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Evaluation of Herbal Formulation for Therapeutic Management of Epilepsy

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Abstract: *Background: Epilepsy is a prevalent neurological disorder characterized by recurrent, spontaneous seizures resulting from synchronous, abnormal electrical discharges in cerebral cortical neurons. Despite the availability of numerous synthetic antiepileptic drugs (AEDs), over 30% of patients remain resistant to treatment, and standard therapies are frequently hampered by severe side effects. Traditional medicine utilizing medicinal plants offers a cost-effective alternative with lower toxicity and improved patient compliance.*

Objective: This study was designed to perform the pharmacognostical standardization, phytochemical screening, and antiepileptic evaluation of Acacia auriculiformis A. Cunn. ex. Benth. stem bark and Cyperus scariosus R. Br. roots. Furthermore, the study aimed to formulate these bioactive extracts into nanoparticulate drug delivery systems to bypass the blood-brain barrier and enhance their therapeutic efficacy.

Methods: Plant materials were authenticated and subjected to morphological, microscopical, and histochemical standardization. Following successive solvent extractions, preliminary phytochemical analysis and gas chromatography-mass spectrometry (GC-MS) were conducted. Antiepileptic activity was evaluated in vivo using pentylenetetrazole (PTZ)- and isoniazid (INH)-induced seizure models in mice. Brain neurotransmitter levels (GABA) and oxidative stress markers—including reduced glutathione (GSH), malondialdehyde (MDA), catalase, and nitrites—were measured biochemically. The most potent extracts (ethyl acetate extract of A. auriculiformis and essential oil of C. scariosus) were fabricated into solid lipid nanoparticles (AEASLN) and chitosan nanoparticles (COCNP), respectively. Formulations were optimized, evaluated for in vitro drug release, and analyzed via Transmission Electron Microscopy (TEM). Brain permeability was validated through behavioral, biochemical, and histopathological assessments.

Results: Preliminary phytochemical and GC-MS profiles revealed an abundance of bioactive constituents, including therapeutic flavonoids, phenols, and terpenoids (such as stigmast-4-en-3-one and limonene). Both plants significantly prolonged seizure latency and decreased seizure duration and mortality by augmenting GABA levels in the brain. They successfully diminished oxidative stress and neuroinflammation, as evidenced by restored GSH and catalase activities, coupled with diminished MDA, nitrite, and TNF-\alpha levels. The optimized nanoformulations (AEASLN and COCNP) displayed sustained-release profiles and demonstrated a highly pronounced antiepileptic and neuroprotective effect compared to their crude counterparts, significantly minimizing hyperchromatic nuclei and perinuclear vacuolization in the cerebral cortex.

Conclusion: The findings suggest that A. auriculiformis stem bark and C. scariosus roots possess robust antiepileptic properties driven by GABAergic facilitation, antioxidant mechanisms, and anti-inflammatory pathways. Engineering these extracts into nanoparticulate systems successfully increases their brain bioavailability and targeted therapeutic efficiency, establishing them as prospective, novel herbal formulations for the clinical management of epilepsy..

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Keywords: Epilepsy, Acacia auriculiformis, Cyperus scariosus, Herbal Nanoformulation, Solid Lipid Nanoparticles (SLN), Chitosan Nanoparticles, Gamma-Aminobutyric Acid (GABA), Oxidative Stress, Pentylenetetrazole (PTZ), Neuroprotection

I. INTRODUCTION

Epilepsy is a neurological disorder caused by repeated occurrence of seizures resulting from recurrent, spontaneous and abnormal electrical discharge from a group of neurons in the brain (Bhardwaj and Kumar, 2016). The term "epilepsy" is derived from Greek word "epilambanein", which means "to seize upon" or "to attack" (Acharya et al., 2008). In Ayurveda, epilepsy is defined as Apasmara: apa, meaning negation or loss of; smara, meaning recollection or consciousness (Manyam, 1992). The estimated population with epilepsy is between 4-10 per 1000 people. However, in low and middle income countries this proportion is much higher, between 7 and 14 per 1000 people. Each year, approximately 2.4 million people are globally diagnosed with epilepsy (WHO fact sheet, 2017). With a conservative estimate of 1% as prevalence of epilepsy, there are more than 12 million persons with epilepsy in India, which contributes to nearly one-sixth of the global burden (Amudhan et al., 2015).

Inhibitory and excitatory neurotransmitters pathways are involved in the underlying neurochemical mechanisms of epilepsy. Previous studies have revealed that decrease in gamma-aminobutyric acid (GABA) receptors and increase in postsynaptic glutamate receptors promote epileptogenesis (Jacobs et al., 1999). Decrease or desensitization of GABA receptors causes decreased release of GABA from terminals resulting inhibition of chloride conductance and thus, alterations occur in the ionic gradient of neuronal membrane. These alterations cause decrease in inhibitory postsynaptic potentials (IPSPs) of cortical circuits leading to epileptogenesis. Glutamatergic synapses also play a major role in the epileptic phenomena. Activation of glutamate postsynaptic receptors (both ionotropic and metabotropic) can cause epileptogenesis (Engelborghs et al., 2000).

Historically, around 2000 BCE, epilepsy was often considered as a witchcraft effect or divine punishment, therefore, treatment for epilepsy included punishment, incantations, and amulets. Epilepsy was considered to be a brain disorder by Indian and Greek doctors around 600 BCE. Then, special diets, living arrangements, mineral, animal and plant products were included in the treatment. The arrival of 20th century led to a thorough understanding of processes underlying disease resulting in the development of effective drugs (Magiorkinis et al., 2011). Since the second decade of 20th century, synthetic drugs, X-ray irradiation, neuro-imaging and surgical methods have been developed (Magiorkinis et al., 2011; Gross, 1992). Many antiepileptic drugs (AEDs) have been introduced in the past 20 years; nevertheless, despite availing many of these drugs either singly or in combination, more than 30% of patients of epilepsy remain unaffected by the treatment and continue to have seizures (Brodie et al., 2016). Antiepileptic drugs also show some interactions and side effects due to inhibition and/or induction of enzymes. The adverse effects of the antiepileptic drugs may affect the quality of patient's life to a greater extent (Kr et al., 2014). In such cases when treatment with antiepileptic drugs are not successful, vagus nerve stimulation, epilepsy surgery, special diet, complementary and alternative medicine based therapy may be recommended (Goldenberg, 2010). Contemplating all these facts, the researchers are motivated to develop novel approaches for treatment of epilepsy, for example, development of herbal medicines by discovering new antiepileptic agents from plants (Zhu et al., 2013). Many plants have been used as traditional medicines to treat seizures and epilepsy. Plants consist of many organic chemical compounds with pharmacological effects on body. Due to presence of these compounds, plants can interact with receptors of central nervous system (CNS) like γ-aminobutyric acid receptor type A (GABAA) and other neurotrophic factors to inhibit the physiologic excitability that occurs in neuronal membrane after seizures (Singh and Verma, 2012). There is also increasing evidence that only symptoms are suppressed by many current drug therapies and the underlying cause of disease is not treated completely. In contrast, natural products appear to treat the cause of diseases. Hence, research is a never ending process in this field (Devi et al., 2010). As there has been continuous effort for development of drugs to inhibit the progression of seizures and plants have antiepileptic potential with cost effectiveness, least side

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effects and better patient compliance, there is a great perspective towards identification of plants and their active constituents (Singh and Verma, 2012).

In an attempt to increase the permeability and persistence of drugs in the brain, several new approaches are also being developed. Development of nanoparticulate drug delivery systems for drugs is one of such main strategies (Nagpal et al., 2013). Nowadays, phytotherapeutic agents are also formulated as nanoparticles to improve their pharmacodynamic and pharmacokinetic profile. Nanoparticles measure approximately 1 - 1000 nm in dimension and exhibit different properties as compared to their macroscale counterparts (Rajendran et al, 2011). A high surface area to volume ratio is achieved due to nanonization of phytoceuticals which leads to enhanced solubility, permeability, bioavailability and physicochemical stability (Jadhav et al., 2014).

With this background, the present study was designed to evaluate the antiepileptic potential of certain medicinal plants and fabrication of their extracts into suitable herbal formulations.

II. REVIEW OF LITERATURE

2.1 Epilepsy

Epilepsy, a serious psychiatric disorder, is defined as repeated occurrence of excessive, synchronous and sudden discharges in cerebral cortical neurons leads to disturbance of movements, impairment of cognitive function and disruption of consciousness (Engelborghs et al., 2000; Nemani et al., 2005). It is also defined as recurrent occurrence of seizures. A seizure is a sudden rush of electrical activity in the brain. There are two types of seizure namely generalised seizure (affect whole brain) and partial or focal (affect one part of the brain) Epileptic seizures of unidentified cause are called as primary or idiopathic epilepsy while epileptic attacks of known causes are known as secondary or symptomatic epilepsy. No single marker or etiopathogenic mechanism can be solely attributed for this disorder; therefore, multiple biomarkers have been suggested for the development of epilepsy. Stress, nutritional deficiency, trauma, brain ischemia, brain tumors, cerebrovascular disease, infection meningitis, metabolic abnormalities, low blood sugar and alcohol withdrawal can cause or worsen epileptic seizure. Neurotransmitters imbalance (GABA and glutamate), oxidative stress and neuroinflammation mainly involved pathophysiolocally in the development of epilepsy (Meurs et al., 2008; Walker et al., 2012; Puttachary et al., 2015)

2.1.1. Types of epilepsy (Gastaut et al., 1975; Loscher, 1985)

1) Generalized epilepsy: It affects whole part of the brain. It can be further classified into a. Tonic clonic seizures (grand-mal type): It starts from both side of brain but it can begin from one side and spread to entire brain.

b. Absence or minor seizures (petitmal): It involve sudden lapse of consciousness. It occurs mainly in children. Seizures usually disappear instinctively after adolescence.

c. Myoclonic seizures: It occurs due to brain damage. This type of seizure increased muscle tone.

d. Infantile spasms: It is rare type of seizures occurs child under one year of age.

It affects memory.

2) Partial or focal epilepsy: It affects localised regions of brain. This type of seizure mainly affects motor, sensory and behavioural phenomena.

a. Complex partial seizures (lobe or psychomotor seizures): It affects anterior temporal lobe of brain and is provoked hallucinations, behavioural and emotional abnormalities.

b. Motor epilepsy: It occurs due to cortical abnormalities. Consciousness is not lost.

c. Sensory epilepsy: Similar to motor epilepsy except that it arises from the sensory cortex area.

d. Akinetic seizures: Superficially no convulsions are observed. Patient suddenly falls down on the ground without the loss of consciousness.

3) Status epilepsy (acute repetitive seizures): It is the condition in which one attack follows another without patient regaining consciousness.

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2.1.2. Pathophysiological hypothesis

a) Glutamatergic hypothesis:

Glutamate is an excitatory neurotransmitter, which play important role to transfer signal one nerve cell to another nerve cell. It is also regulates in motor activity, synaptic plasticity and cognition (Kerner, 2009). It acts by binding N-methyl-D-aspartate, αamino-3hydroxy-5-methyl-4-isoxazolepropionic acid (NMDA) receptors and increases intracellular Ca2+ concentration resulting in the flood of glutamate into the synaptic cleft. Increased activate NMDA and kainate receptors with subsequent influx of Na+ and Ca2+ ions through the ion channels by these receptors leads to neuronal hyperexcitability. Uncontrolled increase in neuronal hyperexcitability is also one of major cause of epileptic seizures (Barker-Haliski 2015).

b) GABAergic hypothesis:

Gamma amino butyric acid (GABA) is an inhibitory neurotransmitter in the cerebral cortex and regulates the inhibitory control on neuronal excitation or excitatory neurotransmission such as glutamate. When GABA and glutamate balance is disturbed then it can cause seizures. GABA is formed within GABAergic axon terminals and released into the synapse, where it acts at on two receptors: GABAA, which controls chloride entry into the cell and GABAB, which increases potassium conductance, decreases calcium entry and inhibits the presynaptic release of other neurotransmitters. Therefore decrease in GABA release or function loss the control on excitatory neurotransmission and calcium influx increases the risk of seizure (Fisher, 1993; Treiman, 2001).

c) Oxidative stress hypothesis:

Generation of free radicals can reduce antioxidant defence systems (Kumar et al., 2017). These free radicals react with mitochondria, proteins, lipids and DNA, which cause neuroinflammation, abnormal neurogenesis and neuronal degradation. Chronic inflammation and mitochondrial dysfunction can also worsen the oxidative damage by producing reactive oxidative species and involved in the pathogenesis of epilepsy (Shin et al., 2011).

d) Neuroinflammation hypothesis:

Brain trauma, ischemia, infections and immunological reactions activate microglial cells by overstimulation of glutamate receptors, which increases intracellular Ca2+ influx. Increased Ca2+ stimulates the release of arachidonic acid, phospholipase, kinases, nucleases, which further increase the generation of reactive oxidative species (ROS). ROS activates microglial cells, which stimulate the release of cytokines like interleukins and TNF-α by activating Matrix metalloproteinases (MMPs) (Kumar et al., 2017). Activated TNF-α, produce overexpression of AMPA receptor which can causes excitotoxicity. MMPs bind to integrin and interfere with its signalling leads to hyperexcitation of NMDA receptor and induce excitotoxicity. Moreover, MMP-9 also causes breakdown of extracellular matrix molecules at the blood brain barrier and involved in the development of epilepsy (Vezzani, 2005).

e) Mitochondrial dysfunction hypothesis:

Mitochondrion is the powerhouse of energy in neuronal functions (Maurer et al., 2001). It serves by producing ATP for neuronal function and also regulates the cell signalling, calcium buffering and neurogenesis (Manatt and Chandra, 2011). Furthermore, mitochondrial dysfunction causes necrosis and apoptosis by activating caspases. It also produces loss of energy, dysregulation of calcium homeostasis, impaired synaptic plasticity, neuroinflammation, production of free radicals and altered gene expression, which leads to neurodestructive changes and apoptosis that increases the risk of epilepsy (Scaglia et al., 2010).

2.1.3. Antiepileptic drugs

These are used to treat the epileptic seizure via reducing excitability of neurons by blocking of sodium channels, potentiating GABA-mediated inhibition (increasing the action of GABA or inhibiting GABA transaminase, or direct GABA agonizing) and inhibiting T-type calcium channels. Number of drugs are available, but these agents also associated with some serious side effects such as gum teratogenesis, hyperplasia, anaemia, skin rashes, gum hyperplasia, skin rashes, ataxia, mental disturbance, baldness, curling of hair. Antiepileptic drugs are listed in Table 2.1

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(Brunton et al., 2007; Goldenberg, 2010). Plants are important sources of medicine and a large numbers of drugs (Fink and Vanecek, 1975; Herranz et al., 1988). Many traditional plants have been proven for their antiepileptic potential. Some of them are listed in Table 2.2. The plant drugs have received considerable preference because of their safe, potential and therapeutic effects.

Table 2.1: Drugs used for the treatment of epilepsy

Types of seizure	Antiepileptic drugs
Partial seizures	
Simple partial	Carbamazepine, phenytoin, valproate, Gabapentin, lamotrigine, levetiracetam, tiagabine, topiramate, zonisamide
Complex partial	Carbamazepine, phenytoin, valproate, Gabapentin, lamotrigine, levetiracetam, tiagabine, topiramate, zonisamide
Partial with secondarily generalized tonic-clonic seizure	Carbamazepine, phenobarbital, phenytoin, primidone, valproate, Gabapentin, lamotrigine, levetiracetam, tiagabine, topiramate, zonisamide
Generalized seizures	
Absence seizure	Ethosuximide, valproate, Lamotrigine
Myoclonic seizure	Valproate, Lamotrigine, topiramate
Tonic-clonic seizure	Carbamazepine, phenobarbital, phenytoin, primidone, valproate, Lamotrigine, topiramate

Table 2.2: List of some traditional plants evaluated for their antiepileptic potential

Plant	Part Used	Model	Reference
Bacopa monnieri linn Schrophulariaceae	Whole plant	Maximal Electroshock (MES), Pentylenetetrazol (PTZ) and Strychnine nduced convulsions	Kaushik *et al*., 2009
Withania somnifera linn Solanaceae	Roots	PTZ Induced Convulsions in mice	Kulkarni *et al*., 2008
Ocimum sanctum linn Lamiaceae	Stem and leaves	MES Induced Convulsions	Jaggi *et al*., 2003
Curcuma longa linn. Zingiberaceae	Rhizomes	Increasing electroshockcurrent method	Bharat *et al*.,2008
Acorus calamus linn. Araceae	Rhizomes	Metrazol Convulsions Induced	Mukherjee *et al.,* 2007
Aegle marmelos linn. Rutaceae	Leaves	PTZ and MES induced convulsions	Sankari *et al*., 2010
Crocus sativus linn. Iridaceae	Stigmas	PTZ induced convulsions	Hosseinzadeh *et al*., 2007
Cuminum cyminum linn Umbelliferae	Fruits (Essential oil)	PTZ and MES induced convulsions	Sayyah *et al*.,2002
Glycyrrhiza glabra linn. Leguminosae	Leaves, roots and rhizomes	PTZ and MES induced convulsions	Yazdi *et al*.,2011
Pongamia pinnata linn. Fabaceae	Leaves	Electric Shock induced convulsions	Manigauha *et al.,* 2009
Berberis vulgeris linn.	Whole plant	MES and Kainic acid (KA)	Bhutada *et al.*

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Berberidaceae.		induced convulsion	2010
Boerhavia diffusa linn. *Nyctaginaceae*	Roots	PTZ induced seizures	Kaur, 2009
Butea monosperma lam. *Fabaceae.*	Flowers	MES induced convulsions	Kasture et al., 2002
Valeriana officinalis linn. *Valerianaceae*	Roots	Electrical kindling induced convulsions	Rezvani, 2010
Nardostachys jatamansi DC *Valerianaceae*	Roots	MES induced convulsions	Rao et al., 2005
Carrisa carandas linn. *Apocynaceae*	Roots	MES, PTZ, Picrotoxin, Bicuculline and N-methyl-dlaspartic acid Induced seizures	Karunakar et al., 2009

2.2. PLANTS SELECTED FOR PRESENT STUDY

2.2.1 Acacia auriculiformis A. Cunn. ex. Benth. (Mimosaceae)

2.2.1.1. Vernacular names

English: Tan wattle, Papuan wattle, Ear pod wattle, Ear leaf acacia, Australian wattle, Darwin black wattle, Northern black wattle

Hindi: Sonajhuri Tamil: Kaththi savukku

Bengali: Bengali jali, Akash-mono (Orwa et al., 2009).

2.2.1.2. Scientific classification (USDA, 2012)

Kingdom - Plantae
Subkingdom - Trachiobionta
Superdivision - Spermatophyta
Division - Magnoliophyta
Class - Magnoliopsida
Subclass - Rosidae
Order - Fables
Family - Mimosaceae/Leguminosae
Genus - Acacia
Species - Auriculiformis

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2.2.1.3. Geographical distribution

Acacia auriculiformis grows between latitudes 9-16° S and longitudes 130-145°E (Hai, 2009). The altitudinal range of species in its native range is from sea level to about 400 m, with the main occurrence below 100 m (Gunn and Midgley, 1991). Natural distribution of Acacia auriculiformis extends through Australia, Papua New Guinea and Indonesia (Turnbull et al, 1983). In most locations, the species occurs on the banks of rivers and streams including areas immediately behind mangroves along saline estuaries. There are very few tree species which are as adaptable as Acacia auriculiformis. It has proved to be especially suitable for rehabilitating and revegetating different sites (Pinyopusarerk et al., 1990). Climatically, the occurrence of plant is in the hot humid and sub humid zones, where the mean maximum temperature of the warmest month is 32-38°C and the mean minimum temperature of the coldest month is 12-20°C (Hai, 2009; Pinyopusarerk et al., 1990). It grows in wide range of soils (Pinyopusarerk et al, 1990). This species is well known as a cultivated tree in Asia, Africa and South America (Gunn and Midgley, 1991).

In India, it has been introduced into semi-arid regions like Bihar, Orissa and West Bengal (The Wealth of India, 1992). It was first introduced to India on plantation scale in West Bengal in 1946. Then, it was introduced in Karnataka in 1964. Its introduction has been extended all over India from coastal sands to high attitude (Kushalpa, 1991). It has been a major species of afforestation for the past 25 years, particularly in the southern states, Andhra Pradesh, Kerala, Tamilnadu, Karnataka, Maharashtra, Goa and Pondicherry. When compared with the local indigenous species, A. auriculiformis is the most important and widely planted in Karnataka, because of its adaptability, utility and economic value (Bulgannawar and Math, 1991). In its natural habitat, A. auriculiformis grows into a tree 25–30 m tall with a straight stem dominant for a greater part of tree height (Doran and Turnbull, 1997).

2.2.1.4. Plant description

It is a straight, medium-sized tree, upto 16 m in height. On favorable sites it can grow to 30-40 m tall and 80-100 cm diameter with a straight, single stem. The bark is gray or brown, more or less smooth in young trees, becoming rough and longitudinal fissured with age. Leaves are 10-16 cm long and 1.5-2.5 cm wide, pinnate with 3-8 parallel nerves, thick, leathery and curved., rachis modified into a phyllodes. Flowers white to rich yellow, fragrant, in slender, axillary spikes, 3.75-6.25 cm long (The Wealth of India, 1985). Inflorescence spike number of whorls was four, namely, calyx, corolla, androecium, and gynoecium justifying its inclusion in polypetalae, and stamens were numerous. The pods are slightly woody, glaucous and hard, about 6.5 cm long and 1.5 cm wide. They are initially straight or curved but become

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very twisted and irregularly coil on maturity. The seeds are broadly ovate to elliptical, about 4-6 mm long and 3-4 mm wide. Each seed is encircled by a long red, yellow or orange funicle (Orwa et al.,2009).

2.2.1.5 Traditional uses

The tree is used as a folk medicine in Australia. A decoction of root is used to treat aches and sore eyes while an infusion of bark is used to treat rheumatism by aborigines of Australia (Girijashankar, 2011). Seeds are used to treat skin diseases like itching, allergy and rashes. The plant is useful as anti-malarial remedy by the Ibibios of Niger Delta region of Nigeria (Okokon et al., 2010).

2.2.1.6. Phytoconstituents

Acacia auriculiformis A. Cunn. ex. Benth. consists of following phytoconstituents. Structure of major constituents are given in Figure 2.1.

Carbohydrates: Plant is rich in glucuronic acid, methylglucuronic acid, galactose, Lrhamnose and arabinose (Anderson, 1978).

Tannins: The bark contains 12-16 % tannin, the content of tannin being more in younger trees. Anthocyanidins: Leucodelphinidins and leucocyanidins are present in the bark, which redden exceptionally in light (The Wealth of India, 1985).

Saponins: Saponins are unique in nature due to presence of tridesmoside saponins i.e. proacaciaside-I, proacaciaside-II and acaciamine. They were isolated from the fruits and their structure has been identified as acacic acid lactone-3-O-β-D-glucopyranosyl(1→6)-β-D-glucopyranoside (proacaciaside-I) , acacic acid lactone-3-O α -L- arabinopyranosyl (1→2)-β-o-glucopyranoside (proacaciaside-II) and acacic acid lactone-3-O-α-L-arabinopyranosyl (1→6)-2-acetamido-2-deoxy-β-D-glucopyranoside (acaciamine) (Garai and Mahato, 1997). Apart from these, two acylated biglycoside saponins called Acaciasides A and B have also been isolated from fruits and their structures have been characterized as 3-O-[β-D-glucopyranosyl (1→6) {α-Larabinopyranosyl (1→2)} -β-D-glucopyranosyl]-21-O-{(6′ S)-2′ -trans-2′ , 6′ dimethyl-6′ -O-β-D-glucopyranosyl-2′ ,7′ –octadienoyl} acacic acid 28-O-Lrhamnopyranosyl (l→6) [β-D-xvlopyranosyl (l→2)]-β-D-glucopyranoside and 3-O-[βD-glucopyranosyl (l 6j {α-L-arabinopyranosyl (1→2) }-β-D-glucopyranosyl]-21-O[(6′ S)-2′ -trans-2′ .6′ - dimethvl-6′ -O-{β-D-xylopyranosyl (l→2)-β-D-glucopyranosyl-2′ , 7′ -octadienoyl]-acacic acid 28-O-α-L-rhamnopyranosyl (l→6) [β-D-xylopyranosyl (l→2)]-β-D glucopyranoside respectively (Mahato et al. , 1992). The seeds of A. auriculiformis are a rich source of Acaciaside-A and Acaciaside-B (Parkashi et al., 1991). A new triterpenoid saponin 3-O-[α-L- rhamnopyranosyl- (1→4)]-α-L- arabinopyranosyl-(1→6)-β-D-galactopyranosyl-3β , 16α , 21β , 22α , 28pentahydroxy-olean-12-ene has been isolated from the methanol extract of the stems of the plant (Asati and Yadava, 2014).

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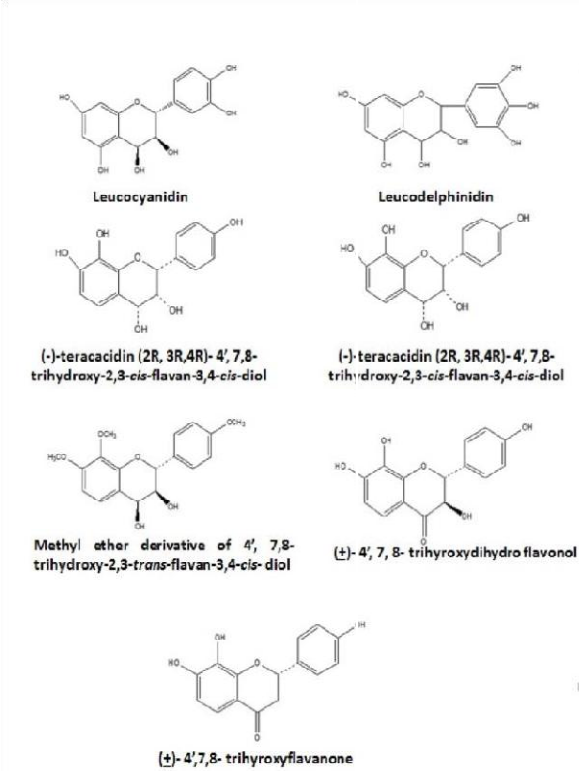

Figure 2.1: Chemical structures of some phytoconstituents present in Acacia auriculiformis A. Cunn. ex. Benth.

2.2.1.7. Pharmacological activities Antifilarial activity

The active principle, isolated from the funicles of A. auriculiformis consisted of two triterpenoid saponins, acaciaside A and acaciaside B which showed antifilarial activity against Setaria cervi. No toxic effect of the saponins was observed in rats. The rise in microfilaria count indicated that the drug induced a very high physiological stress on the adult worms which increased the rate of discharge of the microfilarial density before impending death. The treated rats on autopsy did not show any adult worms (Ghosh et al., 1993). An ethanol extract obtained from the funicles of Acacia auriculiformis was administered orally to 4 pariah dogs naturally infected with Dirofilaria immitis at 150mg/kg/day for 50 days. The treatment resulted in 98% and 99% reduction in microfilarial density on day 45 and 75, respectively following the onset of treatment. No toxic effect in the form of a change in movement, body weight and temperature was observed in the treated dogs. The prolonged maintenance of reduced level of microfilarial density may be ascribed to the partial elimination of adult worms (Chakraborty et al., 1995).

Antimalarial activity

The ethanol extract of leaves of Acacia auriculiformis was proved to have significant antiplasmodial activity which supports its use in treatment of malaria. The antimalarial activity of the ethanol leaf extract of Acacia auriculiformis (350-1050 mg/kg/day) was evaluated for suppressive activity during early infection in 4-day test and curative activity in established infection (Rane's test). Acacia auriculiformis exhibited significant ($p<0.05$) blood schizonticidal activity both in 4-day early infection test and in established infection with a considerable mean survival time though not comparable to that of the standard drug, chloroquine, 5mg/kg/day. The result of this study shows that ethanol extract of the leaves possesses significant antiplasmodial activity and also support the traditional use of this plant in the treatment of malaria (Okokon et al., 2010).

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Antimicrobial activity

Acaciasides A and B, two acylated biglycoside saponins, isolated from the funicles of Acacia auriculiformis, were proved to have antimicrobial activity. Their antibacterial and antifungal activity was investigated using three bacterial and two fungal strains.

Complete inhibition of conidial germination of Aspergillus ochraceous and Curvularia lunata was recorded at 300µg/ml or less whereas to inhibit the growth of Bacillus megaterium, Salmonella typhimurium and Pseudomonas aeruginosa 700 µg/ml or higher concentrations of the mixture was required (Mandal et al., 2005).

Antimutagenic and chemopreventive activity

The barks extracts of A.auriculiformis were found to be potent in suppressing mutagenesis. A correlation of the antimutagenic and chemopreventive activity was studied. Ames anti-mutagenicity assay and the mouse mammary gland organ culture (MMOC) model were used. The plants were extracted with organic solvents to obtain chloroform fractions and acetone extracts. The antimutagenic activity was determined in two different strains using both direct-acting [4-nitro-o-phenylenediamine (NPD) or sodium azide] and indirect-acting [2-aminofluorene (2AF)] mutagens. The anticarcinogenic activity was evaluated based on the development of preneoplastic lesions in response to the chemical carcinogen 7, 12-dimethylbenz[a]anthracene (DMBA). The results showed that the activity resulting from the 2AF mutagen was selectively greater than the activity from the direct-acting mutagens. Moreover, in general, acetone extracts were more potent in suppressing mutagenesis than the chloroform extracts. The antimutagenicity results obtained with extracts using the 2AF–TA100 system were comparable to the chemopreventive results with DMBAinduced mammary lesions. These results exhibited a good correlation between the antimutagenesis assay and the MMOC model, suggesting that these plants may contain active chemopreventive agents (Kaur et al., 2002).

Antioxidant activity

The antioxidant activity of bark and empty pods of A. auriculiformis were investigated using 2,2-diphenyl-1-picrylhydrazyl (DPPH), reducing power, chelating power, 2,2′ azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), superoxide radical scavenging, nitric oxide (NO) scavenging and β- carotene bleaching assays. Extracts of bark and empty pods were analyzed for presence of bioactives like phenolics, flavonoids and proanthocyanidines. The total phenolic content, total flavonoid content and proanthocyanidin content of bark and pod extracts were calculated from the standard calibration curves. Bioactives were found to be higher in bark than empty pods except flavonoids. Iron chelating activity was not observed in both the extracts.

Both extracts showed higher quenching capacity on DPPH and ABTS and lower quenching capacity on OH-. Equivalent quenching was observed on O2- and NO (Sathya and Siddhuraju 2012). The antioxidant potency of acetone extract and fractions of bark powder was also evaluated. Fractions were comparatively more effective than the crude acetone extract in all the assays. The potential was compared with known antioxidants (ascorbic acid and butylated hydroxytoluene) and correlated with the total phenolic content in crude extract and fractions. It was revealed that Acacia auriculiformis could be a valuable source of antioxidants of potential use in different fields such as food, cosmetics, and pharmaceuticals (Singh et al., 2007).

Central nervous system depressant activity

The aerial part of Acacia auricufiformis was evaluated for central nervous system (CNS) depressant activity through barbiturate potentiation test in mice. Ethanol extract was prepared and partitioned with hexane, chloroform, n-butanol and water. The butanol fraction was found to be active and was further resolved into ethyl acetatesoluble and ethyl aceate-insoluble fractions. The activity was confirmed in ethyl acetate-soluble portion. It was found to contain one major constituent, auriculoside, which gave 80% CNS depressant activity (Sahai et al., 1980).

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Cestocidal activity

Saponins obtained from funicles of Acacia auriculiformis have been proved to have cestocidal activity. The cestocidal activity of these saponins and ethanol extract obtained from funicles was evaluated using rats each harbouring a single adult worm of Hymenolepis diminuta. Adult worms were expelled within 5 days from rats treated with the ethanol extract and within 3 days from those treated with saponins. No appreciable side effects were observed in the treated rats (Ghosh et al., 1996).

Hepatoprotective activity

Bark and pod extracts from Acacia auriculiformis were evaluated for their protective effect against paracetamol intoxicated liver injury. Liver function biochemical markers such as alanine transaminase, aspartate aminotransferase, alkaline phosphatise, total bilirubin and total protein were evaluated in the serum obtained from the experimental animals. The hepatoprotection was comparable to the reference drug silymarin. The results signify the importance of bark and empty pod extracts of A. auriculiformis as good therapeutic candidates for liver injury (Sathya and Siddhuraju. 2013).

Antidiabetic activity

Analysis of antidiabetic factors

The acetone extracts of bark and pods of Acacia auriculiformis were evaluated for their inhibitory potential against α-amylase and α- glucosidase enzymes, which are important enzymes in the carbohydrate metabolism and as targets for the therapeutics of diabetes. Both the bark and pods extracts showed dual inhibitory potential against the enzymes. The bark extract showed higher inhibition of (64.55±5.12)% and (95.12±4.75)% on α- amylase and α- glucosidase at a concentration of 50 µg and 2.5 µg respectively as compared to pods which showed (50.57±5.12)% and (79.1±6.5)% inhibition at concentration of 50µg and 5µg against α- amylase and α- glucosidase respectively. It was concluded that the bark and pods can be used as remedy for type II diabetes (Sathya and Siddhuraju 2012).

In vivo antidiabetic activity

The antidiabetic activity of bark and empty pods was investigated using alloxan induced diabetes model in rats. Acetone extracts of both the parts of plant were tested for antidiabetic potential and glibenclamide was used as reference drug. Dose dependent effect was noticed for both bark and pods in lowering the blood glucose levels which was comparable with the reference drug. Similarly, substantial elevations of blood glucose, distorted lipid profile (total cholesterol, triglycerides, high density lipoprotein cholesterol and low density lipoprotein cholesterol) and kidney function signs (creatinine and urea) have been refurbished to the desirable levels as compared to the standard antidiabetic glibenclamide. On the basis of these results, it was concluded that the bark and empty pods of A. auriculiformis can be used as good therapeutic agents to treat diabetes (Sathya and Siddhuraju, 2013).

Memory enhancing activity

The leaves of A. auriculiformis were evaluated for its leaq Qrning and memory enhancing potential. Ethanol extract of the leaves was prepared and assessed using passive avoidance and rewarded alternation tests on inbred albino rats of wistar strain. The activity of brain acetylcholinesterase (AChE) enzyme was measured. The results showed that the extract treated group showed a dose-dependent improvement in memory as compared to control in both the models. Extract treated group also demonstrated inhibition of AChE which was dose-dependent and superior to that produced by rivastigmine. Hence the plant can be added as an adjuvant to existing therapies for the treatment of dementia (Sharma et al., 2014).

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Spermicidal activity

A triterpene glycoside, acaciaside-B enriched fraction isolated from the seeds of A. auriculiformis showed significant spermicidal activity with apparently no possible mutagenic or adverse effects. Significant spermicidal potential with no possible mutagenic effect and adverse impacts on lactobacilli growth attests to the credential of Ac-B-en as a prospective future spermicide (Parkashi et al., 1991).

Wound healing activity

The ointments prepared with ethanol and aqueous extracts of stem bark of Acacia auriculiformis were evaluated for wound healing studies by using excision and incision wound models in Swiss albino mice. Hydroxyproline content determination and histopathological studies of treated groups were carried out. The results showed that both formulations possess significant wound healing activity, which was evidenced by decreased period of epithelialization, increased rate of wound contraction, tensile strength, hydroxyproline content, granulation tissue and collagen fibre formation in all treated animals. The activity of ointment containing ethanol extract was significantly higher than the ointment containing aqueous extract. The wound healing activity of the extracts may be due to presence of various phytoconstituents like phenolic constituents, flavonoids, tannins (Singh and Sharma, 2014).

2.2.2. Cyperus scariosus R. Br. (Cyperaceae)

2.2.2.1. Vernacular names (Chopra et al., 1986) Sanskrit: Chakranksha, charukesara, nagar-mustaka Hindi: Nagarmotha Marathi: Lawla

Tamil: Koraikkilangu Telugu: Kolatungamaste Malyalam: Karakizhanna

Kannada: Konnarigadda, nagarmusthe English: Nutgrass, umbrella sedge, nutsedge Urdu: Sadkofi

2.2.2.2. Scientific classification

Kingdom:Plantae

Order: Poales

Family:Cyperaceae

Genus: Cyperus

Species:C. scariosus

2.2.2.3. Geographical distribution

Cyperus scariosus R. Br. is medium sized sedge found in South Africa, China, India and Pacific Islands. In India, it is widely distributed, especially in Uttar Pradesh, Chhattisgarh, West Bengal, Bihar, Orissa and southern part of India. The plant is also present in Katni, Chattarpur, Jhansi and Banda districts in Madhya Pradesh and adjoining areas of Uttar Pradesh. The plant is wildly present in and around waterfall, rivers, canals and other damp places. It requires sun and moist conditions. It can grow in sandy soil as well as in loamy moist fields and along coastal regions. The plant grows rapidly and the soil is filled with its tangle of rhizomes and roots. It thrives under the upland conditions where water resource is not a limiting factor (Srivastava et al., 2014).

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2.2.2.4. Plant description

Cyperus scariosus is basically perennial, delicate and slender sedge of approximately 45-75 cm. height. The leaves of plant are pointed and with 0.3-0.85 cm width. Flowers are 5-
17.5 cm in length. The rhizomes occur 3-4 cm deep in soil and vary in thickness and size. They are bluntly conical, initially fleshy and white with scaly leaves. With age, they become wiry, fibrous and dark brown. Stolons are 10-20 cm in length with a number of rhizomes crowded together. A number of wiry roots are present with rhizomes and stolons (The Wealth of India, 1950; Srivastava et al., 2014).

2.2.2.5. Traditional uses

The roots of Cyperus scariosus R. Br. are used in folkoric preparations as cordial, emmenagogue, tonic, vermifuge, diaphoretic. The plant is also used in treatment of diarrhoea, syphilis, fever, gonorrhoea, liver damage (Kirtikar and Basu, 1918).

2.2.2.6. Phytochemical Constituents

Cyperus scariosus R. Br. contains numerous valuable chemical constituents. Major chemical constituents of this plant are essential oils, terpenoids, flavonoids, monoterpenes, sesquiterpenes, hydrocarbons (Uppal, 1984), steroids, ketones, saponin, (Neville, 1968), alkaloids and polyphenols. Other main constituents include 1,8cineole, α-cyperone, α-rotunol, β-cyperone, 4α,5α-oxidoeudesm-11-en-3-α-ol, βpinene, β-rotunol, copaene, cyperene, cyperenone, β-selinene, camphene, cyperol, cyperolone, cyperotundone, copadiene, kobusone, isokobusone, limonene, mustakone, linoleic-acid, linolenicacid, γ-cymene, isocyperol, myristic-acid, oleicacid, oleanolicacid, oleanolic-acid-3-o-neohesperidoside, patchoulenone, p-cymol, stearic-acid, sugeonol, sugetriol, selinatriene, sitosterol, cyperotundone, patchoulenone, sugeonol, kobusone, and isokobusone. Stigmasta-5,24(28)-diene-3-β-o-α-l rhamnopyranosyl-o- βd-arabino-pyranoside (Bhatt,1982) and a new glycoside leptosidin-6-O- β -Dglucopyranosyl-O-α -2-rhamnopyranoside (Bhatt,1981) was isolated from the leaves and (–)-β- selinene and a new tricyclic hydrocarbon, isopatchoula- 3,5-diene (Gopichand,1978) isolated from Cyperus scariosus oil. Sesquiterpene (Nerali,1969) patchoulenol, cyperenone, cyperenol, isopatchoulenone, rotundene (Nerali,1970), 2, 3- diacetoxy-19-hydroxy-urs-12-ene-24-O-β-D –xylopyranoside rotundenol (Sahu, 2010) have isolated from rhizomes of this plant.

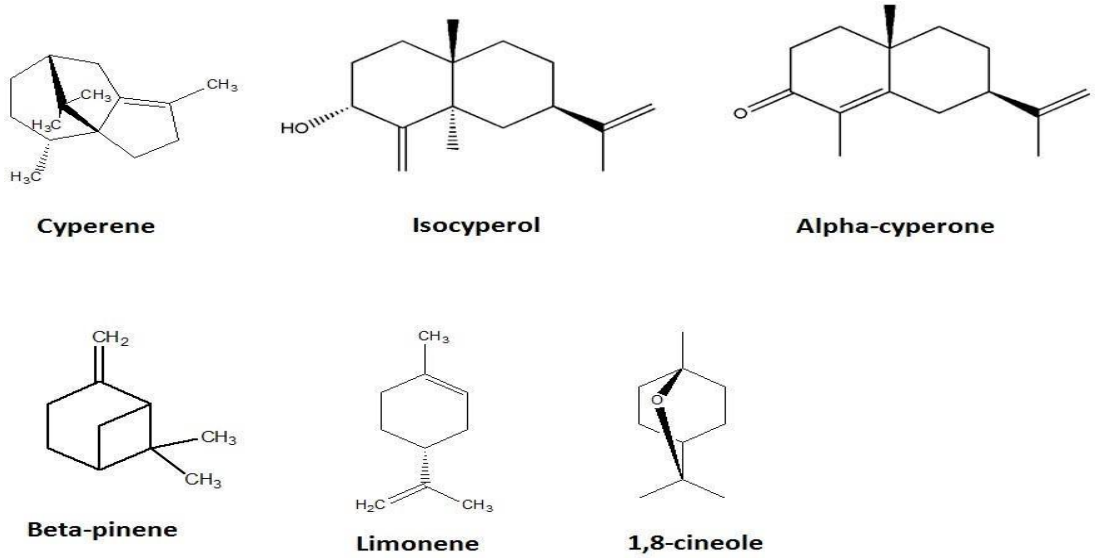

Figure 2.2: Chemical structures of some phytoconstituents present in Cyperus scariosus R. Br.

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2.2.2.7. Pharmacological activities

Anti depressant activity

The anti-depressant like activity of n-hexane extract of Cyperus scariosus oil was evaluated in swiss albino mice using Force swim test (FST) and Tail suspension test (TST). Oil possesses antidepressant effects. As the effect of extract was similar to that of imipramine, it may be concluded that this effect might be due to inhibition of norepinephrine uptake which eventually leads to increased availability of norepinephrine in synapses (Ramesh et al., 2012).

Hypotensive and spasmolytic effects.

The hydro-methanolic extract of dried tubers of Cyperus scariosus were evaluated for hypotensive and spasmolytic effects by in vitro and in vivo studies experiments. in vitro studies were performed on guinea pig atria, rabbit aorta, rat uterus, guinea pig ileum and rabbit jejunum. Wistar rats were used for in vivo studies. The results of in vivo studies showed that The hydro-methanolic extract of Cyperus scariosus (3-10 mg/kg) produced hypotensive and bradycardiac effects in anesthetized rats. It caused a fall in systolic, diastolic and mean arterial blood pressure in a dose dependent manner. Pretreatment of animals with atropine did not alter the cardiovascular responses of the plant extract, whereas both vasodilator and bradycardiac effects of acetylcholine were completely abolished. The results of in vitro studies showed that the plant extract at the concentration of 0.1-1 mg/ml caused aconcentration dependent inhibition of spontaneous contractions of guinea-pig paired atria, rat uterus and rabbit jejunum. In guinea-pig ileum, the inhibitory effect of plant extract was studied against agonistinduced contractile responses. Both acetylcholine and histamine produced submaximal contractions at 0.1 μM. Pretreatment of tissue with plant extract caused concentration dependent inhibition of both acetylcholine and histamine responses. The rabbit aorta preparation was used for the study of mechanism of spasmolytic action. In rabbit aorta, plant extract inhibited norepinephrine or K+-induced contractions in a concentration- dependent manner. The results of the studies clearly indicate that the hydro-methanolic extract of cyperus scariosus tubers produced hypotensive and spasmolytic effects (Anwar et al., 1994).

Antinociceptive and anti-hyperglycemic activity

The antinociceptive and anti-hyperglycemic activites of methanol extract of Cyperus scariosus leaves were investigated. Antinociceptive activity was determined using a model of acetic acid-induced gastric pain in mice and anti-hyperglycemic activity through glucose tolerance test using glucose loaded mice. The result obtained suggests that methanol extract of Cyperus scariosus leaves possess antinociceptive and antihyperglycemic activities (Alam et al., 2011).

Hepatoprotective activity

The hepatoprotective effect of Cyperus scariosus aqueous-methanolic extract was investigated against acetaminophen and CCl4 induced hepatotoxicity in male wistar rats. Plant extract at the dose of 500mg/kg showed 70% protection against lethal effect of acetaminophen. Acetaminophen (640 mg/kg) and CCI4 (1.5ml/kg) induced rise in serum levels of alkaline phosphatase (ALP), glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) as compared to respective control values Pretreatment with plant extract (500 mg/kg) was able to significantly prevent acetaminophen and CC14 induced rise in serum enzymes The serum levels of ALP, GOT and GPT were lower than the values of toxic control and similar to the control values. The effect of plant extract on CCl4 induced prolongation of pentobarbital sleeping time was also studied in swiss albino mice.CCl4 caused prolongation of pentobarbital induced sleeping time whereas prior treatment of mice with plant extract prevent this prolongation. Thus, the study shows the importance of Cyperus scariosus in treatment of hepatic diseases (Gilani and Janbaz, 1995).

Hypolipidemic activity

The hypolipidemic effect of hydroalcoholic extract of Cyperus scariosus root was evaluated in guinea pigs. Rosuvastatin (1.5 mg/kg) was used as standard drug and study was conducted four 60 days. Animals were fed diet according to the diet plan of their groups. The serum levels of total cholesterol, triglycerides, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein cholesterol (VLDL C) were determined. The anthrogenicxc indices, atherogenic coefficient and cardiac risk ratio were also calculated.

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Evaluation of liver Enzymes, Alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (AP) in serum was also done to analyze any functional abnormality of the liver. Serum level of cardiac dehydrogenase (LDH) and creatininekinase MB (CK-MB) was also analyzed to assess cardiac function. In the groups of animals fed with high fat diet, the plant extract (125mg/kg and 250 mg/kg) significantly reduced the serum total cholesterol, triglycerides, LDL-C, VLDL-C, atherogenic coefficient and cardiac risk ratio, as compared to high fat control group. HDL-C level was increased by plant extract and standard drug in the animals fed with high fat diet, AST, ALP and LDH levels were significantly decreased by plant extract (250 mg/kg) while standard drug increased AST and ALP levels in animals fed with high fat diet. However, there was no significant effect of plant extract was observed in lipid profile and serum enzymes of animals fed with normal diet (Chawda et al., 2014).

Antibacterial and cytotoxic activity

The antibacterial activity of a naturally occurring sesquiterpene (longiverbanone) from Cyperus scariosus was evaluated. It was chemically isolated from ethanol extract of the plant and its different concentrations were tested against eleven human pathogenic bacteria using disc diffusion method. Ampicillin was used as standard drug. Minimum inhibitory (MIC) and Minimum antibacterial concentration (MBC) were determined using macrodilution broth technique. The cytotoxic activity (LC50) was determined using new born brine shrimp (Artemia salina). The largest zone of inhibition was observed against Vibrio cohlearae. i.e. diameter of 30, 22, 15 and 8 mm at the concentrations of 160, 80, 40 and 20 µg/disc respectively. The MIC values of longiverbenone were 20 µg/ml against Vibrio cholerae, 40 µg/ml against Bacillus subtilis, Bacillus cereus, Bacillus megaterium and Shigella dysenteriae, 80 µg/ml against Escherichia coli and almonella paratyphi, and 160 µg/ml against Shigella sonnei and Salmonella typhi. Staphylococcus aureus was found to be resistant to the compound. The MBC values of the compound varied between 80 and 320 µg/ml. The LC50 of the compound was found to be 14.38 µg/ml. This study reveals that longiverbenone isolated from Cyperus scariosus gives moderate to good antibacterial activity against the tested organisms (Rahman and Anwar, 2008).

Immunosuppressant activity

Cyperus scariosus roots ethanol extract and its fractions were tested for immunosuppressant activity using male Balb/C mice (Mus musculus).

Cyclophosphamide and cyclosporine A were used as standard drugs. Humoral antibody response and delayed-type hypersensitivity response were determined for the primary screening of extract. Further, the extract was evaluated by skin allograft rejection test and phagocytic response (both in vitro and ex vivo). the extract was fractionated into chloroform, n-butanol and water fractions which were investigated for T-cell specific immunosuppressive potential by flow cytometry. The plant extract showed inhibition of humoral ntibody response by decreasing primary (26.8%) and secondary (29.7%) antibody formation. Cyclophophamide showed 31.8% and 36.4% decrease respectively. The extract also inhibited delayed type hypersensitivity immune response (45.9%). Delay in skin allograft rejection time (45.9%) and decrease in in vitro (37.4%) and ex vivo (37.8%) phagocytic was also observed. Out of the three fractions, only chloroform fraction showed significant suppression of CD8+ T cells (14%) and CD4+ T cells (25.3%) as compared to control values, 22.3% and 38.6% respectively. A significant reduction in intracellular cytokines (IL-2 and IFN- γ) by the chloroform fraction was also observed. the results were comparable to standard drug cyclosporine A. Thus, the results revealed that the plant exhibits a marked immunosuppressive activity (Bhagwat et al., 2009).

Anti-inflammatory activity

Cyperus scariosus rhizomes methanol extract was evaluated for anti inflammatory activity using anti denaturation of bovine serum albumin assay. Diclofenac sodium was used as reference drug. The extract inhibited the denaturation of bovim serum albumin in a dose dependent manner. The percent inhibition of denaturation was increased on increasing concentration of the plant extract. Further, the extract was subjected to gas chromatography- mass spectroscopy

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analysis which revealed the presence of nine compounds. In-silico molecular docking analysis of these compounds was performed and among all, three compounds, N-methyl-1- adamantaneacetamide, 1,5-diphenyl-2H1,2,4-triazine and benzene-1,2-diol,4-(4-bromo-3 chloropheyl iminomethyl) interacted with anti inflammatory COX-2 binding receptors. Therefore, the results showed that Cyperus scariosus rhizomes contains anti inflammatory compounds and justified the use of plant for prevention of inflammatory disorders (Kakarla et al., 2014),

Antioxidant activity

The antioxidant activity of Cyperus scariosus rhizomes was determined by phosphomolybdate method. Methanol extract of rhizomes was used for the study and ascorbic acid was used as standard antioxidant agent. The antioxidant activity of the extract was expressed as µg/ml of ascorbic acid equivalents. The extract increased antioxidant activity in a dose dependent manner. The extract at 100µg/ml showed similar activity to ascorbic acid at 50 µg/ml. Hence, the plant has a strong antioxidant activity.

III. AIM AND OBJECTIVES

The present study was aimed to investigate the antiepileptic effect of Acacia auriculiformis A. Cunn. ex. Benth. stem bark and Cyperus scariosus R. Br. roots using experimental animal models. Moreover, the study aimed at development of herbal formulations using extracts of the selected plants to enhance their therapeutic efficacy in management of epilepsy.

The following objectives were carried out to achieve the aim of present study:

- Pharmacognostical standardization of selected plants as per official guidelines
- Extraction of selected plants with various solvents
- Preliminary phytochemical screening and gas chromatography-mass spectroscopic analysis of extracts for identification of active phytoconstituents.
- Evaluation of extracts for antiepileptic effects using experimental animal models
- Evaluation of possible mechanism of antiepileptic action of the selected plants by carrying out biochemical estimations.
- Design and development of suitable herbal novel formulations from bioactive extracts
- Physicochemical characterization of formulations

IV. MATERIALS AND METHODS

4.1. Selection of plants

Acacia auriculiformis A. Cunn.ex. Benth stem bark and Cyperus scariosus R.Br. roots were selected for the present study.

4.2. Collection and authentication of plant materials

Acacia auriculiformis A. Cunn. was collected from Guru Jambheshwar University of Science & Technology, Hisar (Haryana) in the month of March, 2015. The stem bark of the plant was selected for present research work. The plant was authenticated by Dr. Anjula Pandey, Principal Scientist, National Herbarium of Cultivated Plants, NBGPR, New Delhi, vide reference no, NHCP/NBGPR/2015-3 dated 22.04.2015 and was identified as Acacia auriculiformis A. Cunn. ex. Benth. belonging to family Mimosaceae.

Cyperus scariosus R. Br. was procured by Nature and Nurture Healthcare Pvt. Ltd. (New Delhi) in the month of March, 2015. The root of this plant has been selected for present research work. The plant was authenticated by Dr. Sunita Garg, Chief Scientist, Raw Material Herbarium and Museum, CSIR-National National Institute of Science Communication and Informaion Resources, New Delhi, vide reference no, NISCAIR/RHMD/Consult/2015/2835/28 date 16.06.2015 The plant was identified as Cyperus scariosus R. Br. belonging to family Cyperaceae.

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4.3. Pharmacognostical standardization

Fresh as well as air dried bark and roots were used for pharmacognostical standardization. The air dried bark and roots were pulverized mechanically to coarse powder. This powdered form of plant materials was also subjected to various parameters of pharmacognostical standardization.

4.3.1. Morphological studies

Morphological studies of medicinal plant materials includes the study of colour, size, shape, surface characteristics, texture etc. Substitutes or adulterants may closely resemble the genuine plant material. These parameters help to identify the adulterants. The morphological characters color, odour, taste, texture, shape and size of the selected plant materials were studied by naked eye and with the help of dissection microscope (WHO, 2011)

4.3.2. Microscopical examination

a) Transverse section of bark

The plant material was soaked in water to make it smooth enough for transverse section. Transverse section was cut by free hand sectioning. The transverse sections were cleared in chloral hydrate with gentle warming. Then, sections were stained with phloroglucinol and concentrated hydrochloric acid. Further, the sections were mounted in glycerine, covered with cover slip and studied under light microscope (Carl Zeiss Primo star, Germany) (WHO, 2011).

b) Microscopy of powder

For powder microscopy, small amount of powdered plant material was taken on slide. This powder was stained with phloroglucinol and concentrated hydrochloric acid. Then, it was mounted in glycerine and covered with cover slip. The prepared slides were observed under light microscope (Carl Zeiss Primo star, Germany) (WHO, 2011).

c) Histochemical detection of cell wall and contents (WHO, 2011)

Cellulose cell walls: 1 drop of iodine (0.1 mol/l) was added to thin section of plant, allowed to stand for 1 minute and excess reagent was removed. Cellulose cell walls are stained blue to blue-violet on addition of 1 drop of sulfuric acid TS.

Lignified cell walls: The powder or section on a slide was moistened with a small volume of phloroglucinol TS and allowed to stand for about 2 minutes to dry. 1 drop of hydrochloric acid TS was added, a cover-glass was applied and observed for pink to cherry red stained lignified cell walls.

Suberized or cuticular cell walls: 1-2 drops of sudan red TS are added to the plant section and observed for red stained suberized or cuticular cell walls.

Aleurone grains: A few drops of iodine/ethanol TS are added to powdered plant material and observed for yellowish brown to brown colour of aleurone grains. Addition of about mercuric nitrate TS turns the colour to brick red.

Calcium carbonate: Crystals or deposits of calcium carbonate dissolve slowly with effervescence on addition of acetic acid TS or hydrochloric acid TS.

Calcium oxalate: Sulphuric acid was added to the powdered plant material, allowed to stand for 10 minutes and observed for presence or absence of calcium oxalate crystals.

Fats, fatty oils, volatile oils and resins: 1–2 drops of sudan red was added to powdered plant material, allowed to stand for few minutes and heated gently. Fatty substances shows orange-red to red colour. Irrigation of the preparation was done with ethanol and heated gently. Volatile oils and resins, if present, dissolve in the solvent, while fats and fatty oils remain intact.

Hydroxyanthraquinones: 1 drop of potassium hydroxide was added to the powder. Cells containing 1, 8-dihydroxyanthraquinones are stained red.

Inulin: 1 drop each of 1-naphthol and sulfuric acid was added and observed for the appearance of brownish red colored crystals that dissolves immediately.

Mucilage: 1 drop of chinese ink was added to the dry sample and observed for presence and absence of transparent, spherically dilated fragments on black background.

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Starch: Addition of a small volume of iodine gives blue or reddish blue color in presence of starch.
Tannin: Addition of 1 drop of ferric chloride to the powdered drug gives bluish black or greenish black colour in presence of tannin.

V. RESULTS

Pharmacognostical standardization of Acacia auriculiformis A. Cunn. ex. Benth. stem bark Morphological studies
The stem bark was observed as flat pieces with thickness of 5 to 8 mm with fibrous fracture. Young fresh bark was grey in colour while mature bark was dark greyish brown. External surface of younger bark was smooth while older bark was rough with longitudinal and transverse striations. Internal surface was smooth with light colour bearing few dark brown patches in mature bark. It had characteristic odour and nonbitter in taste. Results are shown in the Figure 5.1.

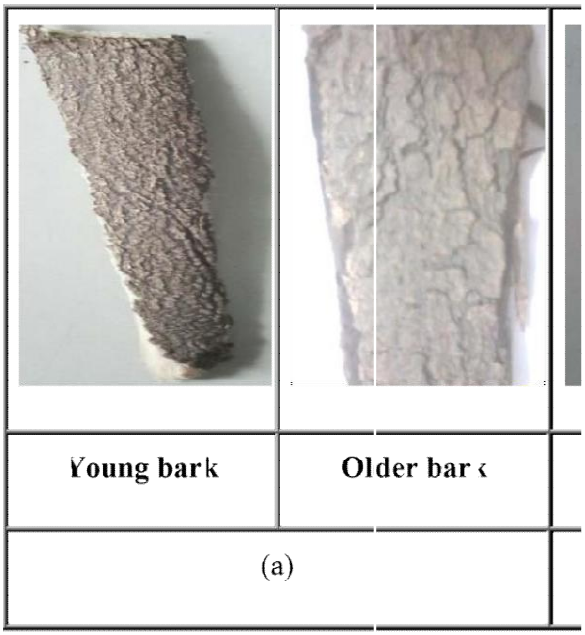

Figure 5.1: Stem bark of Acacia auriculiformis A. Cunn. ex. Benth.
(a) External surface of bark (b) Internal suface of bark Microscopical studies
a) Transverse section
The transverse section of stem bark showed periderm region consisting of cork, phellogen and phelloderm. The outermost multilayered cork cells were filled with black brown content of tannin. A broad phelloderm region was present after 3-4 cell layered phellogen. Phelloderm contains several layers of closely packed cells. The cortex consists of parenchyma and large number of groups of stone cells. In phloem region, sieve tubes were present with companion cells. The radially elongated medullary rays extended to phloem region were biseriate while in outer phloem region few medullary rays were multiseriate. The phloem tissue was associated with phloem parenchyma and phloem fibers. Prismatic calcium oxalate crystals were also present in phloem region. The transverse section is shown in Figure 5.2.

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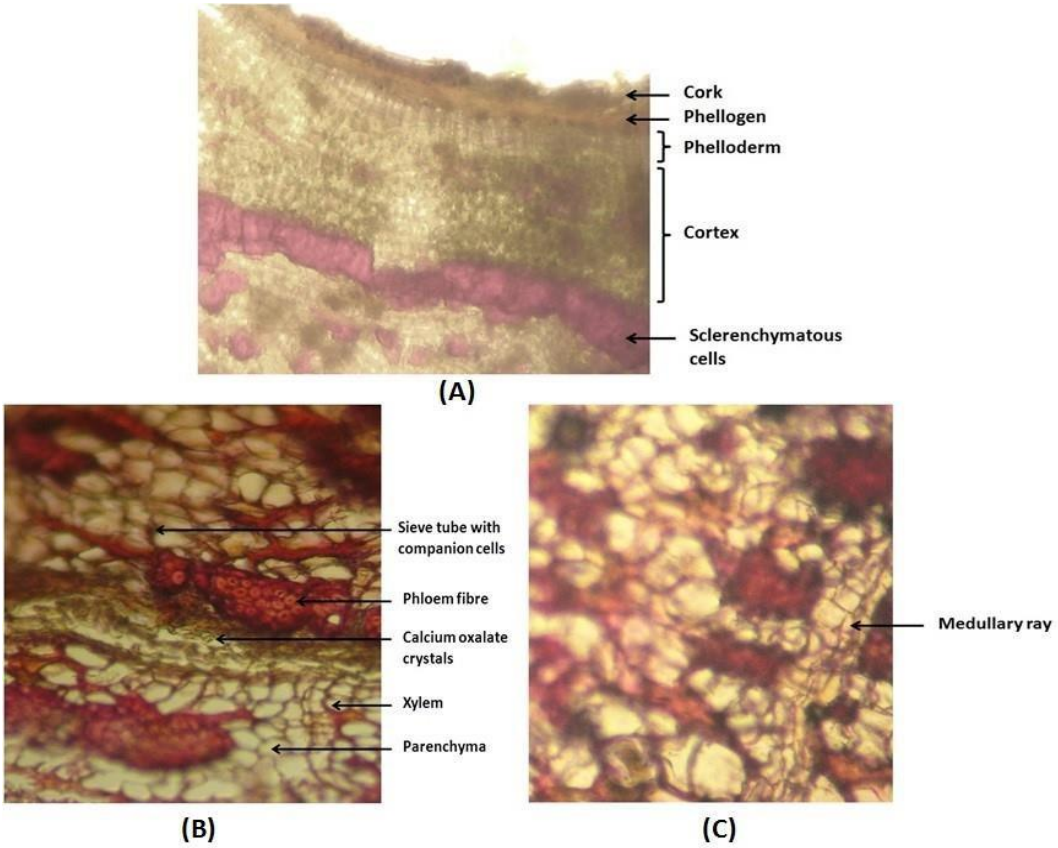

Figure 5.2: Transverse section of Acacia auriculiformis A. Cunn. ex. Benth. stem bark
(A) Periderm and cortex region of stem bark (B) and (C) Phloem region of stem bark

b) Powder microscopy

In powder microscopy, the stem bark showed the presence of parenchyma cells, sclerenchyma cells, cork cells filled with tannin, lignified fibres, tracheids, starch grains, prismatic calcium oxalate crystals, bordered pitted xylem vessel which are shown in Figure 5.3.

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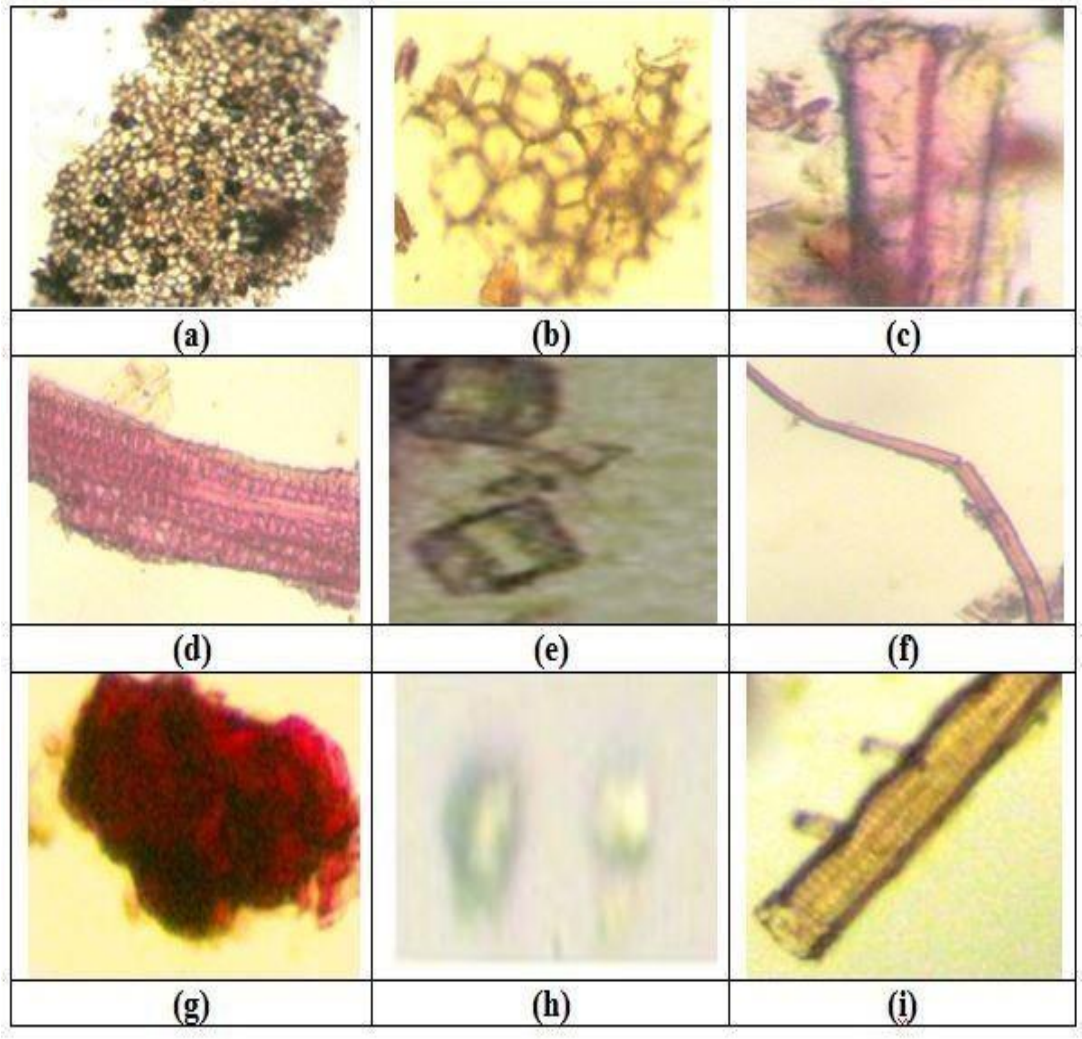

Figure 5.3: Powder microscopy of Acacia auriculiformis A. Cunn. ex. Benth. stem bark
(a) Cork cells filled with tannin (b) Parenchyma cells (c) Tracheids (d) Lignified fibres covered with calcium oxalate crystals (e) Prismatic calcium oxalate crystals (f) Phloem fibre (g) Sclerenchyma cells (h) Starch grains (i) Xylem vessel

VI. DISCUSSION

The present research work was carried out for the development of herbal formulations for therapeutic management of epilepsy. Acacia auriculiformis A. Cunn. ex. Benth. stem bark and Cyperus scariosus R. Br. roots were selected and evaluated for antiepileptic effect. Thereafter, ethanol extract of Acacia auriculiformis A. Cunn. ex. Benth. stem bark and essential oil of Cyperus scariosus R. Br. roots were used to formulate nanoformulations, which were further evaluated for antiepileptic effect. Pharmacognostical standardization is an efficient tool to establish quality control parameters of plants. It helps to assure the authentication of plants and prevention of adulteration (Chanda, 2014; Amponsah et al., 2014). These studies also ensure reproducible quality of plant material and herbal products in trade (Amponsah et al., 2014). Standardization and quality control of plants are also essential for the worldwide acceptance of herbal products in modern system of medicines. In the present study, quality control parameters for standardization

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of the Acacia auriculiformis A. Cunn. ex. Benth. stem bark and Cyperus scariosus R. Br. roots. were done prior to their biological evaluation. Foreign organic matter in both plant materials was within the limits. The ash remaining after ignition of medicinal plant materials measured the physiological ash that is derived from plant tissue and non-physiological ash which is residue of the extraneous matter adhering to the plant surface. Hot extraction of Acacia auriculiformis A. Cunn. ex. Benth. stem bark in ethyl acetate resulted in higher percentage yield than extraction with other solvents. Hot extraction of Cyperus scariosus

R. Br. roots with water showed higher percentage yield than extraction with other solvents. The result of swelling index showed the absence of mucilage in both plants. The heavy metals like arsenic, lead and mercury were found to be within the limits in both plants. Preliminary phytochemical analysis of Acacia auriculiformis A. Cunn. Ex. Benth. stem bark and Cyperus scariosus R. Br. root extracts showed the presence of carbohydrates, anthraquinone glycosides, saponins, phenols, flavonoids, steroids and terpenoids. Total phenolic content in ethanol extracts of both plants was higher than other extracts. The highest concentration of total flavonoid content was found in ethyl acetate of Acacia auriculiformis A. Cunn. Ex. Benth. stem bark and ethanol extract of Cyperus scariosus R. Br. root. GC-MS analysis of ethyl acetate and ethanol extracts of Acacia auriculiformis A. Cunn. Ex. Benth. stem bark revealed the presence of therapeutic active constituents such as stigmasterol, stigmast-4-en-3-one, 7, 22-ergostadienone, betulin, squalene, eremophilane, aromadendrene, lup-20(29)-3,28 diol, γ-Tocopherol, gingerol, 1-hydroxy-3,5,8-trimethoxy-xanthen-9-one and 4-tertbutylcalix[4]arene. GC-MS analysis of Cyperus scariosus R. Br. roots also revealed the presence of pharmacologically active compounds such as ergost-5-en-3-ol, stigmasterol, stigmast-4-en-3-one and rhapontin. Essential oil of roots showed the presence of α-pinene, limonene, cyperene, patchouli alcohol, caryophyllene, rotundene and curcumen-12-ol in GC-MS analysis.

These materials were further used for evaluation of antiepileptic activity against pentylenetetrazole (PTZ) and isoniazid (INH) induced seizures in mice. PTZ is widely used as behavioural models of epilepsy (Corda et al., 1991). It act by blockade of GABAA receptor gated chloride channels, resulting in decrease of GABA binding and GABA stimulated chloride uptake (Corda et al, 1990; Luthman, 1997). Isoniazid (INH) is also used to induce seizures. The mechanism of INH induced seizures is interference with the function and supply of pyridoxine. INH directly or in its hydrazone form binds with pyridoxine causes blockage of pyridoxine phosphokinase and prevents conversion of pyridoxine to active form pyridoxal 5- phosphate, which is required for synthesis of gamma amino-butyric acid (GABA) from glutamic acid. It also causes inactivation of pyridoxal 5-phosphate. GABA deficiency and glutamic acid accumulation lead to central nervous system excitation and produces seizures in animals (Jagannatha, 2015).

In the present study, PTZ induced seizures (jerks, severe straub tail, myoclonic, extensor phase and mortality) and INH induced seizures (tonic clonic), which were decreased with the treatment of ethyl acetate and ethanol extracts of Acacia auriculiformis A. Cunn. ex. Benth stem bark by increasing the latency and decreasing the duration of seizures. Essential oil, ethanol and aqueous extracts of Cyperus scariosus R. Br. roots also showed protection against seizures by increasing the latency. Duration of seizures and mortality were decreased by essential oil and ethanol extract of Cyperus scariosus R. Br. roots. Furthermore, biochemical studies indicated that PTZ decreased brain GABA level, which were increased with the treatment of Acacia auriculiformis A. Cunn. ex. Benth stem bark ethyl acetate and ethanol extracts. This effect may be due to presence of flavonoids in both extracts, which have been reported to possess GABA potentiating effect with their high affinity for the benzodiazepine binding site on GABAA receptor (Hanrahan et al., 2011), which seems that this protective mechanism of action is involved in our study. Essential oil and ethanol extract of Cyperus scariosus R. Br. roots also increased the PTZ decreased GABA level. The effect of ethanol extract was probably due to presence of flavonoids. αpinene, patchouli alcohol and caryophyllene in the essential oil and ethanol extract, which are reported to have GABA potentiating effect (Zamyad et al., 2016; Kessler et al., 2014), these studies support our work. Oxidative stress is also involved in the pathogenesis of epilepsy. Several studies on animal models and genetic studies have suggested that oxidative stress increases in subsequent cell damage after persistent seizures (Aguiar et al., 2012). Free radicals are believed to play an important role in causing oxidative stress. Abnormal structural changes in membrane lipids, cellular proteins, RNA and DNA occur due to accumulation of free radicals in

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brain tissue (Erkec et al., 2015). There are a number of defence mechanisms that neutralize the free radicals. The glutathione redox system is a major component of antioxidant defence system, which act as free radical scavenger (Pence et al., 2009). Reduced glutathione (GSH) is essential to prevent lipid peroxidation and to protect sulfhydryl groups of proteins (Devi et al., 2008). In the present study, PTZ administration decreased GSH level, which was increased with the treatment of ethyl acetate and ethanol extracts of Acacia auriculiformis A. Cunn. ex. Benth stem bark and essential oil, ethanol and aqueous extracts of Cyperus scariosus R. Br. roots. Moreover, PTZ administration also increased in brain malondialdehyde (MDA) level, which is a metabolite of lipid peroxidation. It also affects membrane permeability, fluidity and protein activity (Shin et al., 2011). Both extracts of Acacia auriculiformis A. Cunn. ex. Benth. stem bark, and essential oil of Cyperus scariosus R. Br. roots diminished the PTZ induced increase in MDA level. Catalase is an enzyme that reacts very effectively with H2O2 to form water and molecular oxygen and protects neurons from toxic reactive oxygen species (ROS) such as hydrogen peroxide and hydroxyl free radicals. Thus, catalase has an important role in the acquisition of tolerance to oxidative stress (Mates et al., 1999; Aguiar et al., 2012). Hence, catalase activity was also determined in present research work, PTZ administration diminished the brain catalase activity. Treatment with extracts of Acacia auriculiformis A. Cunn. ex. Benth. stem bark and Cyperus scariosus R. Br. roots effectively increased the catalase activity. Nitric oxide functions as a second messenger in the nervous system. Increased level of nitric oxide is responsible for the hyperexcitation resulting degeneration of neurons (Kumar et al., 2014). Nitrite levels are also measured to determine nitric oxide production as nitrites represent source as well as metabolic end product of nitric oxide (Lundberg et al., 2008). In the present study, administration of PTZ increased brain nitrite level, which was potentially attenuated by ethyl acetate and ethanol extracts of Acacia auriculiformis A. Cunn. ex. Benth. stem bark. Both extracts and essential oil of Cyperus scariosus R. Br. roots were also found to be effective in attenuating nitrite levels in mice brain. The effect of Acacia auriculiformis A. Cunn. ex. Benth. stem bark on oxidative stress may be attributed to the presence of phenols and flavonoids in them. The stem bark also consists of antioxidant phytoconstituents like γ-Tocopherol and gingerol. Cyperus scariosus R. Br. roots also consists of antioxidant phytoconstituents like phenols and flavonoids. Limonene is present in essential oil of roots which is a potent antioxidant agent (Murali et al., 2013).

Both, behavioural and biochemical studies showed the antiepileptic effect of selected plants (Acacia auriculiformis A. Cunn. ex. Benth. stem bark and Cyperus scariosus R. Br. roots). But, ethyl acetate extract of Acacia auriculiformis A. Cunn. ex. Benth. stem bark and essential oil of Cyperus scariosus R. Br. roots showed more pronounced antiepileptic effect among all extracts. Hence, both of these were further selected for the preparation of herbal formulation.

Ethyl acetate extract of Acacia auriculiformis A. Cunn. ex. Benth. stem bark was formulated as solid lipid nanoparticles (SLN). Preparation of SLN is one of the most recent approaches for targeting of drugs to brain. Poloxamer-407 was used as surfactant because it is effective in enhancement of drug permeability to brain. The possible mechanism of poloxamer-407 behind the increased brain permeation includes inhibition of efflux action of P-glycoprotein and solubilization of membrane lipids in brain endothelial cells (Kulkarni and Feng, 2011). Essential oil of Cyperus scariosus R. Br. roots was formulated as chitosan nanoparticles. The chitosan nanoparticles were coated with Tween 80, a non ionic surfactant, in order to target the brain. Tween 80 shows brain specificity by binding with plasma Apo-E lipoprotein that mimics low density lipoproteins (LDL), which further attach with LDL receptors present on brain micro vascular endothelial cells (Kreute, 2001). FT-IR spectroscopic analysis confirmed the incorporation and the absence of chemical interaction. In vitro drug release studies of AEASLN and COCNP showed sustained release pattern with Korsemeyer-Peppas model and Higuchi model as best fit model respectively. TEM analysis of the optimized nanoformulations substantiated the size and shape of the nanoparticles. These two formulations were further evaluated for their antiepileptic effect. The prepared SLN of optimized Acacia auriculiformis A. Cunn. ex. Benth. stem bark ethyl acetate extract (AEASLN) and chitosan nanoparticles of Cyperus scariosus R. Br. roots essential oil (COCNP) showed more pronounced antiepileptic effect as compared to ethyl acetate extract and essential oil in crude form respectively. Both behavioural and biochemical results indicate the increased bioavailability and permeation of extract and essential oil in the brain as compared to their crude form. These results are also supported by

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histopathological studies. Histopathological study showed that the nanoformulation of ethyl acetate extract and essential oil were more effective to decrease the formation of hyperchromatic nuclei and perinuclear vacuolization in cortex part of brain as compared to their effects in crude form. The effect of both formulations on TNF-α was determined to find out their role on neuroinflammation. Oxidative stress stimulates microglial cell activation that increases the release of inflammatory cytokines such as TNF-α, which further increases the oxidative stress leading to neuronal toxicity (Monji et al., 2009). Both AEASLN and COCNP significantly attenuated level of TNF-α in mice brain revealing their anti inflammatory effect and protection against neuronal toxicity. The anti-inflammatory effect of Acacia auriculiformis A. Cunn. ex. Benth. stem bark may be contributed to eremophilane, aromadendrene and lup-ene-20(29)-3,28 diol (Arciniegas et al., 2013; Zhang et al, 2014). Cyperus scariosus R. Br. roots contains limonene as anti-inflammatory agent as supported by Hirota et al., 2010. Possible mechanisms of action of Acacia auriculiformis A. Cunn. ex. Benth. stem bark and Cyperus scariosus R. Br. roots have been portrayed in the Figure 6.1 and 6.2 respectively.

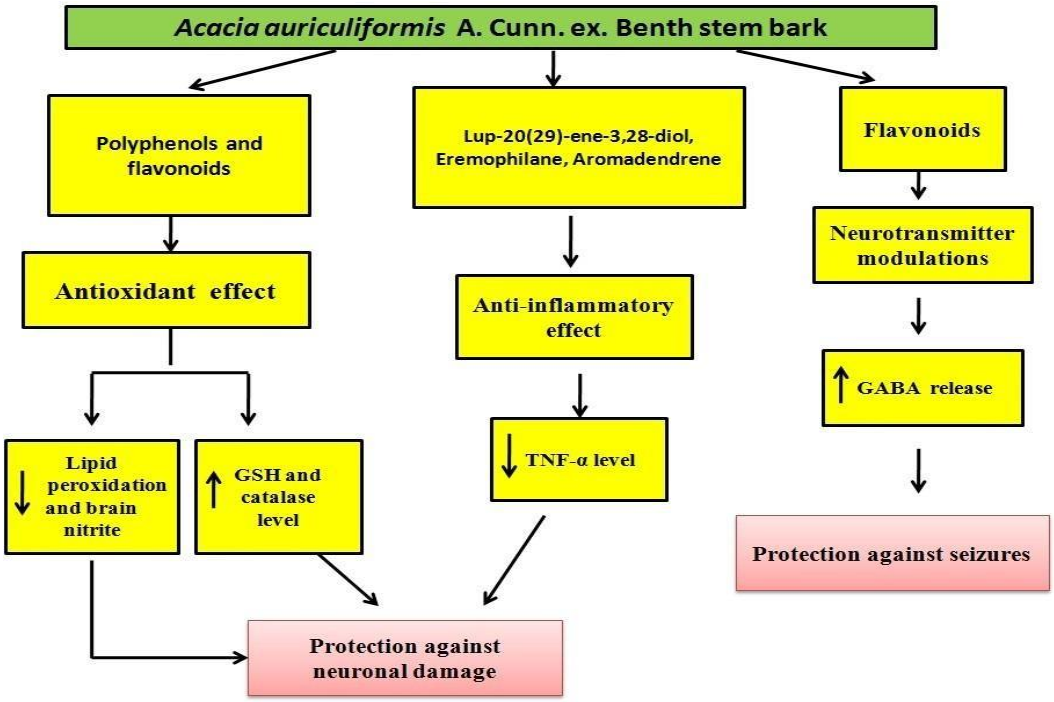

Figure 6.1: Possible mechanism of action of Acacia auriculiformis A. Cunn. ex. Benth. stem bark

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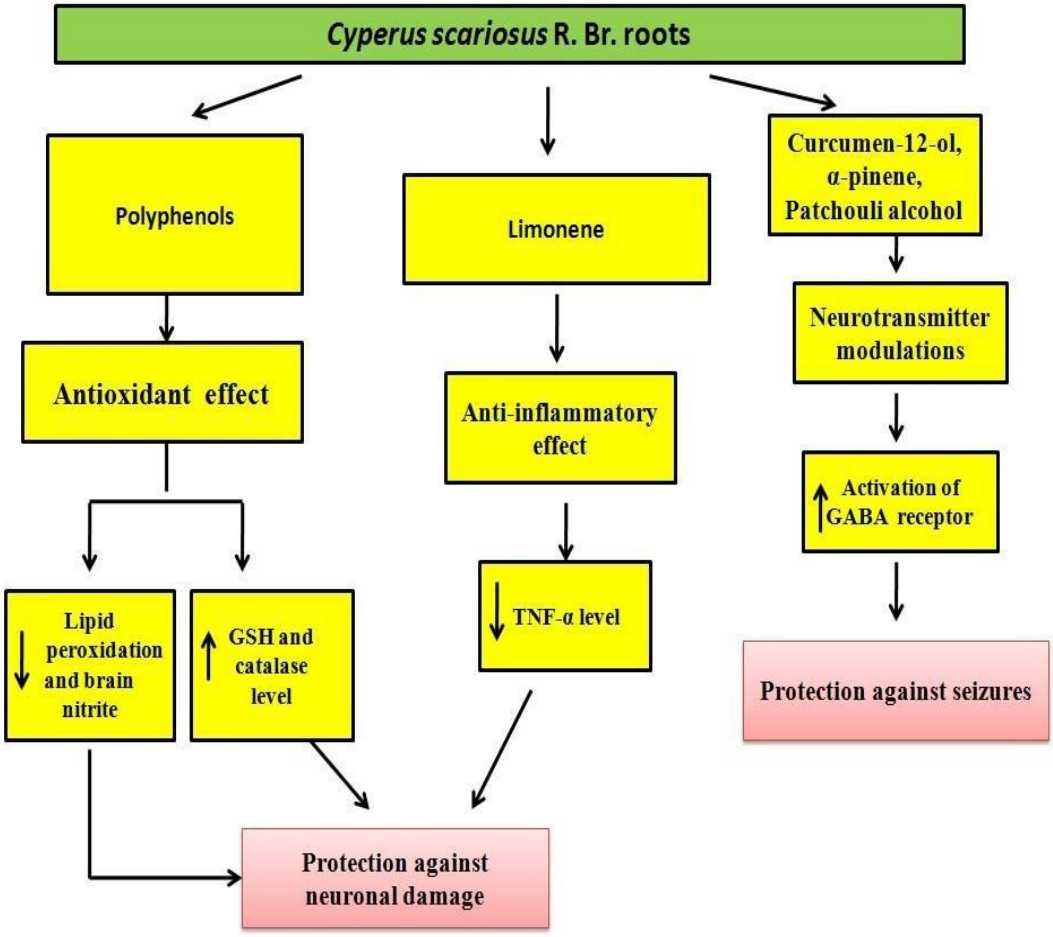

Figure 6.2: Possible mechanism of action of Cyperus scariosus R. Br. roots

VII. SUMMARY AND CONCLUSION

Epilepsy is a neurological disorder resulting from recurrent, spontaneous and abnormal electrical discharge from a group of selected neurons in the brain, which causes occurrence of the epileptic seizures. It is classified into generalised seizure (affect whole brain) and partial or focal (affect one part of the brain) seizure. Generalized seizures include absence, myoclonic, and tonic-clonic seizure. Partial seizures include simple partial seizure, complex partial seizure and partial with secondarily generalized tonic-clonic seizure. Neural mechanisms underlying the occurrence of seizures are imbalance between inhibitory (GABA) and excitatory (glutamate) neurotransmitters pathways. Antiepileptic drugs are used to treat this disorder and these agents are also associated with some serious side effects. Therefore, the researchers are increasing their interest to develop novel therapies for treatment of epilepsy such as herbal formulations. Nanoparticulate drug delivery systems are used to target the drugs to brain for increasing bioavailability, physicochemical stability and better efficiency. Therefore, the present research work was designed to explore the antiepileptic potential of Acacia auriculiformis A. Cunn. ex. Benth. stem bark (Family: Mimosaceae) and Cyperus scariosus

R. Br. roots (Family: Cyperaceae) and formulate them into suitable nanoparticles to maximize their therapeutic efficacy in management of epileptic seizures.

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Pharmacognostical standardization was done for both plant materials and quality control parameters were performed. Toxic heavy metals were absent in both plants. Preliminary phytochemical screening and GC-MS analysis of ethyl acetate and ethanol extracts of Acacia auriculiformis A. Cunn. Ex. Benth. stem bark and ethanol extract and essential oil of Cyperus scariosus R. Br. roots revealed the presence of many pharmacologically active phytoconstituents. These materials were further used for evaluation of antiepileptic activity against pentylenetetrazole (PTZ) and isoniazid (INH) induced seizures in mice. Both plant materials were effective to decrease the PTZ and INH induced seizures probably by facilitating the neurotransmission of GABA. The plant materials also showed antioxidant effect by potentiating catalase activity and GSH concentration and attenuating lipid peroxidation and nitrite level.

These effects contribute to neuroprotective effect of plants against seizures induced neuronal damage. However, ethyl acetate extract of Acacia auriculiformis A. Cunn. ex. Benth. stem bark and essential oil of Cyperus scariosus R. Br. roots showed more pronounced antiepileptic effect among all extracts. Hence, both of these were further used to prepare the herbal formulation.

Ethyl acetate extract of Acacia auriculiformis A. Cunn. ex. Benth. stem bark was formulated into solid lipid nanoparticles and essential oil of Cyperus scariosus R. Br. roots was formulated into chitosan nanoparticles. The formulations were optimized by 32 factorial design using software Design expert® version 10.0.6.0 (Stat-ease Inc., Minneapolis, MN) to minimize the particle size and maximizing the entrapment efficiency. Optimized formulations of Acacia auriculiformis A. Cunn. ex. Benth. stem bark ethyl acetate extract (AEASLN) and Cyperus scariosus R. Br. roots essential oil (COCNP) were used for cumulative percent drug release studies, physicochemical characterization and evaluation of antiepileptic activity. FT-IR spectroscopic analysis confirmed the incorporation and the absence of chemical interaction. In vitro drug release studies of AEASLN and COCNP showed sustained release pattern with Korsemeyer-Peppas model and Higuchi model as best fit model respectively. TEM analysis of the optimized nanoformulations substantiated the size and shape of the nanoparticles. AEASLN and COCNP showed protection against seizures than the crude extract and essential oil respectively as indicated by enhanced latency and reduced duration of seizures. These results were confirmed by biochemical studies as indicated by increased GABA, GSH and catalase activity and diminished lipid peroxidation, TNF-α and nitrite levels. Histopathological studies revealed diminished perinuclear vacuolization and hyperchromatic nuclei in AEASLN and COCNP treated mice brain as compared to ethyl acetate extract and essential oil respectively. All these results show the successful permeability of AEASLN and COCNP into brain.

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