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RESEARCH ARTICLE

"PRECLINICAL EVALUATION OF POLYHERBAL AS AN ANTI- ALZHEIMER'S, BY USING IN-VITRO ANTICHOLINESTERASE ENZYME MODEL"

Ms. Somvanshi S.R.[1*], Ms. Talware P. R.[2], Mr. Gaikwad Y.S.[3], Mr. Patil R.N.[4], Mr. Vyavhare P.V.[4]

1. Graduate Student, Matoshri College of Pharmacy,Eklahare, Nashik.[1,2,3]
2. Assistant Professor, MET's Institute of D.Pharmacy, Adgaon, Nashik.[4]
3. Principal, D.P. Kharde, Navjeevan College of College of Pharmacy, Nandur Shingote.[5]

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Abstract

Background: Alzheimer's disease is a neurodegenerative brain condition that causes mild cognitive impairment and memory loss, eventually leading to dementia. With developments in medicine, there are now more people in the world trying to treat a wider range of illnesses. It has been discovered that traditional herbal treatment works well with little or no adverse effects.

Materials & Methods: There are certain chemical components in the polyherbal extract of liquorice, ginger, and hibiscus sabadariffa that have anti-Alzheimer's properties.

Results & Discussion: The polyherbal extract of liquorice, ginger, and hibiscus sabadariffa has been shown to have significant inhibition up to 44.02% in the group treated with it, which supports the extract's anti-Alzheimer's activity. This was determined through a dose-dependent study and statistical comparison using Graph Pad Prism, version 10.2.2 by two-way ANOVA.

INTRODUCTION

Neurodegenerative disease

Neurodegenerative disease is a major global source of illness and death in the senior population. Even while each brain degenerative disease has a different underlying physiology and set of clinical symptoms, they frequently have some things in common. Alzheimer's disease, front temporal dementia and its variations, Parkinson's disease, dementia with Lewy bodies, progressive supranuclear palsy, multiple system atrophy, and Huntington's disease are the most common neurodegenerative illnesses.

Alzheimer's disease

In 1907, German psychiatrist and neuropathologistAlois Alzheimer gave the disease its initial description and name. This illness begins off slowly, gets worse over time, and then gets worse. Dementia associated with AD is primarily defined by a deterioration in cognitive functions like memory, praxis, and orientation. Short-term memory loss, an inability to pick up new skills, mood fluctuations, trouble pronouncing words, forgetting names, and misplacing objects are all signs of early disease. Alzheimer's disease patients often exhibit annoyance, aggression, and exasperation. In extreme cases, patients lose all memory, all sense of time and place, and they become completely incontinent. At some point, patients need full care and become dependent on others. The patient will require full-

Corresponding Author:- Ms. Somvanshi Snehal Ravindra.[1*]
Address:- Graduate Student, Matoshri College of Pharmacy Eklahare, Nashik.

time nursing care and transfer to a nursing home due to their utter dependence on others. Consequently, AD poses a significant challenge for patient care as well.[1]

Currently, there are about 50 million AD patients worldwide; by 2050, that number is expected to have doubled every five years to 152 million. Alzheimer's disease does not currently have a cure, however, some therapies can help with symptoms.[2]

Cognitive decline is a hallmark of Alzheimer's disease, a progressive neurological illness. It is the most prevalent kind of dementia that is both pre- and senile. The World Health Organization (WHO) estimates that 6% of women and 5% of men over 60 suffer from Alzheimer's-type dementia globally.

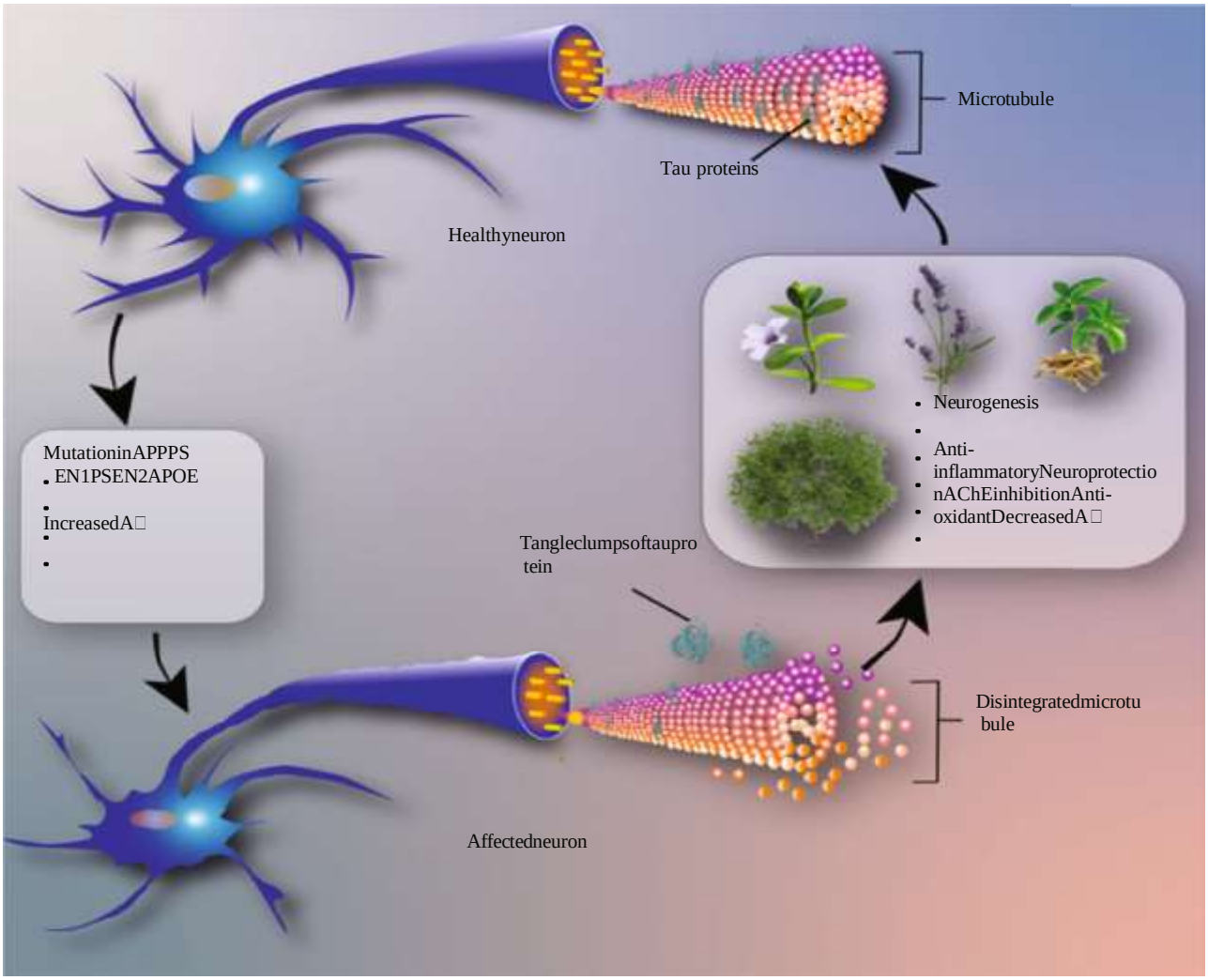

Fig.1.1:- Alzheimer's disease

A disorder caused by duplication or mutation of the genes encoding proteolytic enzymes, APP, apolipoprotein E (APOE-), and presenilin (PSEN1, PSEN2), which results in increased amounts of neurofibrillary tangles and β-amyloid (Aβ). Medicinal plants possess antioxidant and anti-inflammatory qualities, decrease β-amyloid levels, boost neurogenesis, enhance neuroprotection, and inhibit acetylcholinesterase (AChE). Additionally, they lessen Alzheimer's disease symptoms via a variety of postulated mechanisms.[6]

Stages of Alzheimer's disease

The development of Alzheimer's disease usually involves several stages:

1. **Preclinical Alzheimer's:** Years before symptoms manifest, changes in the brain may start.

2. **Mild Cognitive Impairment (MCI):** People with mild cognitive impairment have evident memory issues, but they are nevertheless able to function on their own.
3. **Mild Alzheimer's disease:** Daily duties are affected by substantial memory loss and cognitive deterioration.
4. **Moderate Alzheimer's disease:** Confusion, trouble speaking, and difficulty identifying friends and relatives worsen.
5. **Severe Alzheimer's disease:** Patients lose their capacity for self-care, communication, and recognition of loved ones.[9]

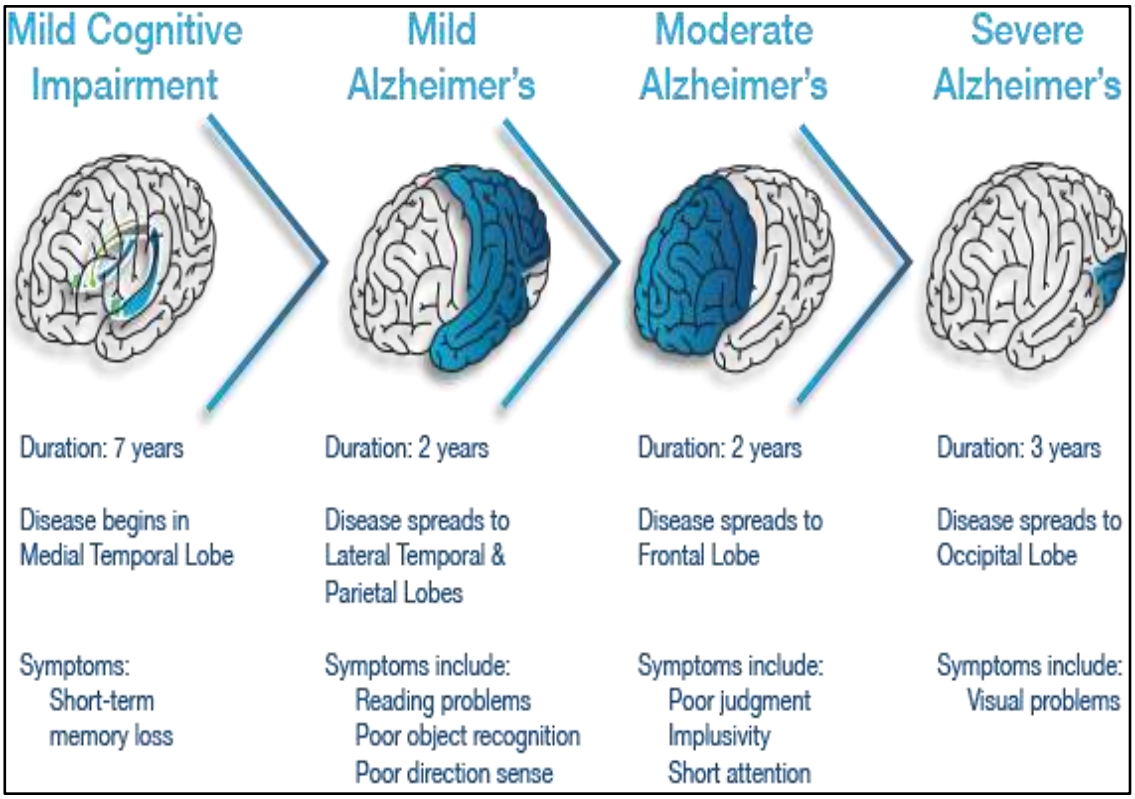

Fig.1.2:-Stages of Alzheimer's disease.

Healthy brain and Alzheimer's disease brain.

The brain's cortex has an accumulation of aberrant protein deposits associated with Alzheimer's disease. These deposits impede brain cell-to-cell contact and lead to the death of nerve cells. Understanding these changes is key to developing effective treatments for Alzheimer's disease. There are two unique features in the brains of AD sufferers.

1. Extracellular deposits of amyloid-beta (Aβ), a peptide formed by breaking down Aβ precursors, are detected in senile plaques (genetic locus 21q21–22). Aβ deposits can also be aberrant in blood vessels.
2. Neurofibrillary tangles, which are dense bundles of abnormal fibres created in the cytoplasm of neurones by a mutated form of the microtubular-associated protein, are seen in AD patients.[5]

Since the fundamental pathological aetiology of Alzheimer's disease is unknown, a significant deal of research is being done to elucidate this process. Given what is known at this time, numerous theories have been put up on the pathophysiology of AD. The amyloid cascade hypothesis, the tau hypothesis, and the mitochondrial cascade hypothesis are the three that are most often accepted.[8]

Causes and risk factors of Alzheimer's disease

AD is considered a multifactorial disease associated with several risk factors (Fig. 1.4), such as vascular problems, infections, ageing, genetics, head trauma, heavy metals, and other environmental variables.The pathogenic changes (Aβ, NFTs, and synaptic loss) linked to Alzheimer's disease are presently unknown in their cause. Though there are several theories explaining AD, only two are considered to be major: one proposes that modifications in the manufacture and processing of amyloid β-protein act as the main cause of the disease, while others argue that

cholinergic dysfunction is a significant risk factor. However, as of right now, no accepted explanation exists to explain the pathophysiology of AD.[2]

Pathogenesis of Alzheimer's disease
Healthy brain

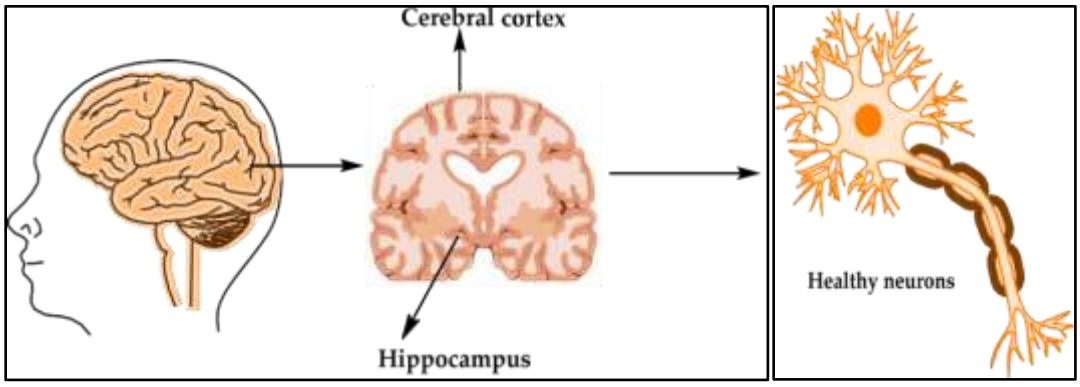

Fig. 1.3:- Healthy brain

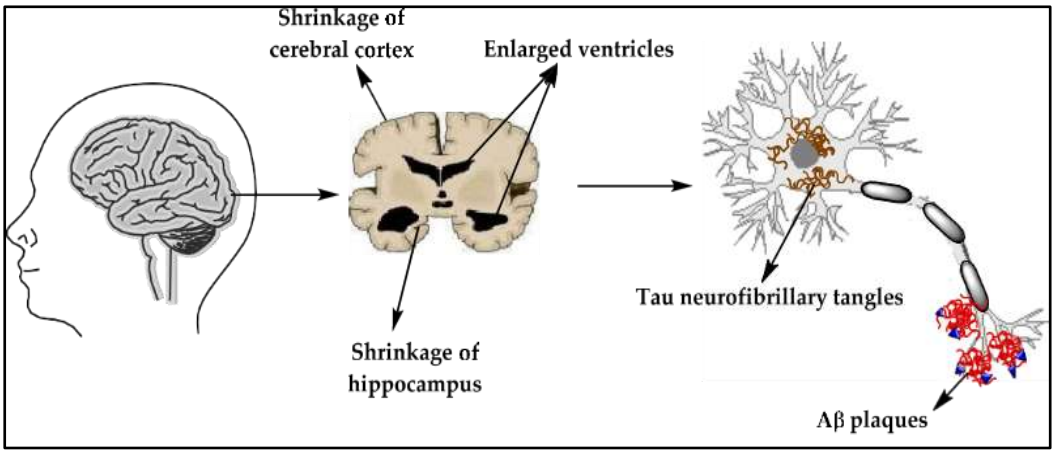

Fig. 1.4:- The physiological structure of the brain and neurons in Alzheimer's disease

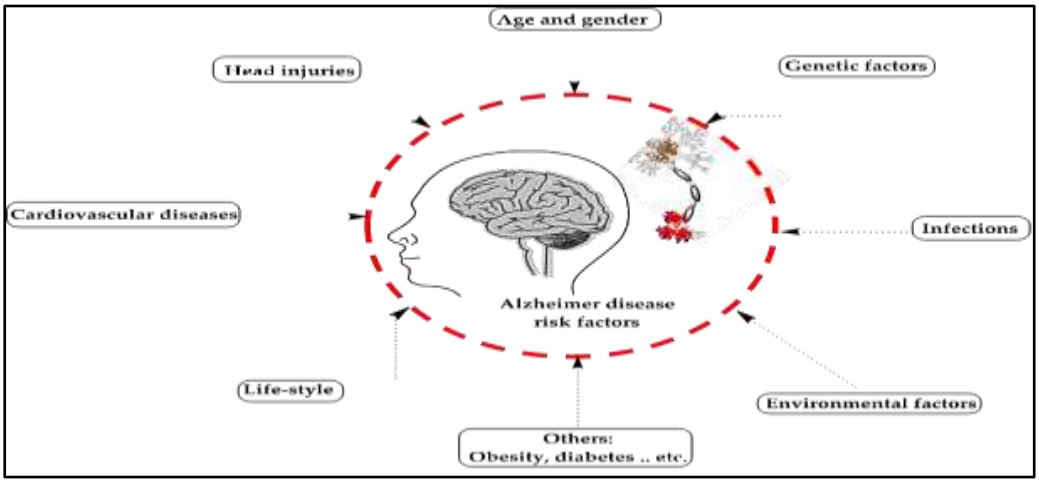

Fig 1.5:- The risk factors for Alzheimer's disease.[2]

Diagnosis of Alzhiemer disease

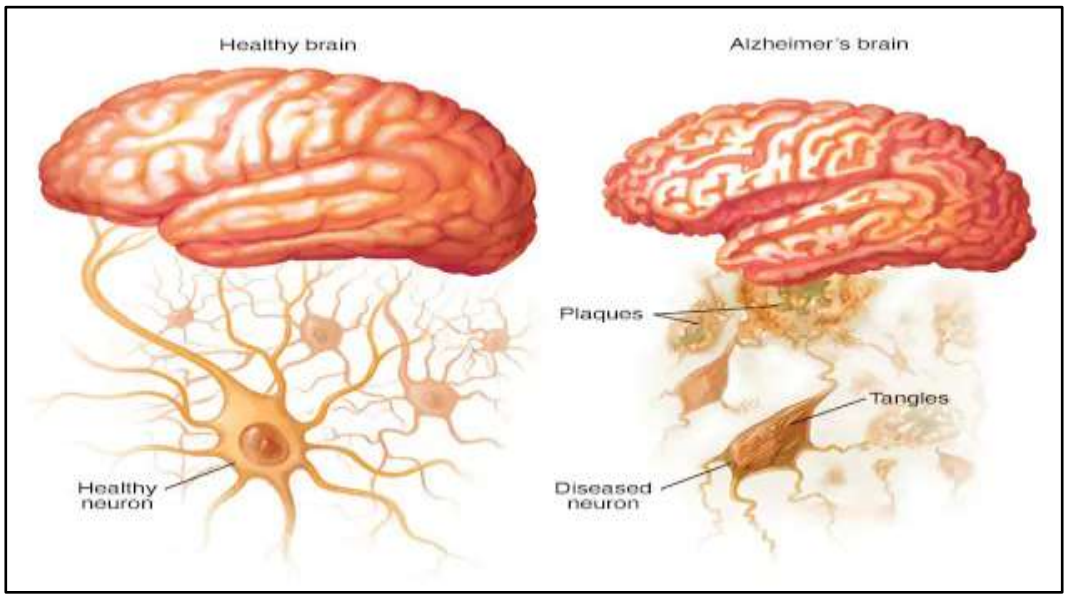

Fig.1.6:- Representation of Healthy Brain & Brain with Alzheimer's Disease.

In clinical settings, the patient's medical history, physical and neurological tests, cognitive testing, and selective auxiliary testing to rule out other aetiologies are the main factors used in the diagnosis of AD. The clinical diagnosis of AD has an accuracy of 70–90% compared to the pathological diagnosis; better accuracy is reached in specialised settings such memory disorder clinics. The clinical diagnosis is based on a set of consensus criteria that were first created in 1984 and most recently updated in 2011 by a workgroup of the National Institute on Aging-Alzheimer's Association (NIAAA). When a patient's cognitive impairment has an unusual clinical course or is believed to be caused by aetiologies other than AD, the diagnosis of "possible" AD dementia is advocated. Physical and neurological exams typically provide normal results for AD patients.

Establishing the Diagnosis of Alzheimer's Disease

1. Clinical signs, Dementia that progresses slowly.
2. Neuro imaging
   a. Diffuse cerebral hypometabolism on PET.
   b. Gross cerebral cortical atrophy on CT or MRI .
3. Microscopic β-amyloid neuritic plaques, intraneuronal neurofibrillary tangles (containing tau protein), and microscopic amyloid angiopathy during postmortem examination. The plaques should stain favourably for β-amyloid and prion antibodies, which are symptomatic of prion disorders. Plaques and tangles have to be more prevalent than in age-matched controls without dementia. For the purpose of quantitatively assessing these changes, there are set guidelines. Alpha-synuclein may also be aggregated by amygdala neurones to form Lewy bodies.
4. The CSF, or cerebrospinal fluid. decreased Aβ and elevated tau and amyloid 42.[8]

Treatment of Alzhiemer disease

1. Alzheimer's disease cannot be cured, although drugs can help control its symptoms.
2. Non-pharmacological methods like cognitive therapy and lifestyle adjustments might also be advantageous.
3. Dementia is the clinical manifestation of Alzheimer's disease (AD), which usually starts as a mild memory loss that is not well recognized at first and gradually gets worse until it is incapacitating.
4. Research is being done to find potential cures and novel treatments for the illness. Current therapies, such acetylcholinesterase inhibitors (Rivastigmine, Galantamine, Donepezil), and N-methyl d-aspartate receptor antagonists (Memantine), target the late stages of the disease and have little effect on it.[9]

Alzheimer's disease medication now being offered by pharmacy

Table 1.1:- USFDA Approved Drugs Used in the treatment of Alzheimer's Disease.[1,9]

Sr.No	Category	Drugs	Brand Name
1.	Cholinergic Activators	Tacrine Donepezil RivastimineGalantamineHuperzine A	Cognex Aricept Exelon RazadyneHup A
2.	Glutamate (NMDA) Antagonists.	Memantine	Namenda
3.	Miscellaneous cerebro-active drugs	PiracetamPyritinolDihydroergotoxin	AlcetamRenervolHydergine
4.	Combination Therapy	Memantine + Donepezil	Namzaric

Role of herbal treatment in Alzhiemer disease

Over the years, AD has continued to be the most common type of dementia, raising concerns worldwide, particularly among the elderly. A significant obstacle is the rise in the population with unworthy medical conditions. Even though Alzheimer's patients receive a lot of medical attention, attempts to treat the neurological condition appear to be ineffective. Nevertheless, many medications can have adverse effects that exacerbate the patient's medical issues. Alzheimer's disease is treated with synthetic medications that regulate neurotransmitter enzymes, such as cholinesterase inhibitors or NMDA receptors, albeit this approach has not yet produced a perfect therapeutic answer. Currently leading the field as an alternative treatment for Alzheimer's disease is herbal therapy. Some herbs' naturally occurring phytochemicals have the power to enhance brain function. These antioxidant-rich plants (beta-carotene, vitamin C, vitamin E, and flavonoids) can counteract oxidative stress, which has been scientifically related to being one of the factors that accelerate the development of neurodegenerative symptoms in neuropsychiatric patients.

Plant profile

Liquorice

Plant Name	Glycyrrhizaglabra L
Common Name	Liquorice, Sweetwood
Synonyms	Mulaithi, Liquorice
Diagram	
Kingdom	Plantae
Division	Angiospermae.
Class	Dicotyledoneae.
Order	Rosales
Family	Fabaceae
Genus	Glycyrrhiza
Species	Glycyrrhiza
Chemical Constituents	The chief constituent of liquorice is a triterpenoidsaponin known as glycyrrhizin (glycyrrhizic acid), which is a potassium and calcium salt of glycyrrhizinic acid. Glycyrrhizinic acid is a glycoside and on hydrolysis yields glycyrrhetinic acid (glycyrrhetic acid), which has a triterpenoid structure. Flavonoid Glycosides, Saponin Glycosides.
Uses	Traditionally, liquorice has been used as an expectorant and demulcent. Shows antigastric effects, Used in the treatment of Neurodegenerative

	disease. This form has a reduced mineralocorticoid activity and feriore used in treating peptic ulcers, for healing purposes. Antispasmodic. Glycyrrhizin is an established anti-inflammatory drug.[24]
Part used	Roots

Ginger

Plant Name	Zingiberofficinale
Common Name	Ginger
Synonyms	Ginger root, Black Ginger, Zingiberic rhizome, Zingiber, Zingiberis.
Diagram	
Kingdom	Plantae
Division	Magnoliophyta
Class	Liliopsida-Monocotyledons
Order	Zingiberales
Family	Zingiberaceae
Genus	Zingiber
Species	Zingiberofficinale Roscoe
Chemical Constituents	Gingerols, Zingerone, Shogaols
Uses	Pain relief from Rheumatoid arthritis (RA)[24,15]
Part used	Rhizomes of root

Hibiscus Sabadariffa

Plant Name	Hibiscus Sabadariffa Linn.
Common Name	Roselle
Synonyms	Hibiscus Cannabinus L.
Diagram	
Kingdom	Plantae
Class	Eudicots
Order	Malvale
Family	Malvaceae
Genus	Hibiscus
Species	Hibiscus Sabdariffa
Chemical	Aliphatic organic acids, phenolic acids, flavonols, anthocyanins, fatty

Constituents	acids. Delphinidin, cyanidin, gossypetin, hibiscetin, kaempferol, myricetin, and quercetin with their respective glycosides.[11]
Uses	It is a tonic, soothing, restorative, and antidepressant, It might have minor laxative effects, diuretic effects, antibacterial effects, anti-spasmodic effects, and gives antioxidant activity.[20,24]
Part used	Flower

MATERIALS AND METHODS

We outline the methodology used in the investigation of herbal plants in this section. Because of their possible therapeutic benefits and medicinal characteristics, herbal plants have been the focus of much research. Comprehending the techniques employed in the study of these plants is essential to appreciating the reliability and repeatability of the results.

Collection and authentication of plant material

Liquorice

The crude powdered drug of liquorice was purchased from DagaduTeliChandwadkar in Nashik, Maharashtra & Authentic from BSI, Koregaon Park, Pune.

Ginger

The Rhizome of Zingiberofficinale was procured from the local market of Nashik (Maharashtra) & Authentic from BSI, Koregaon Park, Pune. Then cleaned and dried for a few days and then convert it into powder & used for further evaluation.

Hibiscus Sabadariffa

The flower of Hibiscus Sabadariffa was collected from local nurseries of Nashik, Maharashtra & Authenticated from BSI, Koregaon Park, Pune. Then the flowers are cleaned and dried for a few days in shade. Then converted into powdered form.

Chemical & reagents

The required quantity of chemicals and reagents are taken from the laboratories of Matoshri College of Pharmacy, Eklahare, Nashik.

Physicochemical evaluation of plant materials

After the Authentication of crude drug, it is tested for physicochemical evaluation, which includes the color, odor, taste, total ash value, acid insoluble ash value, water soluble ash value, loss on drying, alcohol soluble extractive value, water soluble extractive value, foaming index.

LiquoriceGinger Hibiscus Sabadariffa

Fig. 2.1:- Crude Drug Powder of Liquorice, Ginger and Hibiscus Sabadariffa.

Method of extraction of plants materials

Liquorice

1. **Prepare the licorice powder**: A mortar and pestle were used to make the licorice powder.

2. **Bring water to a boil** by heating it to a temperature of around 80-90°C. Enough water is required to submerge the licorice powder completely.
3. **Mix hot water and licorice powder**: Put the licorice powder in a container that can withstand heat. After the licorice powder is completely submerged, pour the hot water over it. To guarantee complete mixing, stir the mixture.
4. **Maceration**: Leave the mixture for a set amount of time, usually a few hours or overnight, at the appropriate temperature. Cover the container to stop heat loss. Occasionally stirring the mixture while it is macerating can improve the extraction process.
5. **Strain the extract**: To separate the liquid extract from the solid licorice powder, strain the mixture following the maceration period. To do this, use filter paper or a strainer with a tiny mesh.

Fig. 2.2:- Extract of Hibiscus Sabadariffa, Ginger and Liquorice.

Ginger

Measure out how much powdered ginger that you want. Add the ginger powder to a mixture of heated (around 80-90°C) water. To help in the extraction of gingerol, let the mixture soak for a predetermined period of time, usually between 30 and 60 minutes. To separate the liquid extract from the solid ginger powder, strain the mixture. To further boost gingerol production, you can choose to repeat the extraction procedure using the filtered residue. To create a more powerful extract, concentrate the liquid extract if needed by freeze-drying or evaporating it.[13]

Hibiscus sabadariffa

For 24 h, the 100 g of air-dried flowers were macerated in 1 liter of boiling water. After 24 h, the macerate was concentrated and freeze-dried after being filtered using filter paper.[19, 22]

Preliminary phytochemical & chemical evaluation of polyherbal extracts

Besides the authentication and physicochemical evaluation, the extract so collected is evaluated for carbohydrates, reducing sugars, phenolic compounds, flavonoids, saponin glycosides, volatile oils, alkaloids & thin layer chromatography was also done.

Fig.2.3:- Phytochemical & Chemical Evaluation of Polyherbal Extracts.

***In-vitro*Acetylcholinesterase Inhibition Activity**

The enzymes that make up the families of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) include serine hydrolases. The different amino acid residues in the active sites of these enzymes account for their varied substrate and inhibitor specificities. They are in charge of destroying acetylcholine at cholinergic synapses. The neurotransmitter acetylcholine is hydrolysed by enzymes, resulting in the termination of the nerve impulse.

Chemicals

1. Acetylcholinesterase (AChE), 200–1000 units/mg, from Electrophorus electricus (electric eels), type VI-S.
2. Acetylthiocholine iodide (ATCI) substrate.
3. Both sodium phosphate dibasic and monobasic are present.
5. -dithiol-bis-[2-nitrobenzoic acid] (DTNB) is the colouring agent.

Procedure:-

Spectrophotometric analysis was used to determine the acetylcholinesterase inhibitory activity in vitro using Ellman's technique. A 96-well plate was used for the experiment, and 200 µL of assay mixture was used overall. 10 µL of U/mL AChE (AChE from Electrophorus electricus (electrical eels), Type VI-S, and 200–1000 unit/mg protein) were added to the specified wells in a 96-well plate after an aliquot of 1 µL of extract (40 mg/mL DMSO) was combined with 179 µL of 0.05 mm phosphate buffer.

The absorption was measured at 415 nm using a Benesphera E21 Avanter Multi Plus Reader. Each run was carried out in triplicate on three different days to determine the percentage of inhibition at 200 µg/mL Afterward, the IC50 value for each sample showing AChE inhibitory activity of 50% or more was determined.[26,27]

The percent inhibition was calculated as under:

% inhibition = Control- test/ control x 100 [26, 27]

Fig.2.4:- The activity of the compound against acetylcholinesterase enzymes.

Statistical analysis

All the data were expressed as ± SEM. Statistical comparison was performed on graph pad prism, version 10.2.2 by using two-way ANOVA method.

RESULTS AND DISCUSSION

Pharmacognostical study

Physiochemical evaluation of polyherbal extract

The physiochemical evaluation of extract showed

Preliminary phytochemical evaluation of polyherbal extract

The phytochemical analysis of extract has confirmed the presence of Flavonoids, Phenolic Compounds, and Anthocyanins in the extract, which mainly shows the Anti-Alzheimer's activity. The Thin Layer Chromatography (TLC) has identified the flavonoids, Phenolic Compounds, and Anthocyanins. The Rf value of Glycyrrhizin (0.32), Glabridin (0.60), Quercetin (0.75) Gingerol (0.41), Anthocyanins (0.28) which matches with that of the standard and confirms the presence of that chemical constituents in the polyherbal extract.

Pharmacological study
***In-vitro*acetylcholinesterase inhibition activity**
Table no. 3.1:- The activity of the compound against acetylcholinesterase enzymes.

Sr. no	Sample	Concentration	OD	Mean OD	% inhibition
1	Control	-	1.197	2.207	-
			2.654		
			2.770		
2	Galantamine	1000 µg/ml	0.489	0.494	77.61
			0.491		
			0.503		
3	Polyherbal Extract	100 µl	1.466	1.416	44.02
			1.402		
			1.382		

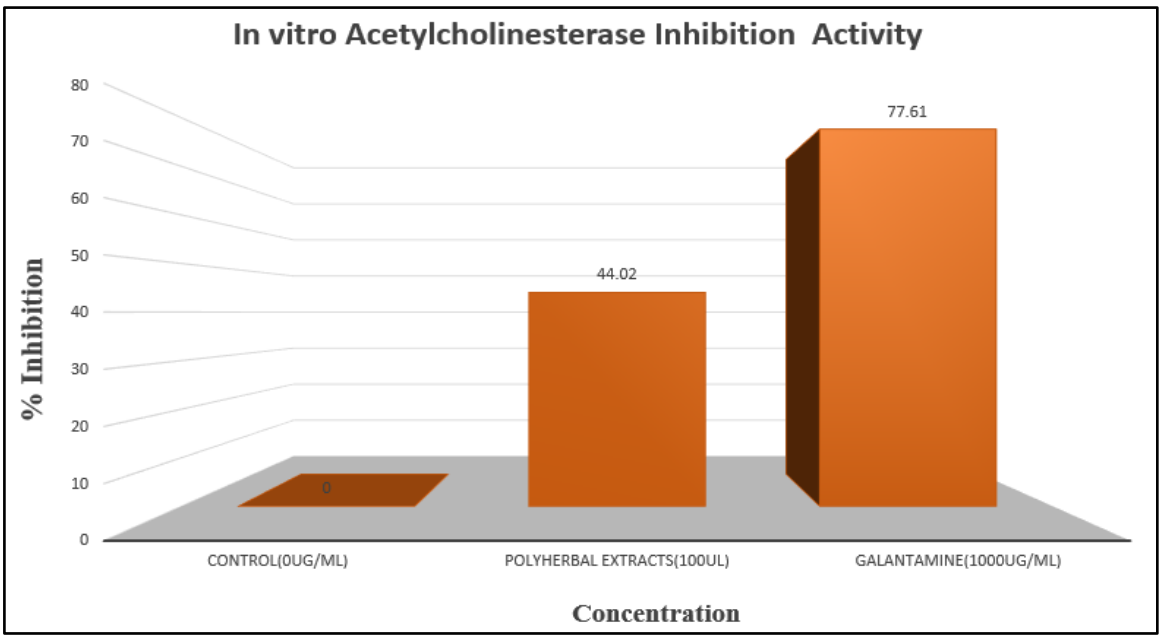

Fig.3.1:- The activity of compound against acetylcholinesterase enzymes (Concentration vs. % Inhibition).

The control sample does not showed inhibition of enzyme acetylcholinesterase. At the different Concentrations of 100 µl, Polyherbal extracts showed Moderate percent inhibition of the enzyme acetylcholinesterase by 44.02% as compared to Galantamine by 77.61%.

CONCLUSION

Based on the literature survey of Liquorice, Ginger, &Hibiscus Sabadariffa, the plant was selected and screened for the In-vitro anticholinesterase enzyme assay. The selected plant extract was analyzed for physical characteristics and phytochemical study.

At the different Concentrations of 100 µl, Polyherbal extracts showed Moderate percent inhibition of the enzyme acetylcholinesterase as compared to Galantamine.

So, it is concluded that this research provides convincing evidence that aqueous extract of Liquorice, Ginger, &Hibiscus Sabadariffa, have anti-Alzheimer's activity and it can act as a potential anti-Alzheimer's agent against Alzheimer's Disease.

FUTURE SCOPE

Polyherbal extracts combining Liquorice, Ginger, and Hibiscus Sabadariffa offer promising potential in the realm of Alzheimer's disease treatment.

1. **Synergistic Effects**: Combining these Herbal extracts may result in synergistic effects, enhancing their individual neuroprotective properties and providing a more comprehensive approach to Alzheimer's treatment.
2. **Clinical Trials**: Conducting rigorous clinical trials to evaluate the safety and efficacy of polyherbal formulations in humans is essential for establishing their therapeutic potential.
3. **Formulation Development**: Optimizing formulations for bioavailability and stability to ensure consistent therapeutic effects and patient compliance.
4. **Personalized Medicine**: Tailoring treatment approaches based on individual patient profiles and genetic factors to maximize therapeutic outcomes.
5. **Combination Therapies**: Exploring combination therapies with existing Alzheimer's medications to potentially enhance efficacy and mitigate side effects.

Conflict of Interest

The author has no competing interests in the study.

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