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A Comprehensive Study on Ethno-Pharmacological Exploration of *Achyranthes aspera* Linn.

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Abstract: Recently, pharmacognosy, exploration of plant and animal based extracts and naturally present bioactive compounds as a source of possible drug has been a central area of research worldwide. In this review, an attempt has been made to list and comprehend various pharmacological potential of a widely available weed, *Achyranthes aspera*, growing in waste places along roadsides in tropical Asia, Africa and America. It has gained much attention in scientific researches due to its ethnomedicinal uses by folks and traditional healers. Its different parts (root, leaf, stem, inflorescence) and their active phytoconstituents have been scientifically investigated for antimicrobial, antidiabetic, anti-inflammatory, anti-asthamic, antilipidemic, anti-oxidant, anti-obesity, nephroprotective, cardio-stimulant, immunostimulant, prothyroidic, hepatoprotective and neuroprotective efficacies and showed positive results in most of the studies. On phytochemical screening, the plant has been reported to be rich in alkaloids, phenols, tannins, glycosides, flavonoids and saponins, which might be responsible for its pharmacological activities. This review provides an insight for various biological activities that have been scientifically explored in the past and led way to bridge the gap required for pharmaceutical utilisation of the herbal product. The plant can be used as a herbal alternative in treatment of diseases in addition to maintenance of a healthy body.

Keywords: *Achyranthes*, Pharmacology, Phytoconstituents, Anti-oxidant, Ethnomedicinal

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Introduction

India is a land of rich biodiversity and possesses a golden history of utilization of natural agents for maintenance of health and treatment of illness. We are having a tremendous diversity of folklore regarding medicinal uses of plants practiced by rural and tribal people as well as traditional healers. These large bulk of traditional knowledge preserved by ethnic and rural people of India have been passing down to generations through words of mouth and are being used in treatment of various disease conditions. But, in recent years, due to over population, urbanization and

continuous exploitation of these herbal reserves, we are facing a day-by-day depletion of natural resources along with their traditional knowledge. Scientific exploration of the pharmacological activities of the herbs and medicinal plants growing in our vicinity can not only help to preserve the traditional knowledge of their uses but also they can provide a novel approach towards formulation of plant based health products by providing additional evidences of their safety and efficacy.

Pharmacological relevance of medicinal plants:

Nature have been a source of medicinal agents for thousands of years. Use of medicinal herbs to treat illness and maintain and promote health can be traced back to the beginning of human civilization. Abundant availability, easy accessibility for all, low cost, effectiveness in treatment of various ailments and little or no side effects make the plant based traditional indigenous medicines an excellent source of primary health care in absence of access to modern medical facilities and a substantial alternative to the modern day expensive drugs having severe side effects (Pandey *et al.*, 2013). According to a report published by WHO, about 70-80 % of the world's population depend on traditional herbal medicine for their primary health care. In the recent past, a tremendous increase in the use of plant based health products globally have prompted a surge in researches on herbals in terms of their safety and effectiveness against altered health conditions. World Health Organization (WHO) has taken an initiative to identify all medicinal plants used globally and accordingly listed more than 20,000 species (Srivastava 2014). The folkloristic concepts of a wide range of traditional herbs and spices have been scientifically explored for their pharmacological activities and a plethora of herbs have been reported with anti-cancer, anti-oxidative, anti-inflammatory, hypoglycemic, cardioprotective, immunemodulatory and hepatoprotective activities, namely, *Ocimum sanctum* (Tulsi), *Aloe barbadensis* (Aloe vera), *Asparagus racemosus* (Shatavaari), *Tinospora cordifolia* (Guduchi), *Centella asiatica* (Brahmi), *Gymnema sylvestre* (Gurmar), *Withania somnifera* (Ashwagandha), *Zingiber officinale* (Ginger), *Camellia sinensis* (Tea plant), *Mangifera indica* (Mango) etc. The broad spectrum of biological activities exerted by the herbal preparations can be attributed to the presence of diverse group of secondary metabolites in them (Kumar *et al.*, 2012; Abou Seif, 2016). *Achyranthes aspera* is one of the widely used folklore herb and has been extensively explored for various pharmacological activities. In the present review, an effort has been made to investigate and summarize the potential pharmacological activities of different parts of *Achyranthes aspera*.

Achyranthes aspera as a promising medicinal herb:

1. *Taxonomic Position*:

Kingdom- Plantae; Subkingdom- Tracheobinota; Super Division- Spermatophyta; Division- Mangoliophyta; Class- Mangoliophsida; Subclass- Caryophyllidae; Order- Caryophyllales; Family- Amaranthaceae; Genus- *Achyranthes*; Species- *aspera.*

2. *Common names of Achyranthes aspera* (*Sharma and Chaudhary, 2015*):

English- Prickly chaff flower, Devil's horse whip; *Hindi*- Latjira, Chirchira, Lamchichra, Sonpur, Onga; *Sanskrit*- Aghata, Apamargah, Khara-manjari; Unani- Chirchitaa; *Marathi*- Aghada, Pandhara- aghada; *Bengali*- Apaang; *Gujarati*- Safad Aghedo, Anghadi, Andhedi, Agado; *Tamil*- Shiru- kadaladi, Nayuruvi; *French*- Achyranth a feuilles rudes, Collant, Gendarme; *Spanish*- Mosotillo, Rabo de gato, Rabo de change, Rrabo de raton.

3. *Geographical distribution*:

Achyranthes aspera is globally available as a medicinal weed in Baluchistan, Ceylon, Tropical Asia, Africa, Australia and America. It is found in field boundaries, road sides and waste places throughout India. It has been reported as an invasive alien species in Northern Bangladesh. The plant grows in absence of shade or in semi- shade

condition. It requires moist soil but prefers light sandy, medium loamy or heavy clay soils for its proper growth. It flowers during July to September and seeds ripe in month of October (Sharma and Chaudhary, 2015).

4. *Botanical description*:

Achyranthes aspera is an erect herb with height ranging between 0.3 to 0.9 m. Stems are stiff, erect, hairy, branched from the base and are often tinged with purple colour. They are generally solid but become hollow when dry. Leaves are simple, opposite, velvety with wavy margins, petiolate and pubescent due to presence of thick coat of long simple hairs. Yellowish- brown, gradually tapering, cylindrical roots are present. The flowers are bisexual, greenish- white, numerous and are arranged in long spike form of inflorescence, generally appearing during summer. They are 8-30 cm long, 3-7 mm wide, bracteate with two bracteoles, actinomorphic, syncarpous and hypogynous in nature. Seeds are endospermic, brown coloured, sub-cylindrical with round at the base and truncate at the apex. Fruits are persistent, perianth and indehiscent dry utricle, enclosed within bracteoles (Sharma and Chaudhary, 2015).

5. *Phytochemistry*:

Different parts of *Achyranthes aspera* have been phytochemically screened for the presence of various metabolites and their active components have also been identified. They have been reported to possess phytochemicals like alkaloids, tannins, cardiac glycosides, steroids, flavonoids, terpenoids, reducing sugar and saponins, which are responsible for their medicinal and pharmacological activities. Whole plant and their aerial parts have been reported to be rich in saponins and alkaloids with ecdysterone and betaine being the active ingredients. Shoots are rich in ketones, long chain compounds and aliphatic alcohol. Presence of Saponin- A and Saponin- B have been reported in seeds of *Achyranthes aspera*, along with long chain fatty acids and glycosides. Roots have been reported to be rich in ecdysterone, ecdysone and oleanolic acid (Sharma and Chaudhary, 2015). The preliminary phytochemical screening of leaf and flower extracts of *Achyranthes aspera* showed the presence of alkaloids, carbohydrates, flavonoids, proteins, amino acids, tannins, phenols, steroids, glycosides and saponins, which were further qualitatively confirmed by HPTLC analysis of extracts (Kamble, 2018).

6. *Ethnomedicinal uses of Achyranthes aspera*:

Different parts of *Achyranthes aspera* have extensively being used by traditional healers. In "Nighantus" of Ayurveda, the *Achyranthes aspera* has been described as purgative, pungent, digestive, a remedy for inflammation of the internal organs, piles, itch, abdominal enlargements and enlarged cervical glands. Diuretic property of the plant has been extensively utilised by Indian and European physicians. The plant has been used by native of Philippines to relieve toothache, dysentery and bowel complaints. Decoction of its roots have been used in treatment of pneumonia, cough and kidney stones. Tribal of Andhra Pradesh, India, use this plant in treatment of epilepsy and the payasam or kheer made of its seeds is used as a good remedy for brain diseases. The plant has also been useful in treatment of asthma, dyspepsia, bronchitis, abdominal tumor and menstrual disorders (Krishnaveni and Thaakur, 2006). The whole plant has been reported to be useful in indigenous system of medicine for treatment of renal dropsy, bronchial affections and leprosy. They have been attributed with abortifacient, contraceptive, cardiac stimulant, astringent and antiperiodic properties (Tahiliani and Kar, 2000). Paste of flowering spikes or seeds of *Achyranthes aspera* has been used as an external application for bites of poisonous snakes and reptiles, night blindness and cutaneous diseases (Nadkarni and Nadkarni, 1976).

7. *Pharmacological activities of Achyranthes aspera*:

Based on the folklore uses of *Achyranthes aspera*,

its different parts (whole plant, root, leaf, stem, inflorescence, seed) and their bioactive metabolites have been scientifically explored by researchers to identify their effects on animal body functioning and metabolism. This study included the part of plant used, the type of extract prepared, the kind of phytochemical identified or extracted, the dosage administered, mode and duration of administration of drugs, type of study made (*in vitro/in vivo*), the experimental animal used, the methodology adopted for pharmacological study and pharmacological effects of the study. A dramatic upswing in the analytical technologies and techniques paved way for scientific study of different biological activities at molecular level with greater precision and accuracy. This study might bridge the gap between what has been explored and what needs to be unveiled further to pharmacologically utilise this widely used medicinal plant for various combination therapy.

7.1. *Anti- fertility activity*:

In context of traditional use of *Achyranthes aspera* as abortifacient and contraceptive agent, its effects on male and female reproductive systems of experimental animals have been explored. Feeding 50% ethanolic extract of root of *Achyranthes aspera* to male rats at the dose of 50 mg/100 g body weight resulted in reduced sperm count, decrease in weight of epididymis and decline in serum level of testosterone and testicular activity of 3β- hydoxysteroid dehydrogenase. This reproductive toxicity in male rats caused by *Achyranthes aspera* might be due to suppression of synthesis of androgen and elevation of catabolism of testosterone as well as its spermicidal activity (Sandhyakumary *et al.*, 2002). Total alkaloids isolated from *Achyranthes aspera* reduced the weight of testes and accessory glands as well as sperm count in male albino rats (*Rattus norvegicus*) in a dose dependent manner, when orally administered at dosage of 50, 100 and 150 mg/kg body weight/day for 30 consecutive days. No alterations in haematological and serum parameters in experimental rats at the tested dose levels showed anti-fertility effect of alkaloid fraction of *Achyranthes aspera* without any toxic side effects (Kumar *et al.*, 2010). Spermatotoxic effect of a 58- kDa protein isolated from root of *Achyranthes aspera* was reported by Anuja *et al.* (2010). They reported a significant decrease in sperm motility, sperm count and activities of 3β-HSD, 17β-HSD, glucose-6-phosphate dehydrogenase and serum testosterone level as well as an increase in total number of abnormal sperm following oral administration of protein at the dose of 25 mg/kg body weight/day for 35 days in sexually mature male albino mice. No significant change in serum and liver level of AST (Aspartate aminotransferase) and ALT (Alanine transaminase) in the experimental mice as compared to control group showed non- toxic effect of protein isolated from *Achyranthes aspera* root (Anuja *et al.*, 2010). Sarwar *et al.* (2014) reported various alterations in histological sections of male reproductive organs such as, degeneration of leydig cells, spermatogenic disruption and shrinkage of seminiferous tubules along with reduction in secretion of seminal vesicle and prostate and a lesser population of spermatozoa in epididymis after treatment with hydroethanolic extract of root of *Achyranthes aspera*. They have also reported a drastic reduction in implantation sites of fertile female rats mated with treated male rats, advocating for suppressive effect of *Achyranthes aspera* on fertility in males. The ethanol extract of root of *Achyranthes aspera* was screened for its anti-fertility activity in proven fertile albino rats. Oral administration of extract at 200 mg/kg body weight on days 1-7 of pregnancy exhibited 83.30% anti-implantation activity and the rats which continued their pregnancy, failed to deliver any litters after their full term, showing 100% abortifacient activity. The extract also exhibited estrogenic activity tested in immature ovariectomised female albino rats, as shown by significant increase in uterine weight, diameter of uterus, thickness of endometrium and vaginal epithelial cornification (Vasudeva and Sharma,

2006). On the other hand, administration of *Achyranthes aspera* leaves methanolic extract at the dosage 25 and 50 mg/kg body weight were reported to have anti-ovulatory and estrogenic activities, but failed to show any teratogenic, anti-implantation and abortifacient activity. At these dose levels, the *Achyranthes aspera* leaves showed safety in efficacy and might be used as an herbal contraceptives in females (Reddy *et al.*, 2016). Teratogenic toxicity of oral administration with ethanolic extract of leaves of *Achyranthes aspera* (250, 500 and 1000 mg/kg) from day 6-12 of gestation was evident in terms of reduction in number of implantation sites, retardation in development of embryo and foetus, elevation in rate of foetal resorption and death as well as significant reduction in litter weight and Crown-Rump Length (CRL) (Teshome *et al.*, 2021).

7.2. *Hepatoprotective and anti- ulcer activities*:

Achyranthes aspera has been studied for its protective effects against drug as well as metal compounds induced toxicities to the hepatic cells. Methanolic extracts of aerial parts of *Achyranthes aspera* significantly reduced the elevated level of serum liver function markers [SGOT (Serum glutamic oxaloacetic transaminase), SGPT (Serum glutamic pyruvic transaminase), ALP (Alkaline phosphatase), total bilirubin, cholesterol, protein and albumin) in paracetamol and rifampicin treated rats in a dose dependent manner. The extract treatment was reported to exhibit protection against acute hepatocellular damage and biliary obstructions (Bafna and Mishra, 2004; Kumar *et al.*, 2012). Methanolic root extract of *Achyranthes aspera* showed anti- hepatotoxic effects against aflatoxin, a carcinogenic and toxic secondary metabolite of fungus, *Aspergillus* species, through regulation of abnormal activities of serum and liver enzymes as well as enzymatic antioxidants. The findings were comparable to silymarin, a known hepatoprotectant (Pappa and Padmalatha, 2014). In a similar way, aqueous root extract and methanolic extracts of roots and barks of *Achyranthes aspera* exhibited hepatoprotection against sodium arsenate and carbon tetrachloride induced damages to hepatic tissues, advocating for the hypothesis that herbal products from plant sources can be a great alternative for treatment of hepatic diseases (Sharma and Chaudhary, 2016; Fahim and Sathi, 2018). The anti-ulcer activity of ethanolic extract of *Achyranthes aspera* leaf was assessed by pylorus ligation and chronic ethanol induced ulcer model. Pre-treatment with extract reduced the gastric volume and pH in induced ulcer models and provided protection against ulcer in a dose dependent manner. This gastric anti-secretory activity of *Achyranthes aspera* could be due to the presence of flavonoids and tannins, which are responsible for maintenance of efficient gastric mucosal microvascular supply (Das *et al.*, 2012).

7.3. *Diuretic and Nephroprotective activities*:

Diuretic activity by oral administration of aqueous extract of seed of *Achyranthes aspera* at the dose level of 3 g/kg body weight was investigated in goats and results were compared with a standard diuretic drug, furosemide, orally administered at the dose of 5 mg/kg body weight. The effects were studied on various blood and urine parameters; such as, diuresis, pH, creatinine, urea and electrolytes (Na^+, K^+, Cl^-, HCO_3^-). A high diuretic effect and significant renal clearance of electrolytes were observed in seed extract treated and furosemide treated goats as compared to control group (Jahan *et al.*, 2002). Due to having diuretic effects, methanolic extract of whole plant of *Achyranthes aspera* might be useful for the management of hypertension, renal and cardiovascular diseases (Srivastav *et al.*, 2011). Nephroprotective role of methanolic extract of whole plant of *Achyranthes aspera* (200 mg/kg body weight) was evaluated against toxicity induced by administering lead acetate (0.2 %) in drinking water for 6 weeks. The rats showed improvements in body weight, kidney weight, serum and urinary level of urea, uric acid, creatinine and protein, when treated with extract along with induction of toxicity by lead. Activities of urinary enzymes were also increased along with complete amelioration of lead induced

histopathological damage to renal tissues (Jayakumar *et al.*, 2009). Antilithiatic activity of ethanolic leaf extract of *Achyranthes aspera* was supported by reduced urinary level of creatinine, calcium and oxalate ion along with elevated magnesium level in rats treated with 0.75 % of ethylene glycolated water (Awari *et al.*, 2009). Aqueous extract of root of *Achyranthes aspera* has been reported to be efficient in prevention and reduction of the growth of calcium oxalate stone in nephrolithiatic model of mouse treated with ethylene glycol (Aggarwal *et al.*, 2012). Treatment with alcoholic and aqueous extracts of root of *Achyranthes aspera* (300 mg/kg body weight) significantly reversed the serum biochemical alterations induced by adenine (200 mg/kg), showing nephroprotective effect against adenine induced chronic kidney disease (Gehani *et al.*, 2019).

7.4. *Anti-hyperlipidemic, anti- obesity and cardioprotective activities*:

Hypolipidemic activity is characterized by a decrease in serum level of total cholesterol, triglycerides, VLDL (Very low density lipoprotein) cholesterol and LDL (Low density lipoprotein) cholesterol along with an increase in HDL (High density lipoprotein) cholesterol and phospholipid level. Treatment with saponins and ethanolic seed extract of *Achyranthes aspera* showed hypolipidemic activity in rats fed with high fat diet as well as in diabetic rats. Reduction in food efficiency ratio, body weight gain and visceral organ weight indices predicted the weight reducing effects of extracts on high fat diet fed rats, mediated through reduced absorption and elevated excretion of lipids (Latha *et al.*, 2011). Anti-obesity potential of aqueous extract of *Achyranthes aspera* was investigated by Athesh *et al.* (2020) in obese rats induced by high fat diet. In *in-vitro* condition, the extract partially inhibited the activity of pancreatic lipase. In *in-vivo* study, extract treatment significantly reduced the daily food intake, body weight, fat pad weight, size of adipocytes, body mass index, waist circumference and serum level of glucose, insulin and leptin in a dose-dependent manner. Restoration of serum, hepatic and cardiac lipid profiles and atherogenic index showed cardioprotective activity of the extract (Athesh *et al.*, 2020). Ethanol extract of *Achyranthes aspera* seed husk and its crude saponin were also reported to have anti-atherosclerotic potential by regulating lipid profile values and atherogenic index in high fat diet induced hyperlipidemic rats, suggesting for its probable use as a promising cardioprotective drug in management of coronary artery diseases (Sharma *et al.*, 2013).

7.5. *Anti- diabetic activity*:

The glucose lowering or hypoglycemic activity of *Achyranthes aspera* extracts have been studied in diabetic rats induced with either drugs (Alloxan, Streptozotacin) or high fat diet. Oral administration with aqueous and methanolic extract of whole plant of *Achyranthes aspera* (4 g/kg) was reported to reduce blood glucose level in experimental rabbits which were artificially made diabetic by intravenous administration of alloxan monohydrate at the dose of 150 mg/kg (Akhtar *et al.*, 1991). This antidiabetic activity of plant extract can be attributed to the presence of high quantity of mineral elements such as Zinc, Copper, Manganese, magnesium and Calcium (Akhtar *et al.*, 1991). Significant amelioration of alloxan induced diabetic condition by *Achyranthes aspera* was reported in various studies; oral administration with ethanolic extract of stem and leaves (Talukder *et al.*, 2012), aqueous extract of leaves (Kamalakkannam and Balakrishnan, 2015) and ethanolic extract of leaves (Geetha, 2016). In all the previous reports, *Achyranthes aspera* was found to exert its anti- diabetic efficacy on account of its free radical scavenging property which reduces the oxidative stress caused by diabetic condition. The extract not only lowered the level of blood glucose, but also altered the activity of enzymes related with glucose metabolism, such as, glucokinase, glucose-6-phosphatase, glucose-6-phosphate dehydrogenase and the level of hormones related with glucose homeostasis, insulin and glucagon. The anti- diabetic and

related anti-oxidative property of methanolic and petroleum ether extract of leaf of *Achyranthes aspera* was reported in an *in vitro* study also (Priyamvada *et al.*, 2021). A herbal tea formulation prepared from leaves, stem and flowers of *Achyranthes aspera* showed lowering of blood glucose level in alloxan-induced diabetic rats. This anti-diabetic potency of *Achyranthes aspera* tea can be attributed to its phytoconstituents, such as, alkaloids, saponins, flavonoids, phenolics, tannins and phytosterols. Administration of tea (1, 2 and 3 mg/100 ml) also reduced the serum level of total cholesterol and triglycerides that were elevated following diabetic induction with Alloxan (125 mg/kg body weight) (Njideka *et al.*, 2019). Other reports showed anti- diabetic/ anti-hyperglycemic, anti-oxidative as well as hypolipidemic activity of plant extracts in streptozotacin treated animals, seed extract (Vijayaraj and Kumaran, 2018), leaf extract (Lakshmi *et al.*, 2018; Une and Deshpande, 2021) and ethanolic extract of root of *Achyranthes aspera* (Lakshmi and Srivastava, 2019).

7.6. *Anti- inflammatory and Anti- arthritic activities*:

The ethyl alcohol extract of *Achyranthes aspera* root was evaluated for anti- inflammatory activity in Wistar rats using the carrageenan-induced paw edema test (acute inflammatory model) and cotton pellet granuloma test (chronic inflammatory model). Oral administration of extracts at dosage of 50, 100 and 200 mg/kg body weight showed promising anti- inflammatory activity as evident from marked reduction in paw edema (32-40.5%) and suppressed granuloma formation as compared to control group (Kumar *et al.*, 2009). Aqueous, n-butanol and chloroform fractions of *Achyranthes aspera* leaf extract exhibited anti- inflammatory activity against carrageenan-induced paw edema and cotton-pellet induced granuloma in albino rats (Mengie *et al.*, 2021). Silver nanoparticle of ethanolic extract of seed of *Achyranthes aspera* showed better efficacy in anti- inflammatory activity, comparable to the standard drug, Indomethacin (Vijayaraj *et al.*, 2016). Saponin rich fraction of whole plant of *Achyranthes aspera* significantly slowed down the arthritic progression and reversed the pathological changes resulting from Fraud's complete adjuvant induced arthritic development (Kothavade *et al.*, 2015). Ameliorative potential of aqueous leaf extract of *Achyranthes aspera* against subplantar administered formaldehyde induced arthritis was studied in Swiss albino mice and Wistar rats. Oral administration of different dosage of extract for 4 weeks provided dose-dependent inhibition of arthritic symptoms like joint swelling, narrowed joint spaces, increased joint diameter, severe bone erosion with the presence of neutrophil infiltration, increased pannus formation and joint inflammation. The extract treatment also increased the blood haemoglobin level and decreased Erythrocyte sedimentation rate, a good indicators of formaldehyde arthritis (Chinnasamy *et al.*, 2019).

7.7. *Anti- carcinogenic activity*:

Oral treatment of 50 % ethanolic extract of whole plant of *Achyranthes aspera* for 28 days was reported to have ameliorating effects against N-nitrosodiethylamine; a potent hepatocarcinogen present in various food stuffs like milk and meat products, soft drinks and alcoholic beverages, and CCl_4 induced carcinogenic toxicity in Swiss albino rats. This protective effect of *Achyranthes aspera* extracts might be attributed to its free radical scavenging and anti-oxidant potential, as NDEA has been reported to induce carcinogenesis by formation of DNA adduct, induction of gene mutation and oxidative stress by impairment of mitochondrial respiration by free radicals (Kartik *et al.*, 2010). Methanolic leaf extract of *Achyranthes aspera* was reported to have a well defined differential, time and dose- dependent anti-proliferative activity on a panel of cancer cell lines assessed *in vitro*. It also inhibited the expression of pro- metastatic and angiogenic genes (Metalloproteases and VEGFs) in tumor cells (Subbarayan *et al.*, 2010). *In vivo* anti- cancer activity of methanolic leaf extract of *Achyranthes aspera* was reported to be mediated via induction

of apoptosis in human pancreatic cancer cell that was transplanted subcutaneously in athymic mice (Subbarayan *et al.*, 2012). The anticancer potential of 70% ethanol root extract of *Achyranthes aspera* against liver (HEP-2) and colon (HT-29) cancer cell lines might be due to presence of phytoconstituents, like alkaloid, phenolics, flavonoids and terpenoids, which have been reported to have cytotoxic activity. Inhibition of growth of cancer cell lines by root extract treatment can be utilized to formulate herbal anticancer composition that can be used alone or in combination with other chemical drugs to reduce toxicity and side effects of the drug (Singh *et al.*, 2017). Methanolic leaf extract of *Achyranthes aspera* inhibited the proliferation of Dalton's Lymphoma cells by inducing mitochondrial apoptotic cascade mediated through suppressed PKCα signalling pathway, suggesting for its use as a supplementary dietary substance as well as functional food (Singh *et al.*, 2021).

7.8. *Effects on central nervous system*:

Methanolic extract of aeriolar parts of *Achyranthes aspera* showed antinociceptive, analgesic and central nervous system depressant activities by inducing a dose- dependent inhibition of writhing response generated by acetic acid as compared to reference drug; diclofenac sodium, production of rapid onset and maximized duration of sleeping time, decreased motor activity and exploratory behaviour of experimental mice (Alam *et al.*, 2008). Presence of alkaloids, steroids and triterpenes was reported to be responsible for anxiolytic activity of oral administration of methanolic leaf extract of *Achyranthes aspera* in Swiss albino mice (Barua *et al.*, 2012). Significant enhancement in the seizure latency in PTZ (Pentylenetetrazole), Picrotoxin and Bicuculline induced models showed possible anticonvulsant activity of *Achyranthes aspera* methanolic root extract. Increased level of GABA (Gamma aminobutyric acid) in hippocampus and cortex area of brain demonstrated an involvement of GABAergic neurotransmission as a probable mechanism for its anticonvulsant effect (Gawande *et al.*, 2017). A significant reduction in catalepsy, increase in retention time on the rotating rod and improvement in fall-off time along with elevated level of dopamine and DOPAC (3,4-Dihydroxyphenylacetic acid) in brain samples advocated for neuroprotective efficacy of hydroalcoholic extract of whole plant of *Achyranthes aspera* on haloperidol-induced Parkinson's disease in Wistar rats (Chitra *et al.*, 2017). Methanolic extract of aerial parts of *Achyranthes aspera* exhibited significant cerebroprotective activity against ischemia-reperfusion-induced brain injury, possibly mediated through antioxidant and anti-inflammatory actions of the extract (Viswanatha *et al.*, 2019). Hydroethanolic leaf extract of *Achyranthes aspera* exhibited nootropic activity, evaluated using elevated plus maze and radial arm maze tests. Oral administration of extracts for 4 weeks clearly demonstrated the improvement in hippocampus-dependent spatial learning and memory (Bhargavan *et al.*, 2018). Swiss albino mice treated with methanolic extract of root of *Achyranthes aspera* exhibited better cognitive activity on account of modulation in glutamatergic and cholinergic neurotransmission in brain (Gawande *et al.*, 2015). Methanolic root extract of *Achyranthes aspera* and its active component, betaine was found to be beneficial in regulation of stress induced neurobehavioral as well as comorbid complications. Intraperitoneal administration of *Achyranthes aspera* (2.5, 5 and 10 mg/kg) and betaine (20 mg/kg) significantly ameliorated the depression symptoms in CUMS (Chronic unpredictable mild stress) mice. Antidepressant activity of *Achyranthes aspera* was further supported by decreased plasma level of corticosterone and reversal of CUMS induced decline in BDNF elevation in TNF- α and IL- 6 in hippocampus and prefrontal cortex in experimental rodents. These results were comparable with a commercially used anti-depressant drug, fluoxetine (Gawande *et al.*, 2022).

7.9. *Wound healing and anti-oxidant activities*:

Tropical application of aqueous and ethanol extracts of *Achyranthes aspera* leaves at the wound site in an excision as well as incision wound healing model produced significant wound healing activity. The enhanced wound healing by the extract might be due to free radical scavenging and immune-enhancing property of the plant extracts, an outcome of presence of various phytochemicals such as tannins, flavonoids, glycosides and alkaloids (Edwin *et al.*, 2008). In an another study conducted by Barua *et al.* (2012), tropical application of ointment prepared by mixing 5.0 g of methanolic leaf extract of *Achyranthes aspera* in 95 g of white soft petroleum jelly, twice daily for 7 consecutive days, significantly reduced the area of burn wound experimentally created by exposure to aluminium metal rod heated to 85℃ in adult albino rats. This ointment promoted the deposition of collagen fibers, proliferation of fibroblast and epidermis as well as angiogenesis in the wound region. Increased expression of matrix metalloproteinases (MMP-2 and 9) and increased level of hydroxyproline, vitamin C and protein in granulation tissue showed wound healing activity of the ointment applied. Furthermore, the granulation tissue also showed higher activity of antioxidant enzymes, such as, reduced glutathione, SOD (Superoxide dismutase) and CAT (Catalase), advocating for the free radical scavenging property of the ointment applied, that promoted the wound healing in the experimental rats (Barua *et al.*, 2012). The herbal ointment prepared using methanolic leaf extract of *Achyranthes aspera* exhibited wound healing properties in excision as well as incision wound in experimental rats (Fikru *et al.*, 2012). Ethanolic seed extract of *Achyranthes aspera* possessed promising wound healing potential as evidenced by enhanced rate of wound contraction and epithelisation period in excision and thermal burn wound model as well as increased wound breaking strength in incision wound model. The restoration of normal architecture of skin in healed excised wound and enhanced level of antioxidant enzymes (SOD and CAT) and connective tissue marker (hydroxyproline) in dead space wound model also supported the wound healing activity of *Achyranthes aspera*, which are mediated through individual or additive effects of their phytoconstituents (Mondal *et al.*, 2016).

Treatment with herbal ointment prepared with chloroform and n- butanol extract of leaf of *Achyranthes aspera* showed wound healing property in excision wound model (Mengie *et al.*, 2021). Different parts of *Achyranthes aspera* have been explored for its anti-oxidant and free radical scavenging activities studied both *in vitro* and *in vivo*. Oral administration of methanolic leaf extract of *Achyranthes aspera* at dosage of 100 and 200 mg/kg exhibited protection against ethanol induced oxidative damage to the hepatic tissues by reducing the rate of lipid peroxidation as well as restoring the activity of liver function marker enzymes [AST, ALT (Alanine transaminase) and ALP] and enhancing antioxidant defense status [SOD, GST (Glutathione), GR (Glutathione reductase) and GSH (Glutathione)] (Sudha *et al.*, 2012). Tahiliani and Kar (2000) also reported a reduction in hepatic LPO with no alteration in activities of antioxidant enzymes, SOD and CAT after treatment with aqueous leaf extract of *Achyranthes aspera*; advocating for a peroxidative and free radical scavenging activity of the extract. Treatment with aqueous extracts of root and leaf of *Achyranthes aspera* resulted in an increase in antioxidant enzymes [CAT, SOD, GPx (Glutathione peroxidase) and GST (Glutathione S-transferase)] and a decrease in LPO (Lipid peroxidation) in liver tissue homogenate of mice treated with arsenic in a dose dependent manner (Shakeel *et al.*, 2015). Aqueous and alcoholic extracts of root, stem, leaf and seed of *Achyranthes aspera* significantly increased the SOD activity in thymus, spleen and head kidney regions of Asian catfish, *Clarias batrachus* (Jurry *et al.*, 2019). Extracts of *Achyranthes aspera* prepared in different solvents exhibited potential DPPH scavenging activity, suggesting efficient antioxidant capacity of the plant parts (Nechete *et al.*, 2009; Sharma *et al.*, 2014). Methanolic extract of stem- leaves of

Achyranthes aspera showed potential DPPH scavenging activity with IC_{50} value of 30.5 µg/ml, advocating for its potential use as natural antioxidants (Raut *et al.*, 2021).

7.10. *Pro-thyroidic activity*:

Oral administration of aqueous leaf extract of *Achyranthes aspera* not only increased the level of thyroid hormones (T3 and T4) in healthy adult wistar rats, but also exhibited ameliorating effects in Methimazole- induced hypothyroidic rats (Tahiliani and Kar, 2000; Khan *et al.*, 2021).

7.11. *Larvicidal and anti- malaria activities*:

The saponins extracted from ethyl acetate extract of leaf of *Achyranthes aspera* showed larvicidal activity against *Aedes aegypti* and *Culex quinquefasciatus* and can be used as environmentally safe alternative to chemical larvicides to control the vector of dengue and lymphatic filariasis (Bagavan *et al.*, 2008). Green synthesized silver nanoparticles of *Achyranthes aspera* were more efficient than aqueous leaf extract in larvicial activity against three major mosquito vectors belonging to genera *Anopheles*, *Culex* and *Aedes*. Phytochemical analysis of the leaf extract showed its possession of carbohydrates, saponins, coumarins, tannins, glycosides, steroids and flavonoids; most of which have been reported to have detrimental effects on mosquitoes (Elumalai *et al.*, 2016). Although the silver nanocomposites synthesised from *Achyranthes aspera* leaf extract showed excellent larvicidal potential against *Aedes aegypti*; vector of dengue, Chikungunya and Zika, they were found to be non-toxic to non-target organisms including *Gambusia affinis*, *Daphnia magna* and *Moina macrocopa*; thus providing a cost-effective and eco-safe alternative to conventional insecticides (Sharma *et al.*, 2020).

Aqueous extract of *Achyranthes aspera* shoot was evaluated for its potency as an alternative therapy for malaria. *Achyranthes aspera* did not exhibit any toxicity up to a single dose of 5000 mg/kg body weight. Administration of aqueous extract at the dosage of 400 and 600 mg/kg significantly reduced the parasitemia, comparable to the chloroquine and artemisinin combination therapy. The extract treatment not only prolonged the survival time of mice, but also prevented the *Plasmodium berghei* induced body weight loss and temperature reduction (Mankilik *et al.*, 2021).

7.12. *Antimicrobial and antihelminthic activities*:

The water and acetone extracts of *Achyranthes aspera* leaves collected from two different geographical conditions; Ciaat, Eritrea and Ukulinga, South Africa, were evaluated for antibacterial activities against gram negative bacteria (*Escherichia coli* and *Klebsiella pneumonia*), gram positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*), anti- fungal activity against a filamentous yeast- like fungus (*Candida albicans*) and anti- helminthic activity against a free living nematode (*Caenorhabditis elegans*). The variation in level of functional phenolic compounds, protein binding capacity and bioactivities assayed by extracts of different geographical conditions might be associated with varying environmental conditions of habitat, developmental stage and genetic polymorphism (Ndhlala *et al.*, 2015). *In vitro* antihelminthic activity of aqueous and methanolic leaf extracts of *Achyranthes aspera* against Indian adult earthworm, *Pheretima posthuma*, was evident from the induction of paralysis in the worms, ranging from loss of motility to loss of response to external stimuli, gradually leading to death. Methanolic leaf extract showed better antihelminthic activity, when compared to the activity of aqueous extract and reference standard drug, Albendazole (Jadhav *et al.*, 2017). Aqueous extracts of root and stem of *Achyranthes aspera* were assessed for their antibacterial activity against *Vibrio alginolyticus*, a pathogen causing life- threatening infections in immune-compromised individuals. Significant zones of inhibition portrayed by extracts can be attributed to the presence of alkaloids and tannins in the plant extracts (Unissa *et al.*, 2017).

Two-dimensional ultrathin sharp- edges magnesium oxide nanosheets were synthesized using aqueous extract of leaf of *Achyranthes aspera*

and were evaluated for their anti- bacterial and anti-fungal potential against *Eggerthella lenta*, *Enterobacter aerogenes*, *Candida albicans* and *Aspergillus niger* using Agar well diffusion method. A considerable antibacterial and fungicidal efficacy of nanosheets might be due to active free radicals rendered by MgO that lead to formation of inhibition halo around the MgO nanomaterial (Pavithra *et al.*, 2020). Silver nanoparticles synthesized using fresh leaves of *Achyranthes aspera* showed anti- fungal efficacy against *Aspergilus niger*, *Aspergilus flavus* and *Fusarium oxysporum*. Smallest myecilum diameter in agar dishes treated with *Achyranthes aspera* silver nanoparticles (20 and 40 ppm) after 96 h of inoculation advocated for its application as alternative eco-friendly fungicide in fields of medicine and agriculture (Le *et al.*, 2020).

Methanolic root extract of *Achyranthes aspera* and one of its pure compound oleanolic acid exhibited antiviral activity against herpes complex virus type 1 (HSV-1) and type 2 (HSV-2), with higher efficiency shown by oleanolic acid. The time-course study between 0 and 6 h post-infection showed inhibitory effects of extracts on early stage of viral replication rather than inhibition of viral adsorption or penetration. The non-amplification of DNA isolated from infected cultures treated with extracts also advocated for the above finding. Thus, *Achyranthes aspera* could be a potent herbal alternative for treatment of HSV infections due to its inhibitory ability, high SI value and ability to induce release of proinflammatory cytokines, IL-6 and IL-12 (Mukherjee *et al.*, 2013). A molecular docking study done by Dutta *et al.* (2021) reported significant inhibitory activity of 8 anti-HIV compounds isolated from different parts of *Achyranthes aspera* against SARS-Co-2. Out of them, oleanolic acid showed highest binding affinity with the targeted proteins (ACE-2, Mpro, RdRp) and these results were comparable with the conventionally used drugs (Chloroquine, Hydroxychloroquine and Remdesivir) for treatment of SARS- Co- 2 symptoms. These compounds were found non- toxic for human consumption. Antihepatitis B activity of methanolic extract of *Achyranthes aspera* leaves were also reported. The extract showed antiviral activity in a dose- dependent manner and the findings were comparable to reference drug, Elan-PA001. Findings suggested the involvement of polar active molecules, such as, alkaloids, flavonoids and other ingredients with hydroxyl groups, in the inhibitory activity of extracts against HBV multiplication (Bhavya *et al.*, 2021).

7.13. *Immunomodulatory activity*:

Immunomodulatory activities of *Achyranthes aspera* have been scientifically explored in mammalian model as well as in fishes. Alcohol, petroleum ether and 50 % aqueous alcohol fractions of seeds and raw seed of *Achyranthes aspera* were investigated for their effects on growth and immune system of larvae of common carp, *Cyprinus caprio*. The parameters studied were survival and specific growth rate of fish, serum level of protein, albumin and globulin, SGOT (Serum glutamic oxaloacetic transaminase) and SGPT (Serum glutamic pyruvic transaminase), assessment of haemagglutination titer against chicken red blood cells and myeloperoxidase activity. Highest survival and immune enhancement was observed in the raw *Achyranthes aspera* seed incorporated diet fed group fishes. The active components responsible for growth and immune stimulating effects were found to be ecdysterone and two essential fatty acids, linolenic acid and oleic acid (Chakrabarti *et al.*, 2012). Adult *Catla catla* and *Cyprinus carpio*, fed with *Achyranthes aspera* seed incorporated diet for 4 weeks were reported to have increased immunity and enhanced antigen clearance, determined by presence of Bovine Serum Albumin (BSA) particles in electron micrographs of spleen sections (Chakrabarti and Vasudeva, 2006; Chakrabarti and Rao, 2012). A potentiation of specific and non- specific immunity was observed in Indian major carp, *Labeo rohita*, fed with *Achyranthes aspera* root incorporated experimental diet. A significant increase in hemagglutination antibody titer, serum level of globulin and protein as well as elevation of

RNA/DNA ratio in spleen were observed in fishes immunized with chicken RBC (Rao *et al.*, 2004). In a subsequent study, experimental diet incorporated with *Achyranthes aspera* leaves and ripe seeds not only decreased the cumulative mortality rate but also increased the serum myeloperoxidase and nitric oxide synthase activity in head kidney and hepatopancreas of *Labeo rohita* fry, challenged with *Aeromonas hydrophila* in natural pond condition. Level of TBARS (Thiobarbituric acid reactive substance) and carbonyl proteins were decreased in herb fed rohu. The expression of immune related genes were also altered, supporting the fact that this herbal formulation can exhibit protection against bacterial pathogen to all age groups of *Labeo rohita* in laboratory as well as natural pond condition (Kumar *et al.*, 2019). Dietary supplementation of seeds of *Achyranthes aspera* for 50 days significantly increased the respiratory burst activity, bactericidal activity and antibody response in *Catla catla*, immunized with heat-killed *Aeromonas hydrophila*. Experimental diet also regulated the expression of immune related genes, such as, pro-inflammatory cytokine IL-10, lysozyme c and g, TNF-α in kidney, gill and hepatopancreas of fish (Chakrabarti *et al.*, 2014). Asian catfish, *Clarias batrachus*, fed with supplementary feed composed of leaves and seeds of *Achyranthes aspera* showed higher serum level of lysozyme, myeloperoxidase and nitric oxide synthase, higher expression of immune related genes, TNF- α, iNOS and NF- κB along with elevation in haemagglutination titer against immunization with chicken RBC, when compared to control group fish. Additionally, lower level of TBARS and carbonyl protein in liver and kidney tissues of fish fed with enriched diet showed immunostimulatory activity of the plant parts (Kumar *et al.*, 2022).

Intraperitoneal administration of *Achyranthes aspera* extract enhanced the induction of Ovalbumin (OVA)- specific humoral antibody response in mice in a dose dependent manner. It significantly elevated the serum level of IgM, IgG1 and IgG3 antibodies, but anti- OVA IgE antibody response was suppressed. Administration of seed and root extracts showed higher immune stimulatory activities as compared to stem and leaf of the plant (Vasudeva *et al.*, 2002). Immunomodulatory activities of methanolic and aqueous extracts of whole plant of *Achyranthes aspera* were investigated in Swiss albino mice and Wistar albino rats. Acute oral toxicity assessment of extracts suggested the safety of use at the dose of 2000 mg/kg. Pre-treatment with extracts at the dose of 200 mg/kg orally for 13 days before intravenous administration of cyclophosphamide on 11th, 12th and 13th day, exhibited protection from myelosuppression by increasing the level of RBC, haemoglobin, platelets and total WBC as well as per cent count of neutrophils, lymphocytes, monocytes, eosinophils and basophils in Swiss albino mice. The treatment with extracts also showed protection from Arthus type immediate hypersensitivity by producing marked decrease in paw edema in Wistar albino rats, comparable to standard drug, prednisolone. This immunomodulatory activity might be due to presence of alkaloids, steroids (β-sitosterol), glycosides, terpenoids and flavonoids in leaf extracts of *Achyranthes aspera* (Kolli *et al.*, 2021).

7.14. *Miscellaneous activities*:

Achyranthes radix extracts administration resulted in stimulation of proliferation and activity of bone forming osteoblasts and inhibition of generation of bone resorbing osteoclasts; thus having potential to enhance the bone metabolism in osteoporosis (Kim *et al.*, 2005). The bronchoprotective effect of ethanolic extract of *Achyranthes aspera* was studied in toluene diisocyanate induced occupational asthma in wistar rats. Intranasal application of 10 % TDI (Toluene diisocyanate) was used to induce airway hypersensitivity. Treatment with *Achyranthes aspera* did not show any airway abnormality. The extract treatment significantly inhibited the symptoms of TDI induced asthma by reducing the level of neutrophils and eosinophils in blood and bronchoalveolar fluid and restoring the

histopathological changes in lung tissues. Amelioration of oxidative stress might be responsible for its activity against asthma symptoms (Goyal *et al.*, 2008). Aqueous stem extract of *Achyranthes aspera* exhibited inhibitory activity against *Bitis arietans* venom protease and Phospholipase A2 activity, supporting the folklore of its use in treatment of snake bites (Nwune *et al.*, 2017). Petroleum ether, chloroform and methanolic extracts of whole plant of *Achyranthes aspera* showed anti- allergy activity against heavy metal potassium dichromate induced allergy in albino mice in a dose dependent manner. Extract treatment for 7 days after induction of allergy gradually decreased the total and differential WBC count, serum immunoglobulin titres of IgG and IgM, which were increased during the allergic reaction. Highest anti- allergy effect was seen with methanolic extract treatment (Kamble, 2018). Ingestion of methanolic leaf extract of *Achyranthes aspera* for 4 days resulted in a dose dependent changes in haematological parameters, such as Total White Blood Cells, Lymphocyte, Neutrophil, Monocyte, Platelet, Red Blood Cells, Haemoglobin and Packed Cell Volume; advocating for its potential of being effective in the treatment of diseases caused by thrombocytopenia and lymphocytopenia. Acute toxicity test showed that the methanolic extract of leaf may be toxically and clinically safe for intake by mammals and mice at dosage below 4000 mg/kg body weight (Etim *et al.*, 2019). Pharmacokinetics and molecular docking studies of 19 phytocompounds of *Achyranthes aspera*, selected through GC-MS analysis, showed potential anti-tuberculosis activity by inhibiting binding with *Mycobacterium tuberculosis* H37Rv proteins; which are promising targets for developing therapeutics against the disease (Beg *et al.*, 2020). *Achyranthes aspera* can be used in dentistry, as it has been extensively used by people in various forms to brush the teeth, to relieve toothache and treating pyorrhea (Jaisankar *et al.*, 2020). Aqueous extract of stem and root of *Achyranthes aspera* have been reported to exhibit anti- bacterial activity against *Streptococcus mutans*, an oral pathogen responsible for dental caries and periodontal diseases (Yadav *et al.*, 2016). The protective effect against bacterial growth can be due to presence of alkaloids and tannins in the plant extract (Yadav *et al.*, 2016). Anti-thrombosis or thrombolytic activity exhibited by methanolic extract of stem-leaves of *Achyranthes aspera*, assessed through *in vitro* clot lysis in human blood provided evidence for its effectiveness in prevention and treatment of disease conditions associated with obstruction in blood flow, such as heart attack and massive pulmonary embolism (Raut *et al.*, 2021).

7.15. *Clinical studies*:

Efficacy of *Achyranthes aspera* was clinically tested in the management of reactions in leprosy. A decoction of whole plant of *Achyranthes aspera*; prepared by Ayurvedic classic method, was orally infused in 12 patients of leprosy, twice daily at the dose of 1 oz. After 30 days of treatment, the patients not only showed marked improvement in the reaction, but also there was a fall in bacterial index and complete subsidence of oedema (Ojha *et al.*, 1966). Aqueous- based gel prepared by using pure root extract of *Achyranthes aspera* was used to test its efficacy in the management of chronic periodontitis, an inflammatory disease of microbial origin. Thirty patients with chronic periodontitis were taken into consideration and 15 of which were treated with scaling and root planing followed by subgingival application of *Achyranthes aspera* gel, and they exhibited good prognosis without any side effects. Antimicrobial, antioxidant, anti-inflammatory and immune-stimulant properties of *Achyranthes aspera* might be playing role in its beneficial activity as a non-surgical local drug delivery system in management of periodontitis (Boyapati *et al.*, 2017).

Conclusion

Achyranthes aspera has potential to strengthen every possible biological activity necessary for functioning of a healthy body. These activities have been scientifically explored and proved by many means and in different experimental animals. But there is still very few evidences on

actual mode of operation of these extracts and how these can affect an individual on cumulative basis. The plant has a potential of being a profound source of naturally available active pharmaceutical product that can be used for easily accessible, cheaply available herbal supplement for healthy functioning of the body as well as in combination therapy for treatment of deadly diseases.

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