in treating psychiatric and neurological disorders, such as depression and Parkinson's disease. This study aimed to evaluate the MAO inhibitory effects of Eulophia guineensis extracts using cell lines and to identify the specific phytochemicals responsible for this activity. The research involved extracting compounds from Eulophia guineensis using solvents such as methanol, ethanol, and aqueous solutions. The methanolic extract were then tested for their ability to inhibit MAO-A and MAO-B using standard enzymatic assays performed on cell lines that express these enzymes. Phytochemical tests are performed through which we identified various phenolic compounds and alkaloids in the extracts. The phenolic compounds, which are among these extract, were divided into several sub-groups, including phenolic acids, flavonoids, tannins, stilbenes and lignans, based on the changes in their chemical structures. Polyphenols with potent antioxidant properties are suggested for the treatment of different conditions, including degenerative, cardiovascular and gastrointestinal disorders. Flavonoid type compounds are generally known for their hepatoprotective, antioxidant, antibacterial, anti-inflammatory and antiviral activities.
The mechanism behind the inhibition likely involves the interaction of phenolic hydroxyl groups with the active site of MAOs, while alkaloids might bind to the enzyme, altering its conformation and reducing its activity. These findings suggest that Eulophia guineensis could be a valuable source of natural MAO inhibitors, providing a basis for developing new antidepressant and neuroprotective agents. However, further in vivo studies and clinical trials are necessary to validate these findings and ensure the safety and efficacy of these extracts in humans.

CONCLUSION

In conclusion, Eulophia guineensis holds promise for providing natural MAO inhibitors that could offer safer and more effective treatments for neuropsychiatric and neurodegenerative disorders such as depression and Parkinson's disease. The identification and characterization of these bioactive compounds not only have the potential to enhance current medical treatments but also validate the traditional medicinal use of the plant. Furthermore, this research underscores the importance of biodiversity conservation and the sustainable use of medicinal plants. Therefore, investigating the MAO inhibitory properties of Eulophia guineensis is a vital step towards discovering novel therapeutic agents and promoting ecological sustainability.

Future research should focus on isolating and characterizing individual active compounds, exploring the synergistic effects of multiple phytochemicals, and investigating the impact of these compounds on neurotransmitter levels in animal models. This study provides a promising foundation for the therapeutic application of Eulophia guineensis in managing mental health disorders.

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