ACHYRANTHES ASPERA - A PHYTO-PHARMACOLOGICAL REVIEW

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Summary

Achyranthes aspera Linn., belonging to family Amaranthaceae, is commonly found as a weed on way side and at waste places throughout India. Phytochemical studies show that *A.aspera* contains water soluble base betain and chloroform soluble base is mixure of alkaloids. Various parts of the plants, viz, seeds, stem, leaves and roots are reported to contain ecdyterone. The food value of seeds in terms of its protein content is 24.8 and calorific value is 3.92/g. It is widely used for asthmatic cough, snakebite, hydrophobia, urinary calculi, rabies, influenza, piles, bronchitis, diarrhea, renal dropsy, gonorrhea and abdominal pain. A powder of dried leaf mixed with honey is useful in the early stages of asthma. One of the drugs from Siddha system of medicine, Naayuruvi kuzhi thailum has *A. aspera* as the primary constituent is reported to be quite effective in the management of asthma. This review describes the phytochemistry and pharmacological aspects of this plant.

Introduction

Achyranthes aspera is known as Apamarg in Sanskrit, Aghedo and Aghedi in Gujarati, Chirchira and Chirchitta in Hindi and Prickly chaff flower in English.

***Figure: Achyranthes aspera* Linn.**

Phytochemical Studies

The plant is reported to yield a water-soluble base and a chloroform soluble base. The former was earlier designated as achyranthine[8]. and was characterized as a betaine derivative of N-methylpyrrolidine-3-carboxylic acid[9]. Later studies by Kapoor and Singh[10] showed that the water soluble base was betaine and not achyranthine. The chloroform soluble basic fraction was shown to be a mixer of two uncharacterized alkaloidal entities[11]. The ethanol extract of the plant contained alkaloids and saponins while flavonoids and tannins were found absent [12].

The shoot yielded a new aliphatic dihydroxyketone, characterized as 36,47- dihydroxyhenpentacontan-4-one together with tritriacontanol[13]; an essential oil; a new long chain alcohol characterized as 17-pentatriacontanol[14]; four new compounds

characterized as 27-cyclohexylheptacosan-7-ol, 16-hydroxy-26-methylheptacosan-2-one[15], 4-methylheptatriacont-1-en-10-ol and tetracontanol-2 [16].

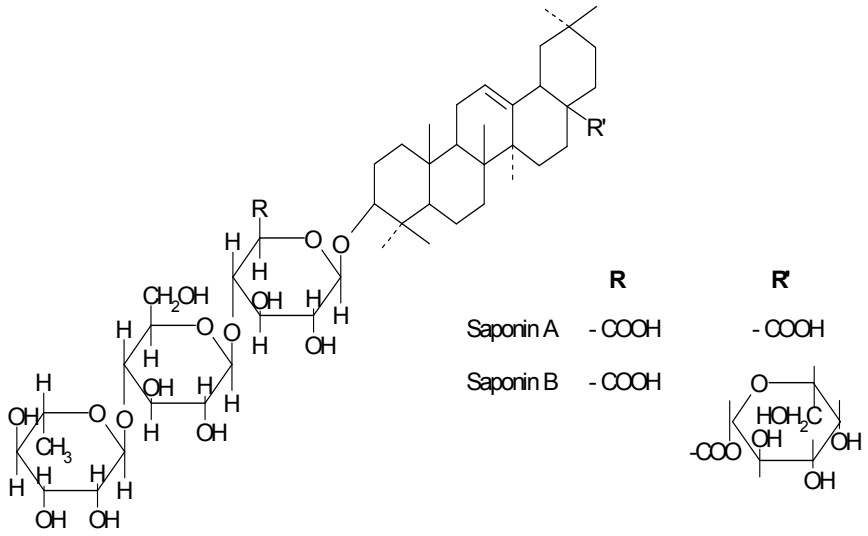
Various parts of the plant, viz., seeds, stem, leaves[17] and root[18] are reported to contain ecdysterone. The chloroform extract of the stem led to the isolation of pentatriacontan, 6-pentatriacontanone, hexatriacontane and triacontane[19]. The inflorescence is reported to contain flavonoids and alkaloids[20].

The food value of the seeds in terms of its protein quality is also reported. The composition of the seeds has close similarity to Bengal gram with a protein content of 24.8 and calorific value of 3.92/g. The hydrolysate contains the usual amino acids. The values obtained for ten essential amino acids and cystine shows that the seed protein can be compared favorably with Bengal gram in its leucine, isoleucine, phenylalanine and valine content, while its tryptophan and sulphur amino acid (methionine and cystine) content are higher than most of the pulses. It is however, deficient in arginine, lysine and threonine as compared to the whole egg protein [21].

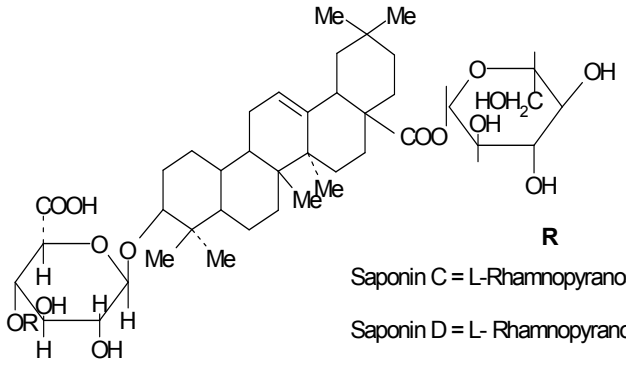
The defatted seeds are reported to yield a saponin in a yield of 2 %, which was identified as oleanolic acid-oligosaccharide. The sugar moiety of the saponin was composed of glucose, galactose, xylose and rhamnose[22, 23]. Khastgir and associates[24] isolated a crude sapogenin fraction from the seeds, which yielded oleanolic acid. Later, investigation led to the isolation of two oleanolic acid based saponins, saponin A and saponin B which were characterized as α–L-rhamnopyranosyl (1→4)-β-D-glucopyranosyl (1→4)-β-D-glucuronopyranosyl (1→3)-oleanolic acid and β-D-galactoyranosyl (1→28) ester of saponin A, respectively[25]. In another study, the total saponins were hydrolysed with acid and the genin was identified as oleanolic acid[26]. A rapid procedure for the separation of triterpenoid saponin based on partition chromatography from the plant has been described[27]. The seeds are reported to contain hexatriacontane, 10-octacosanone, 10-triacosanone and 4-triacontanone[19]. The unripe fruit is reported to yield two new saponins (C and D), which were identified as β-D-glucopyranosyl ester of α-L-rhamnopyranosyl (1→4)- β-D-glucuronopyranosyl (1→3)-oleanolic acid and β-D-glucopyranosyl ester of α–L-rhamnopyranosyl (1→4)-β-D-

glucopyranosyl (1→4)-β-D-glucuronopyranosyl (1→3)-oleanolic acid[28].

The chemical constituents of the root varied in different preliminary studies carried out. The root was found to contain oleanolic acid as the aglycone from the saponin fraction[24]. Both root and shoot of the plant were found to contain saponin and alkaloids but no flavonoids[20]. In another study, the root of the plant was found to contain alkaloids but indicated absence of saponin and tannins[29]. In yet another preliminary chemical study, the root was reported to contain alkaloids, flavonoids, saponins, steroids and terpenoids. Glycosides were found to be absent[30]. Isolation of β-sitosterol was also reported from the root[15].



Structure formula of Saponin A and B



Structure formula of Saponin C and D

Pharmacological Studies

Anti inflammatory activity

An alcohol extract of *Achyranthes aspera* showed the anti-inflammatory activity on carrageenan-induced hind paw oedema and cotton pellet granuloma models in albino male rats[31]. It is also reported that the ethanolic extract of *A. aspera*, in the doses of 100-200 mg/kg possess anti-inflammatory and anti-arthritic activity[32]. The water soluble alkaloid achyranthine isolated from *A. aspera* was screened for its anti- inflammatory and antiarthritic activity against carrageenan-induced paw oedema, granuloma pouch, formalin induced arthritis and adjuvant arthritis in rats. It showed significant anti-inflammatory activity in all the four models employed but was less active than phenylbutazone and betamethasone. Further, achyranthine significantly reduced the weight of adrenal gland, thymus and spleen and raised the adrenal ascorbic acid and cholesterol contents. The effects were qualitatively similar to betamethasone. All the three drugs tested reduced food intake but had no significant effect on urinary and faecal output and mortality rate. Incidence of gastric ulcers was maximum with betamethasone and minimum with achyranthine.

Anti-microbial activity

The aqueous solution of the base achyranthine as well as the entire plant of *A. aspera* showed antibacterial activity against *Staphylococcus aureus, Streptococuss heamolyticus* and *Bacillus typhosus*[33]. While the alcoholic and the aqueous extract of the leaves showed antibacterial activity against *S. aureus* and *E. coli*[34]. The seeds growing on cattle dung revealed antibacterial activity against bacterial strains of *B. subtilis, Pseudomonas cichorii* and *Salmonella typhimurium*[35]. In another study, the 80 percent ethanolic extract of the leaves and stem of the plant inhibited *B. subtilis* and *S. aureus* bacterial strains at a concentration of 25 mg/ml[36].

Anti fertility activity

The ethanol extract of the root was screened for antifertility activity in proven fertile female albino rats at 200 mg/kg body weight and given orally on days 1-7 of pregnancy. The ethanol extract exhibited 83.3 % anti-implantation activity when given orally at 200 mg/kg body weight. The rats, which continued their pregnancy, did not deliver any litters after their full term. Hence the combined antifertility (anti-implantation and abortifacient) activity of ethanol extract was 100 %. The ethanol extract also exhibited estrogenic activity tested in immature ovariectomised female albino rats[37]. The methanolic extract of the root revealed 60 percent anti-implantation activity in rats while the acetone extract of the root prevented implantation in 50 percent of rats[38]. The effect of a composite plant extract of the leaf of *Stephania hernandifolia* and the root of *Achyranthes aspera* on sperm motility and function in a ratio of 1:3 by weight at different concentrations was studied. At a concentration of 0.32 g/ml, this composite extract showed the promising results by complete sperm immobilization within 2 min after the application of the extract. The effects were spermicidal but not spermiostatic as sperm immobilization effect was found to be irreversible. Sperm viability was decreased significantly and was found to be nonviable after 30 min when treated with the composite extract at a concentration of 0.32 g/ml. The hypo-osmotic swelling of these sperm was reduced significantly at this highest concentration, indicating that the crude extract may probably cause injury to the sperm plasma membrane[39]. The methanolic leaves extract of *Achyranthes aspera* on some indicators for anti-fertility

activities such as abortifacient, estrogenesity, pituitary weight, and ovarian hormone level and lipids profile in female rats was investigated. The extract showed significant abortifacient activity and increased pituitary and uterine wet weights in ovarectimized rats. The extract, however, did not significantly influence serum concentration of the ovarian hormones and various lipids except lowering HDL at doses tested[40].

The benzene extract of stem bark at 50 mg/kg prevented pregnancy (100%) in mice when given orally either on day 1 or 6 post-coitum[41]. The crude benzene extract of the stem was found to have potent abortifacient effect in mice[42]. In an attempt to locate the active principle, various chromatographic fractions were tested for anti fertility activity in female mice. The maximal activity was found to be located in the fraction eluted with 50 percent benzene in petroleum ether[43]. The ethanolic extract of the plant (excluding root) at a dose of 100-200 mg/kg body weight administered orally revealed 60 percent anti-fertility activity on early pregnancy in rats. Further, the plant also showed potent activity at secondary testing level[44]. The n-butanol fraction of the aerial parts prevented pregnancy in adult female rats when administered orally at a daily dose of 75 mg/kg or more on day 1-5 post coitum, but was ineffective in hamsters up to 300 mg/kg dose. No anti-fertility activity was observed in the aqueous fraction in either rats or hamsters. In ovariectomized immature female rats, the extract exhibited potent estrogenic activity at a dose of 75 mg/kg. It induced a marked stimulation in uterine weight. Marked uterotrophic effect was discerned even at a dose of 3.75 mg/kg[45]. In another study, it was found that feeding 50% ethanolic extract of *A. aspera* to male rats resulted in reduced sperm counts, weight of epididymis, serum level of testosterone and testicular activity of 3 beta-hydroxysteroid dehydrogenase, while motility of the sperm and activity of the HMG CoA reductase were not affected. Cholesterol level in the testis, incorporation of labeled acetate into cholesterol, 17-ketosteroids in urine and hepatic and fecal bile acids were increased suggesting reproductive toxicity in male rats and the action may be by suppressing the synthesis of androgen[46].

Extracts of the whole plant had shown an abortifacient effect in mice. Maximal activity was in the benzene extract which was tested. Ovaries contained prominent corpus luteum, indicating that

the drug had prevented pregnancy. In rats, no effect was observed. Progesterone or pituitary extract given along with the drug did not prevent abortions in mice suggesting that drug is species-specific in that no abortifacient effect was found in rats[64]. A benzene fraction of the benzene extract of the whole plant showed abortificient activity in rabbit at a single dose of 50 mg/kg[47].

Immunomodulatory activity

The extract of *Achyranthes aspera* Linn. was found to enhance the induction of ovalbumin (OVA)-specific humoral antibody response in mice, on intra peritoneal injection of extract along with OVA. Furthermore, the plant extract was found to increase the induction of OVA-specific antibody response in a dose-dependent manner. A significant elevation of IgM, IgG 1 and IgG 3 antibodies was observed; however, interestingly, the anti-OVA PCA titers were suppressed. The adjuvant property of the extract was further examined in different strains of mice and a significant elevation of the OVA-specific IgG antibody response in all strains tested was found. When the extracts of different parts of the herb were tested, the seed and root extracts appeared to exhibit relatively higher activity[48]. *Catla catla* were fed a diet containing seed of *Achyranthes aspera* (0.5 %) and control diet without *A. aspera* for four weeks prior to and after i.p. injection with chicken erythrocytes. Hemagglutination antibody titers, anti-trypsin activity due to total serum protease inhibitors, alpha-1-antiprotease, RNA/DNA ratio of spleen and kidney were significantly higher in the test group of fishes compared with the control group. Serum globulin levels were significantly higher in the test group than control group on days 14 and 21. All these results confirm that *A. aspera* enhances the immunity of catla[49]. Immunomodulatory activity of *Achyranthes aspera* seed was studied by incorporating it in the diets of *Labeo rohita*, rohu fingerlings. Superoxide anion production, serum bactericidal activity, lysozyme, ALP, serum protein, albumin : globulin ratio (A/G) were enhanced in Achyranthes treated groups compared to the control group. SGOT and SGPT levels were elevated in control group, but in Achyranthes treated groups the levels were similar to the uninfected-control group. Higher cumulative mortalities were observed in the control group (77 %) up to day-9 after infection. This gradually decreased with increasing dose of Achyranthes indicating that *Achyranthes aspera* stimulates

immunity and increases resistance to infection in *L. rohita*[50]. In another study, *Achyranthes aspera* was incorporated in artificial fish diet, and fed to *Catla catla*. Achyranthes has significantly ($P<0.05$) enhanced the BSA-specific antibody titers than the untreated control group throughout the study period. The efficiency of antigen clearance was also enhanced in *C. catla* treated with Achyranthes [51].

Anti-hyperlipidemic activity

The alcoholic extract of the plant *A. aspera* at 100 mg/kg dose lowered total serum cholesterol (TC) and phospholipid (PL), triglyceride (TG) and total lipids (TL) levels by 60, 51, 33 and 53 percent, respectively in triton-induced hyperlipidemic rats. The chronic administration of the extract at the same doses to normal rats for 30 days, lowered serum TC, PL, TG, and TL by 56,62,68 and 67 %, respectively followed by significant reduction in the levels of hepatic lipids. The possible mechanism of action of cholesterol lowering activity of the plant might be due to rapid excretion of bile acids causing low absorption of cholesterol[52].

Anti-diabetic activity

The 50 % ethanolic extract of entire plant[53] was screened for preliminary biological activities. It showed hypoglycemic activity in rat. It was devoid of anti bacterial, anti fungal, anti protozoal, anthelmintic, antiviral and anticancer activities and effects on isolated guinea pig ileum, respiration, CVS and CNS in experimental animals. The MTD on the extract was found to be 1000 mg/kg b.wt. orally in mice[53]. In another study, it was found that oral administration of 2-4 g/kg of whole plant powder produced a significant dose-related hypoglycemic effect in normal as well as alloxan treated diabetic rabbits. The aqueous and methyl alcohol extracts of the plant also decreased blood glucose levels in normal and alloxan induced diabetic rabbits[54].

Diuretic activity

The saponin isolated from the seeds of *A. aspera* in 10-20 mg/kg i.m. doses in rats caused significant increase in urine output after 2, 6 and 24 hour as compared to untreated rats. The diuretic effect was comparable to that observed with 3 mg/kg dose of

mersalyl. The optimum dose of the saponin was 10 mg/kg. After oral administration of the saponin (5-10 mg/kg) in rats, a significant increase in urine output was observed which was comparable to that of 10 mg/kg oral dose of acetazolamide. The diuretic effect of saponin, like acetazolamide, was associated with an increase in the excretion of sodium and potassium in the urine[55].

Activity on Cardiovascular system

The mixture of saponins isolated from the seeds of *A. aspera* caused a significant increase in force of contraction of the isolated heart of frog, guinea pig and rabbit. The stimulant effect of the lower dose (1 to 50 μg) of the saponins was blocked by pronethol and partly by mepyramine. The effect of higher dose was not blocked by pronethol. The saponin increases the tone of the hypodynamic heart and also the force of contraction of failing papillary muscle. The effect was quicker in onset and shorter in duration in comparison to that exerted by digoxin[55]. The effect of saponin on the phosphorylase activity of the perfused rat heart has been investigated and compared with that of adrenaline. The saponin has been found to stimulate the phosphorylase activity of the heart and its effect was comparable to that of adrenaline[56]. In a preliminary study, the aqueous and alcoholic extracts of the roots of *A. aspera* caused a sharp and transient fall in blood pressure without any significant action on the respiration of anaesthetized dogs. In higher doses, there was slight respiratory depression. Atropine sulphate blocked the hypotensive effect of the extracts. On frog's heart the extracts had a temporary negative ionotropic and chronotropic effects. The extracts produced spasm of isolated rabbit's ileum, increased the tone and amplitude of contractions in gravid and non-gravid uteri of albino rats, guinea pigs and rabbits. Oral administration of the drug significantly increases the urine output in rabbits[57]. The total chloroform soluble basic fraction (alkaloidal residue) obtained from the plant *A. aspera* raised the blood pressure of anaesthetized dog, caused initial transitory stimulation of respiration and increased the amplitude of cardiac contractions of isolated guinea pig heart[11]. The water-soluble alkaloid, achyranthine isolated from the plant was found to lower blood pressure, depress the heart, dilate the blood vessels and increase the rate and amplitude of respiration anaesthetized dogs[33, 58].

Anti-carcinogenic activity

Achyranthes aspera leaves have been assessed for chemopreventive activity. The methanolic extract, alkaloid, non-alkaloid and saponin fractions exhibited significant inhibitory effects (concentration 100 μg) on the Epstein-Barr virus early antigen activation induced by the tumor promoter 12-O-tetradecanoylphorbol-13-acetate in Raji cells. In this in vitro assay the non-alkaloid fraction containing mainly nonpolar compounds showed the most significant inhibitory activity (96.9 %; 60 % viability). In the in vivo two-stage mouse skin carcinogenesis test the total methanolic extract possessed a pronounced anti-carcinogenic effect (76 %)[59].

Miscellaneous

The effects of *Achyranthes aspera* leaf extract on body weight, hepatic protein content, lipid peroxidation (LPO), superoxide dismutase (SOD) and catalase (CAT) activities and on serum triiodothyronine (T3), thyroxine (T4) and glucose levels were evaluated. The extract exhibited significant prothyroidic activity as it enhanced the levels of both the thyroid hormones along with an increase in serum glucose concentration, body weight and hepatic protein content. On the other hand, it decreased hepatic LPO without altering the activities of the two antioxidant enzymes, SOD and CAT significantly, suggesting a direct free radical scavenging activity of the extract[60]. Fresh leaf extracts of *Achyranthes aspera* were tested against Alternatia alternate causing leaf spot disease of *Vicia faba*. Inhibition of growth was recorded[61]. The alkaloidal fraction obtain from the alcoholic extract of the root bark of *A. aspera* inhibited the response of oxytocin in isolated rat uterus. This fraction did not inhibit the response to serotonin and acetylcholine in rat uterus and to histamine in guinea pig uterus[62]. The total chloroform soluble basic fraction (alkaloidal residue) obtained from the plant *A. aspera* showed spasmolytic action against various spasmogens on intestine and uterine muscles of guinea pigs and a slight anti-diuretic action in rats. No specific CNS effects were observed in mice. The fraction did not possess analgesic activity in rats[11]. The water-soluble alkaloid, achyranthine isolated from the plant showed spasmogenic effect on frog's rectus muscle and diuretic as well as purgative action in albino rats. No effect was observed on isolated rabbit,

guinea pig and rat ileum and on CNS. The drug exerted a slight antipyretic effect[33, 58]. The ethanolic extracts of leaves[53] were screened for preliminary biological activities. The leaf extract was found to be devoid of anti protozoal and antiviral activities and effects on respiration, pre-ganglionically stimulated nictitating

membrane, CVS and CNS in experimental studies. The LD_{50} of the latter extract was >1000 mg/kg i.p. in mice[63].

Clinical Studies

The plant was subjected to wide clinical evaluation with special reference to its use in leprosy, fistula-in-ano and bronchial asthma. Diuretic activity could not be confirmed.

Leprosy

The effect of oral decoction of *A. aspera* in the treatment of leprosy was studied (uncontrolled) in 19 patients who were found to have positive stain smears at the S. S. Hospital, Varanasi. Fourteen patients were in stage of reaction and rest of them had active lesions but none of them was in quiescent stage. The study revealed encouraging results in both lepra reaction as well as the quiescent stage of lepromatous leprosy[64]. In an attempt to get additional data on the efficacy of the decoction of *A. aspera*, it was observed that the decoction was useful in the treatment of reaction in leprosy particularly in subacute and mild type. When administered in conjunction with the antileprosy drug diaminodiphenylsulphone (DDS), it was found that the chance of reaction became less and rate of improvement was faster. No toxic manifestation, which could be attributed to *A. aspera* was noted during the trial[64].

Fistula-in-ano

The studies revealed that the long term use of 'Kshaarasootra' (a medical thread prepared by coating the latex of *Euphorbia neriifolia*, alkaline powder of *A. aspera* and *Curcuma longa*) was quite effective in treatment of various fistulous tracks[67-73]. The Indian Council of Medical Research has carried out a multicentric randomized controlled trial to evaluate the efficacy of 'Kshaarasootra' in the management of fistula-in-ano (265 patients)

in comparison with the conventional surgery (237 patients). The results have revealed that the long-term outcome with 'kshaarasootra' (recurrence 4 percent) was better than with the surgery (recurrence 11 percent), although the initial healing time was longer (8 wk with thread and 4 wk with surgery). "Kshaarasootra' offered an effective, ambulatory and safe alternative treatment for patient with fistula-in-ano. "Kshaarasootra' has also been found to give encouraging results in 5 patients of chronic non healing milk-fistula 'stannadi-vrana' with additional local application of 'jatyaditaila' and oral administration of ' shigru guggulu' (two tablets t.i.d.) during the course of treatment[74].

Bronchial Asthma

A pilot study was carried out at the Central Research Institute for Siddha in Madras on 15 cases of bronchial asthma. The oil obtained from the root of *A. aspera* soaked in cow urine was smeared on betel leaf and administered thrice a day to these patients. In most of the cases symptoms like wheezing, gasping, dyspnoea, sneezing and cough disappeared. A fall in total WBC, eosinophil counts and ESR was observed[7].

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