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Review Article

Therapeutic and Pharmacological Efficacy of *Achyranthes aspera* Linn.: An Updated Review

Senthil Kumar Raju* , Shridharshini Kumar , Praveen Sekar , Maruthamuthu Murugesan , Mohanapriya Karthikeyan , Anjana Elampulakkadu , Mythili Arthanari

Department of Pharmaceutical Chemistry, Swamy Vivekanandha College of Pharmacy, Tiruchengode – 637 205, Tamilnadu, India

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Abstract



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*Address for Correspondence:

Dr. R. Senthil Kumar, Professor & Head,
Department of Pharmaceutical Chemistry, Swamy
Vivekanandha College of Pharmacy,
Tiruchengode, Tamilnadu, India

Achyranthes aspera is an erect perennial herb that comes under the family Amaranthaceae. The plant and its parts are traditionally used to cure several diseases such as renal dropsy, hemorrhoids, dysentery, asthma, cough, stomach ache, skin eruptions, diarrhea, dysentery, stimulating labor pain, nose-bleeding, snake-bites, nose bleeding and dilating blood vessels. Secondary metabolites such as alkaloids, saponins, tannins, flavonoids, glycosides, steroids, essential oil and fatty acids play a significant role in exhibiting increased bioactivity against a variety of diseases. Particularly, the presence of secondary metabolites including achyranthine, ecdysterone, oleanolic acid, spinasterol, apigenin, achyrantheric acid, ursolic acid, corrosolic acid, betaine are playing an important role in producing the potent pharmacological actions. *A. aspera* is widely used as purgative, laxative, astringent, diuretic and also as digestive. This review is particularly focused on the pharmacological and therapeutic potential of *A. aspera* and critically analyse the bioactivities such as antioxidant, antibacterial, tuberculocidal, antifungal, antiviral, anticancer, anti-diabetic, anti-hyperlipidaemic, anti-diuretic, anti-obesity, anti-arthritis, anti-inflammatory, periodontitis, cerebro-protective, antiepileptic, anti-depressant, anti-parkinson, anxiolytic, bronchodilator, hepato-protective, haemorrhoids, reproductive and anti-venom activity along with the toxicity studies.

Keywords: *Achyranthes aspera*, Amaranthaceae, phytoconstituents, antioxidant, anticancer, anti-inflammatory, wound healing, biological activity.

INTRODUCTION

Natural resources are the country's most valuable asset and supply crucial healthcare services to the people. Secondary metabolites include alkaloids, flavonoids, glycosides, phenol, phlobatannin, tannins, terpenoids, volatile oils and other metabolites that enable plants to treat a wide range of ailments, from minor headaches to life-threatening diseases. The fundamental benefit of employing plant products as folk remedies is that they contain enormous phytochemical constituents, which help to neutralize negative effects while also enhancing synergistic effects. Screening must be done to determine the active principle of a medicinal plant by isolating and characterizing the phytoconstituents present in order to validate its specific usage. The main advantage of plant-based medications are less expensive and safer for humans than modern synthetic medicines^{1,2}.

Achyranthes aspera (Family: Amaranthaceae), is an important perennial herb widely distributed in the tropical and subtropical regions of India, Afghanistan, Africa, Algeria, Australia, Bahamas, Bolivia, Bhutan, Cameroon, Caribbean, Colombia, Cook Islands, Cuba, Fiji, Florida, Hawaii, Guam, Indonesia, Italy, Jordan, Laos, Lesotho, Malaysia, Mauritius, Myanmar, Mexico, Namibia, Pakistan, Philippines, Saint Lucia, Singapore, Sri Lanka, Spain, Syria, Tunisia, Tanzania, Uganda,

and Zimbabwe. Almost all the parts including leaves, seeds and roots of the plant are highly responsible for the medicinal uses. It is mostly grows as a weed along roadsides, on uncultivated plains and on the outskirts of cultivated grounds in India. *A. aspera* is used to treat renal dropsy, piles, stomach aches, skin eruptions, diarrhoea, dysentery, promoting labour pain, nosebleeds, snake bites, asthma, gonorrhoea, wound healing, cancer and menorrhagia. Achyranthine, a water-soluble alkaloid present in *A. aspera* is highly responsible for dilation of blood vessels, cardiac depression, blood pressure reduction and increasing the amplitude and rate of respiration³⁻⁷.

Ethnopharmacological claims revealed that *A. aspera* has been used to treat a wide range of ailments since ancient times. Many countries employ it in their traditional medical systems. It's used to treat asthma, abdominal tumours, haemorrhoids, gynaecological diseases, ophthalmia, odontalgia, snake bites and wound healing in India. It is used in Bangladesh to treat skin injuries and abdominal tumors. It's also used to treat malaria symptoms in Kenya. It is used to treat cardiac edema, dermatological diseases, diabetes mellitus, and renal edema in Sri Lanka and Pakistan. *A. aspera* is used to treat arthritis, contraception, delayed menses, induced absorption and osteoarthritis in South Korea⁸.

An extensive literature survey was performed and we found that a review that partially focused on the pharmacological activities including anti-allergy, anti-arthritis, anti-cancer, anti-depressant, anti-inflammatory, anti-microbial, thrombolytic activities, etc.^{6,7} One more review article which was published in 2017, reported the phytochemistry and pharmacological activities for the entire genus *Achyranthes*⁸. Rafia rehman et al., in 2018 published a review that is mainly focused on the pharmacognostical aspects of *A. aspera*⁹. This review aims to provide a summary of existing knowledge on pharmacology and toxicity of the Indian medicinal plant, *A. aspera* and to critically analyze the reported studies. The present review focused on the collection of data in the time range 2011-2021 from standard kinds of literature that are indexed in ScienceDirect, PubMed, Springer, Google Scholar,

ResearchGate and EMBASE databases using the keyword *Achyranthes aspera*.

PHARMACOLOGICAL APPLICATIONS

A. aspera is a highly impactable medicinal plant that contributes to its various biological applications by the existence of secondary metabolites in the different parts of the plant including leaves, herb, root, stem and seeds. The biological applications are unique to that particular species of the plant due to the presence of particular secondary metabolites constantly^{9,10}. The various pharmacological applications of *A. aspera* are discussed as follows.

Table 1: Antioxidant activity of *A. aspera*

S.No	Part Used	Solvent Used	Concentration (µg/ml)/ Time	Method Used	Scavenging Activity (%/µg/ml)
1.	Inflorescence	Methanol	700/60 min	DPPH free radical scavenging assay	93.05±0.18 ¹⁰
2.	Whole plant	Ethyl acetate	100 µg/ml	DPPH free radical scavenging assay	25.12 ¹⁵
3.	Whole plant	Infused extract	100 µg/ml	DPPH free radical scavenging assay	70.75±1.22 mgTE/g
				ABTS scavenging assay	84.48±3.14 mgTE/g ¹⁶
4.	Leaves	Methanol	400 µg/ml	DPPH free radical scavenging assay	150 µg/ml
				ABTS scavenging assay	60 µg/ml
				FRAP scavenging assay	34 µgTE/mgDE ¹⁷
5.	Leaves	Ethanol	150/20 min	DPPH free radical scavenging assay	62.24 (µg/ml)
				Hydrogen peroxide scavenging assay	68.32 (µg/ml) ¹⁸
6.	Root	Ethanol	100/30 min	DPPH free radical scavenging assay	92.75
	Inflorescence	Chloroform			84.80 ¹⁹
7.	Leaves	Aqueous	100/3 h	Nitric oxide scavenging assay	71.1±0.37
			100/10 min	Lipid peroxidation assay	74.6±0.1 ²⁰
8.	Whole plant	Hexane	100/2 h	DPPH free radical scavenging assay	953.16±72.58 (µg/ml) ²¹
9.	Leaves	Methanol	100/40 min	DPPH free radical scavenging assay	80.00 ²²
10.	Stems and leaves	Ethanol	400/15 min	DPPH free radical scavenging assay	243.7 (µg/ml)
				Nitric oxide scavenging assay	39.0 (µg/ml)
				Hydrogen peroxide scavenging assay	69.9 (µg/ml) ²³
11.	Root	Ethyl acetate	200/90 min	DPPH free radical scavenging assay	93.00 ²⁴
12.	Leaves	Methanol	50 µg/5 min	DPPH free radical scavenging assay	45.41
	Stem				22.52
	Root				33.07 ²⁵
13.	Leaves	Ethanol	500 µg/ml	DPPH free radical scavenging assay	6.41±0.11 (mg/ml) ²⁶
14.	Whole plant	70 % aqueous ethanol	100 µg/ml	FRAP scavenging assay	2.408±0.002
				DPPH free radical scavenging assay	28.051±0.057
				ABTS scavenging assay	59.782±0.207 ²⁷
15.	Leaves	Petroleum ether	250 µg/ml	DPPH free radical scavenging assay	63.06±0.56
		Methanol			68±0.44 ²⁸

Antioxidant activity

Antioxidants are the essential sources for human beings on maintaining a balanced healthy lifestyle which is highly present in *A. aspera*⁶. Oxidation is a reaction, it is a highly important one but unfortunately due to the generation of reactive oxygen species (ROS), it gets damaged. ROS is a by-product of air pollution, herbicides, metabolic processes, pesticides and immunoreactions toward the pathogens. In scavenging these ROS, phytoconstituents including flavonoids and phenolic contents play a vital role. The antioxidant potential of the methanolic extract of the prickly *A. aspera* was found to be more in the inflorescence part (93%). For n-hexane extract of *A. aspera*, the antioxidant potential was more in the root. For chloroform extract of *A. aspera*, antioxidant activity was more in stem whereas the ethyl acetate extract of inflorescences showed high radical scavenging ability. On comparing the antioxidant potential of these extracts, the results showed that the scavenging potential was more in methanol extract¹⁰.

UPLC-PDA and MALDI-TOF-MS were used to assess the antioxidant capacity of phenolic compounds such as quinic acid, chlorogenic acid, kaempferol, quercetin, and chrysin in a hydromethanolic extract of *A. aspera*. Phenolic compounds have a wide range of activities, including free radical scavengers, antioxidants, and oxidative degradation inhibitors. Based on the results of UPLC-PDA and MALDI-TOF-MS, the hydromethanol extract was found to be rich in phenolic compounds, indicating that it has great antioxidant potential by raising the activity of CAT, GST, GR, and SOD while lowering the activity of LDH¹¹. The antioxidant potential of the ointment of *A. aspera* was determined by applying externally to the experimental burn wound of rats by using gelatin zymography and histopathology. The result indicated that 5 % (w/w) ointment of methanolic extract of *A. aspera* showed an increased concentration of the antioxidant parameters including catalase (CAT), superoxide dismutase (SOD) and glutathione (GSH) in the granulation tissues of the rat comparatively¹².

Saponins identified in the seeds of *A. aspera* result in exhibiting greater free radical scavenging potential by the reports of ABTS, FRAP and SOD assay. FRAP radical scavenging assay indicated greater antioxidant potential which is due to the reduction of ferric ions, since antioxidants are reducing agents. Antioxidant serum levels showed better improvement with the saponins. Because of the existence of phenolics and flavonoids, *A. aspera* seed saponins ameliorate oxidative damage¹³. The enzymatic antioxidants SOD, CAT, glutathione peroxidase (GPx), and GSH were measured using an ethanolic extract of *A. aspera* seeds. The results showed that the ethanolic extract of the plant boosted SOD and CAT activity in the diabetes-induced rat's liver tissues. The actions of SOD, CAT, and GPx are also responsible for the increased ability to scavenge free radicals¹⁴. The antioxidant activities of various extracts of the plant *A. aspera* are given in Table 1.

Antibacterial activity

Because of the high number of early deaths around the world, there is a constant need to identify new antimicrobial agents with novel mechanisms. The antibacterial activity of *A. aspera* leaves was investigated using the microdilution bioassay method using aqueous and acetone extracts against gram-positive organisms such as *Bacillus subtilis* and *Staphylococcus aureus* and gram-negative organisms such as *Escherichia coli*

and *Klebsiella pneumoniae*. The results indicated that the acetone extract had a high antibacterial capability against the gram-negative *E. coli* and *K. pneumoniae*, with a MIC of 0.78 mg/ml^{10,29}.

Plants are found to be the best source which has a large amount of anti-microbial agents. According to several reports, it is proved that the extracts of the various plants possess bactericidal and bacteriostatic effects. Antibacterial activity of the seeds *A. aspera* was studied using various solvents namely hexane, chloroform and ethanol using the hole-plate diffusion method against *B. subtilis*, *Micrococcus luteus*, *S. aureus*, *E. coli*, *Pseudomonas aeruginosa* and *Salmonella choleraesuis*. Comparatively, the chloroform seeds extract of *A. aspera* exhibited greater activity towards *B. subtilis*, *E. coli* and *P. aeruginosa* which is rich in saponins, hydrocarbons and amino acids³⁰.

Antibacterial potential of the root extract was determined against *B. subtilis*, *P. aeruginosa*, *E. coli*, *K. pneumoniae*, *Salmonella sp.* and *S. aureus* using the solvents namely hexane, chloroform and water. The results indicated that the ethanolic root extract exhibited strong antibacterial potential towards *K. pneumoniae*³¹. The antibacterial efficiency of *A. aspera* was studied on the dental plaque against *Streptococci*, *Staphylococci* and *E. coli* using the aqueous extract. The extract exhibited about 8 % of susceptibility to all of these organisms³². The absolute alcohol extract of leaves showed 0.25-4 mg/ml of MIC value towards *Xanthomonas vesicatoria* using the broth micro-dilution method³³. For the stem and root extract of *A. aspera*, the antibacterial potential was determined against *Streptococcus mutan* using the agar cup-plate method. The results indicated that about 10 % concentration of the stem and root extract showed 16mm and 21mm of the zone of inhibition. The antibacterial potential of *A. aspera* is highly due to tannins and alkaloids because of its water solubility³⁴.

Nanocomposite cotton fabrics-based copper nanoparticles were synthesized using the leaves extract of *A. aspera* and its antibacterial activity was studied against *S. aureus* and *P. aeruginosa* using the standard disc diffusion method. The results revealed that both the bacteria exhibited strong bacteria-killing activity and indicated that if the concentration increases, the zone of inhibition also increases³⁵. The methanolic leaves extract exhibited better antibacterial potential towards *Streptococcus pyogenes* of about 83.3±28.9 mm zone of inhibition at the concentration of 400mg/ml. Phytochemicals like alkaloids, terpenoids, tannins, flavonoids, steroids and phenols play a responsible role in exhibiting a broad spectrum of activity³⁶. The antibacterial activity of various extracts of plant *A. aspera* are given in Table 2.

Antituberculosis activity

Tuberculosis is a deadly disease caused by a highly professional pathogen namely *Mycobacterium tuberculosis*. *Mycobacterium* primarily causes pulmonary tuberculosis but it results in extrapulmonary TB by spreading to the other parts of the body. Using the molecular docking technique, the tuberculocidal activity of the phytoconstituents of *A. aspera* was evaluated using *M. tuberculosis* H₃₇Rv. Among various constituents, 17-pentatriacontanol, ecdysone, oleanolic acid, spinasterol, β-sitosterol and stigmasta-5,22-dien-3-ol showed greater anti-tuberculosis activity. Particularly, oleanolic acid showed greater activity with the highest docking score⁴⁷.

Table 2: Antibacterial activity of *A.aspera*

S.No	Part Used	Tested organism	Method	Concentration (µg/ml)	Zone of Inhibition (mm)
1.	Root	<i>S.aureus</i>	Agar well cut diffusion method	25 mg/ml	22 ¹⁰
2.	Leaves	<i>S.aureus</i>	Agar well diffusion method	5 mg/ml	21.3±2.65 ¹⁸
3.	Root	<i>K.pneumonia</i>	Agar well diffusion method	50	27±0.26 ³¹
4.	Stem	<i>S.aureus</i>	Agar cup diffusion method	250	40
		<i>E.coli</i>		250	10 ³⁷
5.	Whole plant	<i>B.subtilis</i>	Agar well diffusion method	40 mg/ml	18
		<i>S.aureus</i>		40 mg/ml	16 ³⁸
6.	Leaves	<i>P.aeruginosa</i>	Disc diffusion method	100	12
		<i>S.aureus</i>			13 ³⁹
7.	Root	<i>S.mutans</i>	Disc diffusion method	50 µg/disc	18 ⁴⁰
8.	Seeds	<i>B.subtilis</i>	Agar well diffusion method	50	26
		<i>Proteus vulgaris</i>		50	28 ⁴¹
9.	Whole plant	<i>S.mutans</i>	Agar well diffusion method	125	23±2 ⁴²
10.	Leaves	<i>Salmonella typhi</i>	Agar well diffusion method	0.29 mg/ml	17 ⁴³
11.	Leaves	<i>E.coli</i>	Agar-agar diffusion method	75	18.3±1.3 ⁴⁴
12.	Leaves	<i>Enterobacter aerogenes</i>	Agar well diffusion method	500	11 ⁴⁵
13.	Leaves	<i>Salmonella enteritidis</i>	Agar well diffusion method	100 mg/ml	31±0.37
		<i>S.gallinarum</i>			30±0.26 ⁴⁶

Antifungal activity

Antifungal activity of the methanolic extract of *A. aspera* was determined against *Alternaria sp.*, *Fusarium oxysporum*, *Rhizoctonia solani* and *Sclerotium rolfsii* using the poisoned food technique. The results indicated that if the concentration of plant extract increases, fungal growth decreases. The antifungal activity of *R. solani* was greater (1.2 cm of the zone of inhibition) at 62.5 µg/ml of plant extract concentration. At the concentration of 50 µg/ml, *Alternaria sp.* showed significant (0.3 cm of the zone of inhibition) antifungal potential. But *F. oxysporum* showed less sensitivity as compared to other fungal species¹. The leaf extract of *A. aspera* was evaluated for the antifungal activity towards *C. albicans* using the microdilution method against the standard drug Amphotericin B. The aqueous extract showed 0.79 mg/ml of minimum inhibitory concentration (MIC) whereas the acetone extract showed 0.39 mg/ml of MIC. Both the extracts exhibited greater activity toward *C. albicans*²⁹.

The root extract of *A. aspera* was determined for its antifungal potential against *Saccharomyces cerevisiae*, *C. albicans* and *A. niger* using the agar well diffusion method. The aqueous root extract showed greater inhibition toward *S. cerevisiae* and moderate inhibition toward *C. albicans* and *A. niger*³¹. Prasad and co-workers determined the antifungal activity of leaves extract of *A. aspera* against *Aspergillus ochraceus*, *Aspergillus flavipes*, *Penicillium sp.* and *Fusarium verticillioides* using the broth micro-dilution method. The alcohol and chloroform extract showed about 2.0-4.0 mg/ml of MIC towards all the fungal strains and the benzene extract of leaves showed about 10 mg/ml of MIC towards *Penicillium sp.*³³. The antifungal activity of the acetone and ethanolic extract of *A. aspera* was determined against *Aspergillus niger*, *Verticillium*, *Fusarium oxysporum* and *Candidia sp.* using the agar cup diffusion

method. The acetone extract showed effective antifungal efficacy against *F. oxysporum* with the zone of inhibition ranging about 10 mm (fruit), 15 mm (leaf), 16 mm (root) and 18 mm (stem) than ethanolic extract. The acetone extract of root and fruit controlled the growth of *Verticillium* at 6mm of the zone of inhibition and the acetone extract of leaf and stem inhibited the growth of *A. niger* at 4mm³⁷. The chloroform extract of the *A. aspera* showed moderate inhibition towards *Microsporum canis* with 0.25 mg/ml (Minimum Inhibitory Concentration) whereas the hexane and butanol extracts showed considerable inhibition towards *Aspergillus flavus* and *M. canis*⁴³.

Antidermatophytic activity

Dermatophytic infections are directly connected with the skin fungal infections including ringworm and tinea, due to the temperature. Using various extracts such as hexane, chloroform, methanol and ethyl acetate, the anti-dermatophytic activity of the leaves of *A. aspera*, ethyl acetate showed greater activity with the MFC value of about 15.62 µg/ml at the concentration of 7.81 µg/ml against *Trichophyton rubrum*⁴⁸.

Anthelmintic activity

Several researchers tested the anthelmintic activity of various *A. aspera* extracts. Using a quick colorimetric microdilution test, the anthelmintic activity of the aqueous and acetone extract of *A. aspera* leaves was investigated against a free-living worm, *Caenorhabditis elegans*. The acetone extract exhibited greater activity with the MLC value of about 0.59-0.78 mg/ml²⁹. The ethanolic leaves extract of *A. aspera* was evaluated for the anti-helminthic activity using an anti-helminthic assay against the standard Albendazole. At the highest concentration of about 80 mg/ml, the ethanolic extract

showed greater activity with the paralyzing and death time of about 42.18 ± 0.26 mins and 38.46 ± 0.23 mins against the worm *Phertima posthuma*⁴⁹.

Antiviral activity

The antiviral potential of methanolic root of the plant extract against herpes simplex virus type-1 (HSV-1) and herpes simplex virus type-2 (HSV-2) was investigated. With an EC₅₀ value of 64.4 g/ml for HSV-1 and 72.8 g/ml for HSV-2, the methanolic extract showed weak anti-herpes virus activity. On the other hand, the triterpene acid present in *A. aspera* showed greater anti-herpes virus activity with the EC₅₀ value of 6.8 µg/ml for HSV-1 and 7.8 µg/ml for HSV-2 time-dependently after 2-6 hours of treatment. The result also showed the enhanced level of pro-inflammatory cytokines namely IL6 and IL12⁵⁰. The root infusion or the whole plant decoction of *A. aspera* was used to manage HIV/AIDS-related diseases including oral infections, sexually transmitted infections, skin infections, cough, tuberculosis and diarrhea⁵¹. The methanolic extract of *A. aspera* was found to have greater anti-HIV activity, and the extract showed excellent activity in the TZM-b1 cell line with the lowest IC₈₀ value of about 4.8-53 µg/ml. Against the PM1 cell line, the extract exhibited considerable activity with IC₈₀ values ranging from 1-9 µg/ml. Phytoconstituents namely α-spinasterol, asarone, β-sitosterol, ecdysone, oleanolic acid, sapogenin, spathulenol, stigma-5,22-dien-3-ol were evaluated for the antiviral activity toward SARS-CoV-2 using molecular docking study with the help of three proteins namely ACE 2 (Angiotensin-converting enzyme-2), Mpro (Main protease) and RdRp (RNA-dependent RNA polymerase) as targets. Among these eight compounds, oleanolic acid showed greater activity with the inhibition constant of about 0.30 µM against ACE-2, 2.69 µM against Mpro and 3.77 µM against RdRp protein⁵².

Anticancer activity

Cancer is the major cause of death in India next to cardiovascular diseases. The anticancer potential has been determined by several researchers. Using the annexin-fluorescein isothiocyanate-conjugated assay, the anticancer activity of *A. aspera* towards COLO320DM (Human colon cancer cell line), AGS (Hyperdiploid human cell line), MCF-7 (Human breast cancer cell line) and A549 (Human lung cancer cell line). The hexane extract of *A. aspera* showed a considerably low IC₅₀ value towards MCF-7 (63.43 µg/ml) and AGS (84.27 µg/ml). The mechanisms include preventing cancer cells proliferation through the suppression of proliferative markers²¹. The anticancer efficacy of leaves, stem and root extracts of *A. aspera* was determined by MTT assay against HeLa (Human cervical cancer cell line) using various extracts such as ethyl acetate, acetone, ethanol and methanol. Among various extracts, ethanolic extract showed 90 % of cytotoxicity toward HeLa cancer cells⁵³. The antiproliferation potential of the phytoconstituents present in the root extract of *A. aspera* was determined using an MTT assay against HT-29 (Colon cancer cell line). Three phytoconstituents namely ursolic acid, corrosolic acid and achyrantheric acid were isolated from the root extract and exhibited potent anticancer potential against HT-29 cancer cells⁵⁴.

The alkaloid fraction of leaves of *A. aspera* is widely used in the treatment of cancer particularly in the treatment of cervix and breast cancer. With the apoptosis and necrosis mechanism of the myeloid cells, *A. aspera* produces cell death in association with cyclin-dependent kinase (CDK) and the p53 gene. The results indicated that the alkaloids present in the leaves of *A. aspera* exhibited greater apoptotic activity in the breast cancer cell by inhibiting the level of CDK mammary cancer cells and upregulating the p53 protein expression, as compared to the standard drug Cyclophosphamide⁵⁵. The leaf

extract of *A. aspera* showed the decreased growth of human pancreatic cancer cell BxPC-3-luc-2 which is subcutaneously transplanted to the athymic mice. The mechanism includes inhibition of Akt phosphorylation and caspase-3 activation resulting in cell apoptosis⁵⁶. The polyphenolic compounds of *A. aspera* (PCA) have synergistic antiproliferation potential. Narayan and co-workers determined the anticancer activity of PCA in urethane-induced lung cancer. The anticancer efficacy of PCA was analyzed using RT-PCR at the transcriptional level. The result showed that the downregulates the expression of pro-inflammatory cytokines namely IL-1β, IL-6 and TNF-α (along with NF-κB, STAT-3 and TFS) and enhances the expression of pro-apoptotic proteins namely Bax and p53⁵⁷.

The anticancer activity of the root extract of *A. aspera* was determined against COLO-205 (Human colon cancer cell line) using the ethanolic and aqueous extract. The aqueous root extract exhibited greater cytotoxicity towards COLO-205 cells and increased the expression levels of p16, p21, p27, Bax, caspase-3 and caspase-9 and decreased the expression of Bcl-2. The aqueous extract induces cell death by arresting the cell cycle at S-phase⁵⁸. The *in vitro* cytotoxic potential of the methanolic leaves extract on Non-hodgkin lymphoma was determined using an MTT assay. The results revealed that the methanolic extract exhibited greater cytotoxicity by changing the morphology, decreasing the potential of the mitochondrial membrane and by suppressing cell proliferation. In inducing cell apoptosis, methanolic leaves extract regulated the Bcl-2 family proteins to release cytochrome C and activated the caspase-3 and caspase-9. The methanolic leaves extract suppresses protein kinase Cα (PKC α) signaling pathway by inducing mitochondrial apoptotic cascade in DL cells⁵⁹. In healing the umbilical granuloma by chemical cauterization, *A. aspera* is highly recommended. An umbilical granuloma is a common neonatal umbilical abnormality that occurs because of the umbilical tissue overgrowth during the healing process. The alkaline preparation of *A. aspera* is used in the form of kshara karma (*Apamarga pratisaraneeya kshara*) which is applied over the umbilical granuloma for about 7 days resulting in complete healing of granuloma⁶⁰.

Antidiabetic activity

Diabetes mellitus is one of the world's major causes of death. *A. aspera* is used in the treatment of diabetes mellitus since ancient days without any scientific evidence. The ethanolic seeds extract of *A. aspera* at the dose of 300-600 mg/kg was used to determine the anti-diabetic activity against Streptozocin (STZ) induced diabetes in rats. After 28 days of oral administration of the extract results in reducing the blood glucose level as compared to the standard drug Glibenclamide¹⁴. The plant extract lowers the blood glucose concentration thereby preventing oxidative stress in alloxan-induced diabetes in mice. The presence of triterpenoid namely oleanolic acid in *A. aspera* enhances the insulin secretion in response to INS-1 832/13 cells and islets of rats and it does not increase the concentration of intracellular Ca²⁺ ions and cAMP. The oleanolic acid is responsible for inhibiting α-glucosidase and activating TGR5 G-protein receptors resulting in lowering glucose levels by increasing its sensitivity²³. The methanolic extract of the plant showed 55.0±0.50 % of α-amylase inhibition and 53.06±0.23 % of α-glucosidase inhibition at a dose of 160 µg/ml²⁸.

The anti-diabetic effect of ethanolic extract of *A. aspera* was tested against STZ induces diabetes in the rat. After treatment with the aqueous extract of *A. aspera*, it showed significantly low levels of blood glucose⁶¹. From the seeds of *A. aspera*, the proteinaceous α-amylase inhibitor was identified using electrophoretic analysis. The α-amylase inhibitor which is isolated was found to be resistant to proteolysis and heat⁶². The ethanolic extract of *A. aspera* was evaluated for its anti-

diabetic activity in alloxan-induced diabetic rats. The ethanolic extract showed 95 % of anti-diabetic efficiency by decreasing the BSL of the pancreas at a dose of 400 mg/kg when given orally⁶³. The *in vitro* glucose uptake studies were evaluated in investigating the anti-diabetic activity by determining the inhibitory activity of α -amylase. The methanolic extract of a dose 5-25 mg/ml exhibited 29.75-71.97 % of α -amylase inhibitory potential⁶⁴. The anti-diabetic effect of tea of *A. aspera* was found to have better activity with a fasting blood glucose concentration of about 229.4 mg/dl after one week of administration⁶⁵.

Hypolipidaemic activity

Hyperlipidaemia is a condition that occurs due to increased fat consumption and by altering the levels of triglycerides (TG) and total cholesterol (TC) in plasma and tissues. On the other hand, the reduced level of high-density lipoprotein (HDL-C), increased level of low-density lipoproteins (LDL-C) and TG were found to increase the cardiovascular risk. Hypolipidaemic drugs such as fibrates and statins are used to treat these disorders. Saponins present in the seeds of *A. aspera* showed improvement in serum lipid profile by increasing HDL-C levels and decreasing LDL-C, TG and TC in hyperlipidaemic rats¹³. The herbal tea made from stem, leaves and flowers of *A. aspera* does not show significant activity on HDL and LDL cholesterol but showed a greater reduction in TG level⁶⁵. Saponins present in *A. aspera* was found to possess significant hypolipidaemic and weight-reducing activity on rat by reducing the absorption and increasing the excretion of lipids and the extraction showed a significantly increased level of serum HDL-C⁶⁶.

Thrombolytic activity

Blood clots (thrombus) are generated by homeostasis, on the other hand, it contributes to coronary blood diseases including acute myocardial infarction and cerebral haemorrhage resulting in death. The methanolic extract of *A. aspera* exhibited better clot lysis efficacy at a minimum dose⁶⁶.

Antidiuretic activity

Diuretics are the agents that stimulate the excretion of urine and electrolytes from the body. The crude aqueous extract of *A. aspera* showed greater diuretic, kaliuretic and natriuretic activity in albino rats against the standard drug Furosemide. Lipschitz values revealed that the crude extract exhibited about 46 % of antidiuretic efficacy at the dose of 50 mg/kg with no toxicity even at higher doses².

Anti-Obesity activity

Obesity is the primary cause of many disorders in the world. Several parameters such as consuming fast foods, high-fat diets and sedentary lifestyle results in obesity, which may lead to diabetes, hypertension, myocardial infarction and peripheral vascular diseases. In treating obesity, various anorectic and thermogenic drugs are used which reduce the absorption of nutrients and affect the utilization and mobilization of lipids. The saponin fraction of seeds of *A. aspera* showed a reduction in body weight and BMI in rats by lowering the concentration of the lipid, lowering LDL, VLDL, TG and TC levels resulting in increasing HDL levels¹³. The ethanolic seeds extract of *A. aspera* reduces the enhancement of body weight and retroperitoneal adipose tissue and also reduces the level of LDL, TG and TC by the delayed intestinal absorption and inhibition of pancreatic lipase and amylase activity in mice⁶⁷. The aqueous extract showed anti-obesity activity by delaying the intestinal absorption, inhibiting the action of pancreatic lipase, enhancing the status of antioxidants particularly, flavones and by regulating the metabolism of fat⁶⁸.

Antipyretic activity

Pyrexia is the same as that of fever in which the temperature was 1.8-3.6 °F higher than the normal body temperature. The methanolic leaves extract of *A. aspera* exhibited better activity by reducing the elevated body temperature induced by yeast and by maintaining the normal temperature in a dose-dependent manner. The methanolic extract was found to synthesize a potent anti-prostaglandin that regulates the temperature balance between the loss and production of heat⁶⁹.

Antiarthritic activity

Rheumatoid arthritis (RA) is a chronic inflammatory illness that causes diarthrodial joint stiffness, discomfort, and symmetrical synovitis, as well as subchondral bone abnormalities and continuous articular cartilage destruction. Disease-modifying anti-rheumatic drugs (DMARDs), including sulfasalazine and methotrexate and NSAIDs including naproxen, ibuprofen and aceclofenac in combination with steroidal hormones like prednisone and cortisone are used to treat rheumatic arthritis clinically. The saponin fraction-treated mice showed less inflammation on joints and synovial hyperplasia at the highest dose of about 300 mg/kg. The saponin fraction exhibited better anti-arthritis activity by decreasing the progression of arthritis, inhibiting the production of TNF- α and IL-1 β , by slowing bone erosion and soft tissue inflammation⁷⁰. The anti-arthritis potential of triterpenoid saponins found in *A. aspera* against Freund's full adjuvant-induced arthritis in rats was investigated. After 28 days of treatment, hyperalgesic rats treated with saponin extract (100 mg/kg) showed significant improvement in pain thresholding. The saponin fraction exhibited significant inhibitory activity towards the development of arthritis by decreasing the progression of arthritis, reversing pathological changes and by decreasing the level of cytokines namely TNF- α and some other pro-inflammatory factors⁷¹.

In a dose-dependent manner, the aqueous extract of the plant demonstrated a greater anti-arthritis effect. The extract had a significant inhibitory effect on the course of rheumatoid arthritis at concentrations ranging from 250 to 500 mg/kg. At the concentration of 500 mg/kg, the aqueous extract showed prevention of joint swelling, destruction and narrowing joint space. Phytochemicals such as flavonoids and phenolic compounds are responsible for the suppression of inflammation. At the concentration of 200 mg/kg, the aqueous extract showed inhibition of bone resorption, pannus formation, and joint inflammation with reduced neutrophil infiltration⁷².

Anti-inflammatory activity

Inflammation is a vascularized living tissue response to the local injury caused by free radicals, immunological reactions and microbial infections. Despite the fact that inflammation is a protective response, it is associated with fever, pain and loss of function. To relieve these symptoms, efficient steroidal or non-steroidal anti-inflammatory agents (NSAIDs) are used. But, as per the reports about 33 % of NSAIDs result in peptic ulcers. Therefore, there is an urgent need to design effective and safe anti-inflammatory agents with the least or no side effects. The ethanolic extract of *A. aspera* was used for determining the anti-inflammatory effect on Carrageenan (injected in sub plantar route) induced paw edema using an acute mice model. After one hour of injection, the ethanolic leaves extract showed 25.71 % of maximum percentage inhibition and the ethanolic stem extract showed 35.17 % of maximum percentage inhibition as compared with the standard drug Diclofenac sodium. After 3 hours of injection, the ethanolic leaves extract showed 28.98 % of maximum

percentage inhibition which is more effective than stem extract and the standard drug^{37,73}.

Using the plethysmographic method, the anti-inflammatory activity of the aqueous extract of *A. aspera* was compared with the standard drug Diclofenac sodium in the Carrageenan (injected intraperitoneally) induced edema in mice. Dose ranging from 400-800 mg/kg of the aqueous extract exhibited stronger activity and the maximum percentage inhibition was found to be 35.71-54.76 %⁷⁴. The anti-inflammatory effect of ethanolic extract of *A. aspera* was determined. The ethanolic extract showed up to a 60 % reduction in producing nitric oxide (NO) without toxicity and down-regulating the inflammatory gene's mRNA expression levels. By inducing the activity of TNF- α and MyDss (adaptor molecule), ethanolic extract of *A. aspera* could block the promotion activity of NF- κ B and it also inhibited the nuclear translocation of p65 and I κ B α phosphorylation⁷⁵.

The analgesic activity of the methanolic extract of *A. aspera* on the acetic acid-induced writhing test in mice was determined. The methanolic extract showed significant inhibition on writhing response which is induced by acetic acid at a dose ranging from 50-200 mg/kg as compared to the standard Diclofenac sodium. The methanolic extract at 200 mg/kg and Diclofenac sodium at 10 mg/kg exhibited 78.68 % and 81.09 % of inhibition⁷⁶. Ambreen and co-workers evaluated the anti-inflammatory potential of tannins isolated from the leaf callus culture of *A. aspera* against the Carrageenan-induced paw edema in rabbits. The results revealed that the callus culture extract of *A. aspera* showed maximum percentage inhibition and decreased paw edema size and thickness⁷⁷. The 80 % methanol leaf extract of *A. aspera* was investigated for its anti-inflammatory effect in rats using the Carrageenan-induced paw edema technique. The chloroform fraction of 80 % methanolic leaf extract showed greater inhibitory action (52.50 %) against chronic inflammation proliferation as compared with Indomethacin⁷⁸. The aqueous extract of *A. aspera* at a dose of 800 mg/kg showed greater inhibition of paw edema in mice as compared to Indomethacin⁷⁹.

Effect on Periodontitis

Periodontitis is a severe gum infection that is an array of inflammatory diseases mainly composed of anaerobic gram-negative bacteria. *A. aspera* gel was used in the treatment of chronic periodontitis, combined with scaling and root planing. The extract had a stronger effect with no side effects. PPD (Probing pocket depths) and CAL (Clinical attachment levels) characteristics are assessed in the treatment of periodontitis. The extract showed decreased PPD in the range of 5.96-2.92 mm and increased CAL level in the range of 1.03-0.30 mm⁸⁰.

Cerebroprotective activity

Ischemic stroke is a disability cause of death. Acute ischemic stroke is a serious condition that results in rapidly activating microglia and astrocyte resident cells and infiltration of T-cells, granulocytes and monocytes in the experimental animals. The highest dose of about 800 mg/kg of methanolic extract of *A. aspera* showed greater cerebroprotection towards the ischemic reperfusion-induced neurocognitive alteration in Wistar rats after 7 days of treatment. In the histopathological evaluation of the brain, extract at the concentration 800 mg/kg exhibited moderate protection towards ischemic reperfusion-induced histological brain as compared to the standard quercetin. The presence of phytoconstituents in the methanolic extract including apigenin, acteoside, glucose, gossypin, pentagalloyl glucose and rutin are responsible for producing synergistic cerebral protective activity. Particularly, apigenin, quercetin, rutin, gossypin and pentagalloyl glucose are playing a defensive role in ischemic reperfusion-induced brain injury⁸¹.

Anticonvulsant activity

Epilepsy is a neurological disease that occurs when there is an imbalance between excitatory glutamate-mediated and inhibitory GABA-mediated neurotransmission. Many anti-epileptic agents are used as GABA modulators and GABAergic agonists. The aqueous root extract of *A. aspera* showed greater enhancement in thresholding the seizure and greater anticonvulsant effect by increasing the GABAergic neurotransmission in the brain⁸². The methanolic extract of *A. aspera* showed dose-dependent efficacy, at the concentration of about 400 mg/kg, the extract showed enhancement in GABA levels and GABA_A receptor density⁸³. The dried root powder was given orally with water in the epileptic condition in order to regain consciousness⁸⁴.

Antidepressant activity

Hypnosedation is principally mediated by GABA_A receptor complex in the CNS, which is also engaged in some other neurological and physiological disorders including epilepsy, depression, Alzheimer's disease and Parkinson's syndrome. In treating these diseased conditions, most of the drugs are involved to modify the synthesis level of GABA which induces hypnosis in animals by strengthening GABA-mediated postsynaptic inhibition by allosteric modification of GABA receptors. Barbituric acid is the agent which directly/indirectly enhances the chloride conductance along with simultaneous depression of voltage-activated Ca⁺⁺ currents. The antidepressant activity of the methanolic extract was estimated using a hole cross test, open field test, force swimming test and tail suspension test against the standard Imipramine hydrochloride. The extract showed significant antidepressant activity⁷⁷. The ethanolic extract of *A. aspera* was evaluated for the antidepressant activity using the rotarod method and actophotometer test. The ethanolic extract showed significant activity and triterpenoid saponins A and B played a greater role in exhibiting the pharmacological actions⁸⁵. Due to the presence of long-chain alkanes namely hexatriacontane, the methanolic root extract of *A. aspera* showed greater antidepressant activity by increasing the monoamine levels⁸⁶.

The methanolic extract of *A. aspera* showed potent antidepressant activity by improving the swimming activity through the agonists of GABA (gamma-aminobutyric acid), BDNF (brain-derived neurotrophic factor), NT-3 (neurotrophin), kappa opioid receptor, melanocortin-4, glutamate metabotropic receptors and delta-opioid agonists⁸⁷.

Antiparkinson activity

Parkinson's disease is a CNS disorder that is characterized by the inability in maintaining a normal posture and by degrading the dopamine-carrying neurons in substantia nigra with tremor, rigidity and bradykinesia like extrapyramidal symptoms. Prolonged usage of antipsychotic drugs namely Haloperidol, exhibits dopamine receptor blockade in the corpus striatum and produced extrapyramidal effects like Parkinson's disease. The antiparkinson effectiveness of a hydroalcoholic extract of *A. aspera* was tested in Wistar rats with haloperidol-induced Parkinson's disease. The extract showed a greater antiparkinson effect by significantly reducing lipid peroxidation and enhancing the antioxidants in the brain⁸⁸.

Anxiolytic activity

Anxiety is a type of obsessive-compulsive disease that includes panic attacks, anxiety, post-traumatic stress, and phobias. It is the most common mental illness on the globe. Physical dependence, muscle relaxation, anterograde amnesia, and sedation are the common side effects of benzodiazepines, which are used as first-line anxiolytics. *A. aspera* is found all

over the world and can be used to treat anxiety disorders. The methanolic extract of *A. aspera* exhibited greater anxiolytic activity which is highly based on alkaloids, triterpenes and steroids present in the plant¹². Methanolic leaves extract of *A. aspera* was evaluated for anxiolytic activity using behavioral animal models. The study showed that the test animal mostly passed to the light compartment than the dark compartment. Diazepam is used as a standard and increases the activity of the light/dark test. The estimation of anxiety-related behavior in the animal model is based on the hypothesis that the anxiety of animals was compared with the anxiety of humans⁷⁷.

The 400 mg/kg ethanolic extract of *A. aspera* showed muscle relaxation, decreasing locomotor activity and stronger anxiolytic activity in mice as compared with the standard Diazepam⁸⁵. The Anti-anxiety effect can be identified by increasing the head dipping and earliness of head dipping. The result indicated that in the hole board test, a decrease in the head dipping behavior exhibited anxiogenic and anxiolytic behavior. Most of the first-line anxiolytic agents increase monoamine activity through the inhibition of the enzyme that breaks down monoamine and by blocking its reuptake in the synaptic cleft. Most commonly used anxiolytic agents are Benzodiazepines which amplify GABAergic inhibitory transmission⁸⁷.

Effect on Eye

The eyes are the most vital organ of our body. The eye irritation potential must be evaluated for the eye cosmetic products to provide assurance to the consumers that the product is safe to use. The aqueous leaves extract of *A. aspera* was evaluated for the eye irritation potential in rabbits using an acute eye irritation test and Hen's egg chorioallantoic membrane test. The extract had an irritation score of 0.07 in the *in vitro* Hen's egg chorioallantoic membrane test and 0.55 in the *in vitro* acute ocular irritation test. The results showed that the aqueous extract does not exhibit any eye irritation on both *in vivo* and *in vitro* tests⁸⁹. In the treatment of night blindness, root extract is used in the form of eye drops at bedtime⁹⁰.

Bronchodilatory activity

Bronchodilators are the agents used to breathe easily by widening the bronchi. The crude extract of *A. aspera* showed (100 µg/ml) greater activity by inhibiting carbachol (CCh) induced bronchospasms as compared to Aminophylline. The crude extract relaxed the high K⁺ in CCh (1 µM) induced trachea contraction in the guinea pig. The results showed that the crude extract caused a non-parallel shift at higher concentrations while at a lower concentration, it caused a rightwards parallel shift in CCh concentration-response curves⁹¹.

Hepatoprotective activity

The liver is an essential organ that plays an important role in eliminating and detoxifying the toxic substances and in intermediary metabolism. Hepatic damage is a serious condition associated with decreased metabolic functions. Many synthetic agents were used in the treatment of liver damage but these agents produced undesirable side effects. Therefore, the ethanolic extract of *A. aspera* was used to evaluate the hepatoprotective efficacy using the paracetamol-induced toxicity in rats against the standard silymarin. The functions of the liver can be estimated by the activities of enzymes 5'-nucleotidase, SGOT, SGPT (*Serum glutamic pyruvic transaminase*), ALP and LDH which are highly present in the cytoplasm and also by using the bilirubin level. The ethanolic extract of the plant showed a reduction in the elevated bilirubin and urea levels in the toxicity-induced rat which

confirmed the hepatoprotective action of the extract⁹². The methanolic extract showed greater hepatoprotective by inhibiting cytochrome P450, activating reticuloendothelial functions and by stabilizing the endoplasmic reticulum results in hepatic regeneration in toxicity induced rats without any mortality⁹³. The hepatoprotective activity of methanolic root and bark extract of *A. aspera* was identified against carbon tetrachloride (CCl₄)-induced hepatotoxicity in rats was investigated. Hepatotoxicity biomarkers include SGPT and serum bilirubin levels. The methanolic root extract possessed significant efficiency against CCl₄-induced toxicity. At the doses of 10, 20 and 30 mg/kg, the bilirubin level was found to be 6.05±0.2 mg/dl, 4.04±0.34 mg/dl and 7.5±1 mg/dl and the SGPT level was found to be 223.2 U/L, 167.82 U/L and 190.4 U/L⁹⁴.

Gastroprotective activity

Due to an imbalance between aggressive and gastroprotective forces, gastroesophageal ulcers and hyperacidity are prevalent concern. Various drugs including H₂ receptor antagonists and proton pump inhibitors are generally used but they are found to produce drug interactions and severe side effects. Antiulcer activity of the aqueous leaves extract of *A. aspera* was determined using pH, acidity, gastric volume and ulcer index. When compared to Ranitidine, the aqueous extract demonstrated a substantial reduction in ulcer index and gastric acid output at a dose of 200 mg/kg²⁰. In the chronic ethanol-induced ulcer model, the ethanolic leaves extract showed 59.55 % ulcer prevention at a dose of 600 mg/kg by lowering the gastric acid volume and dramatically elevating gastric pH. Because of the presence of flavonoids, tannins and saponins, ethanolic leaves extract produced significant antiulcer activity⁹⁵.

Anti-diarrheal activity

Diarrhea is a contrary pathophysiological state of the gut. The crude extract of *A. aspera* showed enhanced fecal output at 3-10 mg/kg, on the other hand, the crude extract showed protective activity against the castor oil-induced diarrhea in mice at the higher doses (30-700 mg/kg) in mice. The crude extract possessed significant anti-diarrhoeal potential by mediating the cyproheptadine-sensitive receptors and by blocking the dual cholinergic and calcium channels⁹¹.

Effect on Haemorrhoids

Hemorrhoids are otherwise called piles which result in swelling of the rectum and anus by enlarging the blood vessels. The alkaline form of *A. aspera* (Apamarga Tikshna Kshara) was used topically in the management of 1st and 2nd-degree internal piles. After the second week of treatment for *A. aspera*, bleeding in the rectum stopped completely. The rectum bleeding stopped completely after the third week of sclerotherapy. The direct intravascular coagulation used in sclerotherapy takes some time to halt the bleeding. From the results of clinical trials, alkaline *A. aspera* application showed greater efficacy in the management of 1st and 2nd-degree internal piles than sclerotherapy⁹⁶.

Effect on Reproductive System

The achyranthes protein, 58kDa, was extracted from the ethanolic root extract of *A. aspera* to determine the *in vivo* spermicidal activity. The achyranthes protein showed significant spermicidal activity by decreasing the action of membrane-bound 5'-nucleotidase and acrosin enzyme as compared to the standard Nonoxynol 9. This protein also exhibited greater *in vitro* spermicidal activity toward human sperms⁹⁷. The testicular activity of the methanolic leaves extract was studied in male albino rats. The extract showed a significantly decreased weight of testis of about 0.925±0.015 at the dose of 50 mg/100g of body weight. The results

revealed that the methanolic extract inhibited the testicular function of mice by arresting spermatogenesis without side effects⁹⁸.

The ethanolic extract of *A. aspera* was evaluated in female albino rats for the antifertility potential. The ethanolic extract showed antifertility and anti-estrogenic effects by reducing the weight of the reproductive organ and the number of implants without exerting any toxicity⁹⁹. The extracts of *A. aspera* exhibited greater potential for the gynaecological disorders. During the painful menstruation and amenorrhoea, the leaves decoction was given for 3 mornings orally to relieve the pain and also to facilitate the delivery¹⁰⁰.

Immunomodulatory activity

Immunostimulants are drugs that improve the ability of an immune system to fight infections by increasing specific or non-specific defence mechanisms. Polyphenolic compounds of extract of *A. aspera* were evaluated for cytokine-based immunomodulatory effects and it showed greater immunostimulant activity in mice. The seeds and root extract of the plant were found to possess greater immunomodulatory effects^{11,101}. These immunostimulants can activate several components of the immune system including B-lymphocytes, T-lymphocytes, phagocytes, lysozyme and natural killer cells. The aqueous seed extract of *A. aspera* showed significant immunostimulant activity¹⁰².

Wound healing activity

Wound healing is a multi-phased process that includes granulation, inflammation, wound contraction, epithelialization, neo-vascularization, and fibrogenesis, all of which contribute to the restoration of anatomical function. RNA, DNA, and total proteins are the key biochemical indicators used to assess wound healing. 5 % *A. aspera* ointment treated mice exhibited significantly higher activity in wound contraction than the control animals¹². Using the leaf callus culture of *A. aspera*, the wound healing potential was evaluated for the tannins isolated from the leaf callus culture. The burn wound model showed the best results on wound contraction, hydroxyproline content and tensile strength of dead space⁷⁸. A 10 % (w/w) chloroform extract ointment of *A. aspera* showed greater results on wound contraction and reduced epithelialization period⁷⁹. Hydroxyproline can be tested to determine collagenesis in wound healing. All these characteristics were found to be high in *A. aspera* treated group compared with the standard povidone-iodine treated group¹⁰³.

5.0 % (w/w) ointment of methanol extract of *A. aspera* was studied for wound healing efficacy. The result of wound healing activity in various wounds, including burns, diabetic and immunocompromised wounds, revealed a reduction in wound area as well as an increase in various enzymatic and non-enzymatic antioxidants such as *catalase*, *super oxidase dismutase*, hydroxyproline, reduced glutathione, protein and ascorbic acid¹⁰⁴. The rats treated with 5 % methanolic leaf extract ointment exhibited greater wound healing activity as compared with the normal ointment treated rats. 10 % methanolic extract ointment treated rats showed greater healing potential by increasing the number of fibrocytes, remarkable degree of epithelialization and neovascularisation after 21 days of treatment¹⁰⁵.

Antivenom activity

Snakebite is a common cause of death in rural areas. According to the reports on the global burden of snakebite, about 1.2 to 5.5 million snakebite incidences occur per year in the world. About 35,000-50,000 people are dead per year out of 2 lakhs reported cases in India. The ethanolic and aqueous leaves extract of *A. aspera* showed a significant anti-venom

effect by neutralizing the main enzymatic and toxic efficacy of snake venom of the family Viperidae. Among these two extracts, the aqueous extract showed greater potency than the ethanolic extract in neutralizing the lethality and in inactivating some other pharmacological effects of snake venom¹⁰⁶.

TOXICITY STUDIES

Toxicology approaches such as acute and subacute toxicity are commonly utilized to evaluate natural medications. In the subacute toxicity test, the hydroethanolic leaves extract of *A. aspera* does not cause death in rats, even at a higher dose of 2000 mg/kg. The chronic toxicity studies revealed that the extract of *A. aspera* exhibited detrimental effects in developing rat fetuses and embryos at higher doses. Its teratogenic efficacy was determined by the substantial reduction of embryos and foetal development, decreased implantation sites and resorption of the foetus resulting in foetal death¹⁰⁷.

CONCLUSION

The review highlighted the presence of various phytochemicals present in *A. aspera* used in the treatment of several diseases including hemorrhoids, arthritis, infectious diseases, cancer, diabetes, anxiety, Parkinson's disease, depression, epilepsy, obesity, bronchitis, eye infections, snake-bites and in healing the wounds due to the existence of a wide range of phytochemicals in its stem, root, seeds and leaves. The presence of the phytochemicals such as achyranthine, ecdysterone, oleanolic acid, spinasterol, apigenin, achyrantheric acid, ursolic acid, corosolic acid, betaine, etc. played a responsible role in exhibiting potent activity. Especially, oleanolic acid, achyranthine and ecdysterone contributed their role in various biological applications. Flavonoids and phenolic compounds are found to have better antioxidant potential. The presence of alkaloids, tannins, terpenoids, flavonoids, and steroids in *A. aspera* contributed significantly to its antimicrobial activity. Achyrantheric acid, ursolic acid and corosolic acid are responsible for the treatment of cancer. The presence of triterpenoid namely oleanolic acid showed better potency in the treatment of diabetes and tuberculosis. Furthermore, studies are required to obtain the optimum efficacy of this multidisciplinary plant.

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LIST OF ABBREVIATIONS

ABTS-2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); ACE-2-Angiotensin-converting enzyme-2; ALP-Alkaline phosphatase; Bax-2-BCL2 Associated X; BDNF-Brain Derived Neurotrophic Factor; CAL-Clinical Attachment Levels; CAT-Catalase; CDK-Cyclin-Dependent Kinase; Akt-Ak strain Transforming; DMARDs-Disease-modifying anti-rheumatic drugs; DNA-Deoxyribo Nucleic Acid; FRAP-Ferric Reducing Antioxidant Power Assay; GABA-Gamma-Amino Butyric Acid; GPx-Glutathione Peroxidase; GSH-Glutathione; GST-Glutathione S-Transferase; HDL-High Density Lipoprotein; HIV/AIDS-Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome; HSV-1-Herpes Simplex Virus

type-1; HSV-2-Herpes Simplex Virus type-2; IL-1 β -Interleukin 1 beta; IL-6-Interleukin 6; I κ B α -Nuclear factor of Kappa light polypeptide gene enhancer in B-cells inhibitor, alpha; LDH-Lactate Dehydrogenase; LDL-Low Density Lipoprotein; MALDI-TOF-MS-Matrix-Assisted Laser Desorption/Ionization-Time of Flight mass spectrometry; MIC-Minimum Inhibitory Concentration; Mpro-Main protease; MTT-3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; NF- κ B-Nuclear Factor Kappa B; NO-Nitric Oxide; NT-3-Neurotrophin-type 3; PKC α -Protein Kinase C α ; PPD-Pocket Probing Depths; RdRp-RNA-dependent RNA polymerase; RNA-Ribo Nucleic Acid; ROS-Reactive Oxygen Species; RT-PCR-Reverse Transcription-Polymerase Chain Reaction; SARS-CoV-2-severe acute respiratory syndrome coronavirus 2; SOD-Superoxide Dismutase; STAT-3-Signal Transducer and Activator of Transcription 3; TC-Total Cholesterol; TGR5-G Protein-coupled bile acid receptor; TG-Triglycerides; TNF- α -Tumour Necrosis Factor alpha; UPLC-PDA-Ultra-performance liquid chromatography-photodiode array detection; VLDL-Very Low Density Lipoprotein.

REFERENCES

- Baraik B, Jain P, Sharma HP. *Achyranthes aspera* L.: As a Source of Bio-fungicide. *Am J Adv Drug Deliv.* 2014;2(6):686-96.
- Asif M, Jabeen Q, Atif M, Majid AM, Qamar-Uz-Zaman M. Diuretic activity of *Achyranthes aspera* linn crude aqueous extract in albino rats. *Trop J Pharm Res.* 2014; 13(12):2039-45. DOI: <https://dx.doi.org/10.4314/tjpr.v13i12.14>.
- K. M. Nadkarni, *Indian Materia Medica*, Popular Prakashan, Bombay, Vol. 1, 1976, p.21-22.
- The Wealth of India: A Dictionary of Indian Raw Materials and Industrial Products (Industrial Products—Part I). *Ind Med Gaz.* 1985; (1A):55-57.
- Dhale DA, Bhoi S. Pharmacognostic Characterization and Phytochemical Screening of *Achyranthes aspera* Linn. *Curr Agric Res J.* 2013; 1(1):51.
- Hasan S. Pharmacological and medicinal uses of *Achyranthes aspera*. *Int J Sci Environ Technol.* 2014; 3(1):123-9.
- Srivastava PK. *Achyranthes aspera*: A potent immunostimulating plant for traditional medicine. *Int J Pharm Sci Res.* 2014; 5(5):1601. DOI: <https://dx.doi.org/10.1016/j.jep.2017.03.035>.
- He X, Wang X, Fang J, Chang Y, Ning N, Guo H, Huang L, Huang X. The genus *Achyranthes*: A review on traditional uses, phytochemistry, and pharmacological activities. *J Ethnopharmacol.* 2017; 203:260-278. DOI: <https://dx.doi.org/10.1016/j.jep.2017.03.035>.
- Rehman R, Melki D, Shehzad A, Nadeem F, Khalid T. Commercial importance, medicinal value and therapeutic potentials of chaff flower (*Achyranthes aspera*)—a review. *Int J Chem Biochem Sci.* 2018; 14:62-70.
- Abi Beaulah G, Mohamed Sadiq A, Jaya Santhi R. Antioxidant and antibacterial activity of *Achyranthes aspera*: An in vitro study. *Ann Biol Res.* 2011; 2(5):662-70.
- Narayan C, Kumar A. Identification and characterization of phenolic compounds in hydro methanolic extract of *Achyranthes aspera* (HMEA) by UPLC and MALDI-TOF-MS and in vivo antioxidant activity. *Orient Pharm Exp Med.* 2013; 13(1):51-9. DOI: <https://dx.doi.org/10.1007/s13596-012-0085-z>.
- Barua CC, Talukdar A, Begum SA, Pathak DC, Sarma DK, Borah RS, Gupta A. In vivo wound-healing efficacy and antioxidant activity of *Achyranthes aspera* in experimental burns. *Pharm Biol.* 2012; 50(7):892-9. DOI: <https://dx.doi.org/10.3109/13880209.2011.642885>.
- Khan N, Akhtar MS, Khan BA, Braga Vde A, Reich A. Antiobesity, hypolipidemic, antioxidant and hepatoprotective effects of *Achyranthes aspera* seed saponins in high cholesterol fed albino rats. *Arch Med Sci.* 2015; 11(6):1261-71. DOI: <https://dx.doi.org/10.5114/aoms.2015.56353>.
- Vijayaraj R, Kumar KN, Mani P, Senthil J, Jayaseelan T, Kumar GD. Hypoglycemic and antioxidant activity of *Achyranthes aspera* seed extract and its effect on streptozotocin induced diabetic rats. *Int J Biol Pharm Res.* 2016; 7:23-8.
- Manandhar N, Bajgain K, Neupane A. Study on Phytochemical Profile and Antioxidant Activity of *Achyranthes aspera* Whole Plant. *Medicine.* 2021; 3. DOI: <https://dx.doi.org/10.9734/IJBCRR/2021/v30i230251>.
- Sinan KI, Zengin G, Zheleva-Dimitrova D, Etienne OK, Fawzi Mahomoodally M, Bouyahya A, Lobine D, Chiavaroli A, Ferrante C, Menghini L, Recinella L, Brunetti L, Leone S, Orlando G. Qualitative Phytochemical Fingerprint and Network Pharmacology Investigation of *Achyranthes aspera* Linn. Extracts. *Molecules.* 2020; 25(8):1973. DOI: <https://dx.doi.org/10.3390/molecules25081973>.
- Chaturvedi S, Gupta P. Evaluation of Bioactive Metabolites and Antioxidant-Rich Extracts of *Amaranthus* with Possible Role in Pancreatic Lipase Interaction: In Silico and In Vitro Studies. *Metabolites.* 2021; 11(10):676. DOI: <https://dx.doi.org/10.3390/metabo11100676>.
- Pandey G, Rao CV, Gupta SS, Verma KK, Singh M. Antioxidant and antibacterial activities of leaf extract of *Achyranthes aspera* Linn.(Prickly Chaff Flower). *European J Med Plants.* 2014; 4(6):695.
- Sharma V, Chaudhary U, Singh R, Janmeda P. Evaluation of quantitative and antioxidant activity of *Achyranthes aspera* roots and inflorescences. *Asian J Pharm.* 2014; 8(1).
- Alluri R, Sagi AK, Pasala PK. Evaluation of gastric antiulcer and antioxidant activities in aqueous extracts of *Annona squamosa* and *Achyranthes aspera* in rats. *Int J Phytopharm.* 2011; 2(2).
- Baskar AA, Al Numair KS, Alsaif MA, Ignacimuthu S. In vitro antioxidant and antiproliferative potential of medicinal plants used in traditional Indian medicine to treat cancer. *Redox Rep.* 2012; 17(4):145-56. DOI: <https://dx.doi.org/10.1179/1351000212Y.0000000017>.
- Priya CL, Kumar G, Karthik L, Rao KB. Phytochemical composition and in vitro antioxidant activity of *Achyranthes aspera* Linn (*Amaranthaceae*) leaf extracts. *J Agric Technol.* 2012; 8(1):143-56.
- Talukder FZ, Khan KA, Uddin R, Jahan N, Alam MA. In vitro free radical scavenging and anti-hyperglycemic activities of *Achyranthes aspera* extract in alloxan-induced diabetic mice. *Drug Discov Ther.* 2012; 6(6):298-305.
- Rama P, Vignesh A, Lakshmanan GO, Murugesan K. In vitro antioxidant activity of *Achyranthes aspera* Linn. *Int J Med Pharm Sci.* 2013; 3:67-78.
- Upadhyaya V, Pai SR, Hegde HV. Effect of method and time of extraction on total phenolic content in comparison with antioxidant activities in different parts of *Achyranthes aspera*. *J King Saud Univ Sci.* 2015; 27(3):204-8. DOI: <https://dx.doi.org/10.1016/j.jksus.2015.04.004>.
- Devi KA, Pandey G, Rawat AKS, Sharma GD, Pandey P. The Endophytic Symbiont-*Pseudomonas aeruginosa* Stimulates the Antioxidant Activity and Growth of *Achyranthes aspera* L. *Front Microbiol.* 2017; 8:1897. DOI: <https://dx.doi.org/10.3389/fmicb.2017.01897>.
- Datta S, Sinha BK, Bhattacharjee S, Seal T. Nutritional composition, mineral content, antioxidant activity and quantitative estimation of water soluble vitamins and phenolics by RP-HPLC in some lesser used wild edible plants. *Heliyon.* 2019; 5(3):e01431. DOI: <https://dx.doi.org/10.1016/j.heliyon.2019.e01431>.
- Priyamvada PM, Sha A, Mohapatra AK. Evaluation of antidiabetic and antioxidant activities of *Achyranthes aspera* leaf extracts: An in vitro study. *J Pharmacogn Phytochem.* 2021; 10(4):103-10.
- Ndhala AR, Ghebrehiwot HM, Ncube B, Aremu AO, Gruz J, Subrtova M, Dolezal K, du Plooy CP, Abdelgadir HA, Van Staden J. Antimicrobial, anthelmintic activities and characterisation of functional phenolic acids of *Achyranthes aspera* Linn.: a medicinal plant used for the treatment of wounds and Ringworm in East

- Africa. *Front Pharmacol.* 2015; 6:274. DOI: <https://dx.doi.org/10.3389/fphar.2015.00274>.
30. Qadir MI, Khan TJ, Abbas G, Ahmad B, Janbaz KH, Ali M. Antibacterial activity of Vacuum liquid chromatography (VLC) isolated fractions of chloroform extracts of seeds of *Achyranthes aspera*. *J Chem Soc Pak.* 2012; 34(3).
 31. Owk AK, Mortha KR, Lagudu MN. Evaluation of antimicrobial activity of chemical constituents of *Achyranthes aspera* L. roots against human pathogens. *Indian J Nat Prod Resour.* 2014; 5(3):278-281.
 32. Rao DS, Penmatsa T, Kumar AK, Reddy MN, Gautam NS, Gautam NR. Antibacterial activity of aqueous extracts of Indian chewing sticks on dental plaque: An in vitro study. *J Pharm Bioallied Sci.* 2014; 6:S140-5. DOI: <https://dx.doi.org/10.4103/0975-7406.137426>.
 33. Prasad SH, Swapna NL, Anthonamma K, Rajasekhar D. Antimicrobial activity of *Achyranthes aspera* and *Aerva lanata* leaf and callus extracts. *Biosci Biotechnol Res Asia.* 2016; 6(2):887-91.
 34. Yadav R, Rai R, Yadav A, Pahuja M, Solanki S, Yadav H. Evaluation of antibacterial activity of *Achyranthes aspera* extract against *Streptococcus mutans*: An in vitro study. *J Adv Pharm Technol Res.* 2016; 7(4):149-152. DOI: <https://dx.doi.org/10.4103/2231-4040.191426>.
 35. Seetha J, Mallavarapu U, Mesa A. In situ green synthesis of antibacterial copper nanocomposite cotton fabrics using *Achyranthes aspera* leaf extract. *J App Pharm Sci.* 2020; 10(05):104-9. DOI: <https://dx.doi.org/10.7324/JAPS.2020.10514>.
 36. Nigusie D, Davey G, Legesse BA, Fekadu A, Makonnen E. Antibacterial activity of methanol extracts of the leaves of three medicinal plants against selected bacteria isolated from wounds of lymphoedema patients. *BMC Complement Med Ther.* 2021; 21(1):2. DOI: <https://dx.doi.org/10.1186/s12906-020-03183-0>.
 37. Alkari S, Chaturvedi A. Phytochemical basis of anti-inflammatory and ant-microbial activity of *Achyranthes aspera*. *Biol.* 2014; 1:128-38.
 38. Mishra D. Antibacterial activity of alkaloids present in plant *Achyranthes aspera*. *Pharma Innov J.* 2018; 7:147-53.
 39. Peter Amaladhas T, Usha M, Naveen S. Sunlight induced rapid synthesis and kinetics of silver nanoparticles using leaf extract of *Achyranthes aspera* L. and their antimicrobial applications. *Adv Mat Lett.* 2013; 4(10):779-85. DOI: <https://dx.doi.org/10.5185/amlett.2013.2427>.
 40. Jebashree HS, Kingsley SJ, Sathish ES, Devapriya D. Antimicrobial Activity of Few Medicinal Plants against Clinically Isolated Human Cariogenic Pathogens-An In Vitro Study. *ISRN Dent.* 2011:541421. DOI: <https://dx.doi.org/10.5402/2011/541421>.
 41. Konala G, Seva L, Davuluri SP. Antimicrobial activity of prickly chaff (*Achyranthes aspera*) seed trypsin inhibitor. *Int J Pharm Sci Res.* 2012; 3(9):3241.
 42. Murugan K, Sekar K, Sangeetha S, Ranjitha S, Sohaibani SA. Antibiofilm and quorum sensing inhibitory activity of *Achyranthes aspera* on cariogenic *Streptococcus mutans*: an in vitro and in silico study. *Pharm Biol.* 2013; 51(6):728-36. DOI: <https://dx.doi.org/10.3109/13880209.2013.764330>.
 43. Ahmad L, Hassan M, Khan A, Khan A. Antibacterial and antifungal activities of leaf extract of *Achyranthes aspera* (Amaranthaceae) from Pakistan. *Pak J Pharm Sci.* 2015; 28(5):1797-800.
 44. Haseena S, Shanavas S, Duraimurugan J, Ahamad T, Alshehri SM, Acevedo R, Jayamani N. Study on photocatalytic and antibacterial properties of phase pure Fe₂O₃ nanostructures synthesized using *Caralluma Fimbriata* and *Achyranthes Aspera* leaves. *Optik.* 2020; 203:164047. DOI: <https://dx.doi.org/10.1016/j.ijleo.2019.164047>.
 45. Pavithra S, Mohana B, Mani M, Saranya PE, Jayavel R, Prabu D, Kumaresan S. Bioengineered 2D ultrathin sharp-edged MgO nanosheets using *Achyranthes aspera* Leaf extract for antimicrobial applications. *J Inorg Organomet Polym Mat.* 2021; 31(3):1120-33. DOI: <https://dx.doi.org/10.1007/s10904-020-01772-7>.
 46. Saeed S, Nawaz S, Nisar A, Mehmood T, Tayyab M, Nawaz M, Firyal S, Bilal M, Mohyuddin A, Ullah A. Effective fabrication of zinc-oxide (ZnO) nanoparticles using *Achyranthes aspera* leaf extract and their potent biological activities against the bacterial poultry pathogens. *Mater Res Express.* 2021; 8(3):035004.
 47. Beg M, Athar F. Pharmacokinetic and molecular docking studies of *Achyranthes aspera* phytochemicals to exploring potential anti-tuberculosis activity. *J Bacteriol Mycol Open Access.* 2020; 8(1):18-27. DOI: <https://dx.doi.org/10.15406/jbmoa.2020.08.00268>.
 48. Kalaivanan C, Chandrasekaran M, Venkatesalu V. Screening of selected medicinal plants for in vitro antidermatophytic activity. *J Mycol Med.* 2013; 23(4):247-54. DOI: <https://dx.doi.org/10.1016/j.mycmed.2013.09.004>.
 49. Hasan K, Lakshmi T, Rathinam TK. Preliminary Phytochemical Analysis and In vitro Anti-helminthic activity of *Achyranthes aspera* Leaf extract. *Pharmacogn J.* 2015; 7(6). DOI: <https://dx.doi.org/10.5530/pj.2015.6.14>.
 50. Mukherjee H, Ojha D, Bag P, Chandel HS, Bhattacharyya S, Chatterjee TK, Mukherjee PK, Chakraborti S, Chattopadhyay D. Anti-herpes virus activities of *Achyranthes aspera*: an indian ethnomedicine, and its triterpene acid. *Microbiol Res.* 2013; 168(4):238-44. DOI: <https://dx.doi.org/10.1016/j.micres.2012.11.002>.
 51. Chinsembu KC. Ethnobotanical Study of Plants Used in the Management of HIV/AIDS-Related Diseases in Livingstone, Southern Province, Zambia. *Evid Based Complement Alternat Med.* 2016; 4238625. DOI: <https://dx.doi.org/10.1155/2016/4238625>.
 52. Dutta T, Ghorai S, Khan AA, Baildya N, Ghosh NN. Screening of potential anti-HIV compounds from *Achyranthes aspera* extracts for SARS-CoV-2: An insight from molecular docking study. *J Phys Conf S.* 2021; 1797(1):012042.
 53. Omidiani N, Datkhile KD, Barmukh RB. Anticancer potentials of leaf, stem, and root extracts of *Achyranthes aspera* L. *Not Sci Biol.* 2020;12(3):546-55. DOI: <https://dx.doi.org/10.15835/nsb12310764>.
 54. Anand M, Ranjitha J, Alagar M, Selvaraj V. Phytoconstituents from the roots of *Achyranthes aspera* and their anticancer activity. *Chem Nat Compd.* 2017;53(1):189-91. DOI: <https://dx.doi.org/10.1007/s10600-017-1946-y>.
 55. Adnyana DP, Meles ID, Meles W. Alkaloid fraction of jarong (*Achyranthes aspera* Linn) leaf induced apoptosis breast cancer cell through p53 pathways. *Adv Nat Appl Sci.* 2012; 6(2):124-8.
 56. Subbarayan PR, Sarkar M, Nagaraja Rao S, Philip S, Kumar P, Altman N, Reis I, Ahmed M, Ardalan B, Lokeshwar BL. *Achyranthes aspera* (Apamarg) leaf extract inhibits human pancreatic tumor growth in athymic mice by apoptosis. *J Ethnopharmacol.* 2012; 142(2):523-30. DOI: <https://dx.doi.org/10.1016/j.jep.2012.05.030>.
 57. Narayan C, Kumar A. Antineoplastic and immunomodulatory effect of polyphenolic components of *Achyranthes aspera* (PCA) extract on urethane induced lung cancer in vivo. *Mol Biol rep.* 2014; 41(1):179-91. DOI: <https://dx.doi.org/10.1007/s11033-013-2850-6>.
 58. Arora S, Tandon S. *Achyranthes aspera* root extracts induce human colon cancer cell (COLO-205) death by triggering the mitochondrial apoptosis pathway and S phase cell cycle arrest. *Sci World J.* 2014:129697. DOI: <https://dx.doi.org/10.1155/2014/129697>.
 59. Singh RK, Verma PK, Kumar A, Kumar S, Acharya A. *Achyranthes aspera* L. leaf extract induced anticancer effects on Dalton's Lymphoma via regulation of PKC α signaling pathway and mitochondrial apoptosis. *J Ethnopharmacol.* 2021; 274:114060. DOI: <https://dx.doi.org/10.1016/j.jep.2021.114060>.
 60. Tiwari LN, Vijayalaxmi M, Shailaja U, Bhandari M. Management of umbilical granuloma through chemical cauterization (Ksharakarma)-A case study. *J Ayurveda Integr Med.* 2021; 12(1):169-171. DOI: <https://dx.doi.org/10.1016/j.jaim.2020.10.003>.

61. Kumar A, Gnananath K, Gande S, Goud E, Rajesh P, Nagarjuna S. Anti-diabetic Activity of Ethanolic Extract of *Achyranthes aspera* Leaves in Streptozotocin induced diabetic rats. *J Pharm Res.* 2011; 4(7):3124-5.
62. Hivrale VK, Chougule NP, Giri AP, Chhabda PJ, Kachole MS. Biochemical characterisation of α -amylase inhibitors from *Achyranthes aspera* and their interactions with digestive amylases of coleopteran and lepidopteran insects. *J Sci Food Agric.* 2011; 91(10):1773-80. DOI: <https://dx.doi.org/10.1002/jsfa.4380>.
63. Zambare MR, Bhosale UA, Somani RS, Yegnanarayan R, Talpate KA. *Achyranthes aspera* (Agadha): Herb That Improves Pancreatic function in Alloxan Induced Diabetic Rats. *Asian J Pharm Biol Res.* 2011; 1(2).
64. Srinivasulu N, Mallaiah P, Sudhakar G, Rao BS, Kumari DS. Alpha amylase inhibitory activity and in vitro glucose uptake in psoas muscle and adipose tissue of male wistar rats of leaf methanolic extract of *Achyranthes aspera*. *J Pharmacogn Phytochem.* 2016; 5(3):176.
65. Njideka BE, Theophilus AE, Ugochukwu NT. Use of *Achyranthes aspera* Linn tea as antidiabetic and hypolipidemic herbal tea. *Int J Health Sci Res.* 2019; 9(2).
66. Latha BP, Vijaya T, Reddy RM, Ismail M, Rao SD. Therapeutic efficacy of *Achyranthes aspera* saponin extract in high fat diet induced hyperlipidaemia in male wistar rats. *Afr J Biotechnol.* 2011; 10(74):17038-42. DOI: <https://dx.doi.org/10.5897/AJB11.2100>.
67. Rani N, Sharma SK, Vasudeva N. Assessment of Antiobesity Potential of *Achyranthes aspera* Linn. Seed. *Evid Based Complement Alternat Med.* 2012; 2012:715912. DOI: <https://dx.doi.org/10.1155/2012/715912>.
68. Athesh K, Sivasubramanian R, Jothi G, Brindha P. Evaluation of anti-obesity potential of aqueous extract of *Achyranthes aspera* Linn. in high fat diet induced obese rats. *Clin Phytoscience.* 2020; 6(1):1-3. DOI: <https://dx.doi.org/10.1186/s40816-020-00217-5>.
69. Goli VE, Macharla SP, Gowrishankar NL, Dhanalakshmi C, Bhaskar JI, Bhaskar KV. Anti-pyretic activity of *Achyranthes aspera* Linn. *Int J Adv Pharm Sci.* 2011; 2(3):204-6.
70. Zheng W, Lu X, Fu Z, Zhang L, Li X, Xu X, Ren Y, Lu Y, Fu H, Tian J. Identification of candidate synovial membrane biomarkers after *Achyranthes aspera* treatment for rheumatoid arthritis. *Biochim Biophys Acta.* 2016; 1864(3):308-316. DOI: <https://dx.doi.org/10.1016/j.bbapap.2015.12.010>.
71. Kothavade PS, Bulani VD, Nagmoti DM, Deshpande PS, Gawali NB, Juvekar AR. Therapeutic Effect of Saponin Rich Fraction of *Achyranthes aspera* Linn. on Adjuvant-Induced Arthritis in Sprague-Dawley Rats. *Autoimmune Dis.* 2015:943645. DOI: <https://dx.doi.org/10.1155/2015/943645>.
72. Chinnasamy V, Subramanian V, Chandiran S, Kayarohanam S, Kannian DC, Velaga VSSR, Muhammad S. Antiarthritic Activity of *Achyranthes Aspera* on Formaldehyde - Induced Arthritis in Rats. *Open Access Maced J Med Sci.* 2019; 7(17):2709-2714. DOI: <https://dx.doi.org/10.3889/oamjms.2019.559>.
73. Khuda F, Iqbal Z, Khan A, Nasir F. Antimicrobial and anti-inflammatory activities of leaf extract of *Valeriana wallichii* DC. *Pak J Pharm Sci.* 2012; 25(4):715-9.
74. Bhosale UA, Yegnanarayan R, Pophale P, Somani R. Effect of aqueous extracts of *Achyranthes aspera* Linn. on experimental animal model for inflammation. *Anc Sci Life.* 2012; 31(4):202-6. DOI: <https://dx.doi.org/10.4103/0257-7941.107362>.
75. Lee JO, Yang WS, Park JG, Jeong D, Kim HG, Yoon KD, et al. Src and Syk contribute to the anti-inflammatory activities of *Achyranthes aspera* ethanolic extract. *J Ethnopharmacol.* 2017; 206:1-7. DOI: <https://dx.doi.org/10.1016/j.jep.2017.05.013>.
76. Mannan MA, Abir AB, Rahman MR, Khatun A, Datta B. Analgesic, antidepressant and anxiolytic effects of the methanolic extract of *Achyranthes aspera* leaves in mice. *Stamford J Pharm Sci.* 2019; 9:11-35.
77. Ambreen M, Mirza SA. Evaluation of anti-inflammatory and wound healing potential of tannins isolated from leaf callus cultures of *Achyranthes aspera* and *Ocimum basilicum*. *Pak J Pharm Sci.* 2020; 33:361-369.
78. Mengie T, Mequanente S, Nigussie D, Legesse B, Makonnen E. Investigation of Wound Healing and Anti-Inflammatory Activities of Solvent Fractions of 80% Methanol Leaf Extract of *Achyranthes aspera* L. (Amaranthaceae) in Rats. *J Inflamm Res.* 2021; 14:1775-1787. DOI: <https://dx.doi.org/10.2147/JIR.S298244>.
79. Nigussie D, Makonnen E, Tufa TB, Brewster M, Legesse BA, Fekadu A, Davey G. Systematic review of Ethiopian medicinal plants used for their anti-inflammatory and wound healing activities. *J Ethnopharmacol.* 2021; 276:114179. DOI: <https://dx.doi.org/10.1016/j.jep.2021.114179>.
80. Boyapati R, Gojja P, Chintalapani S, Nagubandi K, Ramiseti A, Salavadi SS. Efficacy of local drug delivery of *Achyranthes aspera* gel in the management of chronic periodontitis: A clinical study. *J Indian Soc Periodontol.* 2017; 21(1):46-49. DOI: https://dx.doi.org/10.4103/jisp.jisp_130_17.
81. Viswanatha GL, Venkataranganna MV, Prasad NBL, Shylaja H. *Achyranthes aspera* Linn. alleviates cerebral ischemia-reperfusion-induced neurocognitive, biochemical, morphological and histological alterations in Wistar rats. *J Ethnopharmacol.* 2019; 228:58-69. DOI: <https://dx.doi.org/10.1016/j.jep.2018.09.018>.
82. Gawande DY, Druzhilovsky D, Gupta RC, Poroikov V, Goel RK. Anticonvulsant activity and acute neurotoxic profile of *Achyranthes aspera* Linn. *J Ethnopharmacol.* 2017; 202:97-102. DOI: <https://dx.doi.org/10.1016/j.jep.2017.03.018>.
83. Viswanatha GL, Venkataranganna MV, Prasad NBL, Godavarthi A. *Achyranthes aspera* Attenuates epilepsy in experimental animals: possible involvement of GABAergic mechanism. *Metab Brain Dis.* 2017; 32(3):867-879. DOI: <https://dx.doi.org/10.1007/s11011-017-9981-8>.
84. Sharma J, Gairola S, Gaur RD, Painuli RM, Siddiqi TO. Ethnomedicinal plants used for treating epilepsy by indigenous communities of sub-Himalayan region of Uttarakhand, India. *J Ethnopharmacol.* 2013; 150(1):353-70. DOI: <https://dx.doi.org/10.1016/j.jep.2013.08.052>.
85. Bhosale U, Yegnanarayan R, Prachi P, Zambare M, Somani RS. Study of CNS depressant and behavioral activity of an ethanol extract of *Achyranthes Aspera* (Chirchita) in mouse model. *Ann Neurosci.* 2011; 18(2):44-7. DOI: <https://dx.doi.org/10.5214/ans.0972.7531.1118204>.
86. Goel RK, Gawande DY, Lagunin AA, Poroikov VV. Pharmacological repositioning of *Achyranthes aspera* as an antidepressant using pharmacoinformatic tools PASS and PharmaExpert: a case study with wet lab validation. *SAR QSAR Environ Res.* 2018; 29(1):69-81. DOI: <https://dx.doi.org/10.1080/1062936X.2017.1408683>.
87. Emon NU, Alam S, Rudra S, Chowdhury S, Rajbangshi JC, Ganguly A. Evaluation of pharmacological potentials of the aerial part of *Achyranthes aspera* L.: in vivo, in vitro and in silico approaches. *Adv Tradit Med.* 2020:1-4. DOI: <https://dx.doi.org/10.1007/s13596-020-00528-5>.
88. Rajpal Deshmukh G, Hema Kumar K, Suresh Reddy PV, Srinivasa Rao B, Venkata Satish Kumar C. Evaluation of Eye Irritation Potential of Aqueous Leaf Extract of *Achyranthes aspera* by In Vitro and In Vivo Method. *ISRN Toxicol.* 2012; 2012:693489. DOI: <https://dx.doi.org/10.5402/2012/693489>.
89. Chitra V, Manasa K, Mythili A, Tamilanban T, Gayathri K. Effect of Hydroalcoholic Extract of *Achyranthes Aspera* on Haloperidolinduced Parkinson's Disease In Wistar Rats. *Asian J Pharm Clin Res.* 2017:318-21. DOI: <https://dx.doi.org/10.22159/ajpcr.2017.v10i9.19285>.
90. Verma S. A review study on *Achyranthes aspera* (Amaranthaceae)-A valuable medicinal herb. *J Med Plant Stud.* 2016; 4(3):6-7.
91. Rahman HMA, Bashir S, Mandukhail SR, Huda S, Gilani AH. Pharmacological Evaluation of Gut Modulatory and Bronchodilator Activities of *Achyranthes aspera* Linn. *Phytother Res.* 2017; 31(11):1776-1785. DOI: <https://dx.doi.org/10.1002/ptr.5907>.

92. Anantha KC, Siva RC, Manohar RA. Hepatoprotective effect of biherbal ethanolic extract against paracetamol-induced hepatic damage in albino rats. *J Ayurveda Integr Med.* 2012; 3(4):198-203. DOI: <https://dx.doi.org/10.4103/0975-9476.104436>.
93. Kumar SS, Chandrika G, Mahesh K, Meghanath PV. Hepatoprotective activity of *Achyranthes aspera* linn against paracetamol induced toxicity. *Int J Pharm Pharm Sci.* 2012; 4(5):299-302.
94. Fahim NF, Sathi ZS. Assessment of hepatoprotective activity of roots and barks of *Achyranthes aspera* in carbon tetrachloride-induced hepatotoxicity in rats. *Malays J Halal Res.* 2018; 1(2):23-6.
95. Das AK, Bigoniya P, Verma NK, Rana AC. Gastroprotective effect of *Achyranthes aspera* Linn. leaf on rats. *Asian Pac J Trop Med.* 2012; 5(3):197-201. DOI: [https://dx.doi.org/10.1016/S1995-7645\(12\)60024-8](https://dx.doi.org/10.1016/S1995-7645(12)60024-8).
96. Shah B, Dudhamal TS. Efficacy of Apamarga Kshara application and Sclerotherapy in the management of Arsha (1st and 2nd degree piles) - An open-labeled, randomized, controlled clinical trial. *Ayu.* 2018; 39(4):213-219. DOI: https://dx.doi.org/10.4103/ayu.AYU_147_18.
97. Anuja MM, Nithya RS, Swathy SS, Rajamanickam C, Indira M. Spermicidal action of a protein isolated from ethanolic root extracts of *Achyranthes aspera*: an in vitro study. *Phytomedicine.* 2011; 18(8-9):776-82. DOI: <https://dx.doi.org/10.1016/j.phymed.2011.01.007>.
98. Kamble A, Reddy C, Patil S. Testicular activity of mice treated with MeOH extract of *Achyranthes aspera* leaves. *J Adv Med Sci App Technol.* 2017; 3(2):93-100. DOI: <https://dx.doi.org/10.18869%2Fnrip.jamsat.3.2.93>.
99. Gurumani M, Balamurugan K. Evaluation of antifertility potential of ethanolic extract of whole plant of *Achyranthes aspera* in female albino rats. *Int J Current Pharma Rev Res.* 2014; 5(1):31-6.
100. Balamurugan S, Vijayakumar S, Prabhu S, Morvin Yabesh JE. Traditional plants used for the treatment of gynaecological disorders in Vedaranyam taluk, South India - An ethnomedicinal survey. *J Tradit Complement Med.* 2017; 8(2):308-323. DOI: <https://dx.doi.org/10.1016/j.jtcm.2017.06.009>.
101. Chakrabarti R, Rao YV. *Achyranthes aspera* enhances immunity and antigen clearance in common carp, *Cyprinus carpio* L. *J Fish Dis.* 2012; 35(5):389-92. DOI: <https://dx.doi.org/10.1111/j.1365-2761.2012.01356.x>.
102. Chakrabarti R, Srivastava PK, Kundu K, Khare RS, Banerjee S. Evaluation of immunostimulatory and growth promoting effect of seed fractions of *Achyranthes aspera* in common carp *Cyprinus carpio* and identification of active constituents. *Fish Shellfish Immunol.* 2012; 32(5):839-43. DOI: <https://dx.doi.org/10.1016/j.fsi.2012.02.006>.
103. Gangopadhyay KS, Khan M, Pandit S, Chakrabarti S, Mondal TK, Biswas TK. Pharmacological evaluation and chemical standardization of an ayurvedic formulation for wound healing activity. *Int J Low Extrem Wounds.* 2014; 13(1):41-9. DOI: <https://dx.doi.org/10.1177/1534734614520705>.
104. Barua CC, Talukdar A, Begum SA, Handique AK, Handique GK, Roy JD, Buragohain B. Impact of *Achyranthes aspera* L. on protein profile in impaired wound models. *Indo Glob J Pharm Sci.* 2011; 1(1):13-24.
105. Fikru A, Makonnen E, Eguale T, Debella A, Abie Mekonnen G. Evaluation of in vivo wound healing activity of methanol extract of *Achyranthes aspera* L. *J Ethnopharmacol.* 2012; 143(2):469-74. DOI: <https://dx.doi.org/10.1016/j.jep.2012.06.049>.
106. Kothapalli L, Gite PR, Thomas A, Nanda R, Salvi N. Evaluation of antivenom potential of *Achyranthes aspera* leaves against snake venom. *Int J Pharm Drug Anal.* 2016:505-13.
107. Teshome D, Tiruneh C, Berhanu L, Berihun G, Belete ZW. Developmental Toxicity of Ethanolic Extracts of Leaves of *Achyranthes aspera*, *Amaranthaceae* in Rat Embryos and Fetuses. *J Exp Pharmacol.* 2021; 13:555-563. DOI: <https://dx.doi.org/10.2147/JEP.S312649>.